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EXTRACTION OF CANOLA OIL WITH SUPERCRITICAL CARBON DIOXIDE

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Extraction of Canola oil with Supercritical Carbon Dioxide

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SUMMARY

The objective of the study was to extract canola oil from seed (*Brassica napus*) by means of supercritical carbon dioxide (*sc*-CO_2). Extractions were performed with a laboratory scale supercritical fluid extractor (LECO TFE 2000) of the latest design and performance. Extracts were analysed by GC-FID and GC-GC/TOF-MS and a total of 49 components in the extracted oil could be identified. The composition of the sc-CO_2 derived canola oil was in excellent agreement with that of canola oil in the marketplace, while the chemical specification of the sc-CO_2 oil was benchmarked against an industrial standard.

The density of *sc*-CO_2 was found to be the variable which controls the extraction as the fluid becomes capable of chemically dissolving the canola oil from the seed matrix once its density (and thus its solvent strength) approaches liquid-like values ($0.8 < \rho < 1.0$ g/mL). The solubility of the oil in sc-CO_2 could be measured at a given set of conditions (0.036 g per gram of sc-CO_2 at 300 atm and 50^{0}C) by utilising the static extraction mode of the supercritical extractor, whereas the oil content of the seed could by determined (0.14 - 0.22 g oil per gram of seed) by exhaustive extraction utilising the dynamic mode of the supercritical extractor.

The conditions at which a maximum yield of oil could be obtained (80°C, 600 atm, 70 min) were determined by performing runs according to a statistical design and processing the data by computer assisted surface response analysis. The temperature and pressure dependencies of the extraction were also studied in order to calculate activation parameters ($E_a \sim 0$ kJ/mol, $\Delta V^{\neq} = -130$ mL/mol) and hence assisted in confirming the mechanistic steps (chemical dissolution, diffusion controlled transport) of the extraction process.

Finally the effect of pre-drying of seed and using different cultivars of seed on the extraction was studied.

OPSOMMING

Die doel van die studie was om kanola-olie uit saad (*Brassica napus*) met behulp van superkritieke koolstofdioksied (sc-CO_2) te ekstraheer. Die ekstraksies is uitgevoer met 'n laboratoriumskaal superkritieke-fluïed-ekstraktor (LECO TFE 2000) van die jongste ontwerp en werkverrigting. Die ekstrakte is geanaliseer met GC-FID en GC-GC/TOF-MS en 'n totaal van 49 komponente in die geëkstraheerde olie is geïdentifiseer. Die samestelling van die sc-CO_2 verkreë kanola-olie het met dié van kanola-olie in die handel ooreengestem, terwyl die chemiese spesifikasies van die olie gemeet is aan dié van 'n nywerheidstandaard.

Dit kon vasgestel word dat die digtheid van sc-CO_2 die veranderlike is wat die ekstraksie beheer aangesien die fluïed in staat is om die kanola-olie chemies op te los vanuit die saadmatrys sodra die digtheid (en dus die oplosmiddelsterkte) van die fluïed vloeistoftipe waardes ($0.8 < \rho < 1.0$ g/mL) aanneem. Die oplosbaarheid van die olie in sc-CO_2 by bepaalde kondisies (0.036 g per gram sc-CO_2 by 300 atm en 50^{0}C) kon gemeet word deur van die statiese ekstraksiemodus van die superkritieke ekstraktor gebruik te maak, terwyl die olie-inhoud van die saad (0.14 - 0.22 g olie per gram saad) bepaal kon word deur uitputtende ekstraksie met behulp van die dinamiese ekstraksiemodus van die ekstraktor.

Die kondisies waarby 'n maksimum olie-opbrengs verkry kon word (80°C, 600 atm, 70 min) is bepaal deur lopies volgens 'n statistiese ontwerp uit te voer en die data met behulp van rekenaar gesteunde oppervlakresponsanalise te verwerk. Die temperatuur- en drukafhanklikheid van die ekstraksie van kanola-olie is ook bestudeer ten einde aktiveringsparameters te bereken ($E_a \sim 0$ kJ/mol, $\Delta V^{\neq} = -130$ mL/mol) ten einde die meganistiese stappe van die ekstraksieproses (chemiese oplos, diffusiebeheerde vervoer) te bevestig.

Ten slotte is die effek van vooraf gedroogde saad en van die gebruik van verskillende saadkultivars op die ekstraksie nagegaan.

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CHAPTER 1

PROJECT DELIMITATION

This study was undertaken to complement the supercritical carbon dioxide (sc-CO_2) plant extractions performed to date within the Separation Science and Technology (SST) focus area of the North-West University Potchefstroom Campus. These extractions include peppermint from *Mentha piperita*[1,2], aspalathin from *Aspalathus linearis*[3] (rooibos tea), sunflower oil from *Helianthus annuus*[4], components from *Melissa officinalis* (lemon balm)[5], artemisinin from *Artemisia annua*[6], components from *Chamaemelum nobile* (Roman chamomile)[7], olive oil from *Olea europea*[8], lutein from *Tagetes erecta* (marigold flower)[9], soybean oil from *Glycine max*[10] and harpogoside from *Harpagophytum procumbens* (devil's claw) [11].

1.1. OBJECTIVES

Objectives were determined prior to starting the study. These included:

1. to extract canola oil from seed by adopting a non-hazardous *sc*-CO_2 based extraction method;

2. to optimise the yield and composition of the extracted canola oil by manipulating the process variables (time, temperature, pressure, flow rate, cosolvent);

3. to identify, establish and implement chemical and instrumental methods of analysis to determine the composition and purity of the extracted oil;

4. to establish the quality of the extracted oil by comparison to commercial canola oil and available benchmark standards;

5. to obtain a better understanding of the mechanism of sc-CO_2 extraction by studying process dependencies and calculating activation parameters;

6. to compare the oil derived from different cultivars of canola seed.

1.2. STRATEGIES

To achieve the above objectives a set of strategies was developed which included:

1. to acquire canola seed of consistent quality and to adopt a method of sample preparation that warrants efficient oil extraction on laboratory scale by using an advanced supercritical fluid extractor;

2. to develop a *sc*-CO_2 based extraction procedure for the production of canola oil from pre-prepared samples of seed;

3. to develop a suitable chromatographic based protocol (GC-FID, GC-GC/TOF-MS) for the analysis of the extracted oil in terms of yield and composition;

4. to prepare a computer assisted statistical design (Statistica for Windows®) which permits optimisation of process conditions by surface response analysis based on a minimum number of extraction runs;

5. to study the dependence of yield and composition on different process variables in order to elucidate the mechanism of extraction as a means of "tuning" the process towards extracts of preferred composition or characteristics;

6. to evaluate *sc*-CO_2 derived canola oil against commercially accepted canola oil or published reference/benchmark figures for oil in the market place;

7. to cooperate with knowledgeable people in the agricultural sector to test *sc*-CO_2 extracted canola oil for hazardous components, environmental contaminants and health detrimental effects.

1.3. FOCUS AREA OBJECTIVES

There were a few focus area objectives which included:

1. to use sc-CO_2 as an alternative to the cold press method in order to obtain higher yields within shorter extraction times and to acquire solvent-free extracts from the residual "cake" that results from cold pressing and is normally subjected to subsequent solvent extraction;

2. to contribute towards the use of clean technology by emphasising the need for sustainable or "green" chemistry in general, and by promoting the use of environmentally friendly solvents, such as CO_2, in the food industry in particular;

3. to add to the development and implementation of supercritical technology as a major technique for botanical extraction.

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CHAPTER 2

INTRODUCING CANOLA OIL

The term "canola"[1] is derived from **Can**adian **o**il **l**ow erucic **a**cid and is a registered trademark of the Canadian Canola Association. It refers to cultivars of rapeseed oil which contains less than 2% erucic acid (22:1) and meals with less than 30 µmol of aliphatic glucosinolates per gram.

There are several terms used to describe rapeseed. These include "canola", "industrial rapeseed" and "specialty canola".

"Industrial rapeseed" refers to cultivars that produce oils with 45% or more erucic acid and seed meals with insufficient glucosinolate levels. Cultivars with these characteristics are used primarily for non-edible purposes such as lubricants and hydraulic fluids.

"Specialty canola" relates to canola with increased heat stability. These oils are used in high temperature or continuous frying. Specialty canola cultivars normally produce oils that contain less than 4% linolenic acid (18:3) and/or more than 70% oleic acid (18:1). Oils from these cultivars have improved temperature stability and shelf life.

2.1. ORIGIN AND TAXONOMY

Brassica crops are among the oldest cultivated plants known with written records dating back to 1500 BC[2]. *Brassica rapa* seems to have had the widest distribution historically. At least 2000 years ago it was distributed from northern Europe to China and Korea, with a primary center of diversity in the Himalayan region[3].

Brassica napus is the direct cross between "birdsrape mustard" (*Brassica rapa*) and "wild cabbage" (*Brassica oleracea*) due to genetic similarities[4]. *Brassica napus* is believed to have developed in the Mediterranean area. It is possible that it originated by cultivation, since no wild forms are known. Production of oil seed *B. napus* probably started in Europe during the middle ages where its oil was used as lamp oil.

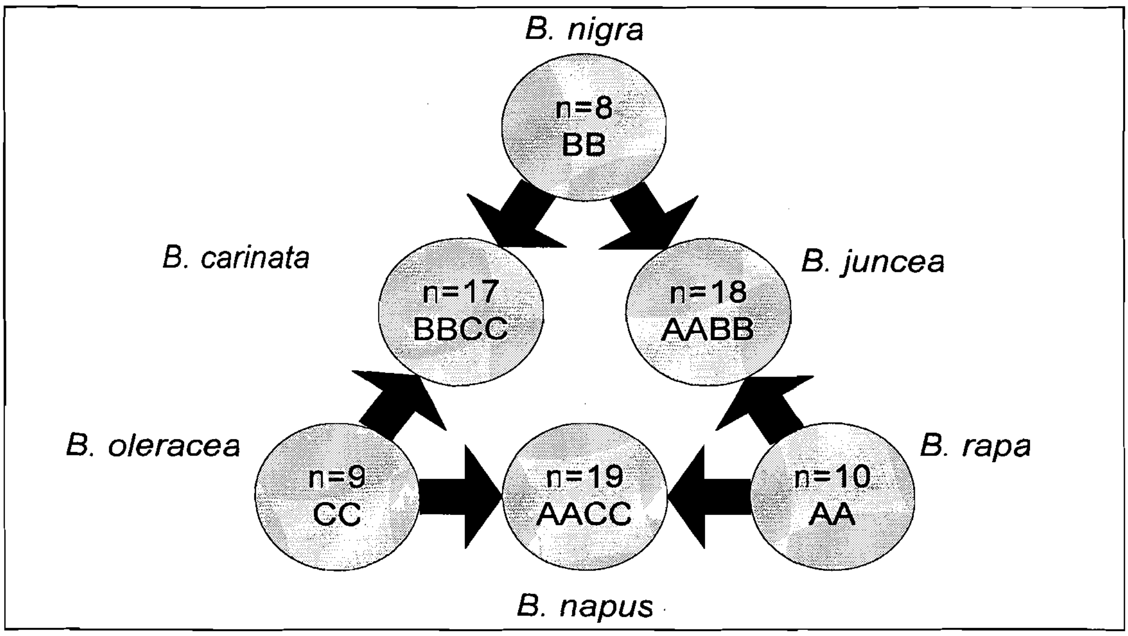

Figure 2.1 "Triangle of U" representing genomic relationship among *Brassica* species

In addition to *B. napus* and *B. rapa*, Brassica species include cultivated species

B. carinata, *B. nigra* and *B. oleracea.* The four most widely cultivated species *B. juncea*, *B. napus*, *B. oleracea*, and *B. rapa* are all highly polymorphic and include oil seed crops, root crops and vegetable crops such as Chinese cabbage, broccoli and Brussels sprouts.

The relationships among the cultivated species as presented in Figure 2.1 were first clarified by Morinaga[5] and verified by U[6]. *B. napus* (2n = 38, AACC), *B. juncea* (2n = 36, AABB), and *B. carinata* (2n = 34, BBCC) are amphidiploid species resulting from combined chromosome sets of the low chromosome number species *B. nigra* (2n = 16, BB), *B. oleracea* (2n = 18, CC), and *B. rapa* (2n = 20, AA).

2.2. HISTORY

The history[7] of canola oil is summarised in Table 2.1. The major stages are listed and mark the development of the canola oil product we have today.

Table 2.1 History of canola

Timeline of Canola History	
1956	The high eicosenoic and erucic fatty acid content of rapeseed oil was first questioned.
1960	Canadian plant breeders first isolated a rapeseed plant with low erucic and eicosenoic acid. The Health and Welfare Department recommended conversion to the production of low erucic acid varieties of rapeseed.
1968	Dr. Baldur Stefansson, a plant breeder of the University of Manitoba, developed a low erucic acid variety of rapeseed.
1970	The development and subsequent release of the first canola quality cultivars by plant breeding programs in Canada created a new, high-value oil and protein crop that has gained acceptance worldwide.[1]
1973	Industry responded with a voluntary agreement to limit erucic acid content to 5% in food products.

1974	Dr. Baldur Stefansson developed the first 'double low' variety, which reduced both erucic and glucosinolate levels. This *Brassica napus* variety was the first to meet the specific quality requirements for canola.
1985	The FDA banned the sale of rapeseed oil for human consumption but granted the GRAS (generally regarded as safe) status for the new genetically improved rapeseed (canola) in the same year.

2.3. CLASSIFICATION AND BOTANICAL DESCRIPTION[4]

Family: *Brassicaceae/Cruciferae*

Genus: *Brassica*

Species: *napus*

The family *Brassicaceae* is also known as the mustards. It includes over 40 genera and 200 wild and cultivated species, including a few weeds. *Brassica* is the genus from which current varieties of rapeseed and canola are developed. Table 2.2 shows a few of the *Brassica* and associated species.

Table 2.2 *Brassica* and associated species

***Brassica* Species**	
Biological name	**Common name**
Brassica kaber	Wild mustard
Brassica hirta	White mustard
Brassica juncea	Indian mustard
Brassica napus	Canola, rape, rapeseed
Brassica nigra	Black mustard
Brassica rapa	Birdsrape mustard
Associated Species	
Biological name	**Common name**
Barbarea vulgaris	Yellow rocket

Camelina microcarpa	Small seed false flax
Capsella bursa-pastoris	Shepherds purse
Chorispora tenella	Blue mustard
Descurainia pinnata	Green tansy mustard
Descurainia sophia	Flixweed
Erysimum cheiranthoides	Wormseed mustard
Raphanus raphanistrum	Wild radish
Sisymbrium altissimum	Tumble mustard
Sisymbrium irio	London rocket
Thlaspi arvense	Field pennycress

Canola, rapeseed and mustard all have very similar genetic properties which make intercrossing among them possible and common.

Brassica napus can easily be distinguished from *Brassica rapa*. *Brassica napus* has hairless, smooth, fleshy, bluish-green leaves, while *Brassica rapa* has yellowish-green leaves and the flower-bearing parts of its stem does not lengthen during flowering. Consequently the flowers are above the buds. The flowers of *Brassica napus* and *Brassica rapa* are both medium-yellow but the latter is slightly smaller and darker.

Brassica napus grows 1.5 m tall. The flower's petals are 7 to 11 mm long and it has pods of about 5 to 10 cm long on pedicels 1 to 3 cm long as shown in Figure 2.2. The beak is 6 to 11 mm long, conical, slender and seedless. The seeds are round, 1.5 to 3 mm in diameter and bluish-black to reddish-brown with a reticulate surface texture as can be seen in Figure 2.3.

