

EXTRACTION OF *HELIANTHUS ANNUUS* (SUNFLOWER) OIL WITH SUPERCRITICAL CARBON DIOXIDE



**Albert Wessels
B. Pharm.**

**Supervisor : Prof. E.L.J. Breet
Co-supervisor : Prof. J.C. Breytenbach**



**YUNIBESITHI YA BOKONE-BOPHIRIMA
NORTH-WEST UNIVERSITY
NOORDWES-UNIVERSITEIT**



**EXTRACTION OF *HELIANTHUS ANNUUS*
(SUNFLOWER) OIL WITH SUPERCRITICAL
CARBON DIOXIDE**

Albert Wessels
B.Pharm. (NWU)

Dissertation submitted in partial fulfillment
of the degree

**Magister Scientae
in
Pharmaceutical Chemistry**

In the school of Pharmacy of the
North-West University

Supervisor: Prof. E.L.J. Breet
Co-supervisor: Prof. J.C. Breytenbach

Potchefstroom
2004

*“The greatest achievement in life is when
you surpass your own previous best.”*

TABLE OF CONTENTS

CHAPTER 0 » ESSENTIA

0.1	WORLDWIDE TRENDS.....	1
0.2	RESEARCH OBJECTIVES.....	2
0.3	REFERENCES.....	4

CHAPTER 1 » HELIANTHUS ANNUUS - SUNFLOWER OIL

1.1	WHAT IS SUNFLOWER OIL.....	5
1.2	BOTANICAL DESCRIPTION.....	6
1.3	THE HISTORY OF SUNFLOWER.....	7
1.3.1	SUNFLOWER SPREADING WORLDWIDE.....	7
1.3.2	SOUTH AFRICAN SUNFLOWER.....	9
1.4	HARVESTING SUNFLOWER SEED.....	12
1.5	SEED.....	12
1.5.1	OIL CONTENT.....	12
1.5.2	OIL COMPOSITION.....	15
1.5.3	OIL QUALITY.....	16
1.5.4	PROTEIN CONTENT.....	17
1.6	GOOD HEALTH THROUGH SUNFLOWER OIL.....	17
1.6.1	MINERAL NUTRITION.....	17
1.7	MEAL.....	19
1.8	MEDICINAL USE.....	19
1.9	VITAMIN E BENEFITS.....	20
1.10	COSMETICS FROM SUNFLOWER OIL.....	22
1.11	INDUSTRIAL USE.....	23
1.12	REFERENCES.....	24

CHAPTER 2 » SUPERCRITICAL FLUID EXTRACTION

2.1	DEVELOPMENTAL HISTORY.....	26
2.2	SEPARATION TECHNOLOGY.....	27
2.3	PHYSICO-CHEMICAL PROPERTIES.....	28
2.4	CHOICE OF SUPERCRITICAL FLUID.....	31
2.5	ROLE OF MODIFIERS.....	33
2.6	APPLICATIONS OF SUPERCRITICAL FLUIDS.....	35
2.6.1	FOOD PROCESSING.....	35
2.6.2	PHARMACEUTICALS.....	35
2.6.3	FRAGRANCES.....	35

2.6.4	ENVIRONMENTAL.....	35
2.6.5	SUPERCRITICAL WATER OXIDATION (SCWO).....	36
2.6.6	METAL PROCESSING.....	36
2.6.7	DEGREASING.....	37
2.6.8	IMPREGNATION.....	37
2.6.9	PARTICLE FORMATION.....	37
2.6.10	BIOTECHNOLOGY.....	38
2.7	FUTURE OUTLOOK.....	38
2.8	REFERENCES.....	39

CHAPTER 3 » EXPERIMENTAL PRINCIPLES AND PROCEDURES

3.1	MATERIALS AND SAMPLE PREPARATION.....	41
3.2	APPARATUS.....	43
3.2.1	OPERATION OF ISCO SFX™ 220.....	44
3.2.2	EXTRACTION WITH LECO TFE 2000.....	45
3.3	OIL ANALYSIS.....	48
3.3.1	CHEMICAL ANALYSIS.....	48
3.3.2	INSTRUMENTAL ANALYSIS.....	49
3.4	PILOT-PLANT SCALE EXTRACTION.....	50
3.5	STATISTICAL DESIGN.....	51
3.6	ACTIVATION PARAMETERS.....	54
3.7	REFERENCES.....	55

CHAPTER 4 » DATA ACQUISITION, PROCESSING AND INTERPRETATION

4.1	EXTRACTION RUN DURATION.....	56
4.2	TEMPERATURE, PRESSURE AND DENSITY DEPENDENCE.....	59
4.3	DRIED SEED EXTRACTION.....	67
4.3.1	OVEN-DRIED SEED.....	67
4.3.2	FREEZE-DRIED SEED.....	68
4.3.3	SUN-DRIED SEED.....	70
4.4	PILOT-PLANT OPERATIONS.....	73
4.5	EVALUATION OF EXTRACTED OIL.....	73
4.5.1	PHYSICAL COMPARISON.....	74
4.5.2	ANALYTICAL COMPARISON.....	76
4.5.2.1	CHEMICAL ANALYSIS.....	76
4.5.2.2	INSTRUMENTAL ANALYSIS.....	77
4.6	REFERENCES.....	81

CHAPTER 5 » STOCK-TAKING OF PROJECT ACHIEVEMENTS

5.1	PROJECT ASSESSMENT.....	82
5.2	FUTURE POSSIBILITIES.....	85
5.3	REFERENCES.....	86

APPENDIX

A1	ANALYTICAL PROCEDURES FOR CHEMICAL ANALYSIS.....	88
A1.1	DETERMINATION OF FREE FATTY ACID (FFA) CONTENT.....	88
A1.2	DETERMINATION OF PEROXIDE VALUE.....	89
A1.3	DETERMINATION OF PHOSPHOROUS CONTENT.....	90
A1.4	DETERMINATION OF SOAP IN OIL.....	94
A2	GC/MS ANALYSIS OF EPKO REFINED SUNFLOWER.....	95
A3	GC/MS-ANALYSIS OF sc-CO ₂ DERIVED SUNFLOWER OIL.....	97
SUMMARY.....		100
OPSOMMING.....		102
ACKNOWLEDGEMENTS.....		104

CHAPTER 0

Essentia

This study was performed within the Separation Science and Technology (SST) research focus area at the North-West University, Potchefstroom Campus. The SST accommodates six subprograms:

1. Catalysis and Synthesis;
2. Supercritical Technology;
3. Membrane Technology;
4. Coal Technology;
5. Advanced Separations;
6. Hydrometallurgy;

and research reported here was done within the supercritical technology group.

0.1 WORLDWIDE TRENDS

Worldwide, people are looking for a better way of living and for better products in the marketplace. They insist on purity and naturalness, and scientists are searching for new ways to "change" products for the better and to give consumers environmentally friendly and natural products [1]. There is concern about vast amounts of

organic solvents needed for solvent extraction, as well as disposal of residues of these solvents in extracts, and regulations for the use of hazardous solvents become stricter [2].

It is with these worldwide trends in mind that the supercritical carbon dioxide (sc-CO₂) extraction of *Helianthus annuus* (sunflower) oil from seed was selected as a research topic. The huge market for sunflower oil poses a challenge for scientists to optimise the yield, quality and extraction time of sunflower oil, and the implementation of supercritical technology could be a preferred way to meet this challenge [3,4].

In the next few chapters the reader will realise why sc-CO₂ extracted sunflower oil meets all the requirements demanded by modern humanity.

0.2 RESEARCH OBJECTIVES

The focus in this research project was on the extraction of a botanical oil which can be performed faster, more efficient, safer and environmentally friendlier with sc-CO₂. The objectives with this project were to:

1. utilise advanced laboratory-scale supercritical extractors to verify the viability of extracting sunflower oil from seed by sc-CO₂ as an alternative to oil acquisition by cold-press crushing of seed;
- 2 optimise the amount of sunflower oil extracted with sc-CO₂ by establishing the most ideal process conditions (temperature, pressure, time, moisture

content, and more) with statistical experimental design and surface response analysis;

- 3 compare oil extracts from seed with natural moisture content and from pre-dried seed as well as from hulled and dehulled seed;
- 4 disclose the principal features of the extraction process by studying various dependencies and by calculating thermodynamic and kinetic quantities in order to maximise process insight, control and tunability;
- 5 produce sc-CO₂ derived extracts for physical-chemical comparison (appearance, composition and quality) to those acquired by mechanical pressing and to a set of standard specifications for marketable oil;
- 6 upscale laboratory-scale operation to pilot-plant scale extraction in order to explore sc-CO₂ extraction of sunflower oil for industry.

0.3 REFERENCES

1. Adamson, S. (2003). Verbal communication with the representative from Natural Ingredients at the Annual Processing Exhibition. Kyalami Race Track. Johannesburg.
2. D. NOBLE, Anal. Chem., 1993, 65, 693A. Also see the *Montreal Protocol*, according to which a few traditional solvents were banned by 1995.
3. E. BREET, R. VAN ELDIK & R. STEINER, Chemical Processing SA, 1996, 3 (6).
4. The classical example of SFE is the extraction of caffeine from coffee beans with $sc\text{-CO}_2$ by the company HAG AG when the use of dichloromethane for this purpose was prohibited in Germany.

CHAPTER 1

Helianthus annuus - Sunflower oil

Sunflower seed contains oil which has become the preferred oil among consumers and chefs, since it is an excellent vegetable oil both in terms of its healthy characteristics and applicability to a wide range of uses. Sunflower oil has the highest level of unsaturated fat of which 72% is linoleic acid, one of the essential polyunsaturated fatty acids required by the human body. Sunflower oil is a rich natural source of vitamin E which is an antioxidant. Sunflower oil is not only valued by the health and medicine industry, but it is also used in the fragrance industry.

1.1 WHAT IS SUNFLOWER OIL?

Sunflower oil is a botanical oil which, depending on the method of acquisition, is a completely natural product. Synthetic oils cannot compete with pure natural oils as modern science still cannot copy it exactly. Scientists find it hard to identify all the different components in pure oils. Since each botanical oil has very specific properties and qualities, each is dealt with on its own [1].

1.2 BOTANICAL DESCRIPTION

FAMILY: Asteraceae
GENUS: *Helianthus*
SPECIES: *annuus*

[SYNONYMS:]

H. indicus L.
H. tubaeformis Nutt.
H. platycephalus Cass.
H. macrocarpus DC.
H. ovatus Lehm.
H. lenticularis Dougl.
H. colossus Kunze.
H. erthrocarpus Barth.
H. multiflorus Hook.
H. grandiflorus Wender.
H. lindheimerianus Scheele.
H. cirrhoides Lehm.
H. aridus Rydb.
H. jaegeri Heiser.
H. annuus var. *macrocarpus* (DC.) Ckll.
H. annuus subsp. *Jaegeri* Heiser.
H. annuus subsp. *Lenticularis* (Dougl) Ckll.
H. annuus subsp. *Texanus* Heizer. [1]

COMMON KNOWN NAME:

Sunflower



Figure 1.1: Sunflowers following the sun.

TAXONOMY:

The sunflower plant varies widely in height, with full-season field crop varieties ranging from 1,5 to 2,0 m. Each plant has a single seed head which consists of 1 000 to 2 000 individual flowers joined together by a receptable base. The true leaves on the stem grow to approximately 4 cm long [2]. The large petals around the edge of a head are actually individual ray flowers, which do not develop into seed (Figure 1.2). Pollination and seed development begin at the periphery of the grain head and move toward the centre [3].

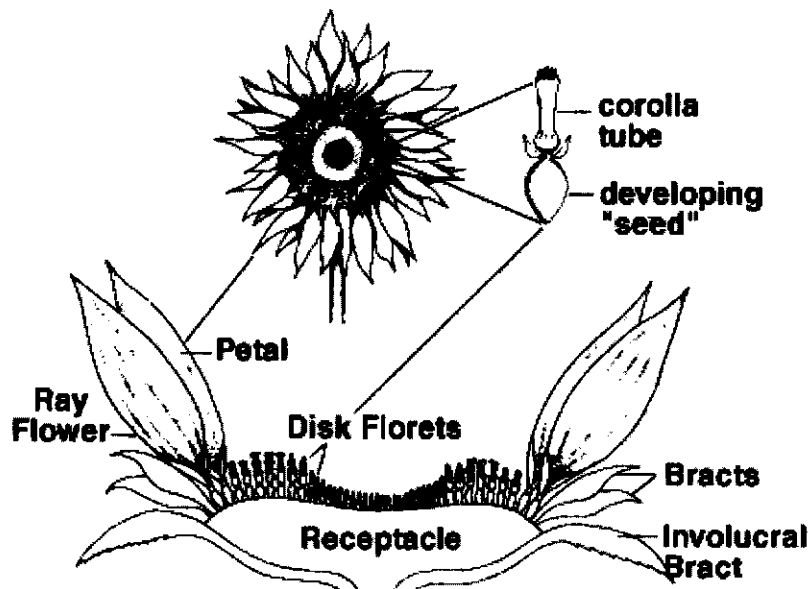


Figure 1.2: Details of the head and other selected parts of a sunflower.

1.3 THE HISTORY OF SUNFLOWER

1.3.1 SUNFLOWER SPREADING WORLDWIDE [4]

The history of sunflower (*Helianthus annuus*) is indeed amazing. The wild sunflower is native to North America, but commercialisation of the plant took place in Russia.

It was only recently that the sunflower plant returned to North America to become a cultivated crop. But it was an american Indian who first domesticated the plant into a single headed plant with a variety of seed colours including black, white, red and black/white striped.

Sunflower was a common crop among american Indian tribes throughout North America. Evidence suggests that the plant was cultivated by Indians in present-day Arizona and New Mexico about 3000 BC. Some archaeologists suggest that sunflower may have been domesticated before corn.

Around 1500, the exotic North American plant was taken to Europe by Spanish explorers. The plant became widespread throughout present day Western Europe mainly for ornamental application, but some medicinal uses were developed as well. By 1916, an English patent was granted for squeezing oil from sunflower seed.

Sunflower became very popular as a cultivated plant in the 18th century. Most of the credit is given to Peter the Great. The plant was initially used as an ornament, but by 1769 sunflower was cultivated for oil production. By 1830, the manufacture of sunflower oil was done on a commercial scale. The Russian Orthodox Church increased its popularity by forbidding most oil foods from being consumed during lent. However, sunflower was not on the prohibiting list and therefore gained immediate popularity as a food.

Canada started the first official governmental sunflower breeding program in 1930. By 1946, Canadian farmers built a small crushing plant, and in 1964 the Government of Canada licensed a Russian cultivar.



Figure 1.3: The sunflower head is known for centuries.

US acreage escalated in the late 70's to over 5 million because of strong European demand for sunflower oil. The Russians could no longer supply the growing demand, and European companies looked to the fledging US industry. Western Europe continues to be a large consumer of sunflower oil today, but depends on its own production.

Americans and Russians completed the early plant genetics and North Americans put the finishing touches to it in the form of hybridisation. Those early ancestors would quickly recognise their contributions to today's commercial sunflower if they were here.

1.3.2 SOUTH AFRICAN SUNFLOWER

In Africa reports have shown a range of 200 000 tons to over 1.2 million tons of sunflower grown. Much of this production occurred in South Africa. Some production was reported in Zimbabwe as well [1]. Figure 1.4 shows South African sunflower production of the last 15 years.

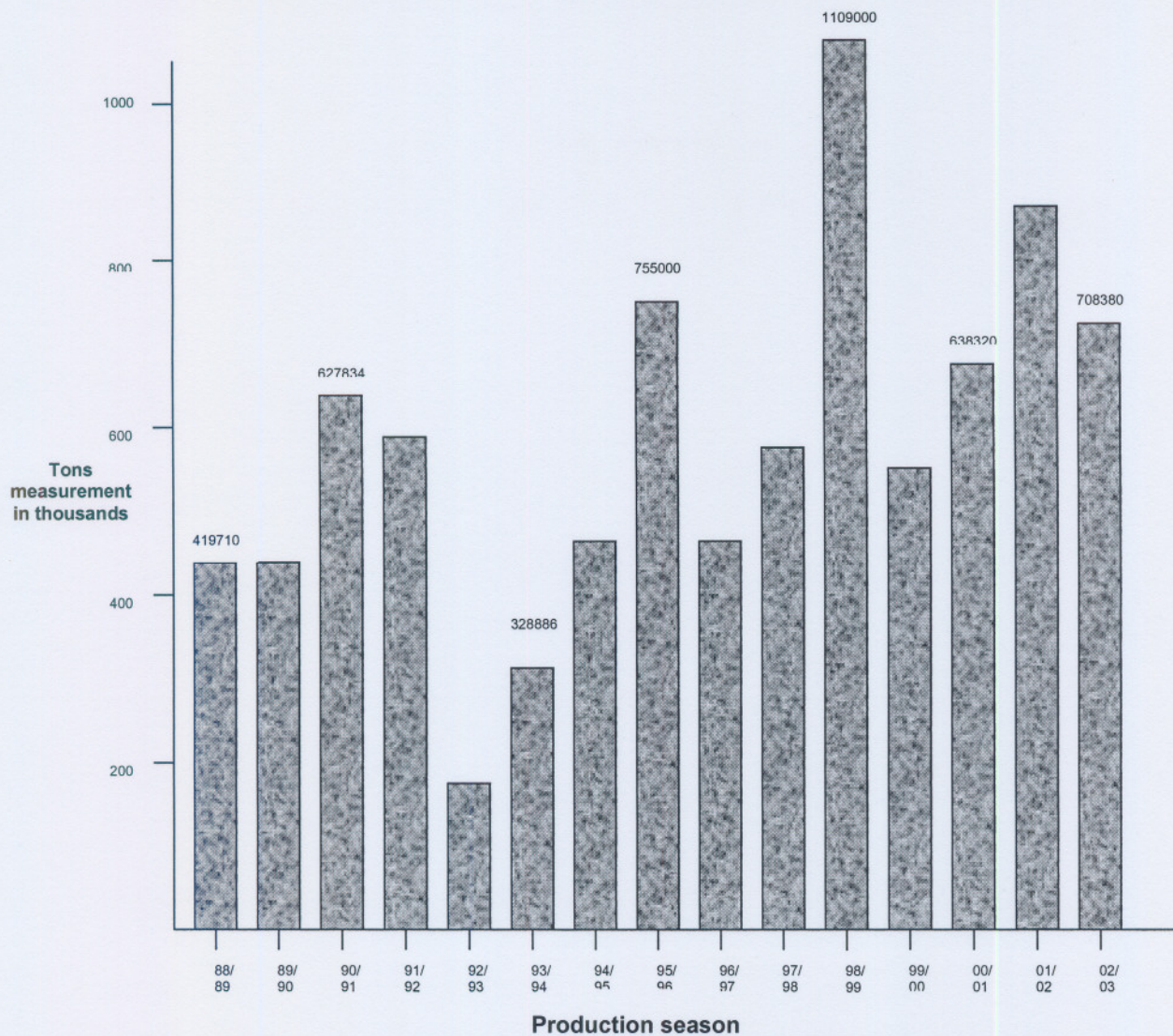


Figure 1.4: Production of sunflower seed in South-Africa

Sunflower is the most important source of plant-like oil in South Africa. During the last few years, sunflower has fallen into the class of main crops in South Africa. Furthermore, it has proven to be very competitive with other main crops. According to Figure 1.5, sunflower is produced mainly in the drier areas of South Africa, in the Free State and North West provinces. It is in these areas where droughts led to such large losses during the last 15 years that farmers could not produce lucratively [5].

Sunflower produces better harvest during drought in comparison to other crops. This is probably the main reason why it is such a favourite crop in South Africa. Unfortunately, sunflowers are particularly sensitive to high ground temperatures during its growth period. The problem is most evident on the sandy soil of western Free State and North West and leads to bad production [5].

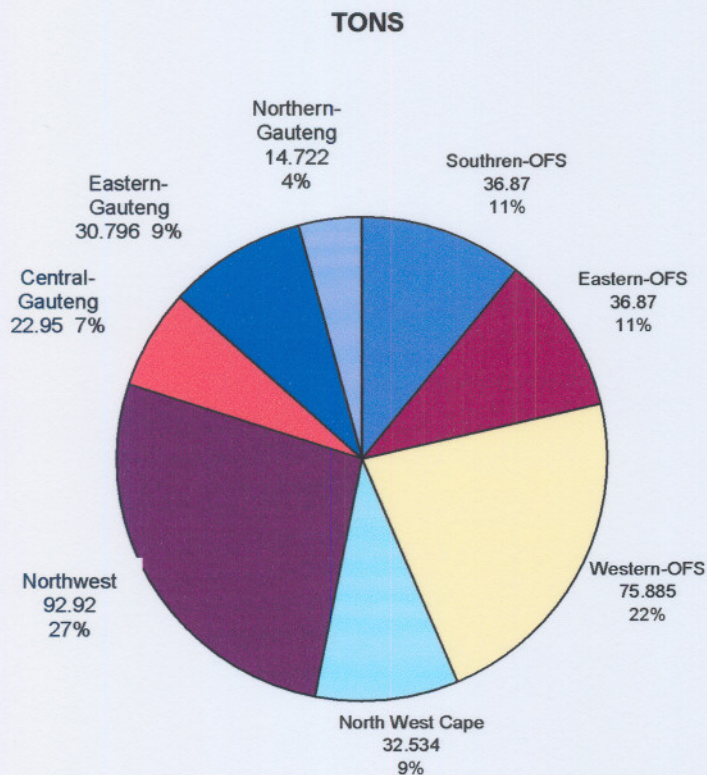


Figure 1.5: Production contributions of different areas in SA.

Sunflower crops also suffer from damage caused by birds and can therefore not be cultivated in certain areas of the country.

On the positive side, sunflower's heat tolerance and low insect cost is an advantage. A short growing season, which leads to a minimum planting period of three months, makes it very appropriate for producers that make use of adaptable rotation of crops [5].

1.4 HARVESTING SUNFLOWER SEED [6]

A growing season is approximately 120 days. It may vary in length, depending on summer temperatures, available moisture, and soil fertility. Sunflower plants are physiologically mature when the back of the head has turned from green to yellow. In some regions, the sunflower plant is frequently desiccated by killing frost. If this occurs before physiological maturity, yield loss results. To reduce seed shattering loss during harvest and loss from birds, many growers harvest sunflower early, with moisture content ranging from as high as 200 to 250 g/kg.

1.5 SEED [6]

Generally, characteristics associated with good vegetative plant growth are correlated with high yield. These include time from sowing to maturity, plant height, head diameter, leaf area, seed number, seed mass, and disease resistance. A high degree of self-fertility is also considered important for high yield in many areas, especially where insect pollinator populations are limiting.

1.5.1 OIL CONTENT

The extractable oil contributes about 80% of the total value of the sunflower crop. Oil content depends on both the percentage of hull and the oil concentration in the kernel.

The rate of increase in oil content has been reduced in recent years and some concern has been expressed that oil content in seeds may be approaching a biological limit. However, it appears feasible to develop lines and hybrids possessing over 550 - 600 g/kg oil and most breeders believe that selecting higher oil content is still an important and realistic objective [6].

Small quantities of lipids (10-30 g/kg) are normally found in all tissues of sunflower, much of it being associated with cellular and sub-cellular membranes. Seed is no exception during its early stages of growth. Rapid deposition of reserve triacylglycerols (TAG) which form the larger part of the oil content of seed only begins days after the start of rapid embryo growth, and little oil is deposited during the first third of the seed-filling period [7].

At maturity, almost all oil present in seed is located in the kernel, as shown in Figure 1.6. The hull contains about 20 to 30 g/kg, explaining the level of wastage if seed is dehulled before crushing. There has been considerable progress in recent years in understanding glycerolipid synthesis and the nature and formation of oil bodies. High population density plantations increased seed oil concentration.

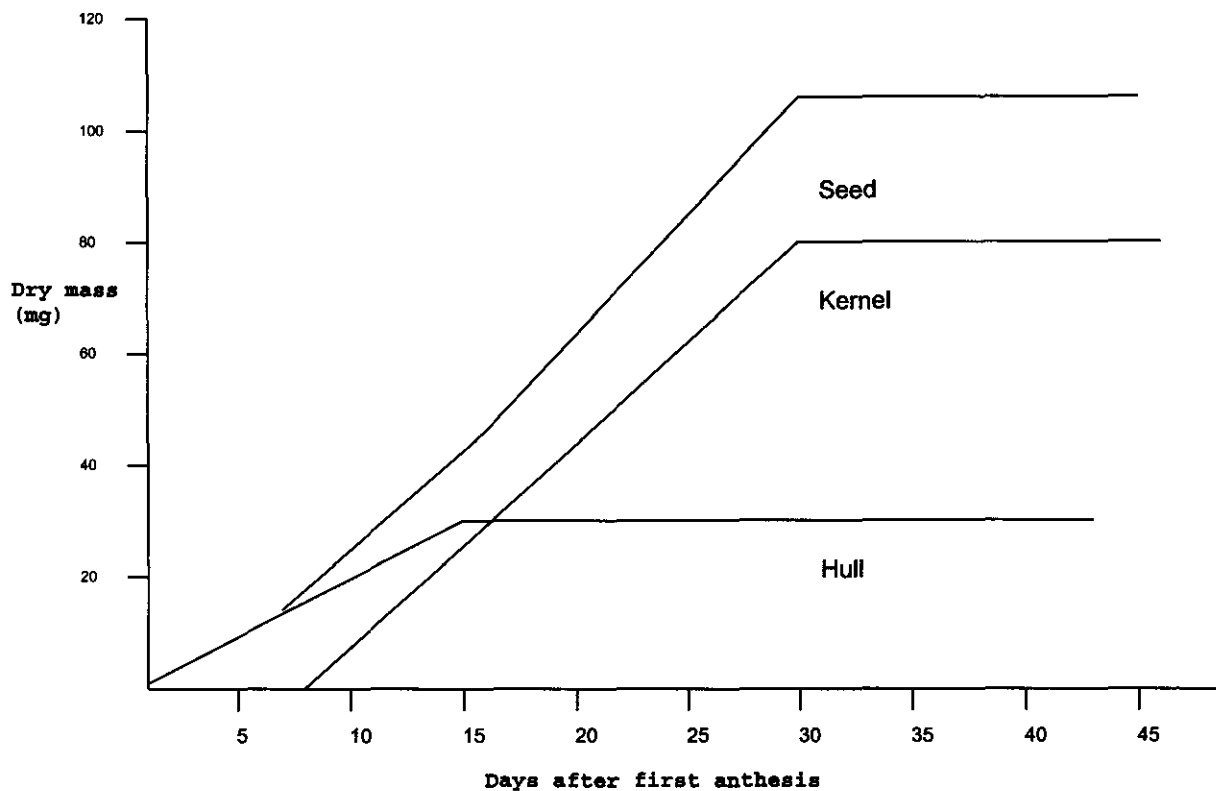


Figure 1.6: Patterns of growth of whole seed, hull, and kernel. Sloping straight lines show regressions fitted to linear-growth phases, horizontal lines to plateau of maximum dry mass.

The preceding discussion emphasises the many gaps in knowledge about oil deposition in seed and the mechanisms through which various environmental factors operate. The temporary differences in patterns of hull growth, kernel and oil, as well as the temporary spread in these patterns probably make an important contribution to these effects. However, present understanding of the physiology of organelles in which oil, protein and carbohydrates are stored in the kernel should be extended if the complex interactions between the various factors are to be understood [7].

1.5.2 OIL COMPOSITION [7,8]

Typically, the oil of mature sunflower seed contains approximately 110 g/kg saturated (mostly 16:0 [palmitic] and 18:0 [stearic]) and 890 g/kg unsaturated (mostly 18:1 [oleic] and 18:2 [linoleic]) fatty acids. The proportion of oleic and linoleic acids is under environmental, mostly temperature, and genetic control. High temperature, especially at night, has been identified as the main environmental factor reducing the ratio of linoleic/oleic acid content of sunflower lipids, although effects of water stress and extremely low irradiance have also been reported.

Triacylglycerol

Sunflower fits the general hypothesis for distribution of fatty acids on the triacylglycerol molecule.

Fatty Acid Composition

The saturated fatty acids, palmitic and stearic, typically comprise about 11% of the total fatty acids.

Phospholipids

Phospholipids are the main nontriacylglycerols or non-neutral oils in sunflower seed. These lipids are composed of glycerol esterified with fatty acids and phosphoric acid.

Waxes

The wax content of sunflower seeds is usually less than 1% of the total lipids. Approximately 83% of the wax and waxlike materials is present in the hull, averaging

between 0,8 and 1,6 g/kg, depending upon cultivar and location. Thus, the wax content of oil will depend more on the efficiency of dehulling than on the cultivar.

Sterols

Sterols are a minor component in sunflower oil and cause few problems during extraction and refining.

Tocopherols

Tocopherols as a group are also known as fat soluble vitamin E. They are important antioxidants and important in diets of cancer patients. There is also a fair amount of water-soluble vitamins in sunflower oil.

1.5.3 OIL QUALITY

Sunflower oil comprises primarily stearic, oleic, and linoleic acids, with oleic and linoleic accounting for about 90% of the total. There is an inverse relationship between oleic and linoleic acid, which is mostly influenced by the environment, especially, as previously mentioned, temperature during the growing season.

Breeding to modify the quality of sunflower oil received little attention until 1976 when genotypes with oleic acid contents as high as 900 g/kg were identified. Breeding objectives for oil quality also include increasing linoleic acid content to 750-800 g/kg for special margarine markets, reducing the palmitic and stearic acids to 60-80 g/kg in order to improve the nutritional value and compete more favourably with rapeseed oil, and increasing levels of palmitic acid to prevent crystallisation in manufacture and storage of margarine.

Increasing the level of alpha tocopherol, a form of vitamin E, has received some attention as a breeding objective [9].

1.5.4 PROTEIN CONTENT

Sunflower proteins are characterised by a moderately low level of albumin and high level of globulin proteins. The values reported for protein concentration vary largely with the source of seed [8]. Breeding to improve protein content and amino acid balance of sunflower meal has received considerable attention, especially in areas where soybean and rapeseed are not grown extensively [9].

1.6 GOOD HEALTH THROUGH SUNFLOWER OIL

1.6.1 MINERAL NUTRITION

Sunflower shares with most higher plants the essential six macronutrients (N, P, K, Ca, Mg and S) and seven micronutrients (Fe, Mn, Zn, Cu, B, Cl and Mo). Uptake depends on root exploration, soil water content, and available, rather than total, nutrient content in the soil [7].

Taste says the most, but health and nutrition rank high for consumers who want foods that are as good for them as they are good to eat. From important vitamins and minerals to convenience of size and cost effectiveness, the amazing sunflower kernel is a powerhouse compared to no other!

Cooking oil

Sunflower has become the premium cooking oil among consumers and chefs, because of its light colour and bland flavour. It has proven to be an excellent vegetable oil, both in terms of its healthy characteristics and its applicability to a wide range of uses: salad dressings, frying and baking [10,8]. Sunflower oil has value as cooking oil because of its high smoke point. Effective degumming of sunflower oil (phosphatide removal) retards oil darkening and break down under sustained frying conditions [8]. Sunflower oil has a very low level of saturated fat which helps to reduce blood cholesterol level [10].

Among all leading vegetable oils, sunflower oil has the highest level of unsaturated fat, of which 72% is linoleic acid, one of the essential polyunsaturated fatty acids required by the human body. These fatty acids have an important role in lowering blood triglyceride level, which decreases coronary heart disease risks [10].

Sunflower oil improves the nutritional quality of a diet with daily intake of sunflower oil packed with healthy fats, protein, minerals and phytochemicals [11]. The oil is rich in vitamin A, B, D and E [11,12,13].

Margarine [8]

The manufacture of margarine requires the physical characteristics of the oil to be modified to raise the melting point. Blending is the process of combining unhydrogenated sunflower oil with a selectively hydrogenated sunflower oil in such a proportion as to give a margarine oil with good body and excellent mouth feel. A high-quality margarine based solely on sunflower oil is difficult to produce.

Natural sunflower oil

Fats don't make you fat! In fact, replacing other fats in a diet with high oleic mono-unsaturated fats can help reduce lipid peroxidation and inhibit fat storage. This high oleic sunflower oil (HOSO) (Figure 1.7) is higher (at 80%) in heart healthy mono-unsaturates than olive oil, and contains no trans fatty acids [14,15]. It is a rich source of essential fatty alpha linoleic acid (ALA), a major building block for immune boosting eicosanoids that add luster to hair, and suppleness and moisture to skin.



Figure 1.7:
High oleic
sunflower oil.

1.7 MEAL [8]

Sunflower is grown principally for its oil, but the meal left behind after oil extraction is a valuable and nutritious by-product. Approximately 8.3 million tons of sunflower meal were produced worldwide during the 1990/1991 market year, which makes sunflower meal the fourth largest source of oilseed meal following soybean, cottonseed, and rapeseed.

The protein and fibre content of the meal depend upon the amount of hull material removed during processing. The oil content of the meal varies with the type and efficiency of the oil extraction process.

1.8 MEDICINAL USE

Sunflower oil has diuretic and expectorant properties and has been employed with success in the treatment of bronchial, laryngeal and pulmonary infections, coughs and

colds. The oil may be given in doses of 10 to 15 drops or more two or three times a day [16,17].

Tinctures of sunflower have been used in malarial fever combat and in combination with balsamics for the treatment of bronchiectasis. Sunflower oil has been used effectively in the treatment of several tar burn patients [18].

1.9 VITAMIN E BENEFITS

Vitamin E, an antioxidant, is a natural cancer fighter found in the germ of wheat, sunflower oil, other grains and nuts. It is a fat-soluble vitamin that is thought to protect the body from diseases such as arthritis, heart disfunctions, diabetes, bowel, lung and renal diseases, and cancer. Vitamin E renders destructive free radicals harmless before harming DNA, therefore preventing mutations and tumour growth. Studies have suggested that if vitamin E is running low, there may be an increased cancer risk [19].

Vitamin E is synthesised only by plants and is found in largest amounts in plant oils. Unlike vitamin A, which is stored in the liver in very large quantities and is easily accessible, vitamin E is kept in fat tissue and is more difficult to retrieve. The human body can go without taking in vitamin A for up to one or two years without suffering from a deficiency, but only two to six weeks without vitamin E consumption [20].

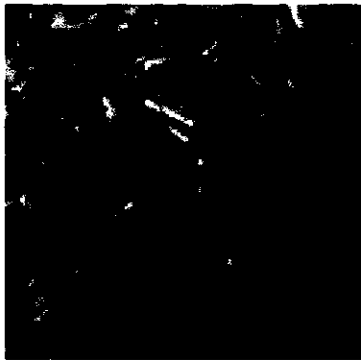
The recommended dietary allowance of vitamin E is 8 milligrams per day for women and 10 milligrams per day for men. To get the best benefit from this cancer-fighting nutrient, plenty of vitamin E rich foods should

be included in a good diet. Choosing a serving or two of vitamin C rich foods daily will help the body to recycle vitamin E and to use it repeatedly.

In Table 1.1 some food sources of vitamin E are listed:

Table 1.1: Most common food sources of vitamin E

Food	Vitamin E (mg/serving)
Almonds, 1 ounce dried	6.7
Avocado, 1 medium	2.3
Broccoli, 1 cup cooked	2.6
Brown rice, 1 cup cooked	1.4
Brussels sprouts, 1 cup cooked	1.3
Canola oil, 1 tablespoon	2.9
Hazelnuts, 1 ounce dry roasted	6.8
Mango, 1 medium	2.3
Mustard greens, 1 cup cooked	2.8
Navy beans, 1 cup cooked	4.1
Olive oil, 1 tablespoon	1.7
Peanut butter, 2 tablespoons	3.2
Pinto beans, 1 cup cooked	1.6
Soybeans, 1 cup cooked	3.4
Spaghetti sauce, 1 cup cooked	3.1
Spinach, 1 cup raw	1.6
Sunflower seeds, 1 ounce	14.3
Wheat germ, 1 ounce toasted	4.0



Sunflower seed (Figure 1.8) has double the amount of vitamin E per serving compared to the second largest plant provider (hazelnuts) [20].

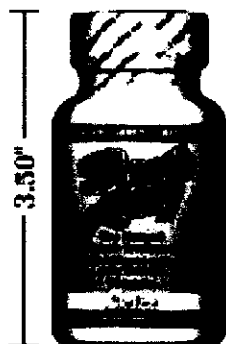
Figure 1.8: Sunflower seed.

1.10 COSMETICS FROM SUNFLOWER OIL

PERFUMES

There is a sunflower perfume range within the Elizabeth Arden products. The company promotes this exiting range with confidence [21].

BREATH FRESHENER



Swiss Herbal's "Breath Away" (Figure 1.9) is a natural breath freshener containing sunflower oil and parsley seed. It helps to clean breath that last for hours and is effective in 30 minutes [22].

Figure 1.9: Breath Away.

AIR FRESHENER

There are several examples of sunflower oil being used as an air freshener. The most common of these is the Glade Secrets Sunflowers air freshener.

1.11 INDUSTRIAL USE

Sunflower oil is not commonly used for industrial purposes because of limited supply and higher prices in relation to soybean oil. Nevertheless, its properties and advanced drying-oil technology would make it possible to adapt sunflower oil to a range of applications. Sunflower oil could find a limited market in oil based pastel paints where yellowing is a problem [8].

1.12 REFERENCES

1. PUTT, E.D. 1997. Early history of sunflower. (In SCHNEITER, A.A., ed. Sunflower Technology and Production. Madison : Wisconsin. P.15-29.)
2. ANDERSON, S.D. 1995. Sunflower Production. North Dakota State University. [Web:] <http://www.ext.nodak.edu/extpubs/plantsci/rowcrops/eb25w-3.htm>
3. MYERS, R.L. 2000. Sunflower: An American Native. University of Missouri. [Web:] <http://muextension.missouri.edu/xplor/agguides/crops/g04290.htm>
4. OPEN TEXT CORPORATION. 2003. Sunflower History. [Web:] http://www.pbench.com/sunflower_history.htm [Date of access: 24 April 2003].
5. NEL, A.A.; DU TOIT, A.P.N.; LOUBSER, H.L. Sonneblomproduksie, 'n Bestuursgids vir die Wenprodusent. 1995. 7 (2).
6. HOFMAN, V.L.; HELLEVANG, K.J. 1997. Harvesting, drying and storage of sunflower. (In SCHNEITER, A.A., ed. Sunflower Technology and Production. Madison : Wisconsin. P.671.)
7. CONNOR, D.J.; HALL, A.J. 1997. Sunflower Physiology. (In SCHNEITER, A.A., ed. Sunflower Technology and Production. Madison : Wisconsin. P.131-160.)
8. DORRELL, D.G.; VICK, B.A. 1997. Properties and processing of oilseed sunflower. (In SCHNEITER, A.A., ed. Sunflower Technology and Production. Madison : Wisconsin. P.713-736.)
9. FICK, G.N.; MILLER, J.F. 1997. Sunflower breeding. (In SCHNEITER, A.A., ed. Sunflower Technology and Production. Madison : Wisconsin. P.397-398.)
10. SLIM. 1999. Sunflower oil. [Web:] http://www.slimoil.com/sunflower_oil.htm [Date of access: 8 May 2003].
11. FOWELL, S.P. 1996. National Sunflower Association. [Web:] <http://www.sunflowernsa.com/health/default.asp> [Date of access: 2 March 2003].

12. QUILES, J.L., HUERTAS, J.R., MATAIX, J. 2002. Role of vitamin E and phenol compounds in the antioxidant capacity, measured by ESR, of virgin olive, olive and sunflower oils after frying. *Food chemistry*, 76(4):461 (8 p.), April. [In SCIENCE DIRECT : Search, Full Text + Links : <http://www.sciencedirect.com>] [Date of access: 13 Feb. 2003].
13. ALT-CANADA. 2000. Sunflower Seed Oil, Grade A WCO161. [Web:] <http://www.essential-oil.org> [Date of Access: 8 May 2003].
14. Life Services Supplements - High Oleic Sunflower Oil. [Web:] <http://www.lifeservices.com/ezStore123/DTPProductZoom.asp?productID=73> [Date of access: 2 March 2003].
15. Sunflower oil (*Helianthus annuus*). Esoteric Oils (Pty) Ltd. [Web:] http://www.essentialoils.co.za/sunflower_oil.htm {Date of access: 8 May 2003}
16. GRIEVE, M. 1995. Sunflower. [Web:] <http://botanical.com/botanical/mgmh/s/sunfl100.html> [Date of access: 6 Feb. 2003].
17. SPELLMAN, J. 1997. Sunflower. Viable Herbal Solutions. [Web:] <http://www.viable-herbal.com/herbdesc4/1sunflow.htm> [Date of access: 9 May 2003].
18. TÜREGÜN, M.; ÖZTÜRK, S.; SELMANPAKOLU, N. 1996. Sunflower oil in the treatment of hot tar burns. *Gülhane Military Medical Academy*, December 3 1996. [In SCIENCE DIRECT : Search, Full Text + Links : <http://www.sciencedirect.com>] [Date of access: 3 February 2003].
19. MINAMOTO T, MAI M, RONAI Z. Environmental factors as regulators and effectors of multistep carcinogenesis. *Carcinogenesis* 1999;20(4):519-27.
20. American Cancer Society. Cancer Facts and Figures. 1997. Atlanta, GA: 1999.
21. SLOAN, P. 1994. Light and fresh scent loud and clear for 90's. *Advertising Age*, 65(35):26, 22 Aug. [In EBSCOHost : Academic Search Premier, Full Display : <http://www-sa.ebsco.com>] [Date of access: 13 April 2003]
22. <http://www.zooscape.com/cgi-bin/maitred/GreenCanyon?quest=pl01143&proclivity=express&specie=US> [Date of access: 10 May 2003].