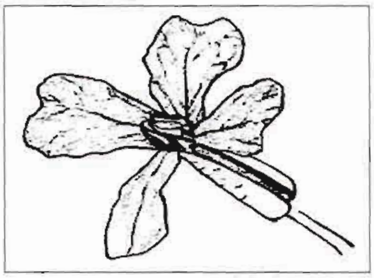

Figure 2.2 Flower of *Brassica napus*

Figure 2.3 Canola seed

2.4. CANOLA PRODUCTION[8]

Canola can be planted in fall or in spring. Winter crops are planted in the fall to provide vegetative soil cover and to assure development of a well established root system that reduces the risk of winter kill. The seedlings must emerge and establish adequate crowns in fall to ensure winter survival. Spring rapeseed is generally planted in September to October with an anticipated harvest in February to March.

The average seed use for a new crop is estimated to be between 5 – 10 kilograms/hectare. Canola is planted 2.5 – 4 cm deep in moist soil.

2.4.1. FACTORS AFFECTING THE GROWTH OF CANOLA

Guidelines[9] have been established to promote successful production of canola. These include:

- select a canola variety
- establish planting dates and methods
- decide which and how much fertilizer to use
- choose when and how to harvest
- determine the advantages of double-cropping
- administer effective pest management

2.4.2. BENEFITS OF GROWING CANOLA[9]

Some of the important advantages of growing canola include:

- larger income than wheat when yields are comparable because of canola's higher value per bushel;
- easy fitting into a double-crop rotation with soybeans, substituting for wheat or barley;
- production with the same equipment required for wheat production;
- reduced weather risks by allowing production of an oil seed crop during winter when drought is not as likely as during summer.

2.5. CANOLA AND THE WORLD

Canola is now second only to soybean as the most important source of vegetable oil in the world as shown in Table 2.3 and Figure 2.4. During the past 20 years, this crop has passed peanut, sunflower and, most recently, cotton seed in worldwide production.

Table 2.3 World oil seed production (1993-2001)[10]

Production (million tons)								
Oil seed	**1993 /94**	**1994 /95**	**1995 /96**	**1996 /97**	**1997 /98**	**1998 /99**	**1999 /00**	**2000 /01**
Soybean	118	138	125	132	158	160	158	166
Cotton seed	28.9	32.3	35.2	33.6	34.4	32.5	33.1	32.9
Peanuts	24.2	27.4	27.5	29.0	27.3	29.8	29.2	29.6
Sunflower seed	20.6	23.3	25.7	23.8	23.2	26.1	26.4	23.8
Canola seed	26.7	30.3	34.4	31.6	33.2	35.9	42.6	38.3
Copra	4.89	5.50	5.13	6.05	5.32	4.32	5.03	5.15
Palm kernel	4.14	4.50	4.87	5.21	5.05	5.62	6.20	6.43
Total	227	261	258	261	287	294	300	303

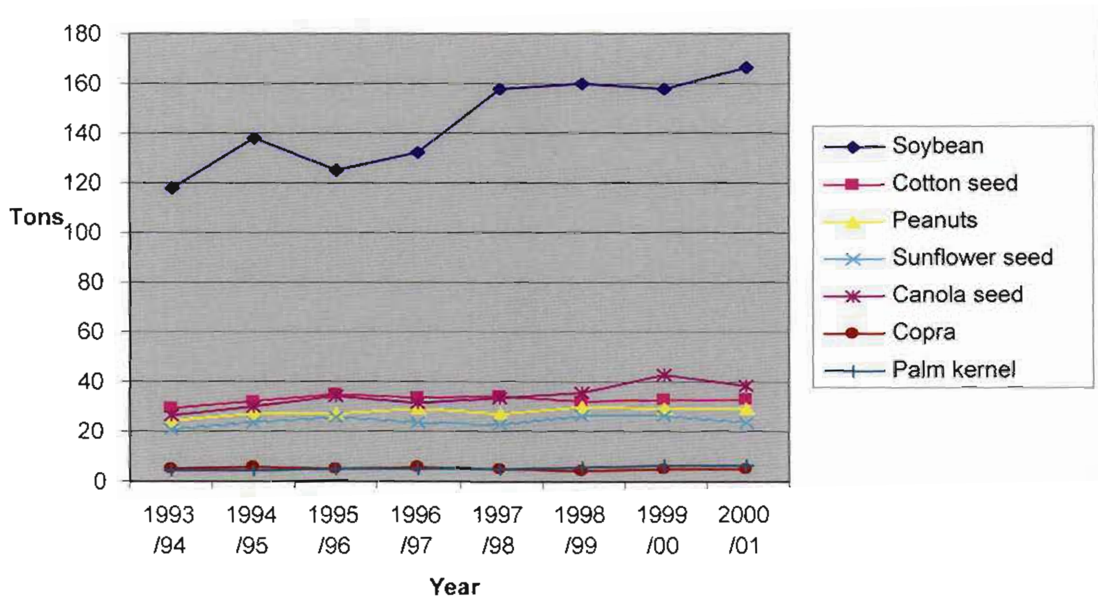

Figure 2.4 World oil seed production (1993-2001)

In 2000 – 2001, world production of rapeseed/canola totalled 33.86 million tons or 13% of oil seeds produced[10].

Canola is produced extensively in Europe, Canada, Asia, Australia, and to a more limited extent, in the United States. The world market is largely supplied by two species, *Brassica napus* and *Brassica rapa*, and to a lesser extent by the mustards, *Brassica juncea*.

Winter type *B. napus* is the main oil seed crop in most of Europe and in parts of China. Spring type *B. napus* is produced in Canada, northern Europe, and China. In Australia and the south-eastern United States, where winters are mild enough, spring type *B. napus* can be grown as a fall-planted winter crop.

Spring *B. rapa* represents a substantial portion of Canadian production and is also grown in northern Europe, China, and India. Spring types of *B. juncea* are dominant in India and are also grown to a limited extent in Canada and Europe for condiment use[11].

2.6. CANOLA COMPOSITION[12]

The main products of canola seed processing, namely oil and meal, are shown in Figure 2.5 below. One group of industries is interested in the quality and composition of the oil, while another group of industries is interested in the quality and composition of the meal.

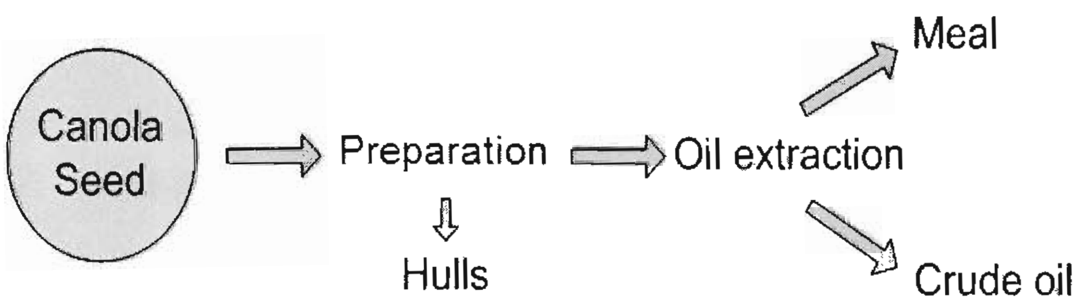

Figure 2.5 Main products from canola seed

2.6.1. MEAL COMPOSITION

The main components found in canola meal can be categorised into the different groups presented in Table 2.4.

Table 2.4 Meal composition (10% moisture basis)[12]

Components	Average
Crude protein	35.0 %
Rumen bypass protein	35.0 %
Oil	3.5 %
Linolenic acid	0.6 %
Ash	6.1 %
Crude fibre	12.0 %
Tannins	1.5 %
Sinapine	1.0 %
Phytic acid	4.0 %
Glucosinolates	1.3 %

2.6.1.1. CARBOHYDRATES AND FIBRE

The levels of starch, free sugars and soluble non-starch polysaccharides in canola meal total about 15%. This should result in a significant contribution to digestible energy. However, the actual contribution is modest due to protection of the cell wall.

The 12% crude fibre is higher than the crude fibre found in soybean meal, because the hull constitutes a relatively high proportion of canola seed. The relatively low NDF (neutral detergent fibre) : ADF (acid detergent fibre) ratio may benefit the feeding of canola meal to ruminants. Table 2.5 shows the carbohydrates and starch composition of the meal.

Table 2.5 Carbohydrates and starch composition[12]

Component	Average (%)
Sugar	8.0
Starch	5.2
Cellulose	4.6
Oligosaccharides	2.3
Soluble non-starch polysaccharides	1.4
Insoluble non-starch polysaccharides	15
Crude fibre	12
Neutral detergent fibre	21
Total dietary fibre	33

2.6.1.2. AMINO ACID

Canola meal has a suitable amino acid profile for animal feeding. It is limiting in lysine but it is noted for having high levels of methionine and cystine. Amino acid content varies with protein content. This relationship has been studied and there are useful equations to predict amino acid content from crude protein. Figure 2.6 shows the amino acid profile.

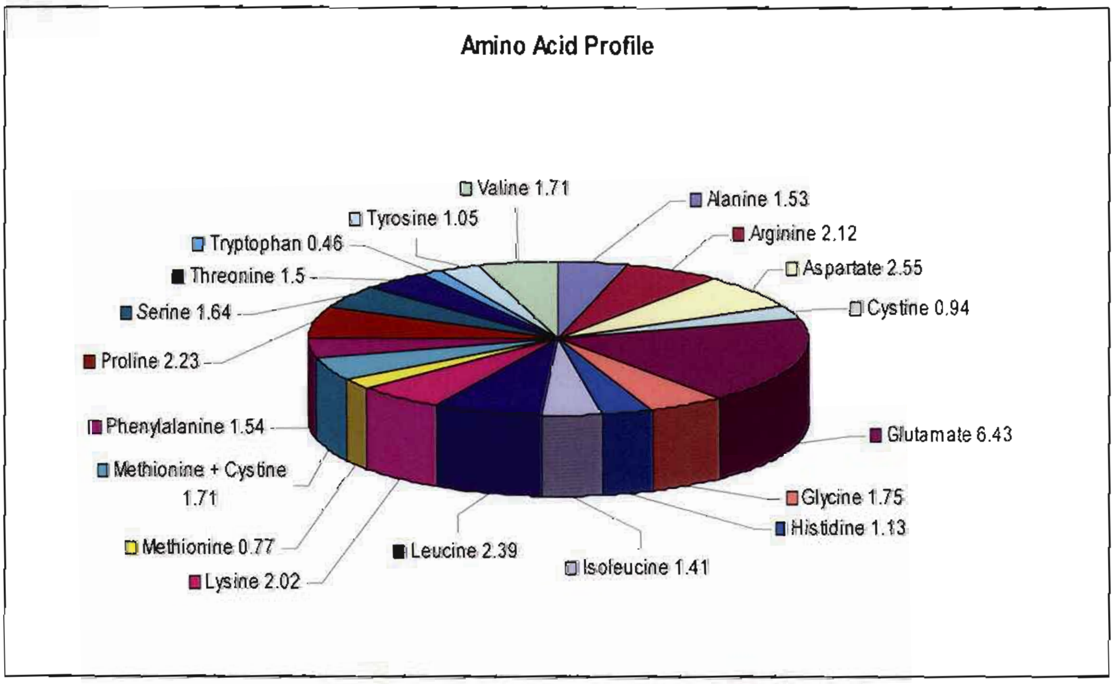

Figure 2.6 Amino acid profile[12]

2.6.1.3. GLUCOSINOLATES

The two main types of glucosinolates are aliphatic and indolyl. Aliphatic glucosinolates remain the predominant form. A problem is that these glucosinolates decompose into toxic aglucones, which have a variety of negative effects on animals – most inhibit thyroid hormone production but others affect the liver. The bitter taste results in reduced feed intake by many animals.

The total glucosinolate content of Canadian canola meal is approximately 16 µmol/g, which is lower than traditional rapeseed meal. The level of glucosinolates in Canadian canola has continued to decrease in recent years due to selection by canola plant breeders.

2.6.1.4. OTHER MINOR COMPONENTS

Tannins, in a range of 1.5% to 3.0% in canola meal, do not appear to have the same negative effects on palatability and protein digestibility that they do in other plants. Sinapine, 0.6% to 1.8%, can result in a fishy flavour. Phytic acid ranging from 3% to 6% is partly digestible by monogastics.

2.6.2. OIL COMPOSITION[13]

According to Figure 2.7 canola oil consists of 30% fats, 50% carbohydrates and 20% protein. The main oil composition does not differ much from the meal composition. The oil contains less fibre elements and more fatty acids.

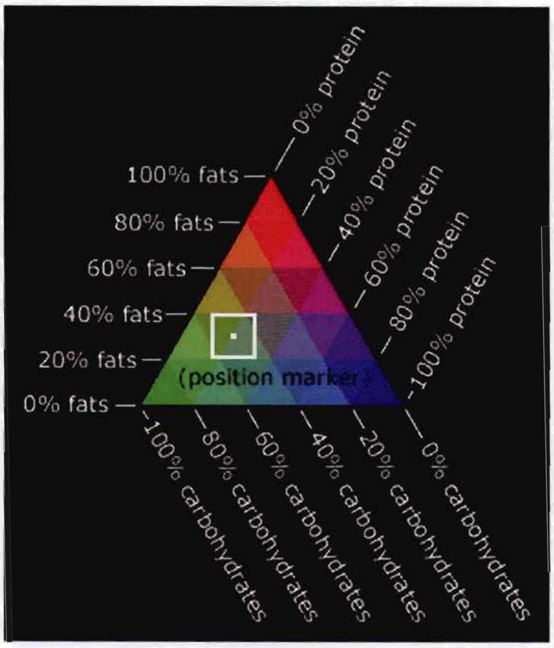

Figure 2.7 Composition of canola oil

The fatty acid profile in Table 2.6 is part of the fat % seen in Figure 2.7. Table 2.7 shows some minor components found in canola oil.

Table 2.6 Canola oil fatty acid profile

Fatty Acid	Name	Common Name	%
Saturated Fat			**7**
16:0	hexadecanoic acid	palmitic acid	4.0
18:0	octadecanoic acid	stearic acid	1.8
20:0	eicosanoic acid	arachidic acid	0.6
22:0	docosanoic acid	behenic acid	0.4
24:0	tetracosnoic acid	lignoceric acid	0.2
Monounsaturated Fat			**60**
16:1 (undifferentiated)	hexadecanoic acid	palmitoleic acid	0.2
18:1 (undifferentiated)	octadecenoic acid	oleic acid	56.1
20:1	eicosenoic acid	gadoleic acid	1.7
22:1 (undifferentiated)	docosenoic acid	erucic acid	0.6
24:1			1.4
Polyunsaturated Fat			**33**
18:2 (undifferentiated)	octadecadienoic acid	linolenic acid	20.3
18:3	octadecatrienoic acid	linolenic acid	9.3
20:2			3.4

Table 2.7 Minor components found in canola oil

Nutrient	Mass (mg) per 10 g of oil	%
Selenium	9.96	0.10
Magnesium	22.28	0.22
Dietary fibre	1080	10.8
Omega 3 fatty acid	200	2.00
Vitamin B3	0.6	0.006
Calcium	38.92	0.39
Protein	1880	18.8
Vitamin E	1.71	0.02
Gamma tocopherol	3.04	0.03
Zinc	0.44	0.004

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CHAPTER 3

SUPERCRITICAL FLUID TECHNOLOGY

A supercritical fluid[1] is a substance at a temperature and pressure above its critical point. The supercritical state of CO_2 occurs at 31°C and 73 atm as shown on its phase diagram in Figure 3.1. Table 3.1 lists the critical constants (T_c, p_c, ρ_c) of a few substances, from which it is evident that CO_2 becomes supercritical at relatively moderate conditions.