CHAPTER 2

SUPERCRITICAL FLUID EXTRACTION

Supercritical fluids refer to substances above their critical temperature and pressure and which cannot be classified as either a gas or a liquid. The capability of a supercritical fluid to dissolve solids was already described in 1879, but it is only in the last 25 years that supercritical fluids have been used in industry. Supercritical fluid extraction has the advantage of being a faster extraction method which produces purer oils and, if carbon dioxide is used, is environmentally friendlier than conventional extraction methods.

2.1 DEVELOPMENTAL HISTORY

The first literature on supercritical fluids appeared in 1822 [1], but only since the nineteen fifties has its industrial significance been considered. The first studies on the application of supercritical fluid extraction (SFE) in the food, petroleum and chemical industries were done by scientists of the *Max Planck Institut für Kohlenforschung*. The company, HAG (Germany) built the first plant for the decaffeination of coffee beans by $sc\text{-CO}_2$ in 1976. Soon thereafter, in 1982, the first extraction plant for hop was constructed in Germany, and later similar plants were erected in Texas and Washington.

Due to potential hazards with the use of organic solvents, SFE progressed from laboratory through pilot plant to industrial scale application. It expanded from the food industry to waste clean-up in the environment. During the last few years, development resulted from changes in environmental regulations with regard to the use of conventional solvents [1].

2.2 SEPARATION TECHNOLOGY

The use of supercritical fluids is fundamental to new separation technology such as supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC).

SFE replaces traditional techniques in many separation operations and in a few cases, especially the food industry, the supercritical fluid extracted product is superior to the organic solvent extracted or steam distilled substances.

SFC uses a supercritical fluid as mobile phase and addresses the shortcomings of both GC (by solvating volatile and thermally unstable compounds) and HPLC (by improving resolution through lower viscosity and higher diffusivity). It has now become a routine analytical tool and many contributions in recent issues of journals, since the early 1990's, should familiarise the reader with the scope of analytical applications of SFC in the field of pollutants, thermolabile compounds, natural oils and polymers, to name a few [2].

2.3 PHYSICO-CHEMICAL PROPERTIES

A supercritical fluid (SCF) is a substance prevailing at a temperature and pressure above the critical point as shown in Figure 2.1. It is neither a gas nor a liquid and is best described as an intermediate to these two extremes. The supercritical phase has solvent strengths close to those of liquids and transport properties common to gases [3].

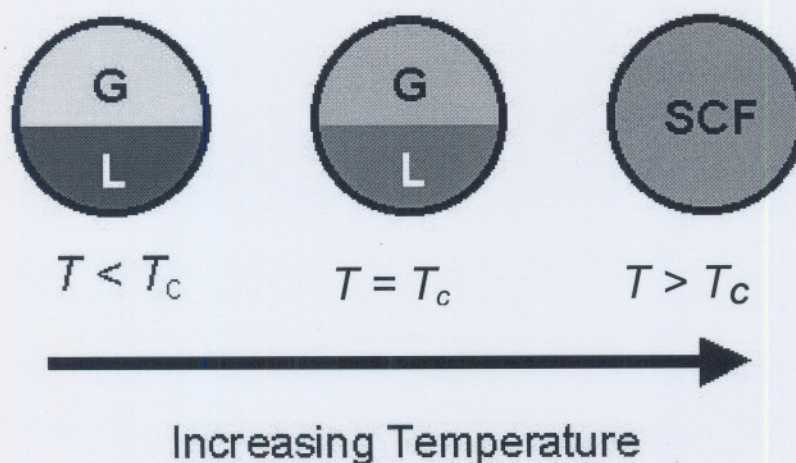


Figure 2.1: Physical properties of SCF falls between those of a liquid and a gas.

A comparison of typical values for density, viscosity and diffusivity of gases, liquids and SCFs is presented in Table 2.1.

Table 2.1: Comparison of physical and transport properties of gases, liquids and SCFs.

Property	Density (kg/m^3)	Viscosity (cP)	Diffusivity (mm^2/s)
Gas	1	0.01	1-10
SCF	100-800	0.05-0.1	0.01-0.1
Liquid	1000	0.5-1.0	0.001

In Figure 2.2 the liquid-gas boundary terminates at the critical point CP above which the supercritical fluid region (blue area) occurs. It is accessed by a combination of an isobaric change in temperature and an isothermal change in pressure. It is possible to convert a pure component from a liquid to a gas (and vice versa) via the supercritical region without incurring a phase transition.

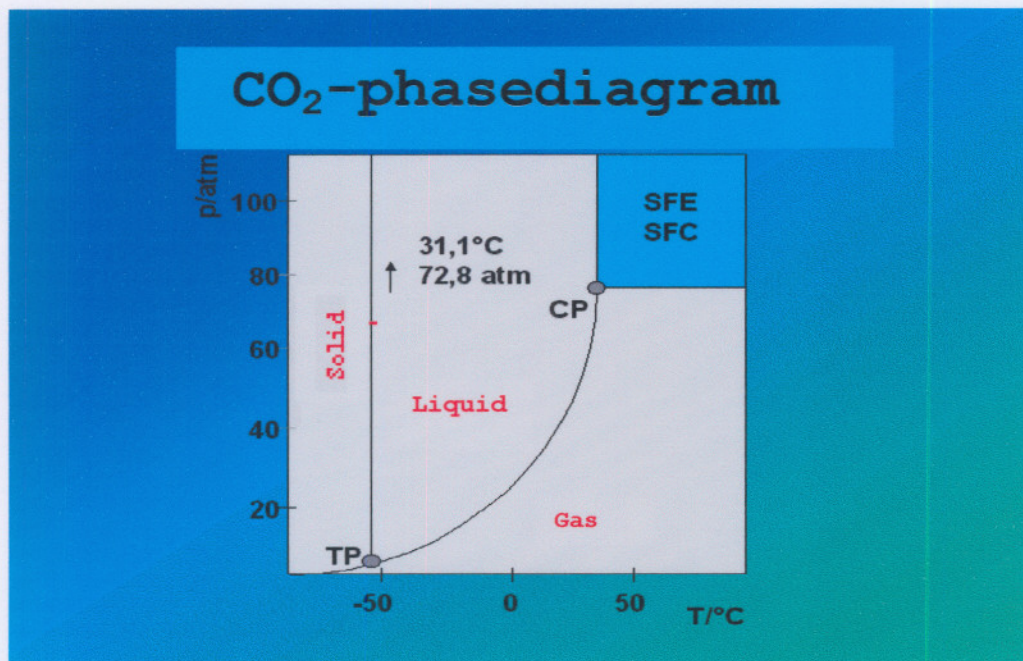


Figure 2.2: Phase diagram of CO₂.

The behaviour of a substance in the supercritical state can be described as that of a highly mobile liquid or a highly condensed gas. As Figure 2.3 shows, the solubility of a substance increases exponentially as the density of the fluid approaches liquid-like values ($800 < \rho < 1000 \text{ kg/m}^3$), while penetration into the solid matrix is facilitated by the gas-like transport properties of the fluid. Consequently, the rate of extraction and phase separation is significantly larger than with conventional extraction processes [3].

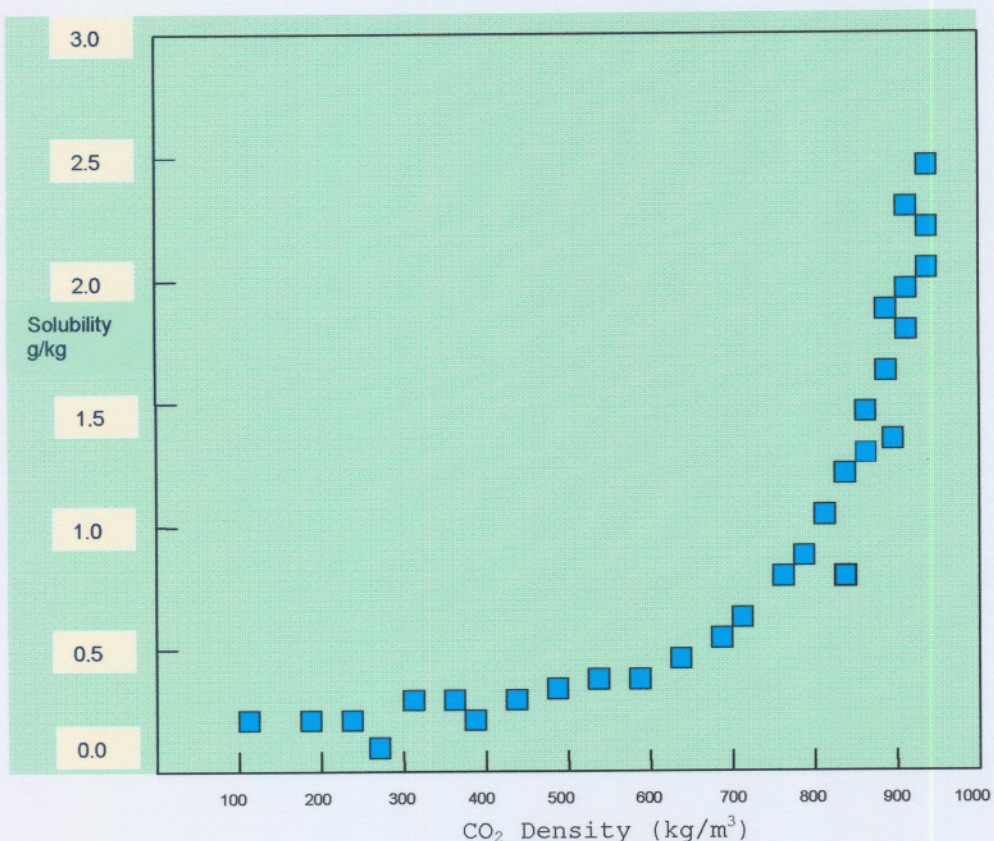


Figure 2.3: The solubility of caffeine in sc-CO₂ at 313K [4]

Supercritical fluids do not only have solvating capabilities similar to those of liquids, but also diffusivities and viscosities comparable to those of gases [5,6]. With zero surface tension, supercritical fluids are able to penetrate microporous materials. These properties are applicable to extraction, purification, fractionation and crystallization of a wide variety of materials [7].

Unlike conventional solvents, supercritical fluids are highly compressible. By changing pressure, the density can be varied over a wide range. By "tuning" the density (and therefore the solvent properties) of supercritical fluids, one can control a number of variables including solubility, phase behaviour, reaction rate and pathway (e.g. stereochemistry product selectivity), and particle size [8].

2.4 CHOICE OF SUPERCRITICAL FLUID [3]

The choice of a supercritical fluid (SCF) is similar to the selection of an appropriate solvent for regular extraction. Principal considerations include

- good solvent strength;
- inertness to product;
- easy separation from product;
- low cost;
- polarity of target substance.

Carbon dioxide is the logical choice, since it has properties most ideal for extraction. It has a relatively low critical temperature (31°C) and critical pressure (73 atm). It is non-toxic, non-flammable, relatively cheap and commercially readily available. CO₂ is regarded as environmentally friendly as it replaces hazardous organic solvents as extractants and results in extracts free from solvent residues [9].

Other substances that have also been used successfully as supercritical fluids include ammonia, argon, propane, freon, xenon and water. Table 2.2 shows the critical temperatures and pressures of a few selected substances.

Table 2.2: Critical parameters of substances.

Fluids	Critical Temperature (°C)	Critical Pressure (atm)
H ₂ O	374	220
CO ₂	31	73
N ₂ O	36	72
NH ₃	132	112
CH ₃ OH	240	78
CClF ₃	29	38
C ₂ H ₆	32	48
C ₂ H ₄	10	51

CO₂ is a superb extractant for non-polar materials, but it is less useful for polar materials. The problem can be solved by using more polar substances like N₂O and CClF₃. However, the destruction of ozone by CCl₃ and the explosiveness of N₂O limit the use of these substances on a routine basis [1].

A few advantages and disadvantages of supercritical fluids are listed below:

ADVANTAGES

- Solvent strength of SCFs is adjusted by pressure and/or temperature variation.
- SCF is easily recoverable from the extract due to its gas-like nature.
- Extract is close to natural since no solvent residue is left.
- Non-toxic solvents leave no harmful residue.
- High-boiling components are extracted at relatively low temperatures.

- Enable separations not always possible with traditional processes.
- Extract thermally labile compounds with minimal damage in view of low extraction temperatures.

DISADVANTAGES

- Expensive equipment is required.
- Elevated pressure is needed.
- Extractor personnel must be trained to acquire necessary skills.
- Compression of solvents requires elaborate recycling to reduce energy costs [3].

2.5 **ROLE OF MODIFIERS** [10]

SFE yields are significantly higher with chemically modified CO₂ than with CO₂ only. Figure 2.4 shows that toluene is one of the most effective modifiers. The CO₂/toluene mixture (90/10) has an extraction efficiency of 94.3% compared to 22.4% with CO₂. Using 10% methanol also improves extraction efficiency of CO₂ considerably from 22.4 to 77.3%. Binary modifiers, such as methanol/toluene and IPA/toluene mixtures, show improvements averaging those of toluene and methanol. A light aromatic hydrocarbon mixture (LAH) as modifier is a viable alternative to the solvents mentioned.

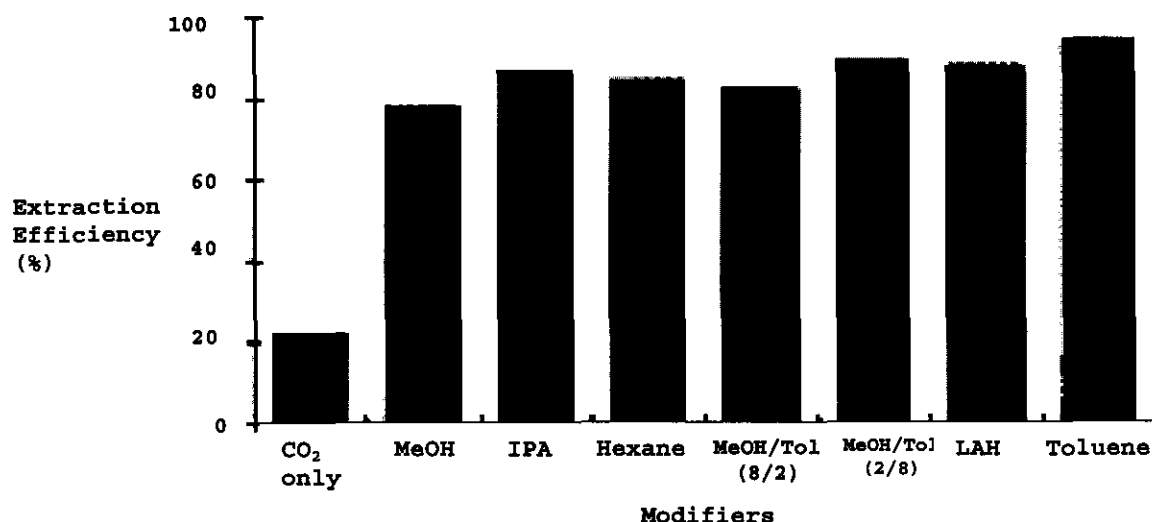


Figure 2.4: Positive effect of chemical modifiers (10% in CO₂) on extraction efficiency of oil from dolomite using sc-CO₂ at 80 atm and 35°C.

Chemical modifiers make the pressure requirement for CO₂ flow less stringent than for CO₂ alone since the threshold pressure for obtaining a miscible phase is reduced. At higher pressures, the degree of improvement in CO₂ extraction efficiency by adding chemical modifiers is greatly reduced, although the improvement is still significant.

The volume of CO₂ has a large effect on the extraction efficiency when CO₂ only is used. In contrast, the extraction efficiencies of CO₂ with chemical modifiers are not very sensitive to the volume of CO₂. The extraction yields only slightly decrease when the volume of extractant is reduced. This implies that extractions with chemically modified CO₂ can save large volumes (amounts) of CO₂ while still giving more satisfactory yields than CO₂ only.

2.6 APPLICATIONS OF SUPERCRITICAL FLUIDS

The growing demand for supercritical fluid based processes has caused various new technologies and developments to emerge. A few of these are briefly discussed in the following paragraphs.

2.6.1 FOOD PROCESSING [11]

The main commercial success of sc-CO₂ is in the food industry. Large decaffeination plants are in operation in both Europe and the US. Extraction of hop, spices and flavours are a few food processing applications of sc-CO₂.

2.6.2 PHARMACEUTICALS [11]

The extraction of natural products with sc-CO₂ has resulted in new health supplements such as an anti-cancer agent extracted from the bark of *Taxus brevifolia* and gamma-lanoline acid extracted from Evening primrose oil seed.

2.6.3 FRAGRANCES [11]

Numerous flavours and fragrances can be extracted with sc-CO₂, including celery, ginger, paprika, rosemary, sunflower, sage and vanilla which are already available commercially.

2.6.4 ENVIRONMENTAL

The majority of sc-CO₂ applications can be considered environmentally friendly since there is no net increase in the amount of CO₂ as it is removed from the environment to

be used as a solvent in a chemical process and returned to the atmosphere. Environmental applications of supercritical fluids fall in both the areas of pollution prevention and resource remediation.

2.6.5 SUPERCRITICAL WATER OXIDATION (SCWO)

Supercritical water (sc-H₂O) is a non-polar solvent since the dipole-dipole forces and hydrogen bonding are depleted under the stringent supercritical conditions (647 K, 218 bar). Water in the supercritical state is therefore able to completely mix with both oxygen and organic compounds and thus lead to fast oxidation through intimate contact of the substances involved [12].

During SCWO organic compounds react completely with O₂ to form CO₂ and H₂O. The hetero atoms chlorine, sulphur or phosphor present in organic wastes are transformed into the mineral acids HCl, H₂SO₄ or H₃PO₄, respectively. Organic bound nitrogen predominantly forms N₂ and small amounts of N₂O [12].

2.6.6 METAL PROCESSING [11]

With the addition of appropriate chelating agents, metals can be extracted in good yield from a variety of matrices with sc-CO₂. The underlying principle is to form electrically neutral complexes which are soluble in non-polar sc-CO₂. This procedure is handy for the analysis of environmental samples, the remediation of metal contaminated premises, the processing of metal ore and the separation of mixtures of metals.

2.6.7 DEGREASING [11]

sc-CO₂ is particularly suited to clean precision machinery as it is capable of dissolving greases and oils and removing these without leaving any residue on the object.

2.6.8 IMPREGNATION [13,14]

The extraction technique can be reversed to impregnate materials. Applications are found in the food (tea with lemon) and material (paper with antioxidant) industries. Timber is permanently coloured, while textiles are dyed with essentially 100% uptake and almost no waste water.

2.6.9 PARTICLE FORMATION [11,15]

Different supercritical processes have been developed for particle formation, of which RESS (rapid expansion of supercritical solution) and SAS (supercritical anti-solvent) are the most important.

RESS causes solvent-free particles with a narrow size distribution to separate from a supercritical solution through a rapid drop in pressure and thus a sudden decrease in solubility. The process has potential for micro-encapsulation, which is of interest for controlled release of ingredients as found in the drug industry.

With SAS, a supercritical fluid acting as an anti-solvent is added to precipitate a solid by reducing (through dilution) the dissolving capability of the solvent. This method has been used to precipitate a wide variety of products including food stuff, proteins and explosives.

2.6.10 BIOTECHNOLOGY [11]

The use of sc-CO₂ for bioseparations has been initiated by the discovery that proteins can be solubilised in reverse micelles formed in the fluid. This breakthrough ends a decade-long quest for a system that can form reverse micelles with an aqueous core similar enough to ambient water to solubilise higher polar compounds. Bioseparations may offset the higher cost of the pressures associated with the use of sc-CO₂.

2.7 FUTURE OUTLOOK

With numerous new applications under consideration to enhance the competitiveness of industry and the need for "clean" technology to warrant sustainable chemical development, supercritical technology may progress for years to come.

The combination of supercritical technology with established analytical methods (GC, IR) is a challenge to chemists.

The extraction of natural products from plants with desired fragrance and flavour characteristics will doubtlessly continue.

Studies involving sc-H₂O have just started. Results are promising, but there is still much work to be done to perform, to understand and to manipulate processes in this special reaction medium [14].

It may happen that supercritical technology surpasses its obstacles and become the preferred extraction method of the 21st century [1].