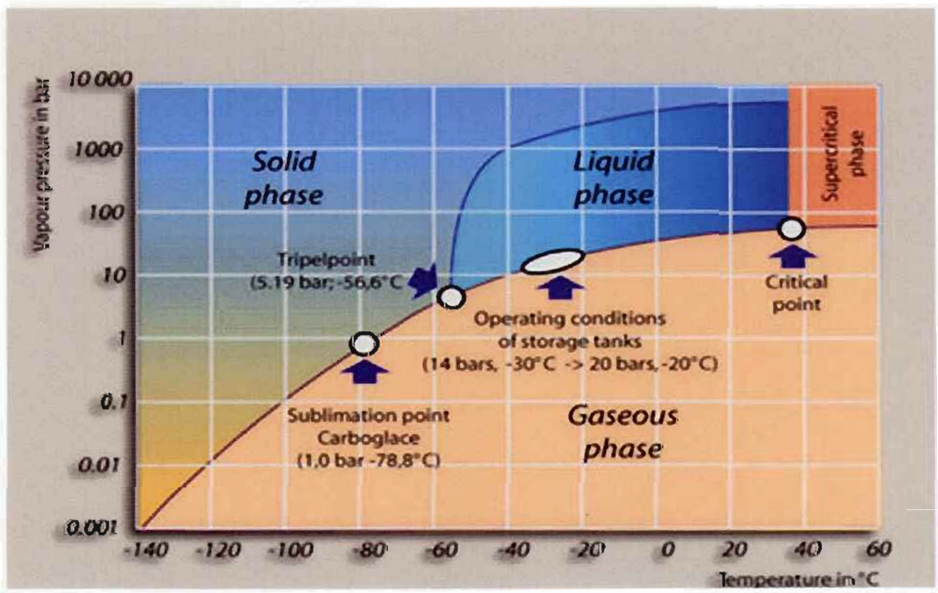

Figure 3.1 Phase diagram of CO_2[2]

Table 3.1 Critical constants of selected substances

Substance	T_c (°C)	p_c (atm)	ρ_c (g/mL)
C_2H_4	9.30	50.4	0.214
Xe	16.7	58.4	1.11
C_2F_6	20.0	30.6	0.622
CHF_3	26.3	48.6	0.528
CO_2	31.1	73.8	0.468
C_2H_6	32.4	48.8	0.203
N_2O	36.6	72.6	0.452
H_2O	374	220	0.322

Supercritical fluids have the advantage of having liquid-like and gas-like properties[3], with the result that they can behave as highly compressed gases or highly mobile liquids.

The application of supercritical fluid technology in industry has endless possibilities, but it is still largely unexplored and much research is still needed to persuade companies to consider it, especially in view of the negative connotation attached to the terms "supercritical" and "extreme conditions".

Supercritical fluid technology is associated with harmless solvents, like CO_2, to yield quality extracts free from solvent residues in a cost-efficient way. sc-CO_2 is regarded as a "green solvent"[4] having environmental benefits above organic solvents such as *n*-hexane or dichloromethane. The implementation of "clean technology" is important to the issue of sustained chemistry worldwide[5].

3.1. PRINCIPLES AND THEORY

The characteristics of a supercritical fluid depend on the prevailing conditions. Temperature and pressure are major parameters, which jointly determine the density of the supercritical fluid. To determine the density of CO_2 at any given time, one has to consult the three-dimensional density-temperature-pressure graph compiled with Statistica for Windows[6] in Figure 3.2.

The density of CO_2 can be calculated for any given temperature and pressure by means of the equation

$$\textit{Density} = 0.771 + 0.0026\,p - 0.0118\,T - (3.5617\times10^{-6}\,p^2) + (1.2783\times10^{-5}\,pT) + (2.204\times10^{-5}\,T^2)$$

which describes the three-dimensional surface plot.

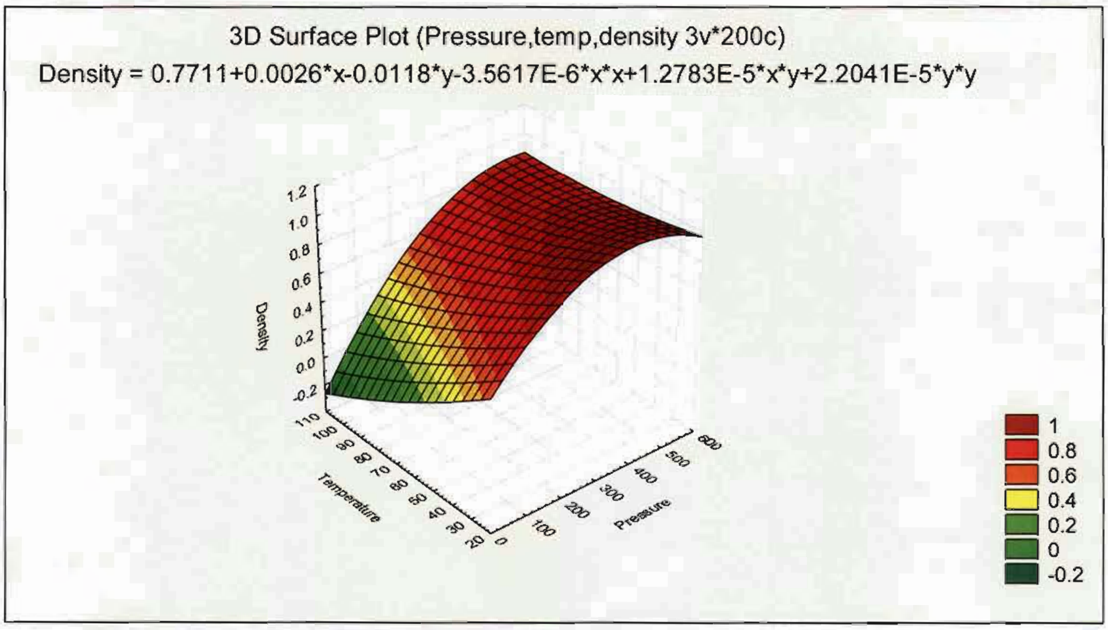

Figure 3.2 Relationship between density, temperature and pressure of CO_2[6]

With densities close to 0 g/mL, CO_2 tends to behave gas-like, whereas at densities close to 1 g/mL it tends to be liquid-like. The liquid-like properties are essential for extraction based on chemical dissolution, since CO_2 then has solvent strengths comparable to those of conventional solvents. It has the added advantage that the solvent strength can be adjusted by varying the density (by changing temperature and/or pressure) to render it more selective for the dissolution of specific substances.

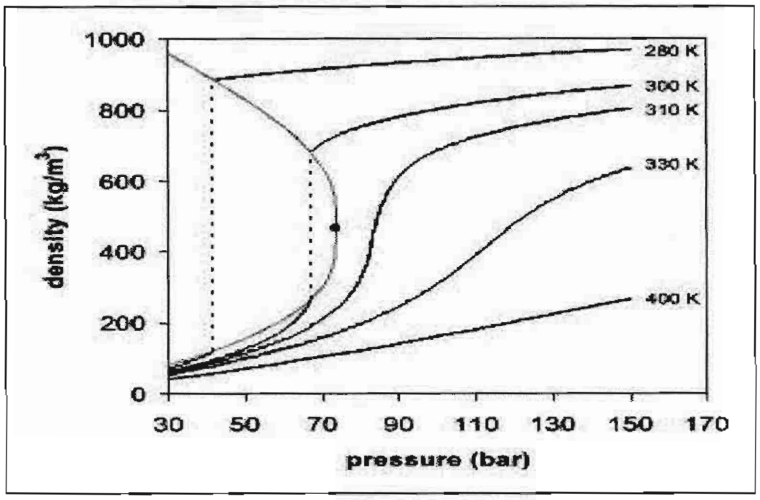

Figure 3.3 Density of CO_2[7]

Increasing temperature[8] at constant pressure results in decreasing density and thus decreasing solvent strength of CO_2, whereas increasing pressure at constant temperature leads to increasing density (thus increasing solvent strength) of CO_2, as shown in Figure 3.3.

Although an increase in temperature lowers the density and thus the solvent strength of sc-CO_2, extraction with sc-CO_2 has a specific energy of activation or energy barrier which needs to be overcome by virtue of a minimum temperature increase. Temperature thus has both a supportive and detrimental effect on sc-CO_2 extraction, the effect being the result of two opposing effects or contributions.

The Arrhenius equation ($k = Ae^{-E_a/RT}$) can be used to determine the energy of activation[8] (E_a) by studying the temperature dependence of the rate constant (k) or any other measurable quantity, such as yield. A graph of ln (yield) versus 1/T based on the logarithmic form ($\ln k = \ln A - E_a / RT$) of the Arrhenius equation allows the value of E_a to be obtained from the slope = $-E_a/R$.

Likewise, an empirical equation ($k = Bpe^{-\Delta V^{\neq}/RT}$) can be used to determine the volume of activation[9] ($\Delta V^{\neq}$) by studying the pressure dependence of the rate constant (k) or any other convenient quantity, such as yield. A graph of ln (yield) versus p based on the logarithmic form ($\ln k = \ln B - (\Delta V^{\neq} / RT)p$) of the empirical equation allows $\Delta V^{\neq}$ to be calculated from the slope = $-\Delta V^{\neq}/RT$.

3.2. SUPERCRITICAL TECHNOLOGY

Supercritical technology[10] can be divided into two subgroups, namely supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC).

3.2.1. SUPERCRITICAL FLUID EXTRACTION (SFE)

SFE[10] produces extracts in a cost-efficient way. The SFE derived product is generally purer than that obtained by classical cold press and steam distillation methods, especially if the additional refining steps associated with the latter methods are taken into account. SFE excludes the use of organic solvents (like *n*-hexane) and thus leads to uncontaminated products free from hazardous solvent residues. SFE products generally compare well with refined cold press products.

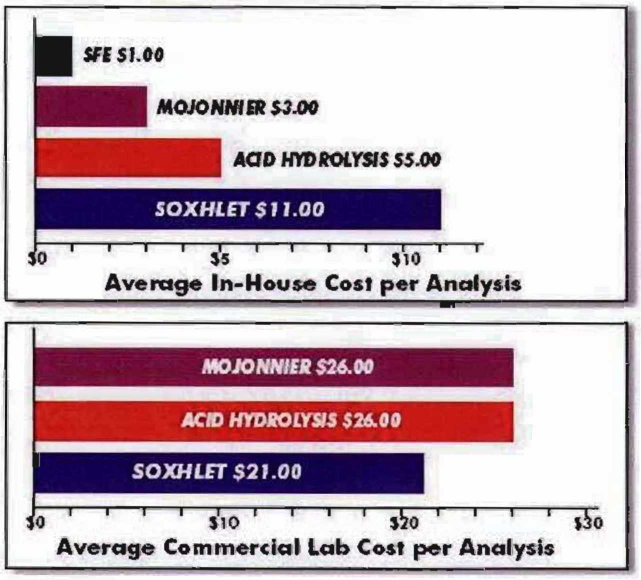

Figure 3.4 Cost Advantage of SFE[11]

Figure 3.4 shows the cost-efficiency of SFE as an alternative for mojonnier, acid hydrolysis and soxhlet procedures in a comparison between in-house and commercial laboratory analysis costs.

3.2.2. SUPERCRITICAL FLUID CROMATOGRAPHY (SFC)

SFC[10] has become a new routine analytical tool. It has additional features to GC and HPLC. SFC uses a supercritical fluid as mobile phase, capable of solvating volatile and thermally unstable compounds and improving resolution by lowering viscosity and increasing diffusivity. These improvements make it a suitable alternative to conventional GC and HPLC methods.

3.3. EQUIPMENT

The supercritical fluid extractor in this investigation is a LECO TFE 2000[11]. It is a unique three channel system with each channel having a thimble and an automated heated variable restrictor (HVR) enabling three different extractions to be run simultaneously. The CO_2 flow is controlled by an integrated microprocessor onto which all operational conditions (temperature, pressure, flow rate, duration etc.) are keyed in. The heated variable flow restrictor (HVR) prevents clogging which results from either viscous materials or Joule-Thomson[12] cooling.

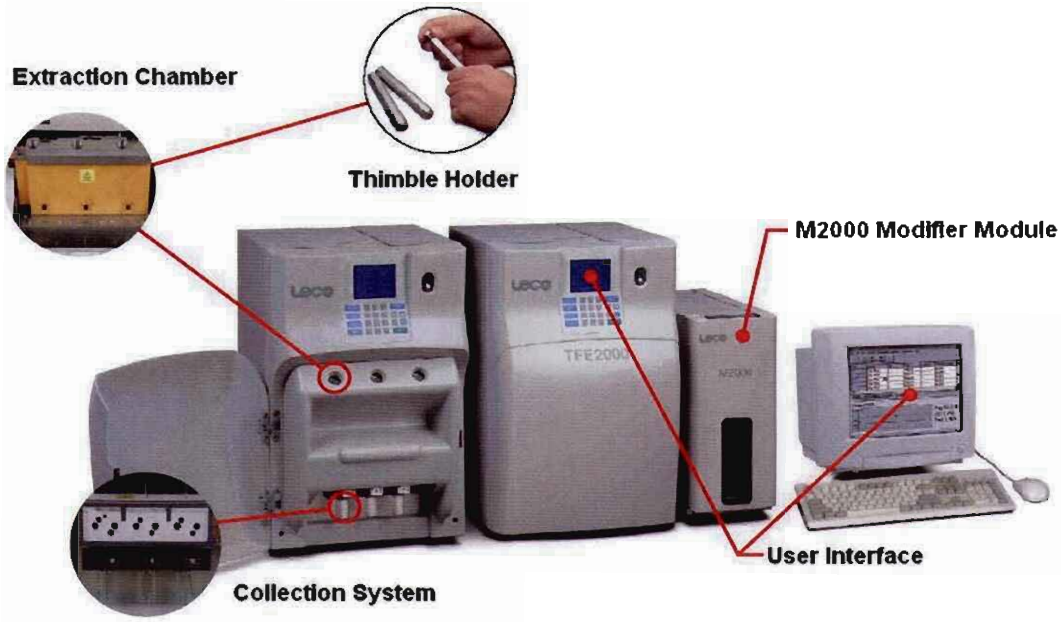

Figure 3.5 LECO TFE 2000 supercritical fluid extractor

The TFE 2000[11] utilises helium compressed CO_2 in a dip-tube cylinder. CO_2 is non-hazardous and excludes the need for fume hoods, extracting chambers, specialised clothing and waste disposal afterwards. The CO_2 is pre-cooled to ease compression and transport of the gas. It is pressurised to the required pressure (p_{max} = 600 atm) and heated to the required temperature (T_{max} = 150°C) before an extraction run is started.

The sample is placed in a thimble and subjected to sc-CO_2 at these set conditions. The CO_2 flow rate can be regulated up to 5 L/min in a dynamic extraction mode or it can be maintained at a zero value during a static extraction run. After extraction the product/extract is collected in collection vials attached to the HVR's. Figure 3.6 presents a flow diagram of the operation of the instrument.

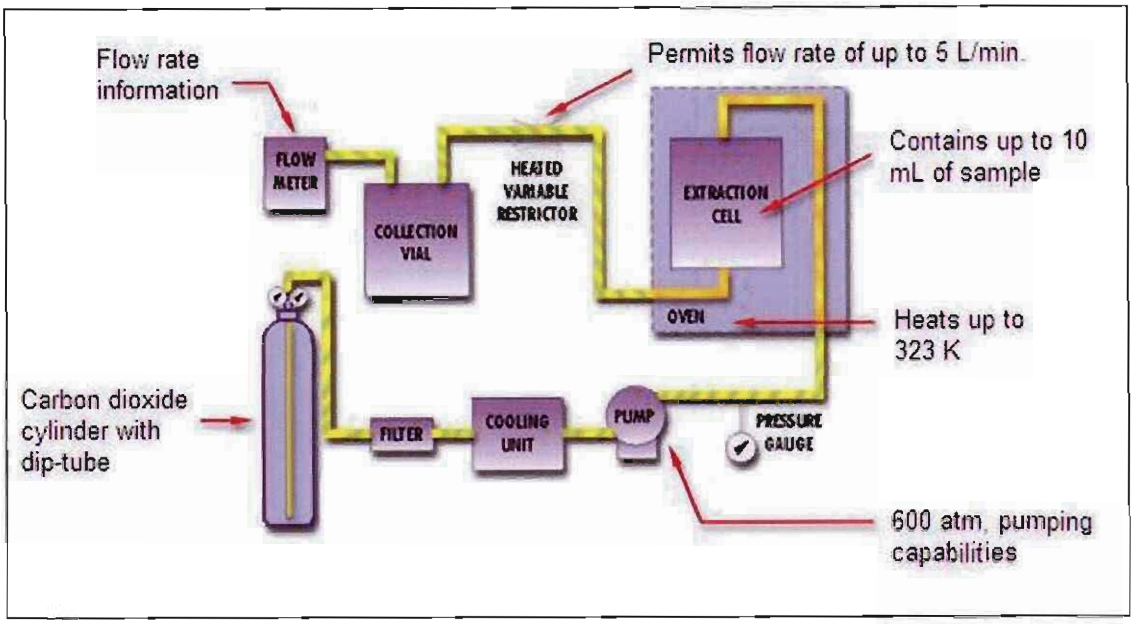

Figure 3.6 Flow diagram of TFE 2000 supercritical fluid extractor

3.4. APPLICATIONS

Supercritical technology is applied to many sectors, including the food industry (extraction of fragrances), the pharmaceutical industry (isolation of active ingredients) and the chemical industry (recovery of resources and impregnation of matrices), to name a few[13].

There are a few fields of application that present major breakthroughs, such as particle formation[15,16], supercritical water oxidation[17,18] and metal processing[13,14].

The implementation of supercritical technology is quite expensive from the point of view of initial capital outlay, but calculations indicate substantial savings in day to day operational costs.

SFE has been subjected to much criticism regarding matrix dependency when applied to real-world samples[11]. Many of the problems associated with SFE development stem from the misconception of a "magic" type of fluid with infinitely adjustable properties capable of isolating any desired substance in a one-step process. SFE requires the same method development applying to conventional techniques like soxhlet extraction. The most popular and successful application of SFE is the extraction of fats/oils from foods, plants and seeds, and the focus of this investigation is on this particular topic.

The batch process used in the majority of extractions can be regarded as a limiting factor in applying supercritical technology in industry. Optimisation of yield and time is crucial in industrial profiting. Therefore, new ways need to be explored to overcome the disadvantages of batch processing with continuous supercritical extraction using an extruder design[19,20].

3.5. REFERENCES

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CHAPTER 4

EXPERIMENTAL PROCEDURES

sc-CO_2 extraction is a viable alternative to traditional extraction methods, especially in view of stricter legislation prohibiting the use of organic solvents in food processing[1]. The methodology related to sc-CO_2 based botanical extractions has been well established, and in this chapter it is outlined in more detail for the purpose of this investigation.

4.1. UTILISED MATERIALS

Canola seed samples used for conducting this research was donated by the Agricultural Research Council (ARC) of Potchefstroom. Cultivars used in the research included AG Outback, Spectrum, 44C73 and 45C05.

Industrial grade CO_2, supplied by AFROX®, was used for extraction.

Materials provided by LECO and used in everyday laboratory sample preparation included glass wool (501-081), LECO-Dry (502-327) and Kimwipe (502-369).

n-Hexane (organic solvent) and helium (carrier gas) were used to perform GC-FID analysis (done by Thebogo Chauke at Nola Industries, Randfontein).

Helium (carrier gas) was the main gas used in GC-GC/TOF-MS analysis (done by Dr. Peter Gorst-Allman at CSIR, Pretoria).

4.2. SAMPLE PREPARATION

Sample preparation of canola seed included crushing of the seed (to obtain optimum surface area), drying of the seed (to investigate the role of moisture content) and sample handling.

4.2.1. CRUSHING OF SEED

Oil cannot be extracted from uncrushed seed. sc-CO_2 can enter the hull of the seed but is unable to transport the dissolved oil through the pores of the hull into the bulk of the sc-CO_2. By crushing the seed with a food processor, one ensures that sc-CO_2 is capable of removing dissolved oil from the matrix as illustrated in Figures 4.1 and 4.2.

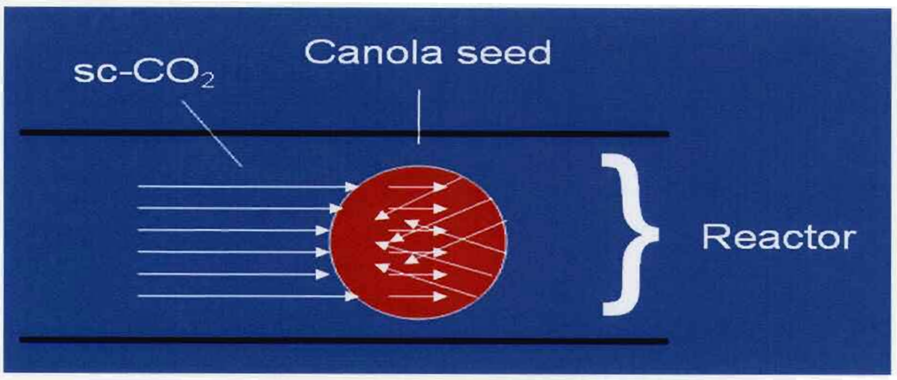

Figure 4.1 sc-CO_2 captured within uncrushed seed

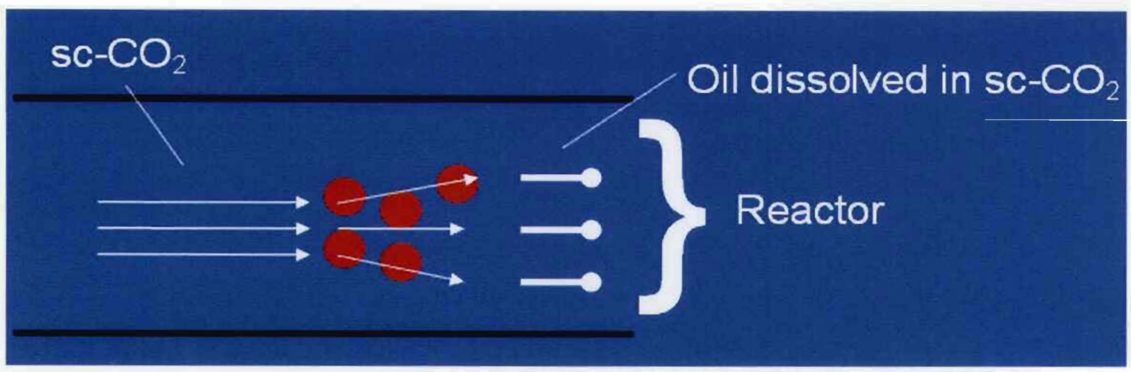

Figure 4.2 Crushed canola seed allowing oil to be transported by sc-CO_2

4.2.2. PRE-DRYING OF SEED

Pre-drying of seed was done to investigate the influence of natural moisture on extraction since the hydrophilic nature of CO_2 could improve its functionality in the presence of moisture. On the other hand the presence of moisture under extreme conditions could lead to the corrosive action of sub- or supercritical water. Moisture present in an extraction process may lead to unexpected high product yield[2].

Three drying procedures were implemented, namely sun drying, oven drying and freeze drying.

4.2.2.1. SUN DRYING

The seed was placed in an open container in the sun for two weeks to dry. The temperature is sufficient to reduce excess moisture.

4.2.2.2. OVEN DRYING

Crushed seed was placed in an electrical oven for four hours to dry at 80°C. A higher temperature may be harmful to certain natural components (fatty acids) of the seed.

4.2.2.3. FREEZE DRYING

Crushed seed was placed in a freeze drier (DURA-DRY-MP) for 12 hours at -50°C and 76 millitorr after 8 hours of normal freezing at -80°C.

4.2.3. SAMPLE HANDLING

Seed preparation was followed by loading a weighed amount of crushed material into a thimble and entering it into the pneumatically controlled extraction chamber of the supercritical extractor.

4.3. APPARATUS[3]

The apparatus was discussed in Chapter 3 as exemplary of equipment used in supercritical technology. The LECO TFE 2000 is a bench-top supercritical fluid reactor with easily adjustable parameters to control an extraction run. The instrument is equipped with an automated valve system ensuring high flow rates, and it features a 3-way flow line for either three different but simultaneous extraction runs or on extraction run in triplicate to test for repeatability. The computer display shown in Figure 4.3 enables the user to set the required variables within the ranges listed in Table 4.1.

Table 4.1 Ranges of variables available for LECO TFE 2000

Variable	Min Value	Max Value
HVR temperature	32°C	350°C
Cell temperature	32°C	150°C
Pressure	50 atm	600 atm
Static time	0 min	60 min
Dynamic time	0 min	99 min
Flow rate	0.1 L/min	5 L/min
Modifier rate	0.1 L/min	5 L/min

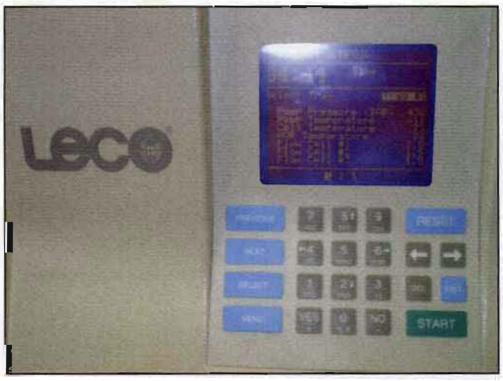

Figure 4.3 Display of supercritical extractor

To start an extraction on the LECO TFE2000, one has to first create a user profile, in this case CANOLA. The profile enables one to store the last settings used in an extraction process. In the SETUP menu one can alter the variables listed above.

The START key activates the solenoid valves to retract the thimbles into the reactor chamber and close it pneumatically for safety.

Once the desired HVR and reactor temperatures have been reached, the pressure pump pressurises the sample holders to the required pressure. The user can select a static or dynamic mode of extraction and can adjust the flow rate for the latter mode.

After an extraction, the fluid is depressurised at the HVR's and the solenoid valves reopen the reactor chamber and lift out the sample holders.

4.4. DETAILED EXPERIMENTAL PROCEDURES

To keep consistency throughout the set of conducted experiments, the experimental procedures recommended by LECO® for canola oil exhaustive extractions (AOCS official SFE methods Am-3-96 and 99.02) and for a standard method of comparison (AOCS method Am-2-93-FOSFA) were adopted[2].

4.4.1. SAMPLE PREPARATION PROCEDURE

1. Grind sample in a mechanical grinder/food processor.
2. Install a lower end-cap assembly on a thimble and place in a thimble stand.
3. Pack 1/4 of a Kimwipe into the bottom of the thimble by folding it once and packing it into the bottom of the thimble with a clean spatula.
4. Place the thimble assembly on a balance and tare.

5. Remove the thimble from the balance and add ~2.0 g of prepared sample using a funnel.
6. Place the thimble on the balance and note the mass.
7. Place thimble in stand, and fill upper portion of thimble with LECO-Dry.
8. Install the upper end-cap assembly on the thimble.

4.4.2. COLLECTION VIAL PREPARATION PROCEDURE

1. Cut 1.3 to 1.5 g of glass wool from the end of a glass wool rope.
2. Pull the compact section of glass wool apart so that the material is loosened considerably.
3. Pack the loosened glass wool into the collection vial with a clean spatula, a little at a time. The goal is to have random, non-vertical orientation of wool strands.
4. Tare the empty balance pan.
5. Weigh collection vial and note the initial mass.
6. Install the collection vial on the instrument collection system.

4.4.3. EXTRACTION PARAMETERS PROCEDURE

1. Set up (or recall and activate) the following instrument parameters:
   Extraction Pressure: 7500 psi (± 520 atm)
   Extraction Temperature: 100°C
   HVR Temperature: 110°C
   Static Time: 0 minutes
   Dynamic Time: 60 minutes
   Flow Rate: 1.3 L/min
   Modifier*: denatured ethanol
   *M2000 modifier module is required for this option.
2. The pump head should be at 0°C or below from the last set of extractions. However, the refrigeration system times out and stops 20

minutes after extractions. If the system has timed out, pre-cool the pump head by pressing any key on the keypad. The thimble and HVR temperatures should also be at set values.

3. Insert the thimbles into the instrument and press the START key. The extraction will automatically take place, and the system will depressurise at the end of the run.

4.4.4. POST-EXTRACTION MANIPULATIONS

1. Remove the collection vials from the instrument.
2. Using the thimble removal tool, remove the thimbles and place them in the thimble stand to cool.
3. Tare the empty balance pan.
4. Weigh each collection vial and note the mass.
5. Further calculations can be done to determine the result with noted masses.

4.5. ANALYTICAL EQUIPMENT

It is essential to analyse the product and compare it to standardised products already available. Analytical equipment used to analyse/evaluate the acquired oil included GC-FID and GC-GC/TOF-MS.

4.5.1. GC-FID ANALYSIS

The GC-FID analysis was done by Tebogo Chauke at Nola industries, Randfontein. The GC parameters are given in Table 4.2.

Table 4.2 Instrumental conditions for GC/FID analysis

Description	
Date	08 December 2005
Operator	Tebogo Chauke
Sample description	Canola oil
Concentration	1:1
Solvent	*n*-Hexane
Volume injected	1 *u*L
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

4.5.2. GC-GC/TOF-MS ANALYSIS

The GC-GC/TOF-MS analysis was done by Dr. Peter Gorst-Allman at the CSIR, Pretoria.

Table 4.3 Instrumental conditions for GC-GC/TOF-MS analysis

Description	
Date	15 July 2006
Operator	Dr. Peter Gorst-Allman
Sample description	Canola oil
Detector	LECO Pegasus 4D Time-of-Flight Mass Spectrometer

Acquisition rate	100 spectra/sec
Stored mass range	35 to 450 u
Transfer line temperature	250°C
Source temperature	200°C
Detector voltage	-1700 Volts
GC	Modified Hewlett Packard 6890N*
Column 1	Rtx-5 Sil MS, 30 m x 0.25 mm ID, 0.25 μm film thickness
Column 2	DB 17.2 m x 0.1 mm ID, 0.1 μm film thickness
Column 1 oven	50°C for 1 min, to 290°C at 10°C/min, hold for 10 min
Column 2 oven	60°C for 1 min, to 300°C at 10°C/min, hold for 10 min
Second dimension separation time	4 sec
Modulator offset	20°C
Inlet	Splitless at 240°C
Injection	1 μL
Carrier gas	Helium, 1.0 mL/min constant flow

* The HP6890N GC has a high-pressure electronic pressure control (EPC) module.

The acquired data were processed with an automated peak finding and spectral deconvolution software package, followed by a library search of the NIST (National Institutes of Standards and Technology) database[4].

4.6. CHEMICAL ANALYSIS

Quality tests are performed on commercial canola oil before it is placed on the supermarket shelf. These include the iodine value, peroxide value and moisture content value. The methods to determine these values are standardised, and these are briefly discussed below.

4.6.1. PEROXIDE VALUE[5]

The peroxide value is an indication of the current state of oxidation of the oil.