2.8 REFERENCES

1. PHELPS, C.L.; SMART, N.G.; WAI, C.M. *Past, Present and Possible Future Applications of Supercritical Fluid Extraction Technology*, J. Chem. Edu, 1996, 73, 1160 p.
2. HAWTHORNE, S.B. *Analytical Scale Supercritical Fluid Extraction*, Anal. Chem., 1990, 62, pA633.
3. BONN, D. 1998. Introduction of the physico-chemical properties of supercritical fluids. *Advanced Chemistry*, 44 (23):88, 22 Aug. [In SCIENCE DIRECT : Search, Full Text + Links : <http://www.sciencedirect.com>] [Date of access : 4 March 2003].
4. BRUNNER, G.; PERRUT, M. *Proceedings of the 3rd International Symposium on Supercritical Fluids*, Vol. 3, Strasbourg, France, 1994.
5. McHUGE, M.A.; KRUSKONIS, V.L. *Supercritical Fluid Extraction*, Second Edition, Butterworth-Heinemann, London, 1994.
6. GOLDMAN, S.; GRAY, C.G.; LI, W.; TOMBERLI, B.; JOSLIN C.G. *Predicting Solubilities in Supercritical Fluids*, J. Phys. Chem., 1996, 100, 7246 p.
7. MALTIN, L. 1995. Development on Extractions, May 1998. [Web:] <http://www.durability.com/home/sfe/introduction.html> [Date of access: 14 March 2003].
8. JESSOP, P.G.; LEITNER, W. 1999. Chemical Synthesis using Supercritical Fluids. *Chemistry Today*, 19 (8):56, 29 Nov. [In SCIENCE DIRECT : Search, Full Text + Links: <http://www.liv.ac.uk/~aicooper/Supercritical.htm>] [Date of access : 4 March 2003].
9. CHESTER, D.F.; PINKSTON, J.D.; RAYNIE, D.E. *Supercritical Fluid Chromatography and Extraction*, Anal. Chem., 1996, 68, 487 p.
10. HWANG, R.J.; ORTIZ, J. 2000. Mitigation of asphaltics deposition during CO₂ solvency with chemical modifiers. *Organic Geochemistry*, 31(12): 1451 (12 p.), December. [In SCIENCE DIRECT: Search, Full Text + Links : <http://www.sciencedirect.com>] [Date of access: 4 March 2003].

11. BRENNECKE, J.F. 1996. New applications of supercritical fluids. *Chemistry and Industry*, p. 831-834, November.
12. DINJUS, E.; KRUSE, A. 2002. Applications of Supercritical Water. (In van Eldik, R. / Klärner, F.G., ed. High Pressure Chemistry. University Erlangen-Nürnberg, Germany. : WILEY-VCH. p. 422-441.)
13. LIESCHESKI, P.B. *J. Agric. Food Chem.*, 1996, 44, p. 823-828.
14. BREET, E. 2003. Verbal communication with the author. Potchefstroom. (Article in possession of the author.)
15. CHESTER, T.L.; Pinkston, J.D.; RAYNIE, D.E. *Anal. Chem.*, 1994, 66, 106R-130R.

CHAPTER 3

EXPERIMENTAL PRINCIPLES AND PROCEDURES

sc-CO₂ is a potentially alternative extractant for botanical extraction and can replace conventional industrial processes such as cold-press and solvent extraction. The use of carbon dioxide is growing due to legislative reduction of the number of permissible solvents in the food industry [1]. Carbon dioxide is non-toxic, unlike hydrocarbon and chlorinated hydrocarbon solvents, and is readily available and affordable. It is used in this investigation to extract sunflower oil from seed. The technical aspects of the investigation are presented in this chapter.

3.1 MATERIALS AND SAMPLE PREPARATION

The sunflower seed used for this research was donated by the Agriculture Research Council (ARC) of Potchefstroom. It comprised AGSUN sunflower seed (code 8251) with a moisture content of 5.6 % and PAN sunflower seed (code 7392) with a moisture content of 5.4 %. The CO₂ used for extraction was supplied by Afrox. Millipore® provided the Milli-Q Plus system used for distilled water production.

Samples of 1.5 to 3.5 grams were prepared. The seed was ground to a fine, uniform consistency using an ordinary food grinder. The samples were thoroughly homogenised before using. Loading the samples into the extractor thimbles was done as follows:

The lower end cap of the thimble was installed and a Kim-wipe plug inserted into the bottom of the thimble. Using a funnel, the sample was loaded into the thimble (Figure 3.1). Finally the upper end cap was installed.

Some samples were dried prior to extraction by various methods (oven-, freeze- and sun-drying) in order to determine the oil yield from seed having different moisture content.

Freeze-dried seed

A DURA-DRY-MP freeze-drier was used. The sample of ground sunflower seed was frozen at -80°C for eight hours before freeze drying for twelve hours at -52°C with vacuum set at 76 millitorr.

Oven-dried seed

A sample of ground sunflower seed was placed in an autoclave for two hours at a temperature of 102°C .

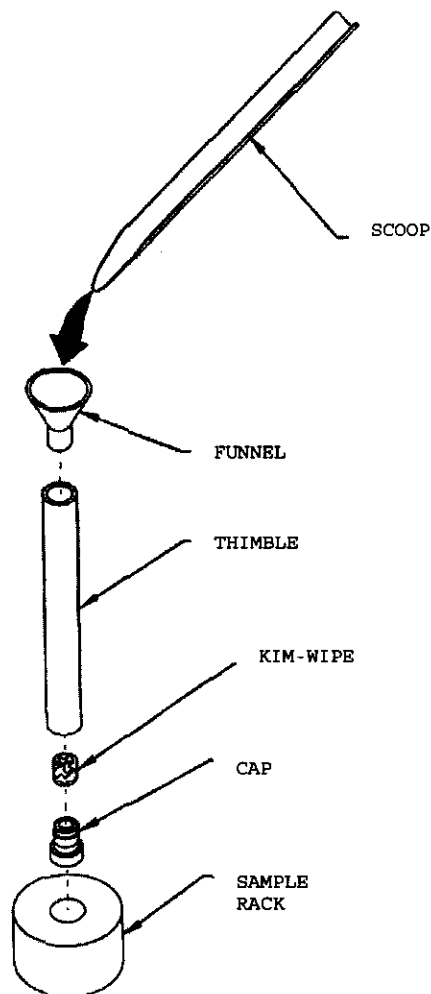


Figure 3.1: Loading sample of seed into thimble

Sun-dried seed

Ground sunflower seed was placed in an open glass container behind a window with eight hours of sun everyday for six weeks.

3.2 APPARATUS

Two types of extractor have been used, a bench-top scale extractor (ISCO SFX 220 and LECO TFE 2000), and a pilot-plant extractor (NOVA SWISS). The basic difference between the two types is that the bench-top extractors have automatic valves so that extractions can be run automatically by computer control, whereas the pilot plant is operated manually. It offers, however, the opportunity to recycle the carbon dioxide for re-use.

The apparatus required for SFE is relatively simple, and its basic components have not changed considerably since the technique became known. Figure 3.2 shows the basic components of a supercritical fluid extractor [2].

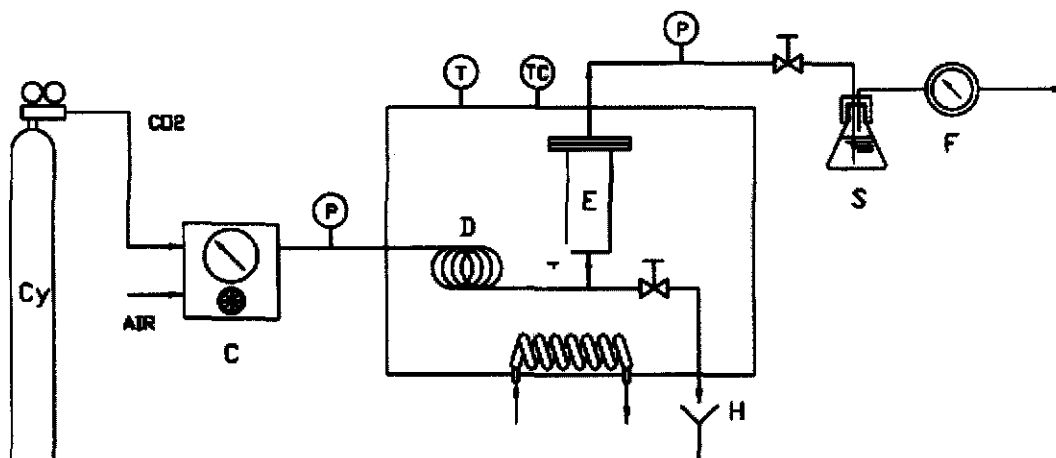


Figure 3.2: Scheme of a basic sc-CO₂ extractor. Cy: CO₂ cylinder; C: air-driven CO₂ compressor; D: heating coil; E: extraction vessel; F: CO₂ totalizer; H: extract drain; S: separator; T: thermostatic coil; TC: Thermocouple [3].

3.2.1 OPERATION OF ISCO SFX™ 220 [4]

A commercial gas cylinder supplies the CO₂ to the extractor. The required pressure is delivered by a CO₂ pump. A continuous flow of the fluid through the reactor (dynamic mode) or a fixed amount of fluid staying within the reactor during extraction (static mode) is regulated manually or by automatic valves. The extraction vessel is held at a given temperature by means of a built-in heating coil. When an extraction run is finished, the sc-CO₂ is relaxed to atmospheric conditions via a restrictor. The extraction product is accumulated in a collection vial.

Figure 3.3 shows the ISCO SFX™ 220, with which several extraction runs were performed, and Figure 3.4 shows the disposable polymer cartridges into which seed was inserted for extraction.



Figure 3.3: ISCO SFX™ 220



Figure 3.4: Disposable polymer cartridges

3.2.2 EXTRACTION WITH LECO TFE 2000

The LECO TFE 2000 supercritical extractor is superior to the previously discussed extractor in a number of ways, including

- (1) high flow rates (L/min instead of mL/min) warranting significantly reduced extraction times;
- (2) split flow line enabling three extraction runs to be performed simultaneously with separate restrictors for each extraction.

Figure 3.5 shows the extractor and Figure 3.6 a flow diagram of its construction.

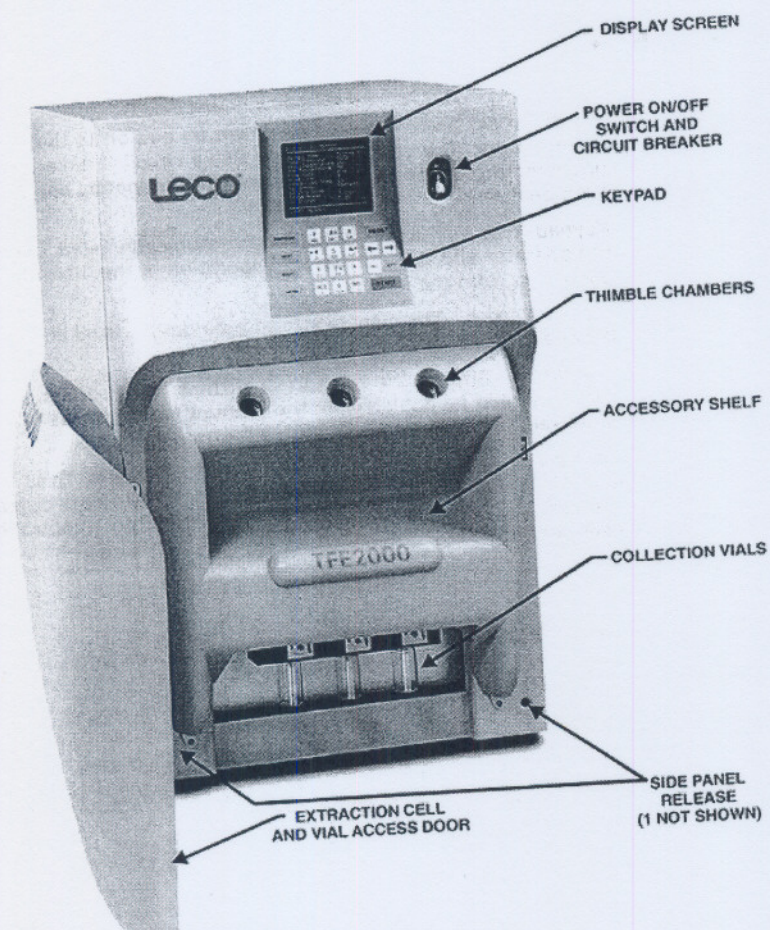


Figure 3.5: LECO TFE 2000 supercritical fluid extractor [5].

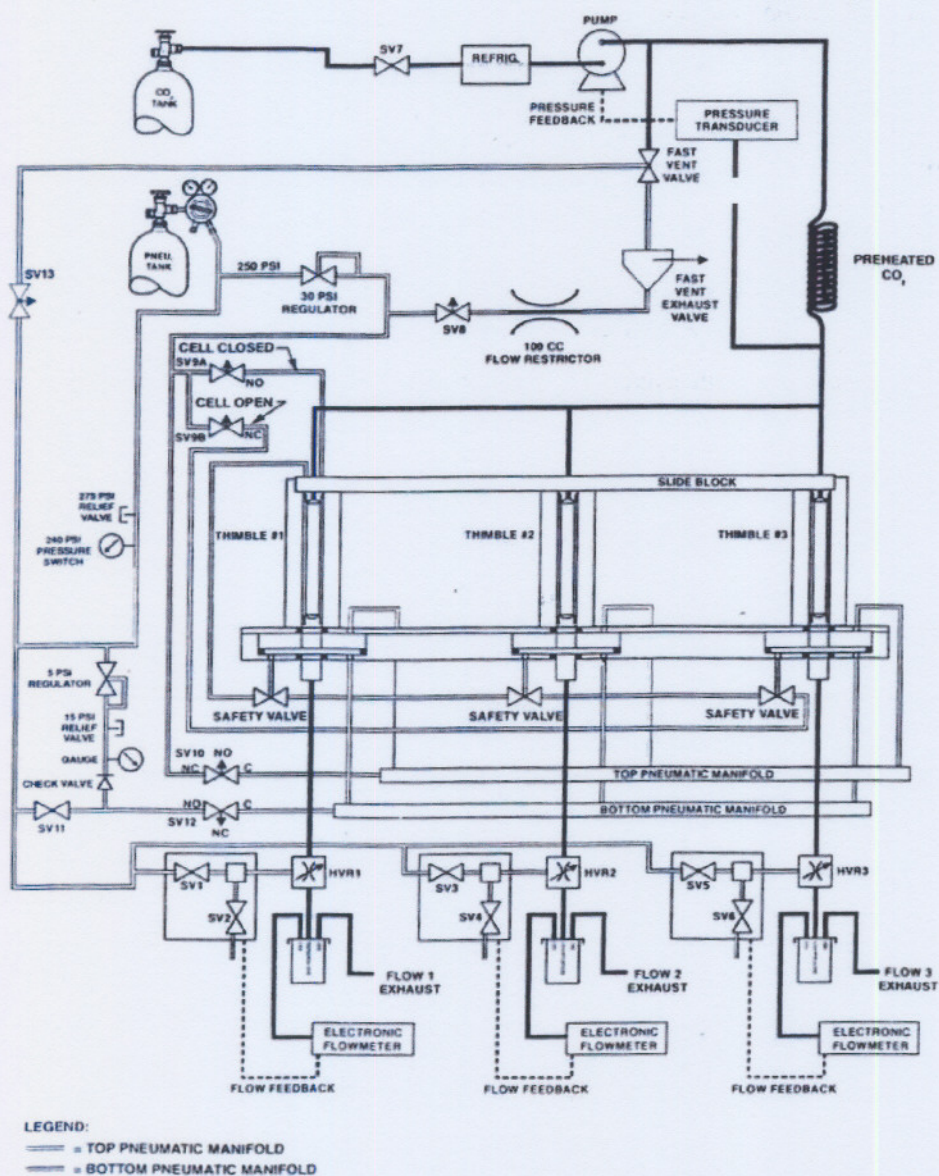


Figure 3.6: TFE2000 Flow Diagram [5].

An extraction run is preceded by weighing a thimble (sample holder) and inserting a sample of plant material into the thimble as described above and weighed again. The mass of the sample of seed in each thimble is obtained by subtracting the two measurements.

A collection vial is weighed next. After an extraction run, the collection vial is weighed again. As with the sample, the difference between the two values is the mass of the oil extracted [6].

After weighing, the thimbles are sealed with caps and inserted into the sample chambers as shown in Figure 3.7. The collection vials are put into place for oil collection.



Figure 3.7: Insertion of thimbles into sample chamber.



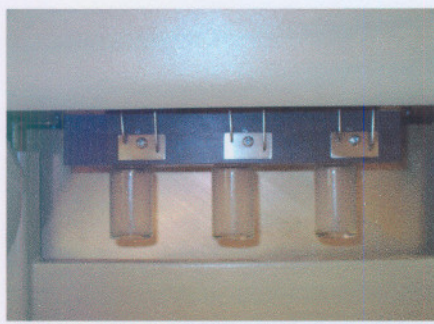
Figure 3.8: Computer control keypad.

The operator starts the extraction run by clicking "analyze" from the "samples" menu after entering the extraction parameters required for the type of sample under investigation (Figure 3.8). Solenoid valves lower the thimbles into the chamber and automatically shut-off the sample compartment.

The thimbles are heated to a temperature default value of 100°C, unless the value is changed beforehand on the "analysis" menu. A pump is turned on to compress the CO₂ to the set pressure. A pump cooler is also turned on, which cools the pump and CO₂ to approximately 0°C to enhance compression and increase the pump flow capacity. Pump pressure (CO₂) continues to increase until the default pressure of 620 bars (9 000 psi) is reached. This value can be also be changed by keying in the desired value prior to the extraction. The pump pressure

is regulated at the set value by variable restrictors (HVRs), electronic flow meters and solenoid valves. As the run continues, oil is extracted from the sample and collected once ambient conditions are restored at the end of the extraction run.

After the extraction run, the flow of CO₂ is stopped and the extraction pressure released to typically 103 bars (1 500 psi) before the CO₂ is released into the atmosphere. The slide block opens and 0.34 bars (5 psi) pneumatic pressure is applied to the bottom of the thimbles to raise them for removal from the sample chambers.



Finally, the collection vials are unscrewed from the instrument (Figure 3.9) and allowed to outgas before final weighing is done.

Figure 3.9: Vials for collection of extracted oil.

3.3 OIL ANALYSIS

The sc-CO₂ derived sunflower oil was thoroughly analysed to assess its quality in comparison to both crude and refined sunflower oil produced by other methods. A kind of benchmarking for the sc-CO₂ extracted oil was regarded an important aspect of the investigation.

3.3.1 CHEMICAL ANALYSIS

The analysis of the sc-CO₂ derived sunflower oil was done by EPKO (Pty) Ltd, Aeroton, Johannesburg. The analytical procedures are listed in the Appendix [7].

3.3.2 INSTRUMENTAL ANALYSIS

Gas chromatography (GC) was used for the analysis of the extracted sunflower oil. It was performed on a Hewlett-Packard 6890 gas chromatograph. The samples of oil were injected with a Hamilton 7105 5 μ L needle equipped with a charney adaptor. The analytical protocol is given in Table 3.1

Table 3.1: GC-parameters.

DATE	Jun 04
OPERATOR	Albert Wessels
SAMPLE DESCRIPTION	Sunflower oil
CONCENTRATION	1:1
SOLVENT	Hexane
VOLUME INJECTED	1 μ L
COLUMN TYPE	Omegawax 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 TEMPO PROGRAM	Yes
INITIAL TEMP	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
PC RUN TIME	55 min

The oil samples were diluted to a 1:1 solution with n-hexane and injected manually through a septum into a flash vaporiser port located at the head of the column. Elution was effected by an inert gaseous mobile phase (mixture of H₂ and N₂) which does not interact with the analyte. A flame ionisation detector was used.

GC-MS analysis was also done. For this purpose organic acid extraction and derivatisation of the samples were done prior to injection into the GC-MS. The organic acid extracts were derivatised with 40 BSTFA and 8 TMS (trimethylsilane) and incubated for 45 minutes at 70°C.

The instrument used was an HP 61999A equipped with a 100% methylpolysiloxane ZEBRON™ capillary GC COLUMN ZB-1. This column features excellent resolving power of critical pairs in complex samples and is suitable for trace analysis due to inertness and low bleed character. It is especially suited for high sensitivity work using GC/MS and can be used for "fingerprinting" and routine quality control analysis (e.g. botanical oils). Table 3.2 lists a few applications of this column.

Table 3.2: Applications of the ZEBRON™ capillary GC COLUMN ZB-1.

APPLICATIONS	
Amines	Drugs of abuse
Ethanol	Essential oils
Gases (refinery)	Hydrocarbons
MTBE	Natural gas odourants
Oxygenates	Pesticides
PCBs	Semi-volatiles
Simulated distillation	Sulfur compounds (light)

3.4 PILOT-PLANT SCALE EXTRACTION

Pilot-plant scale extraction was done on the supercritical extractor (Swiss Nova) shown in Figure 3.10.