REAGENTS USED

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

METHOD

1. Weigh 5.000 – 5.050 g of sample in an Erlenmeyer flask.
2. Add 30 mL of acetic acid/chloroform solution.
3. Stir flask until sample is dissolved.
4. Immediately add 0.5 mL saturated KI solution.
5. Leave the sample for exactly 1 min.
6. Add 30 mL distilled water.
7. Use 0.5 mL 1% starch solution as indicator and titrate with 0.01 N thiosulphate ($HO_3S_2^-$) until the solution turns white. The titration has to be repeated if the titration uses less than 0.5 mL.
8. Take the reading.

CALCULATION

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

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

4.6.2. IODINE VALUE (WIJS METHOD)[6]

This value reflects the unsaturated oil or fat content in terms of the amount of iodine absorbed per centigram.

REAGENTS USED

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

METHOD

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

CALCULATION

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

B – titration of blank

S – titration of sample

N – normality of thiosulphate (0.1 N)

4.6.3. MOISTURE CONTENT[7]

This value indicates the moisture content and volatile substances in the oil sample.

METHOD

1. Weigh container with lid – $W1$.
2. Add 5.0 g of sample and weigh it again – $W2$.
3. Put the open container in an oven at 130°C for one hour.
4. Close the lid and put the container in a desiccator for one hour to cool down.
5. Weigh the container again – $W3$.

CALCULATION

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

4.7. REFERENCES

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CHAPTER 5

RESULTS

sc-CO_2 extraction is influenced by several physical parameters. Temperature and pressure determine the phase of CO_2 at any given time as illustrated by its phase diagram in Figure 3.1. These two variables jointly determine its density, which can be read off directly from Figure 5.1 for any given combination of temperature and pressure. A third variable is extraction time. A suitable combination of these parameters to give an optimum yield of extract or an extract of desired composition was one of the objectives to achieve in this investigation. Other parameters include flow rate of CO_2 and added cosolvents, though these do not influence botanical extraction significantly.

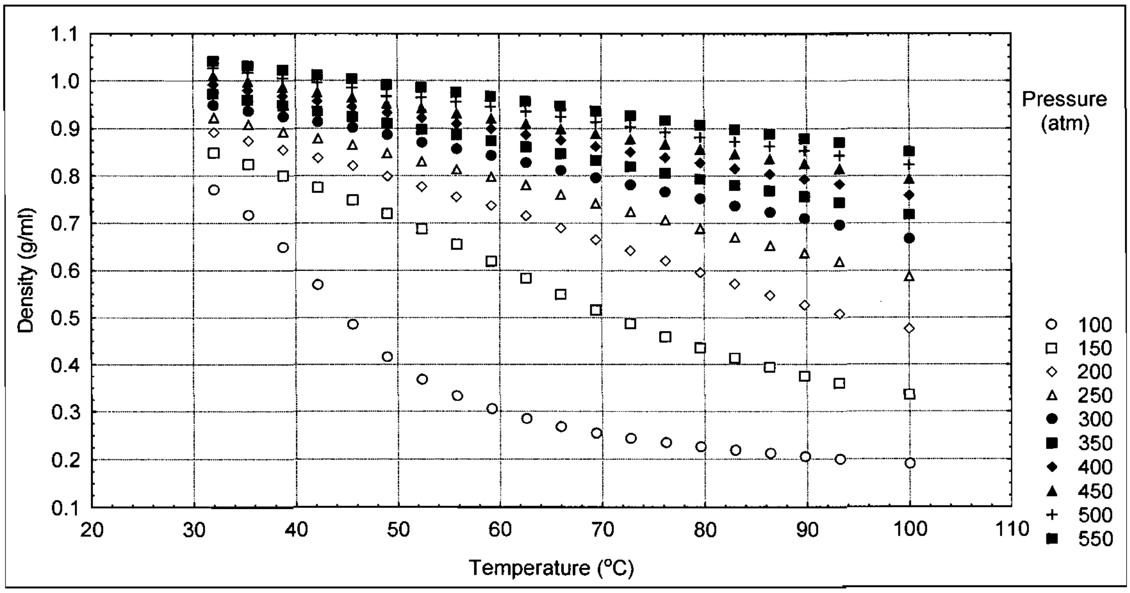


Figure 5.1 Density of CO_2 at different temperatures and pressures

More issues are, however, reported on in this chapter. These include the solubility of canola oil in sc-CO_2, the oil content of the seed used for extraction, the activation parameters and mechanism of extraction, chemical and instrumental analysis of the extracted oil, composition and quality of sc-CO_2 versus cold-press acquired canola oil and comparison of oil extracts from different cultivars of canola seed.

5.1. SOLUBILITY OF CANOLA OIL IN sc-CO_2

This determination required extraction runs at mid-values of temperature and pressure to be performed in static mode. The sample was exposed to sc-CO_2 without any fresh CO_2 flowing through the sample holder. This provides time for the sc-CO_2 to dissolve the oil from the seed matrix. In this mode not all of the oil in the sample is extracted because the fixed volume of CO_2 reaches a point of saturation indicated by the plateau of the yield-time curve and signifies the solubility of the oil in sc-CO_2 as shown in Figure 5.2.

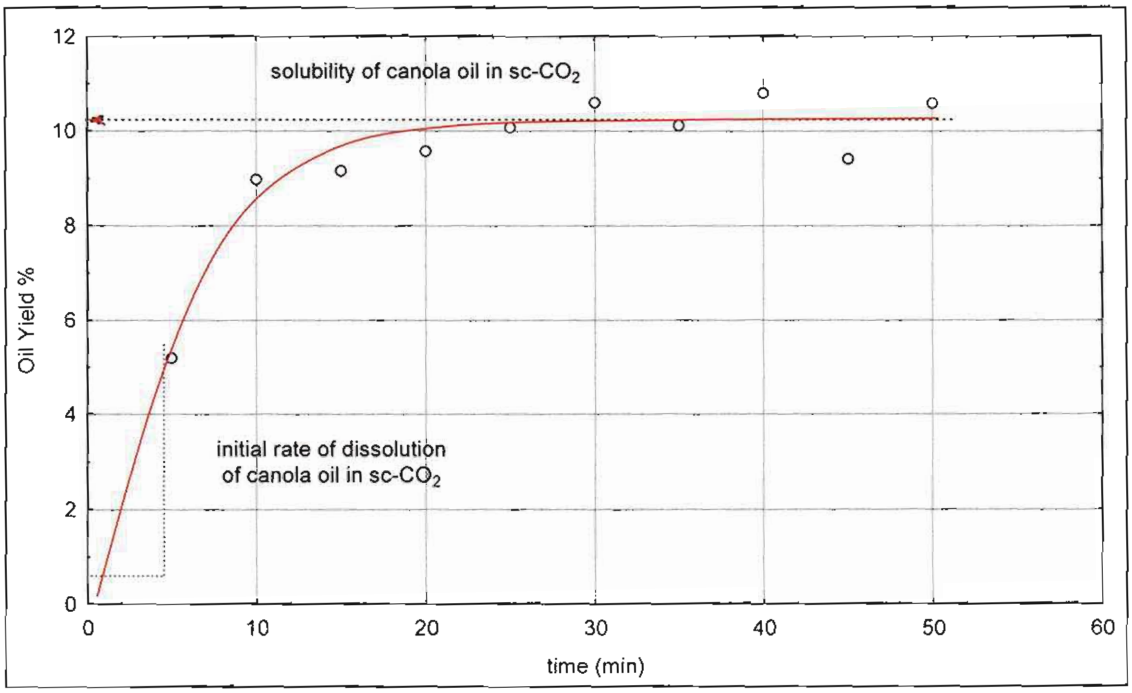


Figure 5.2 Yield-time curve obtained in static mode (p = 300 atm, T = 50°C, ø = 1.5 L/min)

The solubility of canola oil in sc-CO_2 can be determined as outlined below.

Volume of sc-CO_2 used = (volume of thimble) – (volume of seed)

= 10 mL – 3.5 mL

= 6.5 mL

Density of sc-CO_2 at 300 atm and 50°C (conditions at which the static mode runs in Figure 5.2 were performed) is 0.876 g/mL.

Mass of sc-CO_2 used = (volume of sc-CO_2) x (density of sc-CO_2)

= 6.5 mL x 0.876 g/mL

= 5.694 g

Mass of oil = 10.4% of 2.01 g sample as shown in Figure 5.2 = 0.205 g

The ratio of mass of oil produced to mass of sc-CO_2 used is 0.205 g : 5.694 g or 0.036 g per gram of sc-CO_2, which represents the solubility of canola oil in sc-CO_2 at the conditions under consideration.

5.2. OIL CONTENT OF SEED USED

A run in dynamic mode extracts all of the oil in the sample because a continuous flow of fresh CO_2 passes through the sample. The oil is extracted as it dissolves in the sc-CO_2.

The yield-time curve in Figure 5.3 obtained in dynamic mode gives the oil content of the canola seed used in this investigation (almost 14% as read off from the y-value corresponding to the plateau), and indicates the minimum extraction time needed (70 min as read off from the x-value where the curve reaches a plateau) to exhaustively extract the sample of seed at the conditions concerned. The initial slope corresponds to the initial rate of extraction.

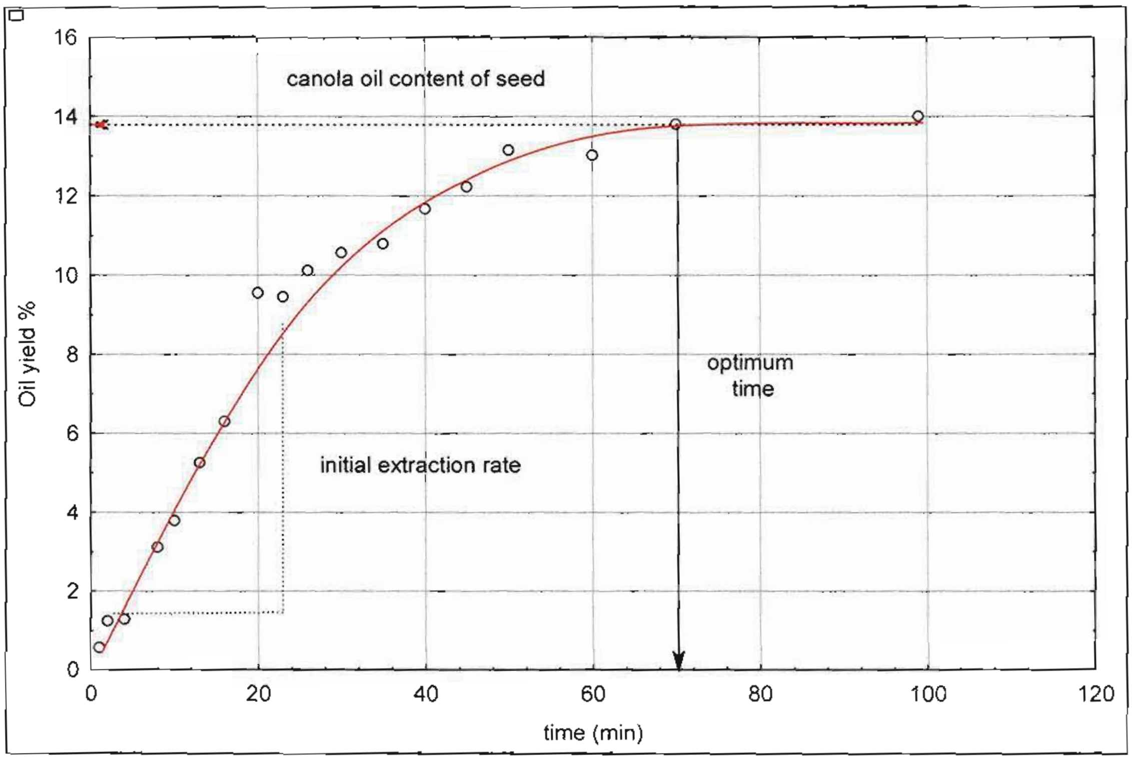


Figure 5.3 Yield-time curve obtained in dynamic mode (p = 300 atm, T = 50°C, ø = 1.5 L/min)

Figures 5.2 and 5.3 illustrate the distinctively different purposes for which a static or dynamic extraction mode is selected. The solubility of the oil in sc-CO_2 and the tamount of extractable oil in the seed are two totally different determinations often confused with one another. The initial rate of extraction is different in the two determinations as dissolution of the oil in a fixed amount of sc-CO_2 proceeds faster than in a stream of sc-CO_2 rapidly passing through the extraction thimble.

5.3. PROCESS DEPENDENCIES

5.3.1. TEMPERATURE

Temperature needs to be considered carefully since it influences the density and thus the solvent strength of the extracting fluid. It can also influence the quality of the oil. Temperatures close to 80°C can harm the natural ingredients of the oil, whereas temperatures lower than 32°C fall outside of the supercritical domain.

The yield-temperature graph in Figure 5.4 shows that temperature has virtually no influence as long as it is varied within the supercritical domain. This means that the energy of activation is practically zero, which is typical of a diffusion controlled process. The high mobility of a supercritical fluid certainly allows diffusion to proceed without resistance, hence resulting in zero energy of activation.

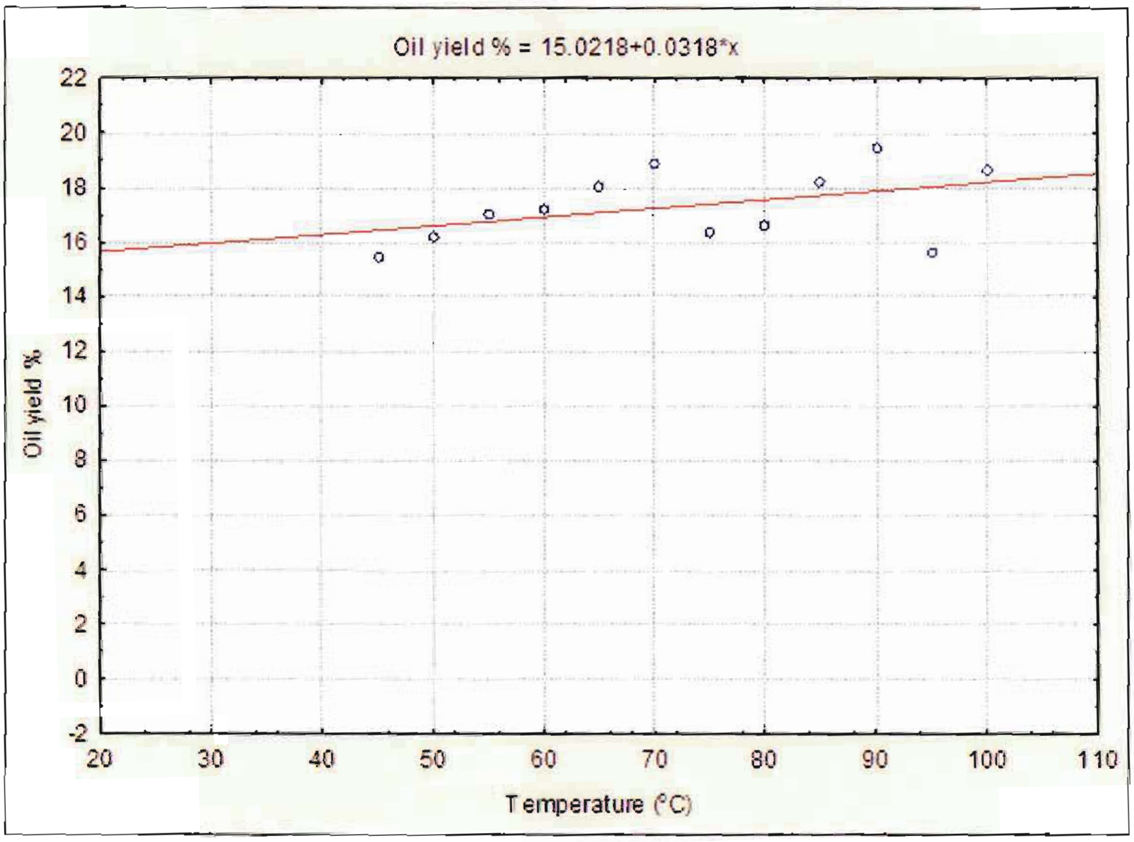


Figure 5.4 Yield-temperature graph (p = 300 atm, t = 30 min, ø = 1.5 L/min, mode = dynamic)

The insignificant effect of temperature can be ascribed to the two opposing effects on sc-CO_2 extraction mentioned previously (Chapter 3). An increase in temperature results in a decrease in the activation energy of the extraction process, but at the same time it decreases the density and thus the solvent strength of the fluid. A decrease in the activation energy affects the yield positively, while a decrease in density has a negative effect on the yield. The two opposing effects largely cancel each other, resulting in the temperature independence observed in Figure 5.4. The very slight increase in yield with increasing temperature in Figure 5.4 is too small to allow for the calculation of the energy of activation from the Arrhenius equation as outlined in Chapter 3.

5.3.2. PRESSURE

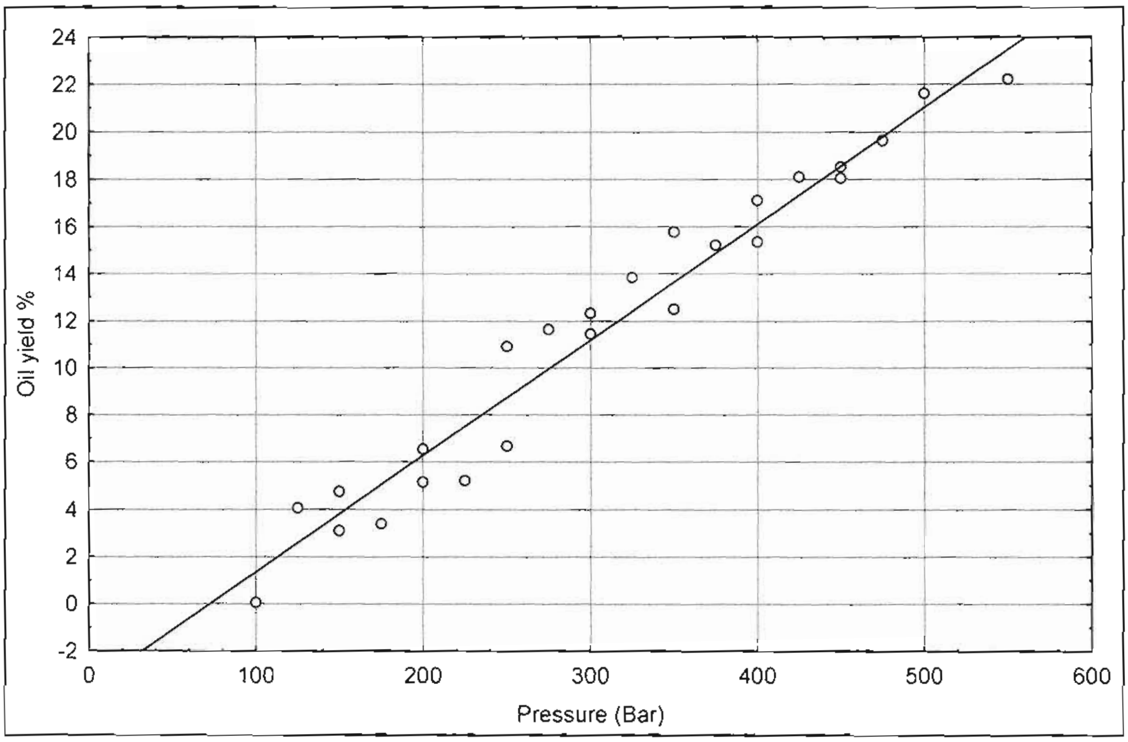


Figure 5.5 Yield-pressure graph (T = 50°C, t = 30 min, ø = 1.5 L/min, mode = dynamic)

The yield of extracted oil increases linearly with an increase in pressure according to Figure 5.5. The reason for this is that the higher the pressure at constant temperature, the higher the density of the fluid and thus the more liquid-like the sc-CO_2 becomes. The solvent strength thus increases with pressure, resulting in larger amounts of dissolved oil.

In Figure 5.6 ln (yield) is plotted against pressure (p) at constant temperature (T

= 50°C) to calculate $\Delta V^{\neq}$ from the slope = $\frac{-\Delta V^{\neq}}{RT}$ as described earlier (Chapter 3). The value turned out to be $\Delta V^{\neq}$ = -130 mL/mol. This large negative value is in agreement with the large decrease in volume in the transition state when canola oil dissolves in the highly compressed sc-CO_2.

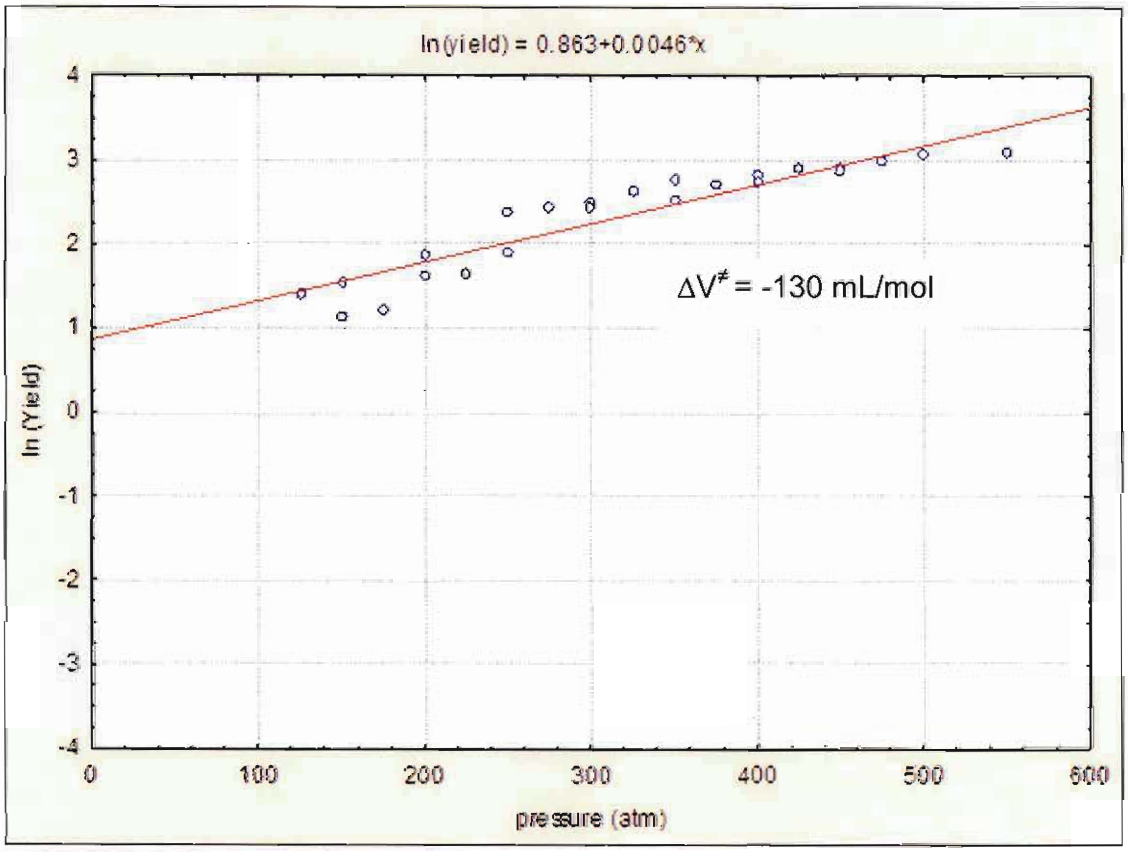


Figure 5.6 Determination of $\Delta V^{\neq}$

5.3.3. DENSITY

As mentioned earlier, the density of sc-CO_2 depends on temperature and pressure. It is the most important parameter of the extraction process. The density of CO_2 determines the extent to which the fluid exhibits liquid-like or gas-like behaviour. When the density is close to 0 g/mL, it has a more gas-like nature, and when the density is close to 1 g/mL, it behaves more liquid-like.

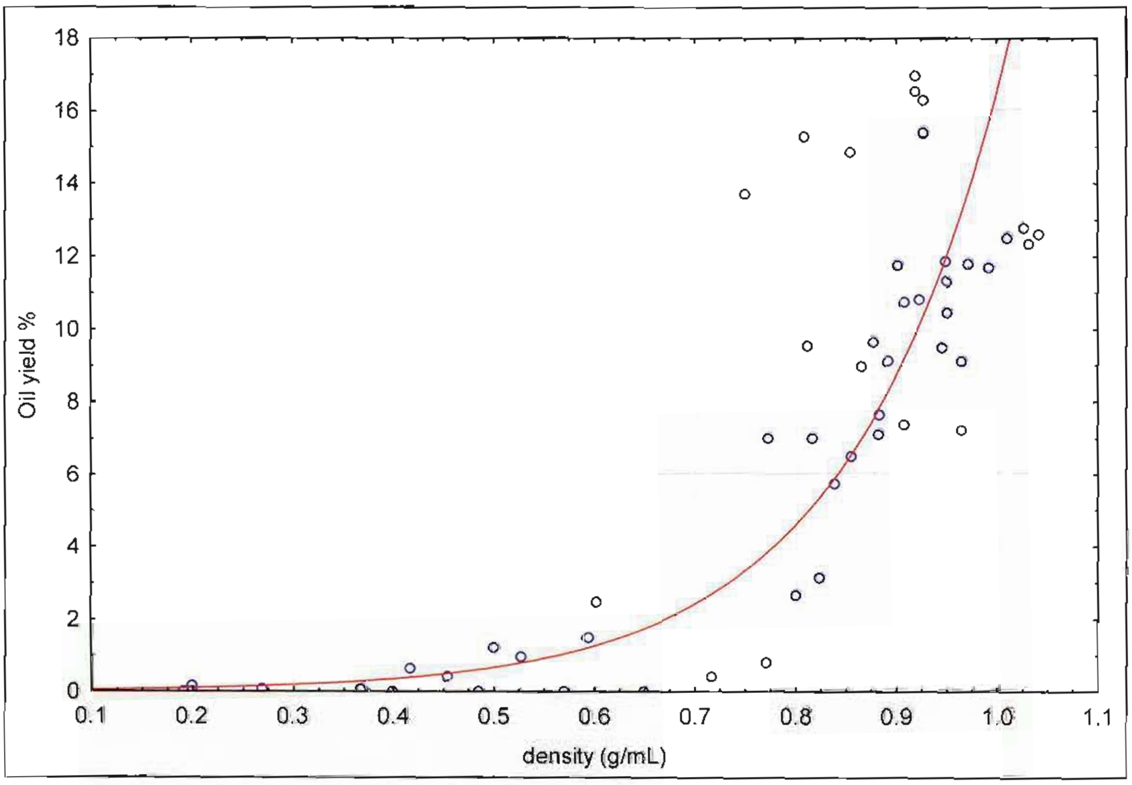


Figure 5.7 Yield-density graph

The yield of extracted oil increases almost exponentially when sc-CO_2 changes from gas-like (0.0 – 0.7 g/mL) to liquid-like densities (0.8 – 1.0 g/mL), proving that the yield of extraction is related to the solvent strength of the fluid and that the mechanism of extraction is principally chemical dissolution. This result has been found for numerous botanical extractions (e.g. caffeine, harpagoside) and relates extraction to the capability of the fluid to dissolve substances when it acquires liquid-like densities.

The statistical design in Table 5.1 was adopted to optimise the yield of extracted oil in terms of the physical parameters dealt with above[2]. It comprised a minimum of 10 runs to establish a relationship among the major parameters (yield, temperature and pressure).

Table 5.1 Statistical model

Run	Variable 1 (Pressure)	Variable 2 (Temperature)
1	-1	-1
2	-1	1
3	1	-1
4	1	1
5	-1.414	0
6	1.414	0
7	0	-1.414
8	0	1.414
9	0	0
10	0	0

The first 4 runs constitute a 2 x 2 orthogonal design. Runs 5 to 8 are the added quadratic components called star points. These added points do not sacrifice the rotatablility and orthogonality of the set of variables. The remaining runs test the linearity and polonomability of the model.

The values in Table 5.1 have no meaning until specific values are substituted. These are calculated by means of several input values within the range of experimental feasibility in Table 5.2, and are listed in Table 5.3.

Table 5.2 Input values for statistical design

Input	Value
Number of factors	2
Number of blocks	1
Number of runs	10 (nc = 4 ns = 4 n0 = 2)
Temperature (low)	45°C
Temperature (centre point)	65°C
Temperature (high)	85°C
Pressure (low)	200 atm
Pressure (centre point)	375 atm
Pressure (high)	550 atm

Table 5.3 Substituted values for statistical design

Run	Variable 1 Temperature (°C)	Variable 2 Pressure (atm)	Density of CO_2 (g/mL)
1	65	622.5	0.97
2	85	200	0.55
3	65	375	0.86
4	45	550	0.98
5	85	550	0.89
6	36.7	375	0.97
7	45	200	0.83
8	65	127.5	0.41
9	65	375	0.86
10	93.3	375	0.76

Figure 5.8 shows the oil yield percentage as a function of temperature and pressure by virtue of a surface response graph resulting from the experiments performed according to the statistical design.

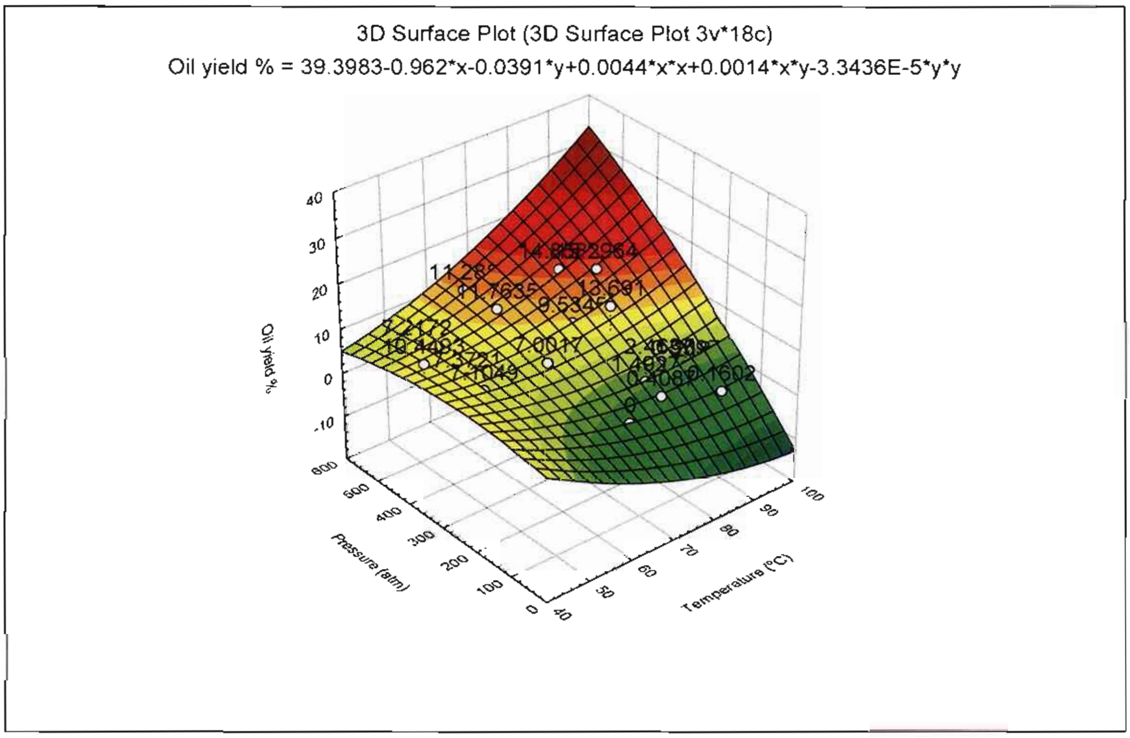


Figure 5.8 3D surface plot based on statistical design

A statistical design of only 10 runs does not compensate for smaller interactions but merely presents an approximate relationship among the three variables. For more precise work, surface response analysis based on 53 data points was performed. The result is shown in Figure 5.9 and illustrates the advantage when extensive analysis is done. The density dependence in Figure 5.7 is clearly echoed, while the dominating role of pressure compared to temperature is also confirmed. Pressure is the density determining factor which, in turn, relates to the solvent strength and thus the amount of oil soluble in sc-CO_2.