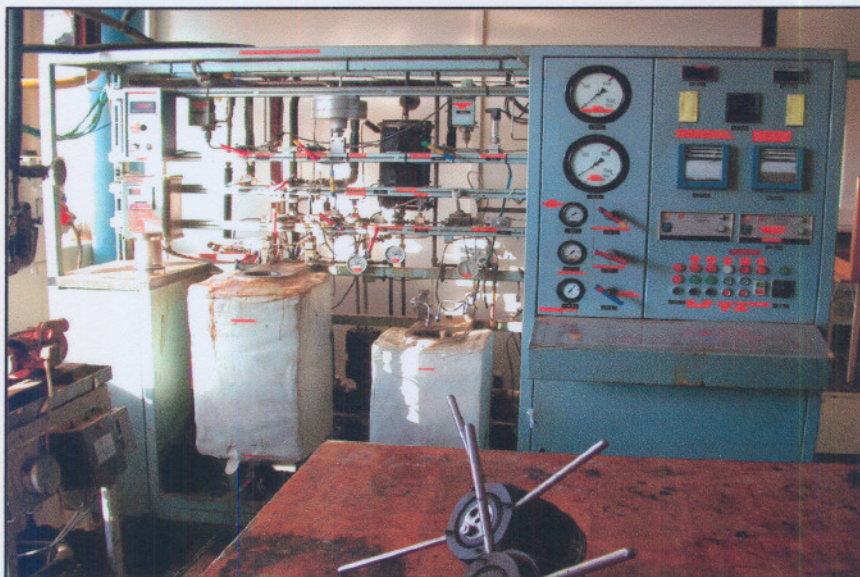


Figure 3.10: Pilot-plant scale supercritical extractor (Nova Swiss).

The same procedures were followed on the pilot-plant as on the bench-top scale instruments (Figures 3.3 and 3.5), except that extraction conditions were adjusted to compensate for larger sample volumes.

3.5 STATISTICAL DESIGN

The effect of independent variables on a dependent variable is important in research. In this particular project the effect of temperature, pressure and time (to name a few) on the amount of extracted oil is important to process understanding and control.

A central composite design based on a statistical method is a scientific approach of planning the extractions to be performed in order to reveal these effects. A prerequisite of such a design is that a maximum amount of reliable information is obtained from a minimum number of observations.

To study the effect of temperature and pressure combinations that may have a substantial influence on the yield of extract, Statistica for Windows® was used to create the experimental design shown in Table 3.3 [8].

Table 3.3: Experimental design

Run	Independent variable 1	Independent variable 2
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

Runs 1 to 4 represent a simple 2x2 orthogonal design, and runs 5 to 8 are so-called star points added to the design to calculate the quadratic components of the relationship among the variables without sacrificing the requirement of orthogonality and rotatability. Runs 9 and 10 are so-called central points which test for a linear or polynomial model [8].

The runs suggested by the central composite design when the low, center and high values of the independent variables are considered, are listed in Table 3.4. These runs were performed with the purpose of establishing the optimum conditions of extraction by virtue of surface response analysis. This entails that the yield of extract (dependent variable) is fitted as a function of the temperature and pressure (independent variables) according to the mathematical model underlying the design to produce a three-dimensional graph. The reliability of the mathematical model can be verified on account of the extent to which observed and predicted values correspond

by plotting them on a common graph. If all data points fall on a straight line, the model can be considered perfect.

STANDARD DESIGN SUMMARY [9]:

2**(2) cube plus star (central composite design)

Number of factors: 2

Number of blocks: 1

Number of runs: 10 nc=4 ns=4 n0=2

Temperature	55 low	70 center point	85 high
Pressure	300 low	425 center point	550 high

Table 3.4: Runs suggested by central composite design.

2**(2) central composite, nc=4 ns=4 n0=2 Runs=10		
	Temperature	Pressure
1	55.00	300.00
2	55.00	550.00
3	85.00	300.00
4	85.00	550.00
5	48.79	425.00
6	91.21	425.00
7	70.00	248.22
8	70.00	601.78
9	70.00	425.00
10	70.00	425.00

The number of extraction runs was expanded significantly in order to support the conclusions drawn from the essential minimum of extraction data. These additional runs did not necessarily comply with the requirement of orthogonality and rotatability and could therefore not be used for surface response analysis, but they indeed gave insight into the intimate nature of the process and its underlying mechanism that would have been impossible to obtain with the results of the 10 basic runs only.

3.6 ACTIVATION PARAMETERS [10,11]

The activation parameters of the extraction can be derived from the temperature and pressure dependence of the yield of extract.

The temperature dependence of the rate constant (k) of a reaction (such as extraction) is given by the Arrhenius equation

$$k = Ae^{-E_a/RT}$$

with E_a the activation energy, $R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$ the universal gas constant, T the temperature in kelvin and A the frequency factor.

The logarithmic form

$$\ln k = \ln A - E_a/RT$$

enables determination of the activation energy (E_a) from the slope of the straight line obtained by plotting $\ln k$ against $1/T$. The rate constant k can be substituted by the yield of extract without changing the magnitude of the slope, and thus the correct value of E_a can be obtained.

The empirical equation

$$\ln k = -\frac{\Delta V^*}{RT}p + \text{constant}$$

can likewise be plotted to determine the volume of activation ΔV^* from the slope of a graph of $\ln k$ against p , where p is the pressure of the extracting fluid and k the rate constant of extraction. The latter can, once again, be substituted by the yield of extract without changing the magnitude of the slope of the resulting straight line.

3.7 REFERENCES

1. KIRIAMITI, H.K.; RASCOL, E.; MARTY, A.; CONDORET, J.S. 2002. Extraction rates of oil from high oleic sunflower seeds with supercritical carbon dioxide. *Chemical Engineering and Processing*, 41 (8):711 (8 p.), Sept. [In SCIENCE DIRECT : Search, Full Text + Links: <http://www.sciencedirect.com>] [Date of access : 4 March 2003].
2. CHESTER, T.L.; Pinkston, J.D.; RAYNIE, D.E. *Anal. Chem.*, 1994, 66, 106R-130R.
3. BRAVI, M.; BUBBICO, R.; MANNA, F.; VERDONE, N. 2002. Process optimization in sunflower oil extraction by supercritical CO₂. *Chem. Engeneering Science*, 57(14):2753 (12 p.), July. [In SCIENCE DIRECT : Search, Full Text + Links : <http://www.sciencedirect.com>] [Date of access : 18 March 2003].
4. BONN, D. 1998. Introduction of the physico-chemical properties of supercritical fluids. *Advanced Chemistry*, 44 (23) : 88, 22 Aug. [In SCIENCE DIRECT : Search, Full Text + Links : <http://www.sciencedirect.com>] [Date of access : 4 March 2003].
5. DAVIES, R. TFE2000 Instruction Manual. Kempton Park, South Africa, 2002, p 5.5 - 5.19.
6. DAVIES, R. TFE2000 Instruction Manual. Kempton Park, South Africa, 2002, p 7.5.
7. ERASMUS, N. 2004. Verbal communication with the manager. EPKO Oil Seed Crushing. Johannesburg. [Telephone to:] Nico Erasmus. (011 494 3991).
8. STATSOFT. 1995. Statistica for Windows (Volume 1-3): Statistics 2, 2nd ed. Tulsa. 3782 p.
9. STATSOFT. 2003. Statistica 6.1^o [CD-ROM].
10. ATKINS, P. 2002. Physical chemistry. 7th ed. Oxford : Oxford University Press. 1149 p.
11. VAN ELDIK, R. 1986. Inorganic high pressure chemistry : kinetics and mechanisms. Elsevier : Amsterdam. 396 p.

CHAPTER 4

DATA ACQUISITION, PROCESSING AND INTERPRETATION

In this chapter the data acquired by performing extraction runs according to a central composite design is processed to determine the conditions at which a maximum yield of sc-CO₂ derived sunflower oil is obtained. The runs were initially performed on sunflower seed with natural moisture content, but were repeated for different types of dried seed as well. In addition to establishing optimum conditions, the data is also utilised to shed light on the intimate nature of the extraction mechanism, among others by considering the temperature, pressure and density dependencies of the yield of extracted oil. For this purpose, several runs have been added to those based on the initial statistical design. Finally, the results of both the chemical and instrumental analysis of the extracted sunflower oil are presented and compared to existing products serving as benchmarks in terms of composition, quality and appearance.

4.1 EXTRACTION RUN DURATION

It is known that the time required to extract sunflower oil by soxhlet extraction until the seed is exhausted of oil content is typically 24 hours. The first important question therefore was what time would be required to obtain a similar yield by sc-CO₂ extraction. The established methodology to determine the required

extraction time is to perform several runs of different duration and to plot the resulting yield of extracted oil as a function of extraction time. The yield versus time curve is initially linear, but it gradually flattens off to a plateau or maximum which signifies the maximum amount of oil that can be derived. The time required to attain this maximum can be considered the optimum extraction time for the conditions at which the extraction was run. A representative example of such a curve obtained in this study with the ISCO SFX™ 220 supercritical extractor and at the conditions specified, is presented in Figure 4.1.

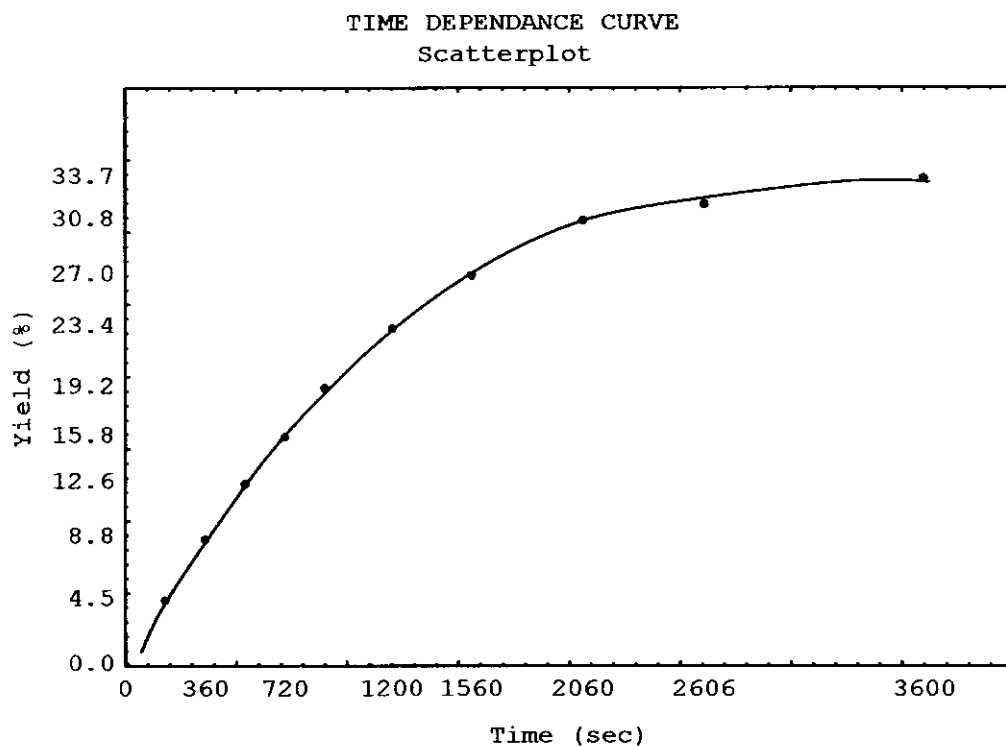


Figure 4.1: Yield against time plot to determine time dependence.

It follows from the figure that an extraction time of 3600 s (60 min) is sufficient to extract all available sunflower oil at the conditions under consideration. Since these conditions represent centre values within the permissible instrumental ranges, it was decided to perform all extraction runs at a fixed minimum time of 60 minutes.

Table 4.1: Extractions done on seed with natural moisture content.

	TEMP	PRESS (bar)	YIELD (%)	DENSITY (g/mL)
1	70	425	33.22	0.873
2	70	248	11.99	0.736
3	85	300	11.02	0.726
4	70	601	35.31	0.954
5	55	300	15.63	0.861
6	55	550	34.64	0.978
7	91	425	12.53	0.806
8	91	425	16.49	0.806
9	85	300	7.71	0.729
10	85	250	3.92	0.666
11	91	300	4.38	0.693
12	85	250	5.01	0.666
13	85	250	4.76	0.666
14	91	300	3.85	0.693
15	70	601	35.67	0.954
16	70	625	35.75	0.96
17	70	650	35.05	0.967
18	85	300	7.22	0.729
19	85	300	10.27	0.729
20	70	350	26.81	0.828
21	70	375	27.21	0.843
22	70	400	28.46	0.86
23	70	400	29.58	0.86
24	70	430	30.34	0.873
25	70	460	29.96	0.884
26	70	460	29.96	0.884
27	70	500	31.66	0.909
28	70	544	32.17	0.929
29	70	600	34.67	0.952
30	70	625	35.75	0.958
31	70	650	35.05	0.964
32	70	450	30.62	0.932
33	95	450	27.51	0.808
34	90	450	26.37	0.889
35	85	450	26.91	0.902
36	80	450	27.26	0.917
37	80	450	29.27	0.917
38	70	450	30.62	0.932
39	75	450	30.6	0.932
40	65	450	30.62	0.947
41	60	450	28.29	0.96
42	55	450	29.95	0.975

Table 4.1 lists 42 runs performed on seed with natural moisture content, 10 runs of which were suggested by the central composite design discussed earlier (Paragraph

3.5). The data in the table are used in the next few paragraphs to highlight the temperature, pressure and density dependence of the sc-CO₂ extraction of sunflower oil.

4.2 TEMPERATURE, PRESSURE AND DENSITY DEPENDENCE

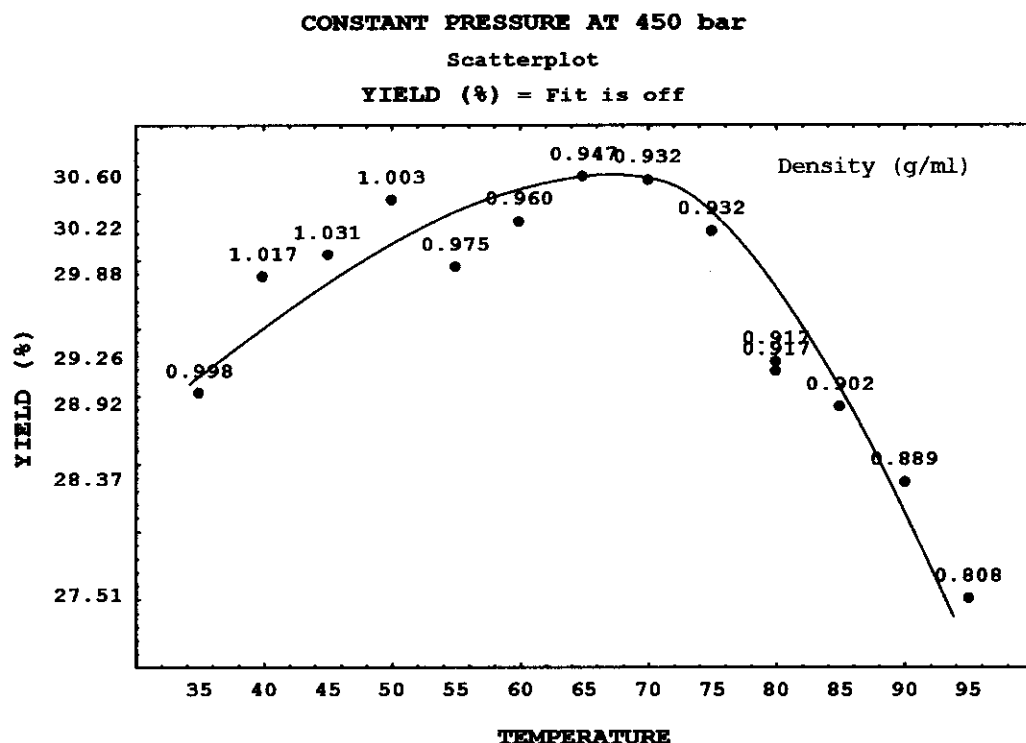


Figure 4.2: Yield against temperature at a constant pressure of 450 bar.

The plot of yield versus temperature in Figure 4.2 highlights the temperature dependence of the sc-CO₂ extraction of sunflower oil. The values of yield % plotted on the ordinate axis were calculated as

$$\text{yield \%} = \frac{\text{mass oil extracted}}{\text{seed sample mass}} \times 100$$

If a smooth curve is drawn through the data, a maximum occurs at 65–70°C. This optimum temperature corresponds to a yield of just over 30%, which is a good value compared to the yield obtained by any other method.

The attainment of a maximum in Figure 4.2 is the result of two opposing effects of temperature on the acquired yield of extract, namely (1) a decrease in the energy barrier for extraction by an increase in temperature (giving rise to a higher yield of extraction) and (2) a simultaneous decrease in fluid density with an increase in temperature (resulting in lower solvent strength and thus a lower yield).

The two opposing effects are such that within a given temperature range one is more dominant than the other, resulting in either an increase or a decrease in yield. In the range of temperatures to the left of the maximum a net increase in yield with an increase in temperature is observed since the lowering of the activation energy exceeds the lowering of density. Likewise, in the range of temperatures to the right of the maximum, the decrease in density exceeds the lowering of the energy barrier and hence a net decrease in yield is obtained. At the maximum, the two opposing effects are equal in magnitude. Here the negative influence of temperature on the density is exactly counteracted by the positive effect of temperature on lowering the activation energy.

An Arrhenius plot can be obtained for both temperature ranges (left and right of maximum) in Figure 4.2 by plotting $\ln(\text{yield})$ against $1/T$ as shown in Figures 4.3 and 4.4. The activation energy for the two regions can then be calculated from the respective slopes as described in Paragraph 3.6. It is calculated as 1.0 kJ/mol for the lower temperature range and 4.2 kJ/mol for the higher temperature range.

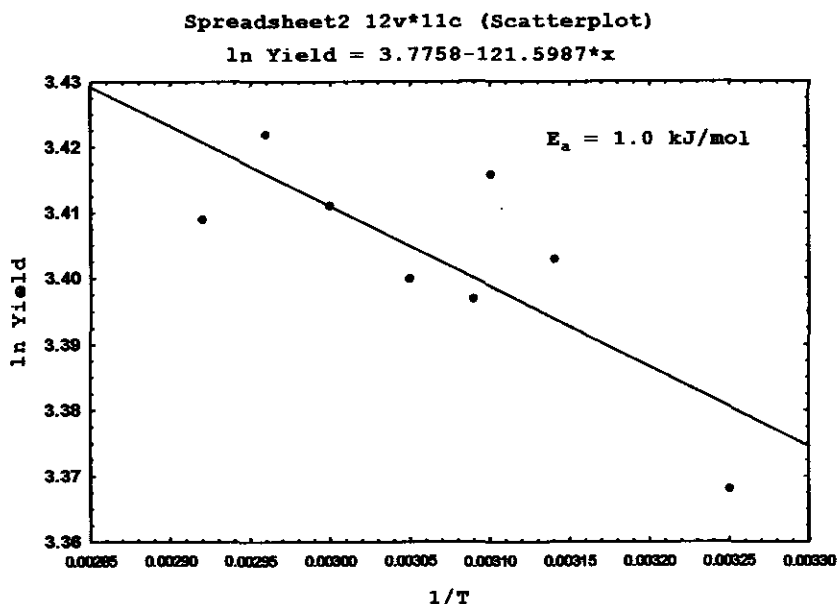


Figure 4.3: Graph of $\ln(\text{yield})$ against $1/T$ to determine activation energy for extractions done at 303–343 K.

The activation energy in Figure 4.3 is positive because the rise in temperature (303–343 K) lowers the energy barrier and leads to higher percentage yield. The activation energy in Figure 4.4 is negative as the increase in temperature (343–368 K) decreases the fluid density and thus its solvent strength, leading to lower yields of sunflower oil extracted by dissolution in sc-CO_2 .

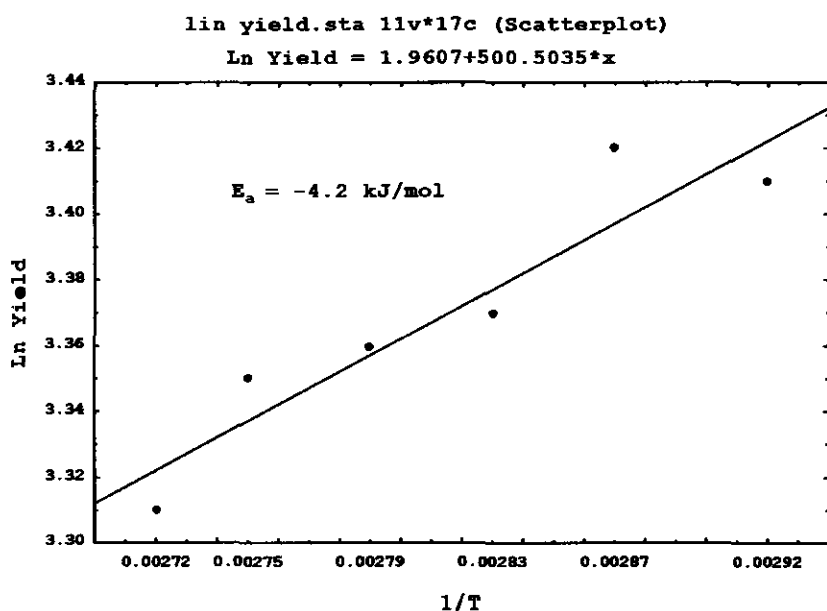


Figure 4.4: Graph of $\ln(\text{yield})$ against $1/T$ to determine activation energy for extractions done at 343–368 K.