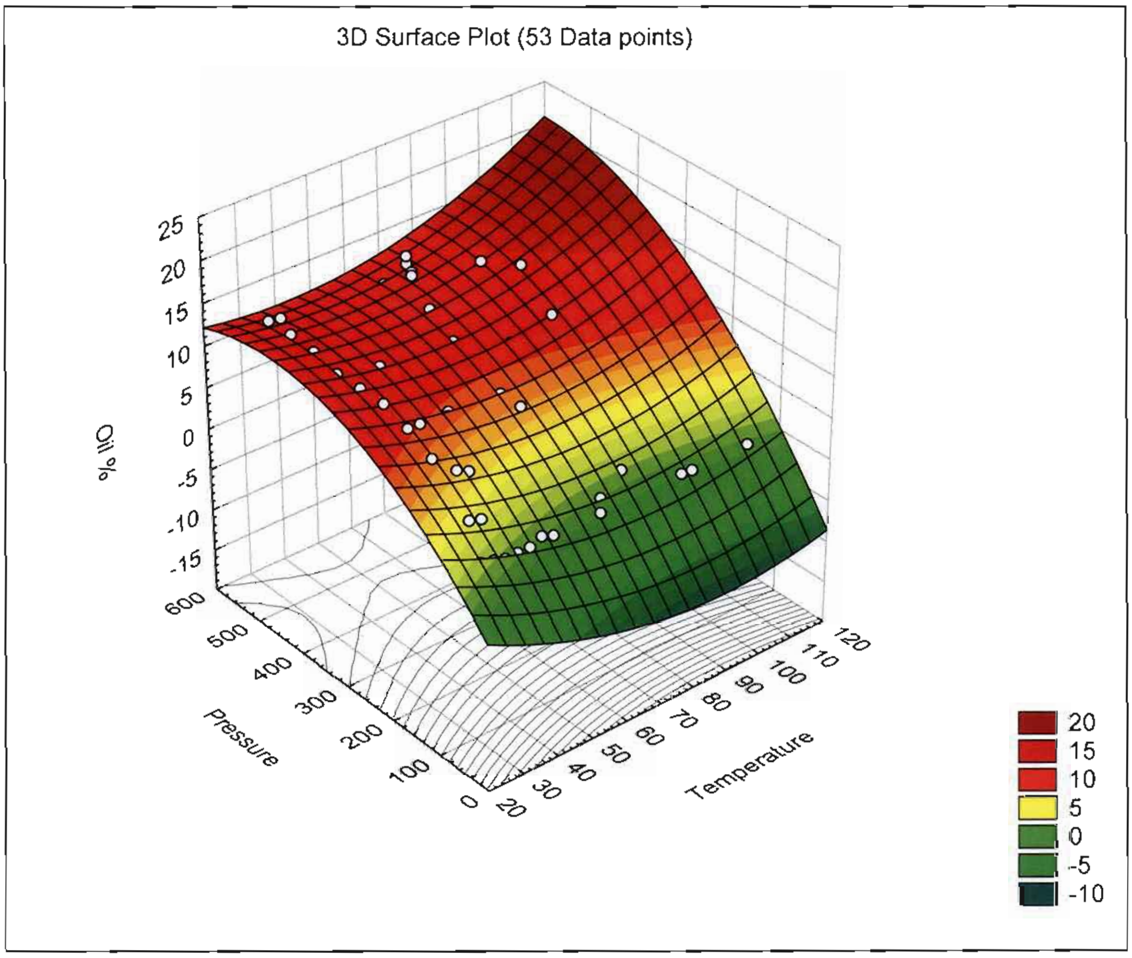


Figure 5.9 Extensive 3D surface plot

5.4. OIL ANALYSIS

5.4.1. GC ANALYSIS

GC-FID analysis was done on a sc-CO_2 extract as well as on a sample of commercially available canola oil. The chromatograms of the extract and commercial sample are compared in Figure 5.10. The components of the sc-CO_2 derived oil are listed in Table 5.4 and those present in the commercial sample are listed in Table 5.5.

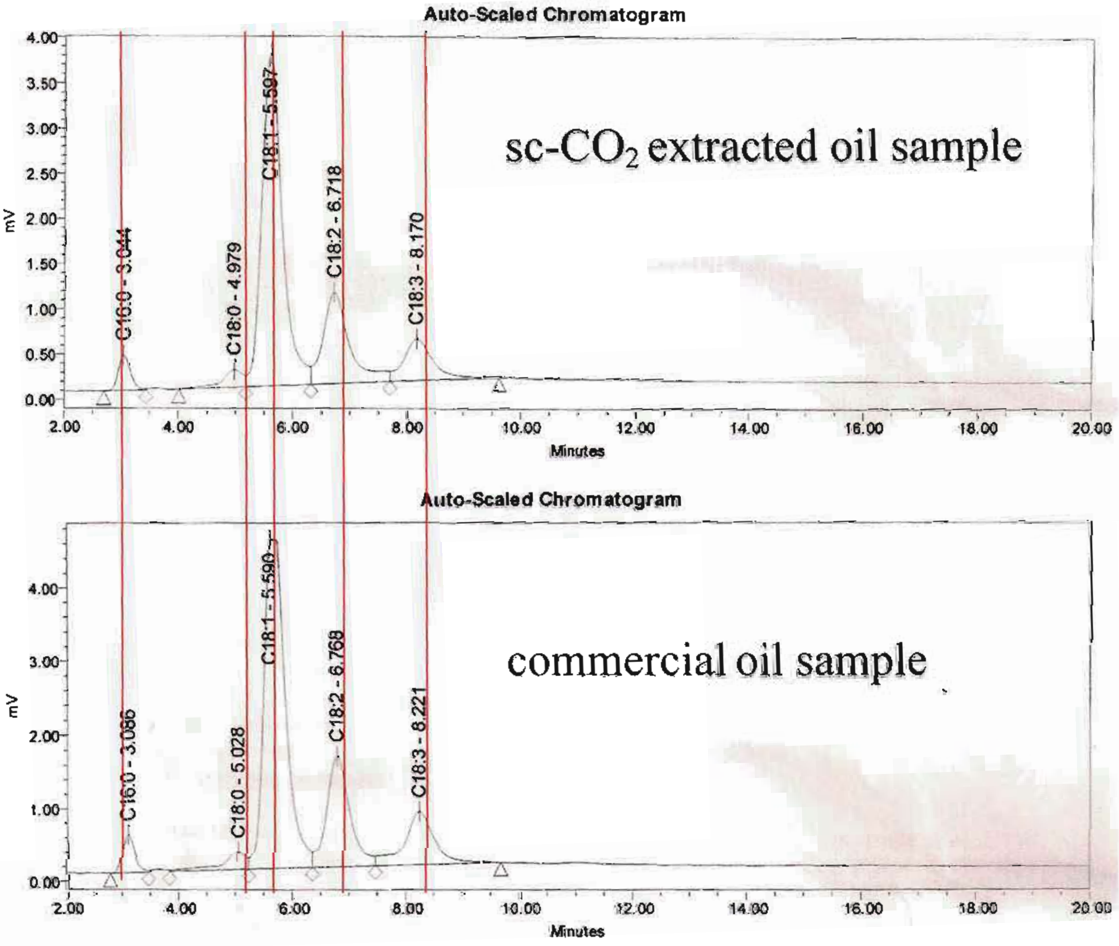


Figure 5.10 GC-FID analysis of sc-CO_2 and commercial sample

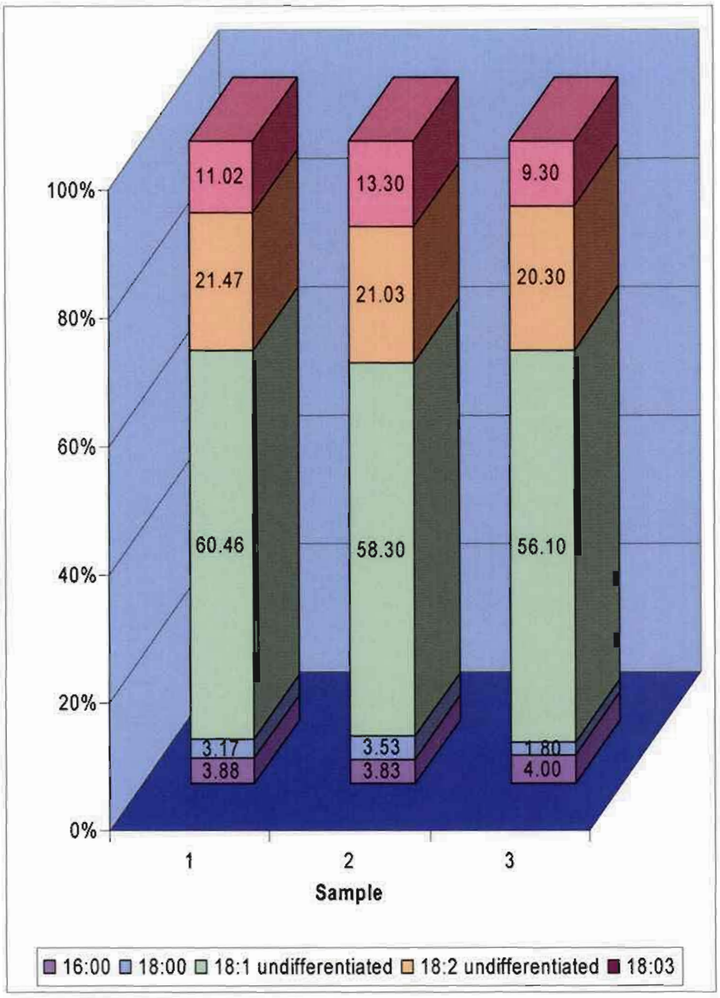
Table 5.4 Compounds in sc-CO_2 extracted oil sample

Peak Results				
	Fatty Acid	**Name**	**RT**	**%Area**
1	C 14:0		1.866	
2	C 16:0	Hexadecanoic acid	3.044	3.88
3	C 18:0	Octadecanoic acid	4.979	3.17
4	C 18:1	Octadecenoic acid	5.597	60.5
5	C 18:2	Octadecadienoic acid	6.718	21.5
6	C 20:0	Eicosanoic acid	7.809	
7	C 18:3	Octadecatrienoic acid	8.170	11.0
8	C 22:0	Docosanoic acid	10.92	
9	C 22:1	Docosenoic acid	11.97	
10	C 24:0	Tetracosanoic acid	14.95	
Sum				100

Table 5.5 Compounds in commercial oil sample

Peak Results				
	Fatty Acid	**Name**	**RT**	**%Area**
1	C 14:0		1.866	
2	C 16:0	Hexadecanoic acid	3.086	3.83
3	C 18:0	Octadecanoic acid	5.028	3.53
4	C 18:1	Octadecenoic acid	5.590	58.3
5	C 18:2	Octadecadienoic acid	6.768	21.0
6	C 20:0	Eicosanoic acid	7.809	
7	C 18:3	Octadecatrienoic acid	8.221	13.3
8	C 22:0	Docosanoic acid	10.92	
9	C 22:1	Docosenoic acid	11.97	
10	C 24:0	Tetracosanoic acid	14.95	
Sum				100.0

From Figure 5.10 and the data in Tables 5.4 and 5.5 it is clear that the composition of the oil obtained by sc-CO_2 extraction largely corresponds to that of commercially available canola oil. Moreover, the comparison in Figure 5.11 of the fatty acid profile of these two oil samples with a profile available in the literature[3] confirms similarity in composition and creates a high level of confidence in the GC-FID analysis.



Sample 1 = sc-CO_2 extracted canola oil (analysed by Nola Industries)

Sample 2 = commercial canola oil sample (analysed by Nola Industries)

Sample 3 = canola oil reported in literature[3]

Figure 5.11 Comparison of fatty acid profiles of sc-CO_2 sample, commercial sample and sample reported in literature

GC-GC/TOF-MS analysis resulted in a contour plot (Figure 5.12) and a surface plot (Figure 5.13) showing the total ion chromatogram of the sc-CO_2 extract. The most intense peaks are shown by the red areas. Very low level peaks are faintly visible as black dots. The x-axis shows the separation on the Rtx5 Sil MS column, which separates on the basis of boiling point. The y-axis shows the separation on the DB wax column, based on polarity.

Figure 5.12 Contour plot

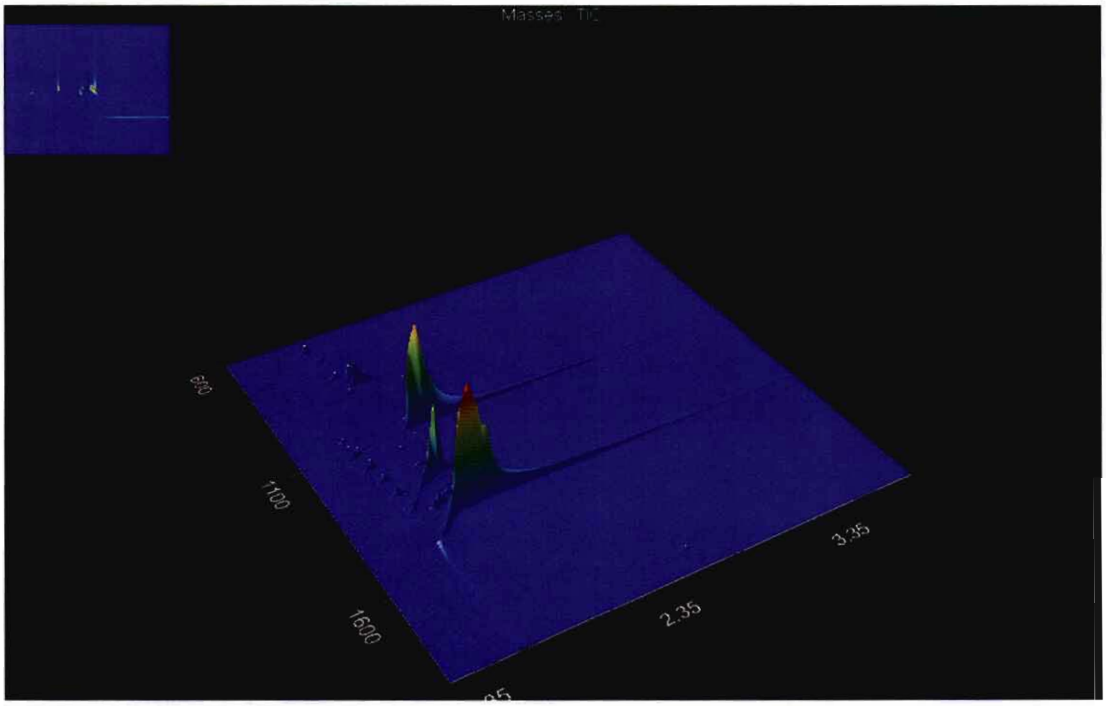


Figure 5.13 Surface Plot

Table 5.6 lists 49 compounds identified with GC-GC/TOF-MS. Every compound was identified with the highest percentage certainty, which exceeded 96% in all cases. In the last column √ indicates compounds reported in the literature and ∞ indicates compounds which were also detected by GC-FID analysis.