The reason why the activation energy is so small (negative or positive) is because the net amount of energy ("average" of positive and negative attributes) is measured, and not the absolute amount of energy needed to initiate the extraction process.

A linear relationship between percentage yield and pressure is found over the limited range of pressure ($350 < p < 650$ bar) as shown in Figure 4.5.

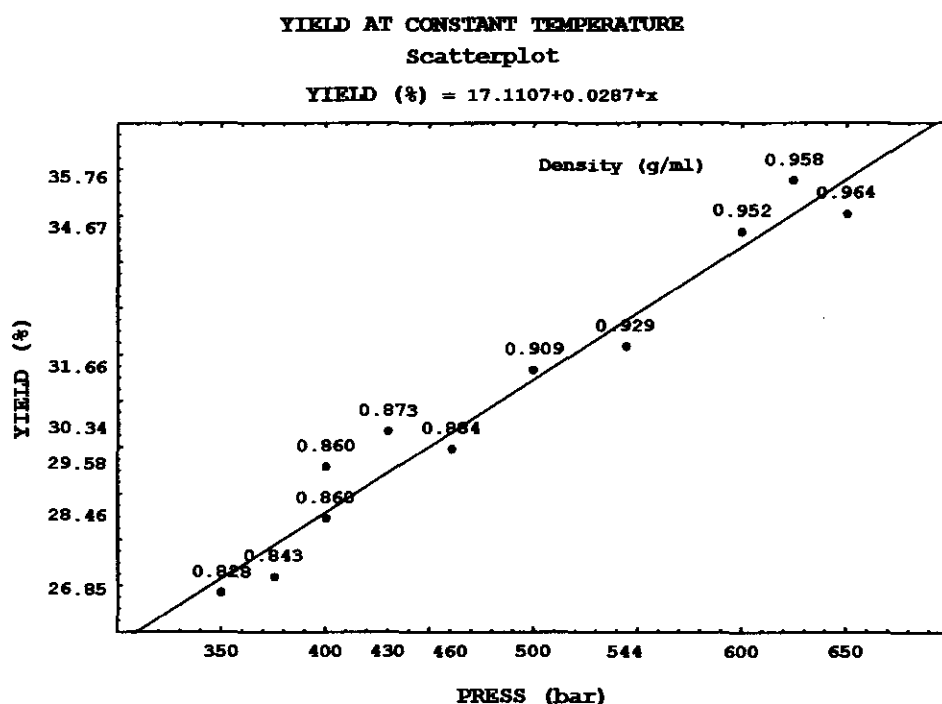


Figure 4.5: Yield against pressure at a constant temperature of 70°.

The result is echoed by the density dependence of the yield as a result of the parallel relationship between pressure and density reflected by the two almost superimposed lines in Figure 4.6. The density, in turn, relates to the solvent strength of the fluid, which is responsible for the extraction of sunflower oil through chemical dissolution in sc-CO₂.

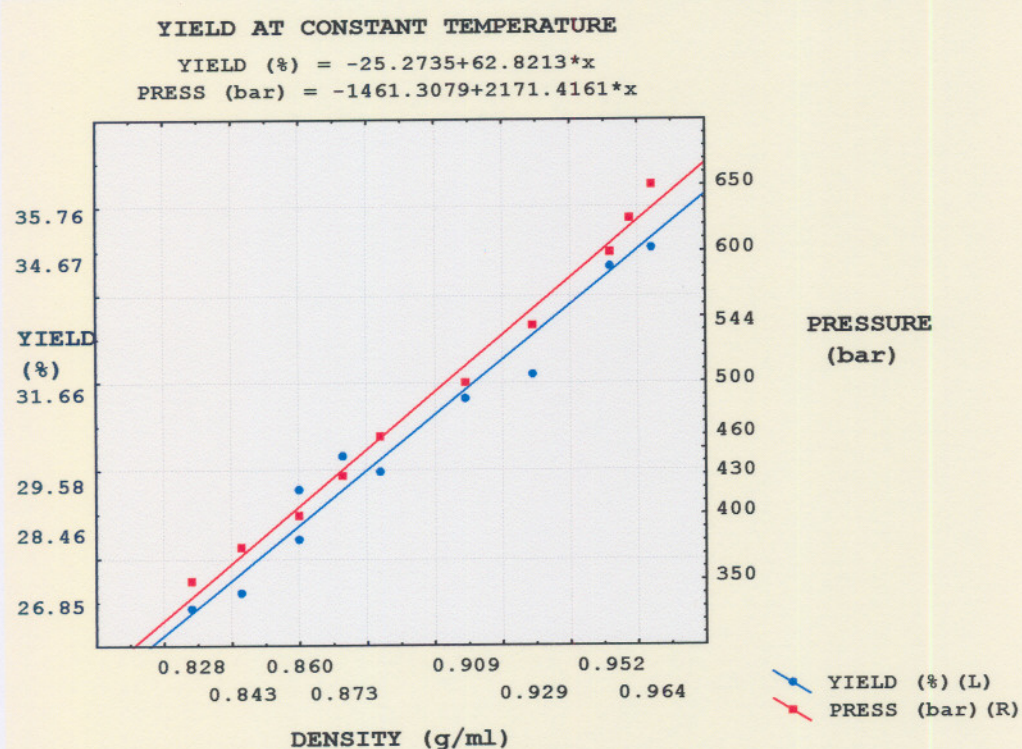


Figure 4.6: Yield against density and pressure at a constant temperature of 70°C.

Figure 4.7 gives a more complete picture of the density dependence. It shows that an exponential increase in yield is effected by shifting (via temperature/pressure control) the density from gas-like ($0,0 < \rho < 0.7$ g/mL) to liquid-like ($0.8 < \rho < 1.0$ g/mL) values. This result is similar to that obtained for many other botanical extractions with sc-CO₂ (extraction of caffeine from coffee beans probably the best known example) and proves that the fluid acts as a solvent of exceptional solvent strength capable of dissolving the oil from the seed matrix. The very low yields obtained at gas-like densities suggest that no volatile components exist which are extracted by desorption (possible by gas-like transport characteristics of fluid) and that dissolution (possible by liquid-like solvating characteristics of fluid) is the limiting mechanism of sunflower oil extraction.

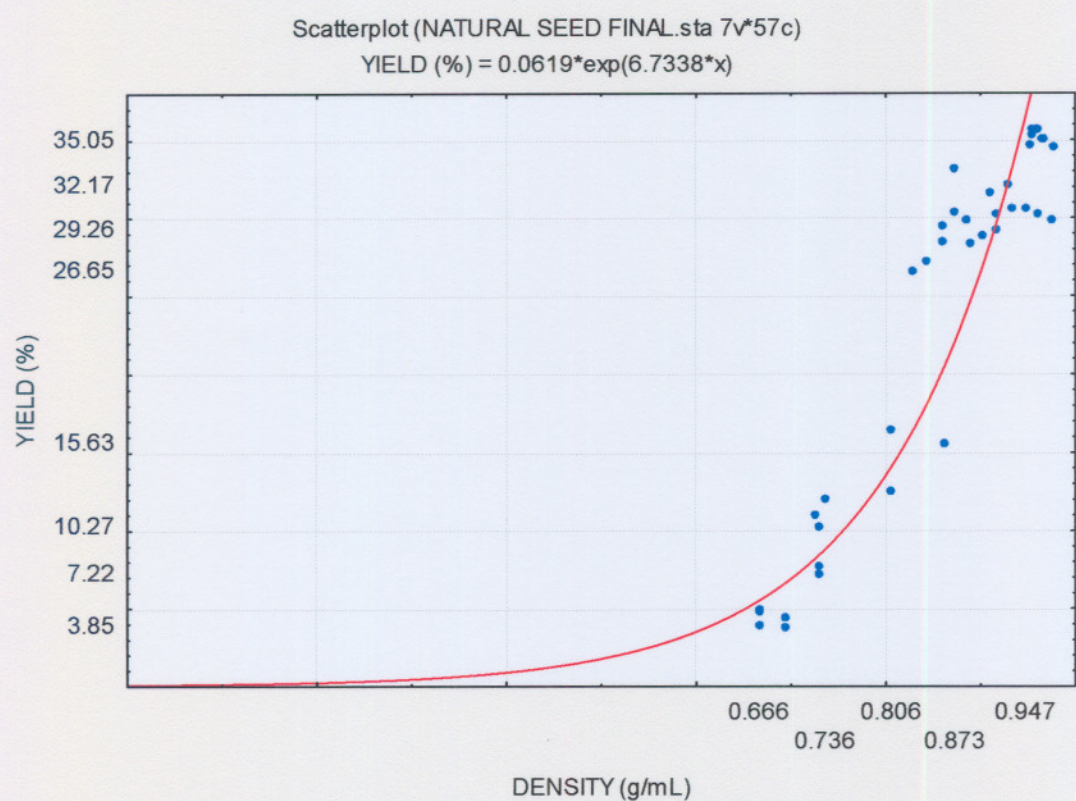


Figure 4.7: Yield against density over entire range.

The yield determining role of density is also reflected by the three-dimensional surface response graph in Figure 4.8 obtained by plotting the yield of sunflower oil (dependent variable) as a function of extraction temperature and pressure (independent variables) as explained in Paragraph 3.5. The density increases ($0.593 < 0.666 < 0.726 < 0.873 < 0.976$ g/mL) along a diagonal from the lowest to the highest yield. The conditions at the highest yield (35,75%) are 343 K (70°C) and 625 bar, illustrating the usefulness of such a surface response graph for optimising experimental conditions.

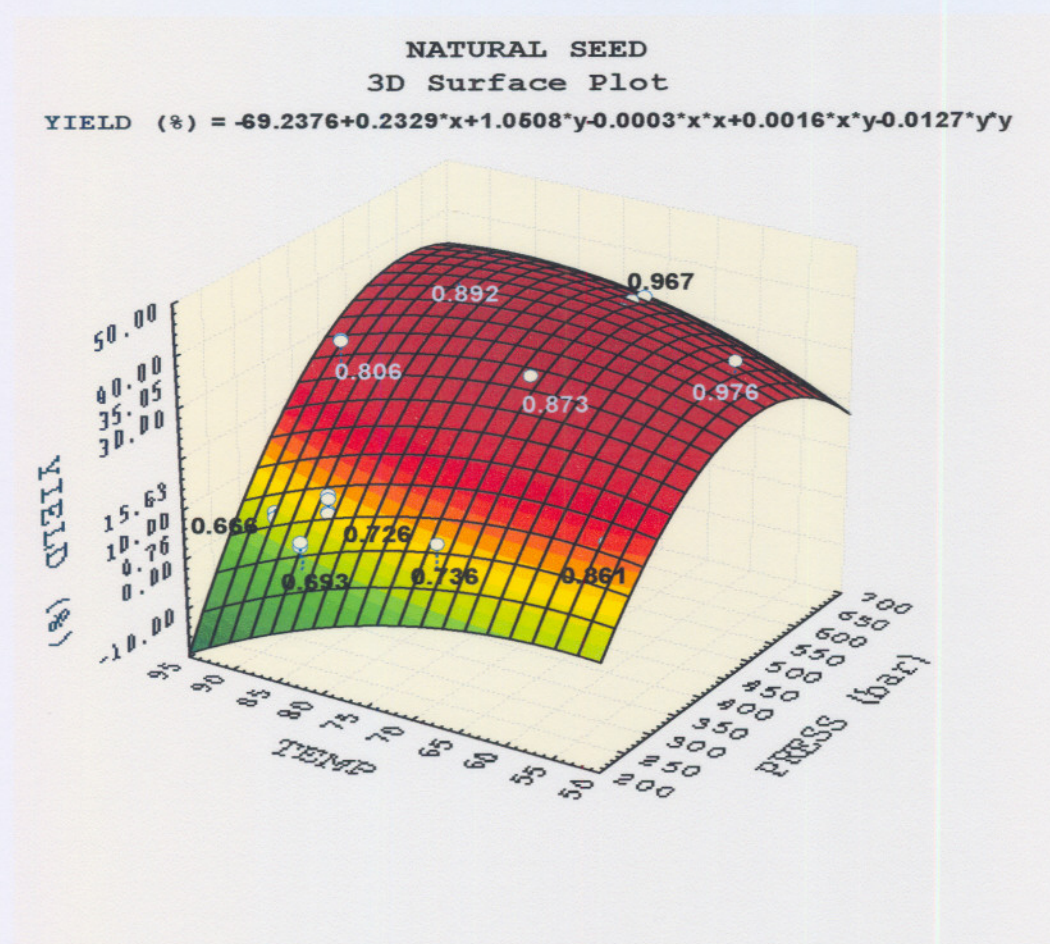


Figure 4.8: 3D surface plot for extraction of oil from natural moisture content sunflower seed.

The reliability of the mathematical model underlying the surface response graph was tested by examining the extent to which the calculated values (model) correspond to the measured (experiment) values. A 96% correlation, displayed in Figure 4.9, was obtained between the observed and the predicted values, which provides confidence in accepting the optimised extraction conditions.

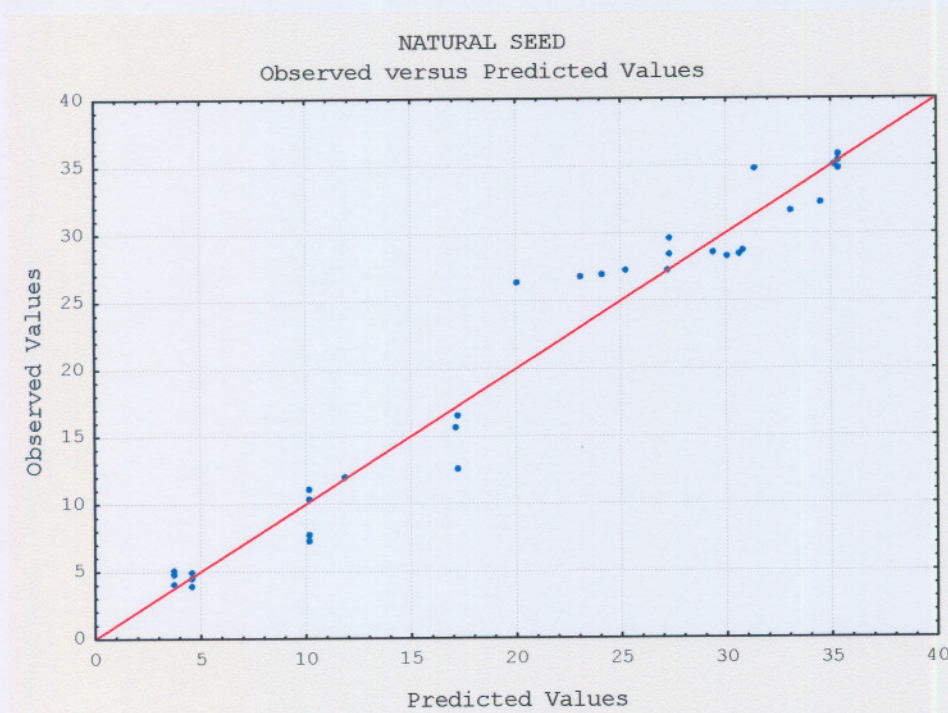


Figure 4.9: Observed versus predicted values for seed with natural moisture content.

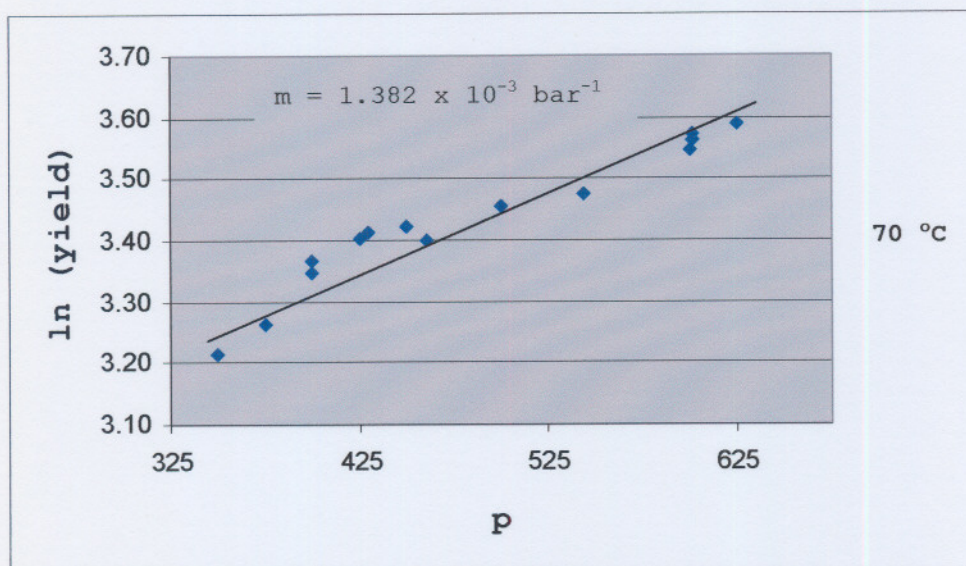


Figure 4.10: ln (yield) versus pressure.

From Figure 4.10 the volume of activation ($\Delta V^\ddagger$) is calculated as -39 mL/mol . This value supports the conclusion above that chemical dissolution is the limiting mechanism of sunflower oil extraction by sc-CO_2 . The solvation of the oil by the highly compressed fluid results in a drastic reduction of volume in the transition state as indicated by the large negative value of $\Delta V^\ddagger$.

4.3 DRIED SEED EXTRACTION

Sunflower seed were dried by different methods as outlined previously (Paragraph 3.1) to compare the respective results to those obtained for seed of natural moisture content covered in the preceding paragraphs.

4.3.1 OVEN-DRIED SEED

Table 4.2 lists the 10 extraction runs performed on ground sunflower seed dried in an oven as described in Paragraph 3.1. The experimental conditions were identical to those proposed by the initial statistical design.

Table 4.2: Extraction of oven-dried seed.

RUN	TEMP (°C)	PRESS (bar)	YIELD (%)	DENSITY (g/mL)
43	49	425	26.86	0.944
44	55	300	19.66	0.861
45	55	550	27.11	0.978
46	70	248	5.84	0.736
47	70	425	24.73	0.874
48	49	425	27.89	0.944
49	70	601	27.87	0.954
50	85	300	7.84	0.729
51	85	550	25.12	0.892
52	91	425	20.11	0.806

A maximum yield of 27% was found for the oven-dried seed, which is less than the yield obtained with seed of natural moisture content. The surface response graph in Figure 4.11 exhibits practically the same tendencies as the previous one in Figure 4.8.

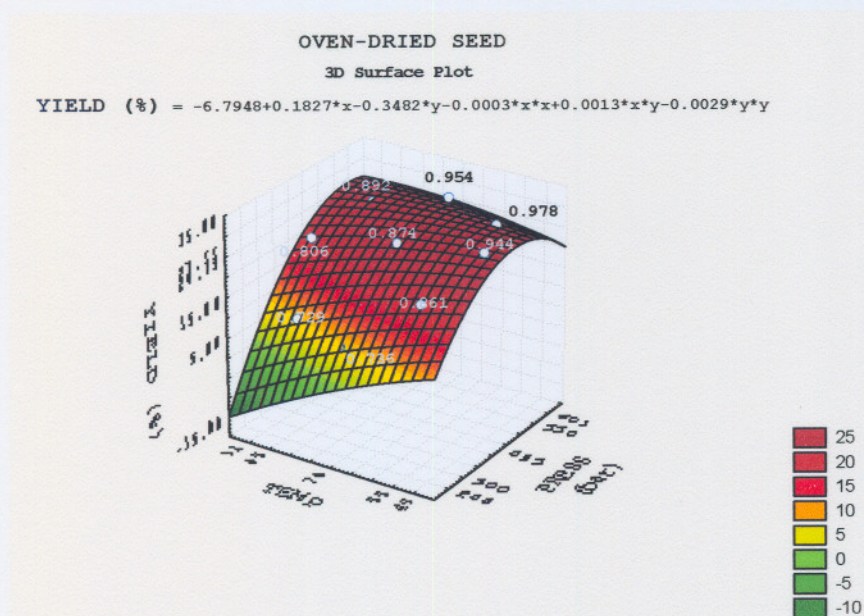


Figure 4.11: 3D surface plot for the extraction of oil from oven-dried sunflower seed.

The extent to which the observed and predicted values mutually agree is shown in Figure 4.12.

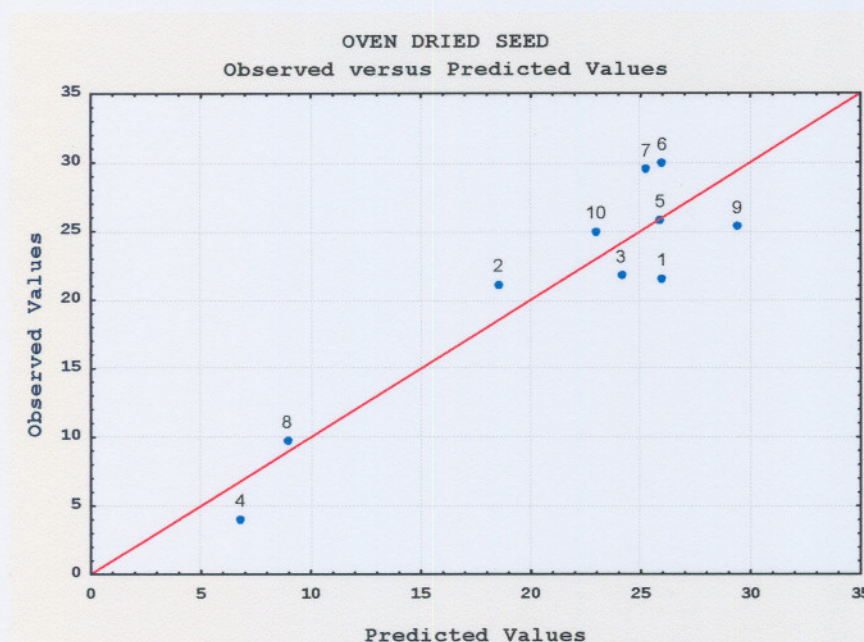


Figure 4.12: Observed versus predicted values for oven-dried seed.

4.3.2 FREEZE-DRIED SEED

Table 4.3 summarises the results of 10 extractions done on ground freeze-dried sunflower seed as described in Paragraph 3.1.

Table 4.3: Extraction of freeze-dried seed.

RUN	TEMPERATURE	PRESS (bar)	DENSITY (g/mL)	YIELD (%)
53	49	425	0.944	27.62
54	55	300	0.861	25.51
55	55	550	0.978	26.9
56	70	248	0.736	4.63
57	70	425	0.874	26.89
58	70	425	0.874	25.68
59	70	601	0.954	29.09
60	85	300	0.729	9.46
61	85	550	0.892	27.47
62	91	425	0.806	22.42

A maximum yield of 29% was found for the extraction of freeze-dried seed, which lies between the yields from natural moisture content seed and oven-dried seed. Freeze-drying is a milder method of drying and therefore somewhat reduces the negative effect of drying on oil yield. Except for the somewhat lower maximum yield, the corresponding surface response graph in Figure 4.13 does not deviate from the others in Figures 4.8 and 4.11.

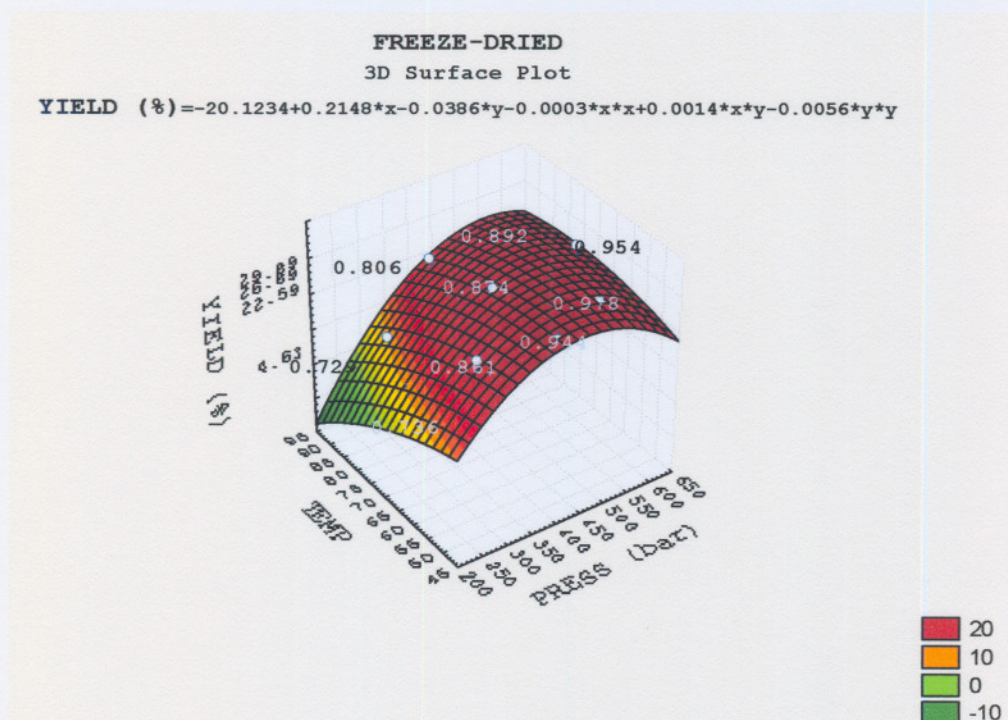
**Figure 4.13:** 3D surface plot for extraction of oil from freeze-dried sunflower seed.

Figure 4.14 is a measure of the agreement between the observed and predicted values. The correlation is fair, taking into account the limited number of data points.

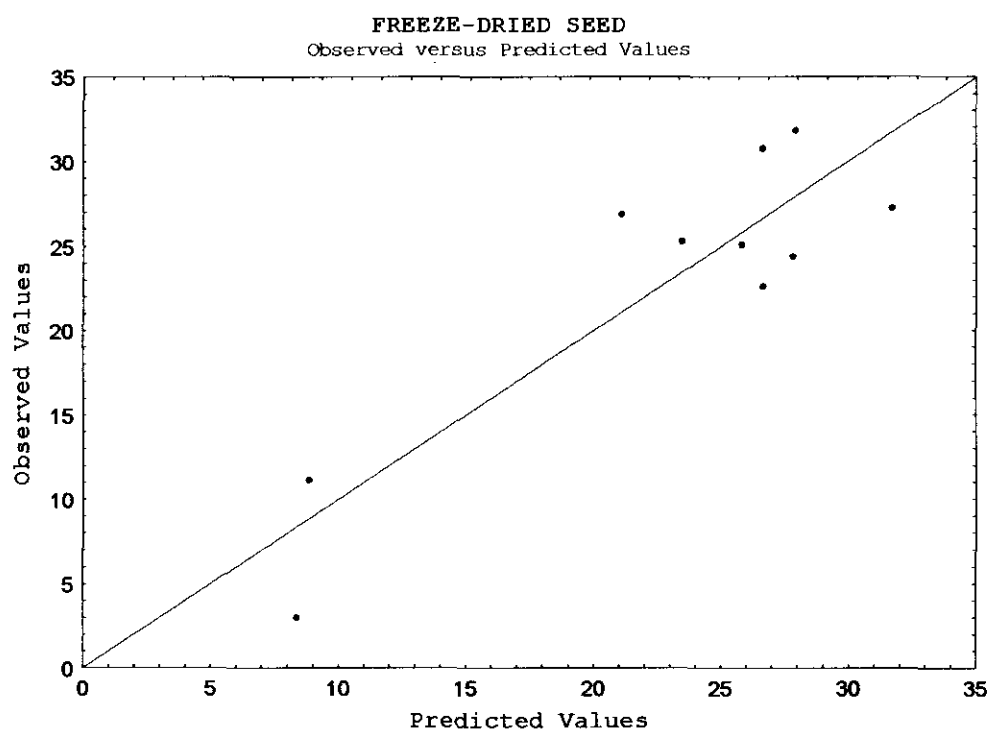


Figure 4.14: Observed versus predicted values for freeze-dried seed.

4.3.3 SUN-DRIED SEED

Table 4.4 shows the extractions done on sun-dried sunflower seed as described in Paragraph 3.1.

Table 4.4: Extraction of sun-dried seed.

RUN	TEMPERATURE	PRESS (bar)	YIELD (%)	DENSITY (g/mL)
63	70	425	20.22	0.873
64	70	248	18.31	0.736
65	85	300	17.96	0.726
66	70	601	21.31	0.954
67	85	550	20.5	0.892
68	55	300	19.94	0.861
69	55	550	20.64	0.978
70	91	425	19.71	0.806
71	91	425	19.21	0.806

The maximum yield of oil extracted from ground sun-dried sunflower seed turned out to be 21%, which is significantly lower than the yield obtained for oven- and freeze-dried seed. A possible explanation for this is that prolonged exposure to sunshine caused a loss of oil attributes by vaporisation and photochemical and/or thermal decomposition. The three-dimensional surface plot in Figure 4.15 does not differ from those for seed dried by other methods.

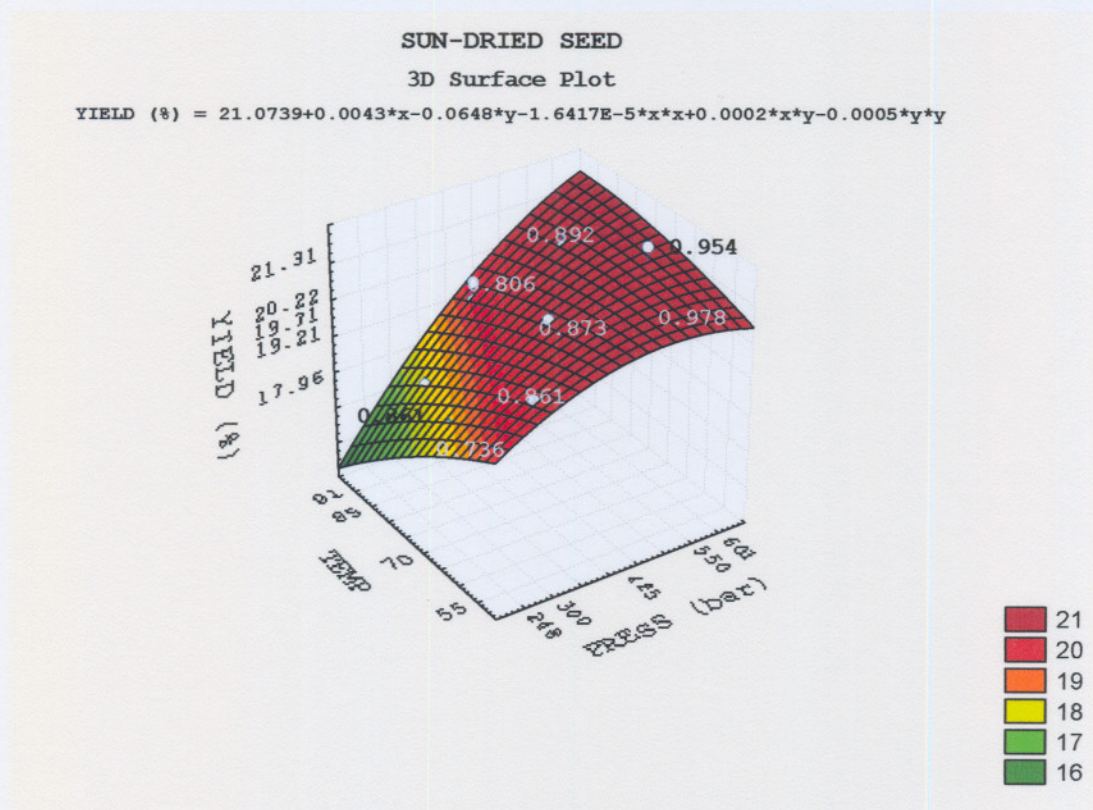


Figure 4.15: 3D surface plot for extraction of oil from sun-dried sunflower seed.

A fair correlation between observed and predicted values was once again obtained as shown in Figure 4.16.

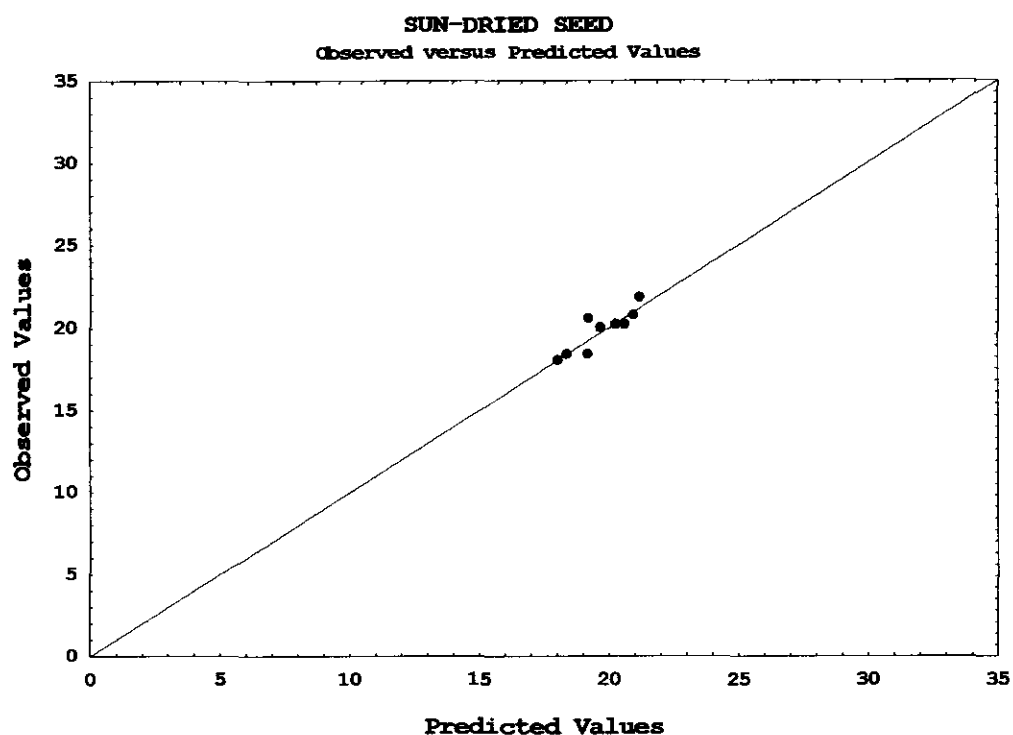


Figure 4.16: Observed versus predicted values for sun-dried seed.

A comparative summary of the yield of extracted oil from natural moisture content and different dried seed is presented in Table 4.5.

Table 4.5: Percentage oil extracted from seed with different moisture content.

Drying method	% Yield
Natural moisture content seed	35%
Oven-dried seed	27%
Freeze-dried	29%
Sun-dried seed	21%

The advantage of natural moisture content seed over pre-dried seed (various methods) is obvious and probably relates to (1) the beneficial hydrophilic character of CO₂ allowing intimate CO₂-H₂O-oil interaction and/or (2) the favourable polarity modifying effect of water on CO₂ as co-solvent.

4.4 PILOT-PLANT OPERATIONS

A kinetic yield versus time curve was also obtained for extractions done on a pilot-plant scale supercritical extractor as shown in Figure 4.17. The extraction conditions were 500 bar and 70°C, and no modifier was added. Extraction runs were performed for different time durations, and the optimum extraction time turned out to be just over 2 000 s (33 min) [1].

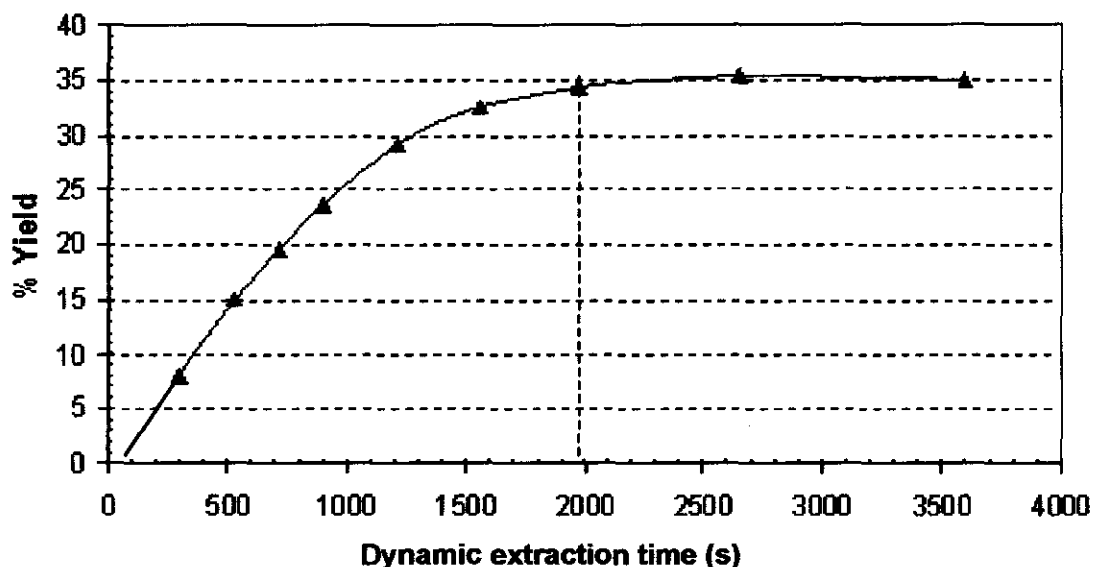


Figure 4.17: Time-dependence of $sc\text{-CO}_2$ extraction with Nova Swiss pilot-plant scale extractor.

4.5 EVALUATION OF EXTRACTED OIL

The most obvious method of evaluation of the $sc\text{-CO}_2$ derived sunflower oil would have been to compare it to commercially available sunflower oil in supermarkets (Pick & Pay, Checkers). However, it was decided to perform more comprehensive evaluation in which the oil produced in this study is compared to crude oil obtained by seed crushing (EPKO, Lichtenburg) and to a standard specification applied countrywide to sunflower oil for the marketplace. The comparison was done in terms of

physical appearance, quality and composition, and the results are presented in the following paragraphs.

4.5.1 PHYSICAL COMPARISON

Figure 4.18 presents examples of sunflower oil produced by seed crushing (EPKO). The crude oil (sample 2) is subjected to three stages of refining (the label on commercially available sunflower oil denotes "triple refined"), the first stage being the neutralisation of free fatty acids. The next stage comprises crystallisation of waxes (sample 3) as a sediment to be separated subsequently. The refined end-product (sample 4) results from a third purification step in which contaminants are removed. This product is too "clear" to be marketed as sunflower oil, and hence a colourant is added to deliver the final marketed product (sample 5).



Figure 4.18: Different stages (crude to final) sunflower oil produced by EPKO.

In Figure 4.19, two samples of sunflower oil extracted with sc-CO₂ in this study are shown, the one being from seed with the hull removed (sample 6) and the other from seed with the hull left intact (sample 7). These two samples are compared to three samples from the previous figure in Figure 4.20 in order to evaluate the oil acquired in this study with respect to physical appearance (clarity, colour, odour, etc.).

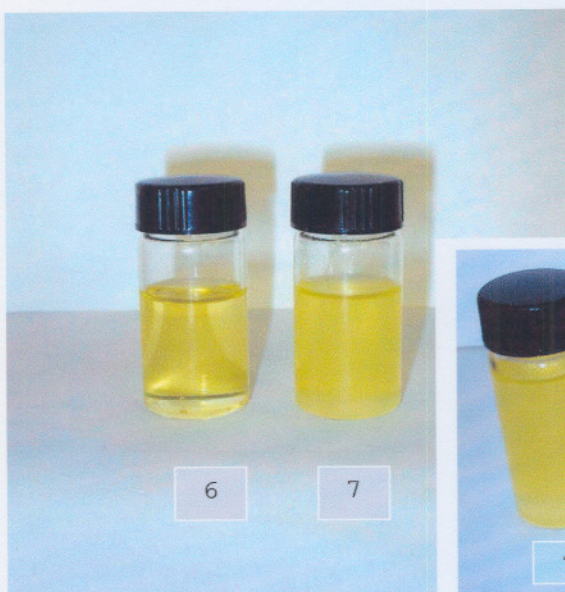


Figure 4.19: Sunflower oil extracted with sc-CO₂ from seed without (6) and with (7) the hull.

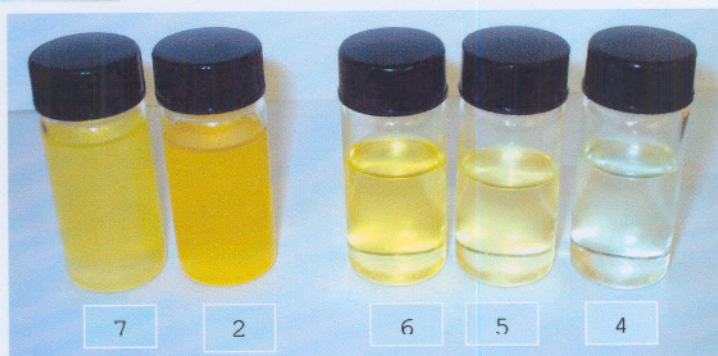


Figure 4.20: Comparison between sc-CO₂ extracted and EPKO produced sunflower oil.

The wax and wax-like material reside almost completely (~83%) in the hull. Therefore, the wax content of the extracted oil depends more on the efficiency of dehulling than on the cultivar [2]. The largest section of the EPKO refining plant is for the removal of wax from the crude oil. It is also the most difficult stage of the refining process due to the viscous nature of the waxy components. The sc-CO₂ extracted oil from dehulled seed (sample 6) compares so favourably to the final products (samples 4 and 5) from EPKO in terms of clarity, colour, flavour and odour that, instead of spending a lot of money on refining crude oil and losing the naturalness of the oil anyway, an efficient method of dehulling seed

should be combined with sc-CO₂ extraction to directly obtain a superior quality sunflower oil.