Table 5.6 Compounds found with GC-GC/TOF-MS

Peak #	Name of Compound	R.T. (s)	Similarity	*
1	Acetophenone	632 , 2.100	953	
2	7-Oxabicyclo[2.2.1]hept-5-en-2one	636 , 1990	764	
3	Nonanal	660 , 1.770	851	
4	Octanoic acid	708 , 1.820	920	√
5	Nonanoic acid	792 , 1.830	944	
6	2-Decenal, (Z)-	800 , 1.820	935	
7	7-Methylene-9oxabicyclo[6.1.0]non-2-ene	824 , 7.930	771	
8	2,4-Decadienal, (E,E)-	828 , 1.880	935	
9	2,4-Decadienal, (E,E)-	848 , 1.900	950	
10	8-Methylene-3-oxatricyclo [5.2.0.0(2,4)] nonane	848 , 1.950	760	
11	2-Undecenal	880 , 1.830	921	
12	2(3H)-Furanone,dihydro-5-penthyl-	884 , 2.140	931	
13	Pentadecane	980 , 1.620	912	
14	Tributyl phosphate	1048 , 1.980	881	
15	Diethyl Phthalate	1048 , 2.330	970	
16	Hexadecane	1052 , 1.630	935	
17	Pentadecane, 2,6,10-trimethyl	1080 , 1.630	917	
18	Tributyl phosphate	1080 , 2.060	700	
19	Tributyl phosphate	1112 , 1.980	804	
20	Heptadecane	1116 , 1.650	950	
21	Tetradecanoic acid	1152 , 1.860	939	
22	Octadecane	1180 , 1.660	937	
23	Germacrene D	1224 , 1.890	778	
24	Nonadecane	1240 , 1.670	941	
25	Cyclopentaneundecanoic acid, methyl ester	1256 , 1.820	887	
26	Hexadecanoic acid, Z-11	1264 , 1.950	928	√,∞
27	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1264 , 2.050	844	√,∞
28	5,9,9-Trimethyl-spiro[3.5]non-	1272 , 2.060	689	

	5en-1-one			
29	Dispiro [2.1.2.1]octane, 1,1,2,2,6,6,7,7-octamethyl-	1276 , 1.770	614	
30	n-Hexadecanoic acid	127 , 1.940	909	√,∞
31	Dibutyl phthalate	1280 , 2.300	935	
32	Eicosane	1300 , 1.690	957	
33	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-	1300 , 2.060	749	
34	Heneicosane	1352 , 1.700	957	
35	n-Hexadecanoic acid	1352 , 1.730	825	√,∞
36	9-Octadecanoic acid (Z)-, methyl ester	1356 , 1.900	947	√,∞
37	Silane, difluorodimethyl-	1372 , 3.140	979	
38	7,11-Hexadecadienal	1380 , 1.830	764	
39	9,12-Octadecadienoic acid, methyl ester, (E,E)-	1380 , 2.050	853	√,∞
40	9,12,15-Octadecatrienal	1384 , 2.120	782	
41	Oleic acid	1392 , 1.980	795	√,∞
42	2-(1-Cyclohexenyl)ethylamine	1392 , 2.030	687	
43	Docosane	1408 , 1.720	925	√
44	Cyclodecene, (Z)-	1412 , 2.030	833	
45	6-Butyl-1,4-cycloheptadiene	1412 , 2.080	789	
46	Oleic Acid	1416 , 1.980	837	√,∞
47	Tricosane	1456 , 1.7410	839	
48	1,2-Benzenedicarboxylic acid, diisooctyl ester	1576 , 2.660	922	
49	Octacosane	1812 , 2.700	948	√

*
√ = Compounds identified in literature
∞ = Compounds identified with GC-FID analysis

The identification of 49 compounds by GC-GC/TOF-MS compared to 10 compounds detected by GC-FID convincingly illustrates the superiority of the first-mentioned analytical technique. The large number of compounds present in the extract demonstrates the capability of sc-CO_2 to yield component rich extracts, though other extraction methods might be equally efficient as no GC-GC/TOF-MS data on canola oil extraction are available to contradict that.

5.4.2. CHEMICAL ANALYSIS

The chemical analysis of both sc-CO_2 and commercial canola oil samples included determination of the peroxide value, iodine number and moisture content, and these values are summarised in Table 5.7.

Table 5.7 Chemical analysis

	Peroxide value (mEq/kg)	Iodine number	Moisture content (%)
MARKET STANDARD[4]	5.0	110-120	< 0.10
Commercial canola oil sample	9.1 (before refining) 2.3 (after refining)	114	0.05
sc-CO_2 oil sample	17.8	111	0.09

The iodine number and moisture content of the two samples are very similar and comply with the market approved standard values. The peroxide value of the sc-CO_2 sample was the only specification which exceeded the allowable figure. It was reduced to 8.6 mEq/kg by passing nitrogen gas through the sample after extraction (to remove dissolved oxygen) and by protecting the sample against exposure to light and oxygen. However, this figure did not match the market approved standard value, but it compared well to the peroxide value for unrefined commercial oil.

5.5. CULTIVAR COMPARISON

sc-CO_2 extraction was performed on different cultivars to determine whether one type of canola seed excels in terms of percentage yield. The results are shown in Figure 5.14.

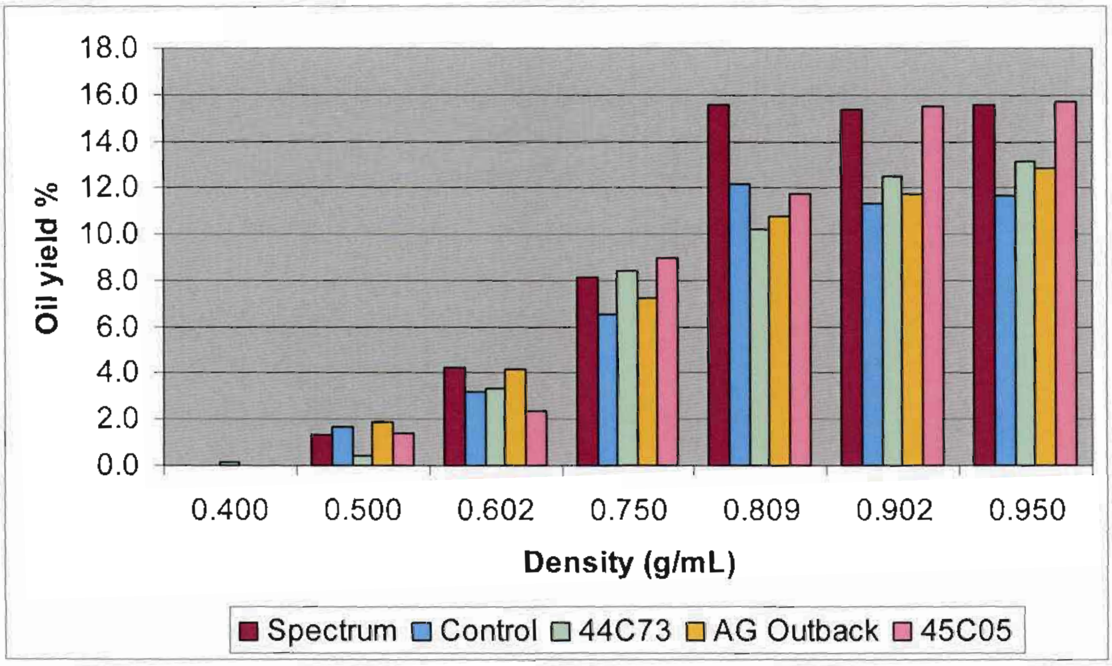


Figure 5.14 Oil yield for different cultivars

The 4 cultivars (Spectrum, AG Outback, 44C73 and 45C05), and a homogeneous mixture of the four referred to as "control", yielded comparable amounts of oil, with Spectrum and 45C05 giving a higher percentage yield at densities between 0.800 g/mL and 1.00 g/mL. The extraction of all the different cultivars exhibited the same density dependence pattern shown in Figure 5.15 and therefore indicates that the same mechanism of extraction is operational throughout the series.

Pre-drying of seed had little effect on the product yield of all the above-mentioned cultivars. Similar tendencies were observed for the different seed drying methods, so that moisture content can be discarded as a major parameter in influencing canola oil extraction.

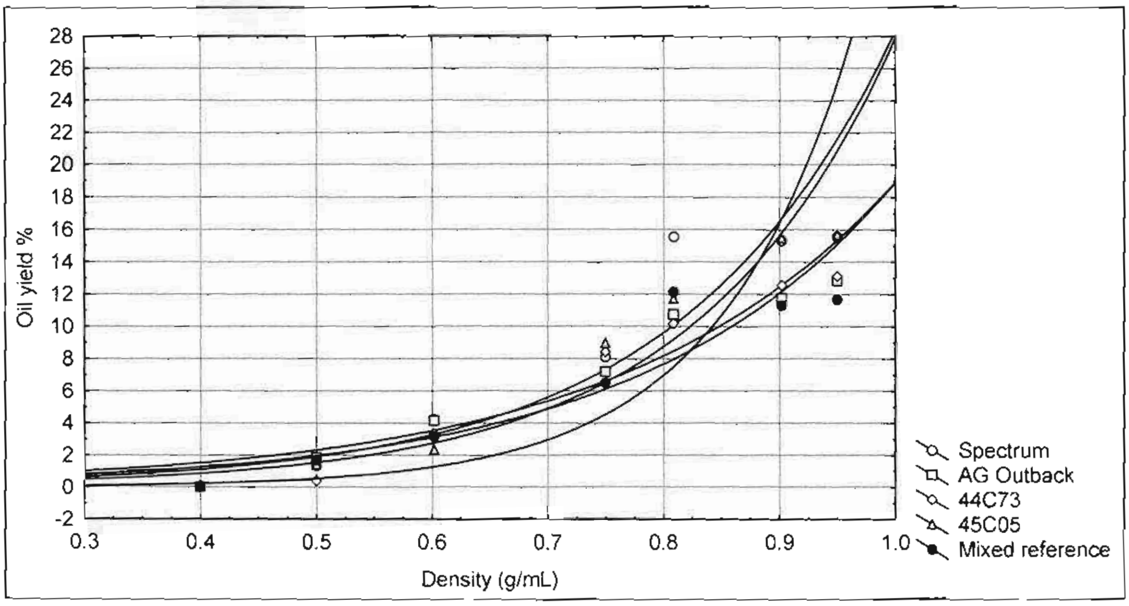


Figure 5.15 Cultivars showing same density dependence

5.6. SEED DRYING

Seed drying seems to have no effect on the mechanism of extraction, but the natural moisture content of the seed enhances the hydrophylic character of sc-CO_2 and thus results in higher oil yield as shown in Table 5.8. Consistency was warranted by using oven dried seed throughout the investigation.

Table 5.8 Comparative data for differently dried seeds

Drying method	% Yield
Undried seed	24
Oven dried seed (from Figure 6.7)	17
Sun dried seed	13
Freeze dried seed	11

5.7. REFERENCES

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CHAPTER 6

PROJECT EVALUATION

The evaluation of the project in this chapter requires the overall outcome to be compared to the objectives stated initially in the first chapter. This is done by considering the achievements and shortcomings of the investigation undertaken. With the evaluation as background, a few perspectives for further research on this topic are presented.

6.1. ACHIEVEMENTS

Canola oil could be successfully extracted from seed using non-hazardous sc-CO_2 and a state-of-the-art supercritical extractor. The composition of the oil could be determined by GC-FID and GC-GC/TOF-MS analysis, and 49 components could be identified. There was excellent agreement between the composition of sc-CO_2 and commercially available cold-press derived canola oil, and both met the quality specifications of a benchmark standard.

The density, and thus the solvent strength, of the supercritical fluid could be shown to be the principal parameter controlling the extraction since an exponential increase in the yield occurred at densities where *sc*-CO_2 becomes liquid-like and exhibits sufficient solvent strength to chemically dissolve the oil from the crushed seed matrix. The solubility of canola oil in sc-CO_2 could be successfully measured by virtue of a yield-time graph based on extraction runs performed in static mode, and the value turned out to be 0.036 g per gram of sc-CO_2 at 300 atm and 50^{0}C.

The oil content of the seed could be determined by a yield-time graph based on exhaustive extractions performed in dynamic mode, and a value of between 0.14 and 0.22 g per gram of seed was obtained, depending on the extremeness of the conditions applied. The oil content was shown to be almost the same for the four cultivars of seed used, and the yield of sc-CO_2 extraction did not change significantly on pre-drying the seed in different ways.

The extraction conditions could be optimised in terms of yield of extracted canola oil by surface response analysis based upon extraction runs performed according to a statistical design. The most favourable conditions turned out to be 80°C and 600 atm for an extraction of 70 min duration. The dependence of the yield of extracted canola oil on temperature and pressure as two major process variables enabled activation parameters to be calculated and conclusions to be drawn about the mechanism of extraction.

6.2. SHORTCOMINGS

It was only possible to perform a limited number of GC-GC/TOF-MS analyses since the equipment belongs to another institution with commitments towards many other customers. The effect of different parameters could therefore not be monitored in terms of the composition of extracts but only in terms of yield of extraction. The selective extraction of specific components by adjustment of the characteristics of sc-CO_2 by varying the principal process parameters could therefore not be demonstrated.

The results of GC-GC/TOF-MS analysis of sc-CO_2 extracts in this study could not be compared to similar results for extracts obtained by other extraction methods as this powerful technique is still fairly new and no published data of this kind could be found. The high peroxide value of sc-CO_2 derived extracts is still a matter of concern since, although it could be counteracted by several precautions, no results are available on the shelf life of sc-CO_2 derived canola oil or the sustainability of the precautions taken to control the peroxide value.

This study demonstrated the feasibility of *sc*-CO_2 extraction of canola oil from seed, but the batch type of extraction rendered the process commercially unfeasible due to the small amounts of oil accumulated and the high capital cost implied by a pilot plant or industrial scale supercritical extractor. A continuous type of extraction catering for high throughput of seed needs to be developed and implemented before industrial scale operation may become reality.

6.3. FUTURE PERSPECTIVE

In a future study efforts should be made to monitor composition as a function of different extraction variables, since these may play an important role to shift the composition into a desired direction. Specifically, a detailed investigation can be done to determine the glucosinolate levels in both sc-CO_2 derived and commercially available oil in view of the importance of glucosinolate levels for health related issues.

Commercial oil samples should also be subjected to GC-GC/TOF-MS analysis in order to achieve a more complete mutual comparison. These analyses should preferably be real-time measurements in order to exclude the detrimental effects of dissolved oxygen and exposure to light and air.

A more detailed study on the role played by natural moisture content should be undertaken, and especially the contribution of the hydrophilicity of sc-CO_2 and the behaviour of sc-CO_2 under subcritical water conditions should be attended to.

Finally, the temperature dependence of canola oil extraction should be studied more comprehensively in a lower region ($30 < T < 40\ ^0C$) and at lower constant pressure where it has more impact on extraction yield so that the activation energy can be determined with more accuracy.