4.5.2 ANALYTICAL COMPARISON

The analytical comparison is divided into two parts. Firstly, chemical analysis on the sc-CO₂ derived oil was performed by EPKO according to well-established procedures in industry. This allowed a comparison in terms of purity (acid value, peroxide value and others) between the sc-CO₂ and EPKO oil samples, measured against the standard specification. Secondly, instrumental analysis of the sc-CO₂ derived oil made it possible to compare the different oil samples in terms of composition.

4.5.2.1 CHEMICAL ANALYSIS

Table 4.7: Comparative chemical analysis of sc-CO₂ extracted oil and EPKO cold-press oil measured against standard specification.

CHEMICAL ANALYSIS	sc-CO ₂ oil	EPKO oil	Standard specification
%FFA	0.032	2.7	0.03 max
PHOSPHOR	0 ppm	0 ppm	5 ppm max
% MOISTURE	0	0	0
% SOAP	0	0	0
IODINE VALUE	154	133	90 - 180
PEROXIDE VALUE	3.5 (24h)	0.90 (refined)	0 - 2 mg/kg max
	27 (3 weeks)	5.7 (refined, 4 months)	0 - 2 mg/kg max
	72 (4 months)	11 (crude, 4 months)	0 - 2 mg/kg max

The results of the chemical analysis in Table 4.7 show that the sc-CO₂ oil complies in all respects to the required specification, except for the peroxide value.

The exceptionally high peroxide values of the sc-CO₂ extracted oil probably results from the lapse of time between extraction and subsequent analysis of the oil. An extended lapse of time causes more oxidation to take place and a higher peroxide value to occur. The same tendency is observed for the EPKO oil (both crude and refined), though the magnitude of the peroxide value is considerably smaller for the cold-press acquired oil. These values are, however, still larger than the standard specification, and the oil therefore needs to be refined further.

4.5.2.2 INSTRUMENTAL ANALYSIS

The primary objective with GC analysis was to compare the composition of sunflower oil obtained by sc-CO₂ extraction and by traditional seed crushing (both crude and refined).

Figures 4.21 4.22 and 4.23 show typical chromatograms, respectively, for a commercial oil purchased in a supermarket, a crude oil acquired by seed crushing and an oil derived by sc-CO₂ extraction.

The retention times were compared mutually as well as to retention times published in the literature in order to identify the components concerned.

Unfortunately, not all instruments worldwide use the same settings or exactly the same types of column. This is the reason why retention times may differ for the same component. The method of extraction also plays a role in GC analysis. A retention time is, however, still useful even if it differs as much as three minutes for the same compound.

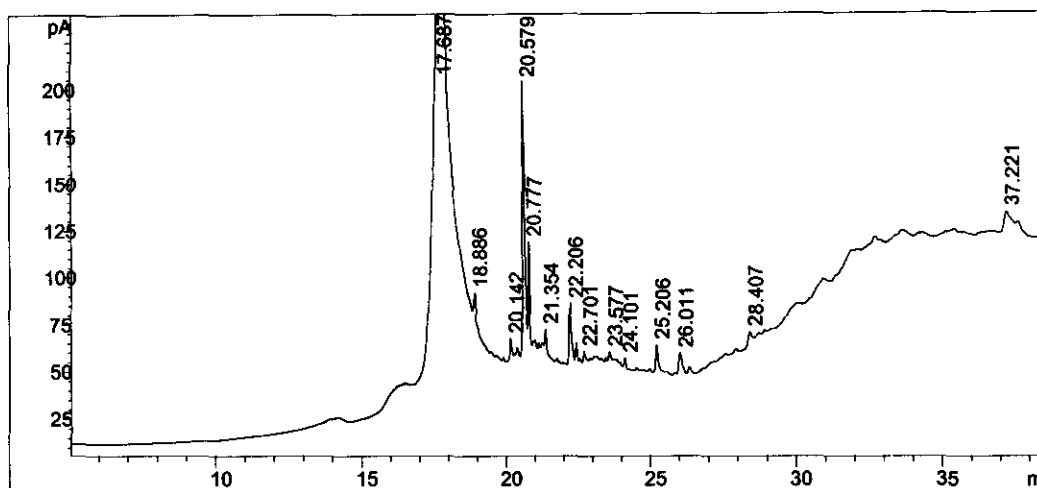


Figure 4.21: Chromatogram of sunflower oil bought at Checkers.

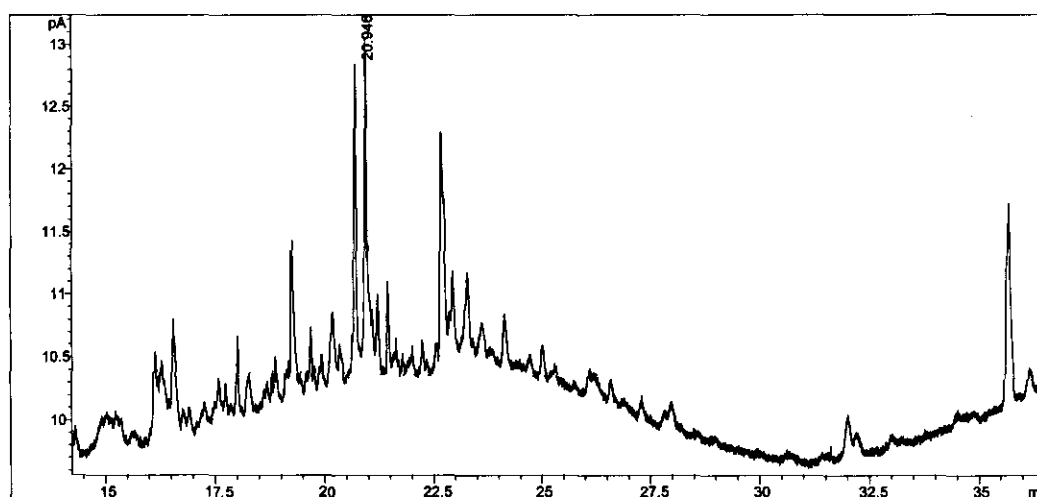


Figure 4.22: Chromatogram of crude sunflower oil from crushed seed (EPKO) .

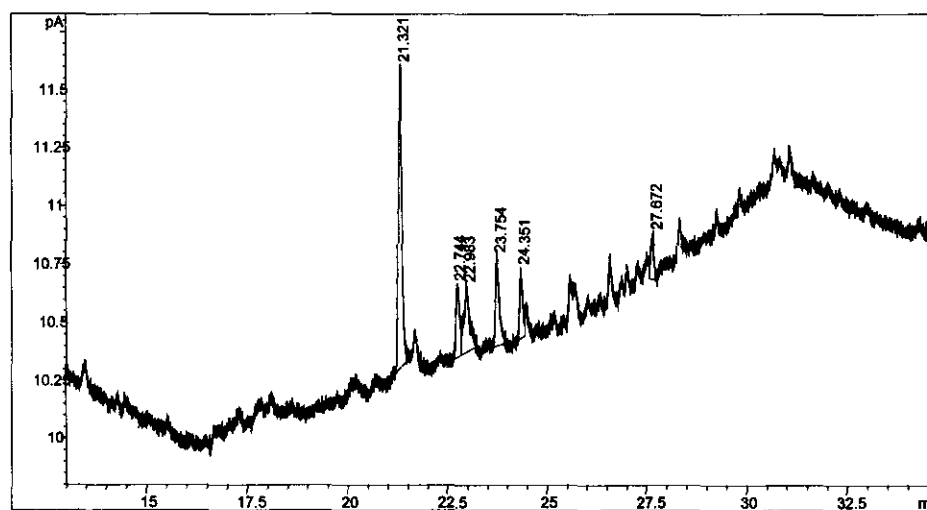


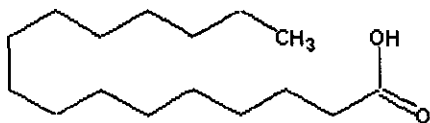
Figure 4.23: Chromatogram of sunflower oil extracted with sc-CO₂.

For all three samples of oil excellent correlation among primary peaks at retention times between 20 and 22 minutes was obtained, proving that with sc-CO₂ all major components of sunflower oil were successfully retrieved. The primary compounds below were detected by GC-MS analysis of both the refined EPKO oil and the sc-CO₂ extracted oil. The complete GC-MS analysis of both types of oil, including peak identification, is provided in the Appendix.

PALMITIC ACID

C₁₆H₃₂O₂

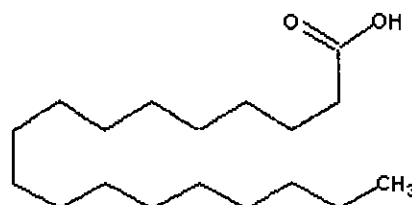
Mol. Mass: 256.424



STEARIC ACID

C₁₈H₃₆O₂

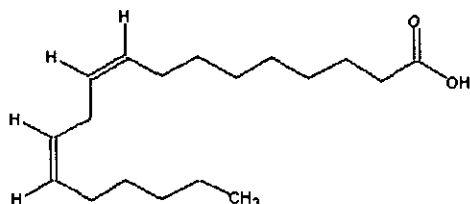
Mol. Mass: 284.477



LINOLEIC ACID

C₁₈H₃₂O₂

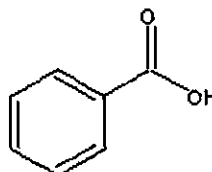
Mol. Mass: 280.445



BENZOIC ACID

C₇H₆O₂

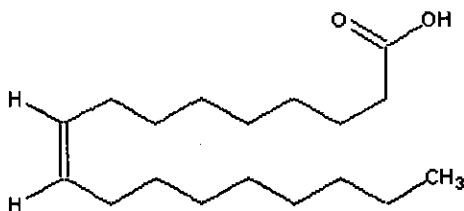
Mol. Mass: 122.121



OLEIC ACID

C₁₈H₃₄O₂

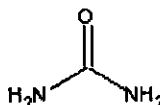
Mol. Mass: 282.461



UREA

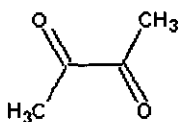
CH₄N₂O

Mol. Mass: 60.055

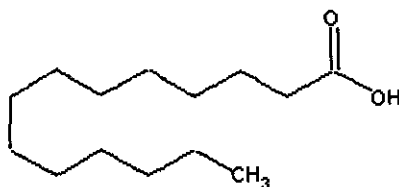


2,3-DIHYDROXYBUTANE $C_4H_6O_2$

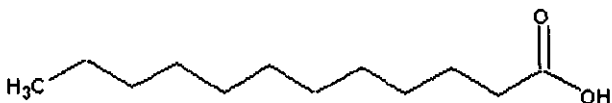
Mol.Mass: 86.082

**MYRISTIC ACID** $C_{14}H_{28}O_2$

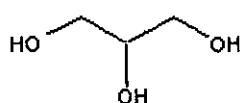
Mol.Mass: 228.371

**LAURIC ACID** $C_{12}H_{24}O_2$

Mol.Mass: 200318

**GLYCERIN** $C_3H_8O_3$

Mol.Mass: 92.094



In the sc-CO₂ extracted oil 484 components were found in comparison to 225 components in the EPKO oil.

The chromatograms show the presence of high concentrations of linoleic and oleic acids which readily oxidise on exposure to air and thereby cause the peroxide value to increase with time [3].

4.6 REFERENCES

1. LE ROUX, C.J. 2003. *sc*-CO₂ Extraction of Sunflower Oil from Seed. Potchefstroom : NWU (Dissertation - B.Sc Honours) 67 p.
2. DORRELL, D.G.; VICK, B.A. 1997. Properties and processing of oilseed sunflower. (In SCHNEITER, A.A., ed. Sunflower Technology and Production. Madison : Wisconsin. P.713-736.)
3. O'NEIL, M.J., SMITH, A., HECKELMAN, P.E., eds. 2001. The Merck Index. New York : Whitehouse Station. P.5527-5529.

CHAPTER 5

STOCK-TAKING OF PROJECT ACHIEVEMENTS

A project evaluation is presented in this chapter. It includes stock-taking of the successes and shortcomings, measured against the objectives stated in the introductory chapter. A few options for future studies on this topic are mentioned in a concluding paragraph.

5.1 PROJECT ASSESSMENT

The primary success of this project was production of a superior quality sunflower oil from seed by sc-CO₂ extraction. This natural oil compared favourably with the crude oil obtained by the cold-press method and, if extracted from dehulled seed, was just as superior as the final cold-press oil after a comprehensive 3-stage refining process.

The quality assessment of the sc-CO₂ derived oil was done with a standard specification as benchmark. It passed all requirements, except for its high peroxide value due to oxidation during prolonged exposure to air. This matter needs further investigation in order to reduce the peroxide value.

The extraction conditions for a maximum yield of sc-CO₂ derived oil were determined to be 70°C and 625 bar. The yield was considerably larger than that obtained by seed

crushing (36% versus 26%) as large amounts of oil stayed behind in the crushed material. n-Hexane is usually used to recover this oil, but the solvent then needs to be separated again from the additional oil obtained. It is during this separation step that the oil loses some of its valuable natural qualities, such as its vitamin E content [1].

The detrimental effect of moisture removal was convincingly demonstrated by repeating extraction runs on natural moisture content seed on oven-dried, freeze-dried and sun-dried seed. It was concluded that natural moisture acts as a modifier which adjusts the polarity of the CO₂ and/or associates with the hydrophilic CO₂ to improve oil extraction.

The time of extraction was drastically reduced to 1 hour as compared to 24 hours with soxhlet extraction and several hours with the cold-press method plus additional extraction time with an organic solvent.

GC and GC-MS analysis facilitated identification of a number of components common to the cold-press and sc-CO₂ oils, which proves that sc-CO₂ extraction is an acceptable alternative to current sunflower extraction methods. Moreover, sc-CO₂ extraction warrants naturalness and quality as no modifier is necessary for the extraction of sunflower oil.

The objectives regarding process understanding and mechanism insight could be successfully achieved by gathering sufficient extraction data to reveal the time, pressure, temperature and density dependencies of the process and to calculate the most important activation parameters. It turned out that density of the extracting

fluid is the process determining factor and that chemical dissolution of the oil in sc-CO₂ is the limiting mechanism of extraction. No attempt was made, however, to mathematically describe, model or simulate the extraction process, as this was not considered to fall within the scope of this investigation.

Supercritical technology has been firmly enucleated within the chemical and engineering research fields through this and other projects at the North-West University, Potchefstroom Campus and other institutions with which collaborative relationships have been established.

This study was done on two cultivars only, and the influence of different soil and farming methods on the quality and quantity of sc-CO₂ derived sunflower oil is still unknown. No large variation is expected though. The effect of different seasonal and harvesting conditions on the quality of the oil has not been examined yet.

The up-scaling of laboratory operations indeed received attention in this investigation by performing a few extraction runs on a pilot-plant scale extractor, but the cost of up-scaling to industrial scale could not be assessed yet. It is accepted that the initial capital output for an industrial sc-CO₂ extraction plant will be considerably higher than that of traditional plants, but in the long run sc-CO₂ extraction may be cost-effective in terms of low operational costs.

5.2 FUTURE POSSIBILITIES

The oilcake is almost as important to the producers of oil as the oil itself since a quarter of their revenue lies in the selling of the cake as feed to cattle and poultry [2,3]. The aim of this study did not include an analysis or evaluation of the oilcake, but it may be investigated in a future study.

The problem of subsequent oxidation of sc-CO₂ derived sunflower oil can probably be solved by passing N₂ or argon through the extract or by mixing N₂ and CO₂ during extraction. This might prevent or reduce oxidation without affecting the naturalness of the oil [4].

Supercritical extraction has until now been performed in a batch mode only because of the difficulty encountered with continuous transport of solid material into, through and out of a high-pressure reactor. There are, however, claims in the literature of extruder designs that could possibly address the problem of batch versus continuous extraction modes [5].

The development of new methods of dehulling which could be incorporated in an extruder design is another challenge for the future to directly produce quality sunflower oil on an industrial scale by virtue of a continuous process.

Finally, a possibility exists to successfully use sc-CO₂ for the extraction of other botanical oils, such as soybean and olive oil.

5.2 REFERENCES

1. American Cancer Society. Cancer Facts and Figures. 1997. Atlanta, GA: 1999.
2. NEL, A.A.; DU TOIT, A.P.N.; LOUBSER, H.L. Sonneblomproduksie, 'n Bestuursgids vir die Wenprodusent. 1995. 7 (2).
3. DORRELL, D.G.; VICK, B.A. 1997. Properties and processing of oilseed sunflower. (In SCHNEITER, A.A., ed. Sunflower Technology and Production. Madison : Wisconsin. p.713-736.)
4. BREET, E.L.J. 2004. Verbal communication with the author. Chemical Processing SA. (Original copy in records of Prof. Ernst Breet at the North-West University in Potchefstroom.) [E-mail to:] Ernst Breet (CHEELJB@puk.ac.za).
5. PHELPS, C.L.; SMART, N.G.; WAI, C.M. *Past, Present and Possible Future Applications of Supercritical Fluid Extraction Technology*, J. Chem. Edu, 1996, 73, 1168 p.

APPENDIX

The appendix includes (1) the analytical procedures followed by EPKO (Pty) Ltd to chemically analyse the sc-CO₂ derived sunflower oil, (2) a chromatogram of the refined sunflower oil produced by EPKO (Pty) Ltd with identified peaks and IUPAC names of the components, and (3) the corresponding chromatogram of the sc-CO₂ extracted sunflower oil together with its identified peaks and IUPAC names of the components.

A1 ANALYTICAL PROCEDURES FOR CHEMICAL ANALYSIS

A1.1 DETERMINATION OF FREE FATTY ACID (FFA) CONTENT

PREPARATION OF REAGENTS

Mentholated spirits

White mentholated spirits is used. Add a few drops of phenolphthalein and a few drops of 0.1 N sodium hydroxide till a light pink colour is obtained.

Phenolphthalein indicator

Dissolve 0.1% phenolphthalein into 95% ethanol. Fill with ethanol to 1000 mL volumetric mark.

0.1 N sodium hydroxide

Use as purchased.

METHOD

1. Weigh out 56.4 g of oil into a 250 mL Erlenmeyer flask.
2. For crude oil, 7.000 g of oil must be used.
3. Add 50 mL of the neutralized mentholated spirits into the flask.
4. Place the flask onto a heater and shake well.
5. When the sample is heated a titration must be done with 0.1 N sodium hydroxide.
6. Add sodium hydroxide until the sample is neutralised. A light pink colour must stay for 30 seconds.
7. Read the titration value from the burette and do the following calculation:
$$\%FFA = \text{titration} \times 2.84 - \text{mass of sample}$$

A1.2 DETERMINATION OF PEROXIDE VALUE

PREPARATION OF REAGENTS

Acetic acid / chloroform solution

Mix 3 parts of acetic acid with 2 parts of chloroform.
Add 400 mL chloroform to 600 mL acetic acid.

Potassium iodide solution (KI)

Add potassium iodide to 50 mL of distilled water until a layer of at least 1 cm of undissolved potassium iodide lies at the bottom. This mixture must be stored in a dark place. Do a blank sample as follows: Add 0.5 mL of the potassium iodide solution to 30 mL of the acetic/chloroform solution. If a blue colour is obtained and more than 1 drop of 0.1 N thiosulphate is used to turn the colour to milky, a new potassium iodide solution must be prepared.

1% Starch solution

Heat 300 mL of distilled water and add 5 g of starch powder (add a few drops of distilled water to the starch to make a paste) and 0.62 g salicylic acid. Add this slowly to boiling water. Mix it very well and then cool it down to room temperature. Fill it up to 500 mL with distilled water.

0.01 N Sodium thiosulphate

Take 100 mL 0.01 N sodium thiosulphate by using a 100 mL pipette and fill it up to 1000 mL with distilled water. Pipette 50 mL 0.01 N sodium thiosulphate and fill it up to 500 mL of distilled water. Pipette 25 mL 0.01 N sodium thiosulphate and fill it up to 250 mL of distilled water.

METHOD

1. Weigh out 5.000 - 5.050 g of the sample into a 250 mL Erlenmeyer flask. Note the mass.
2. Add 30 mL of the acetic / chloroform mixture to the sample.
3. Rotate the sample till the oil is dissolved in the mixture.
4. Add 0.5 mL of the potassium iodide (KI) into the flask.
5. Leave the sample for exactly one minute.
6. Add 30 mL of distilled water to the sample.
7. Add 0.5 mL of the 1% starch solution to the sample. If a blue or black colour is obtained a titration must be done on the sample with 0.01 N thiosulphate until a milky white colour is obtained.
8. If the colour stays milky white the result is 0.0.
9. Take the reading from the burette.

CALCULATION

Peroxide value as milli-equivalent e.g. titration x 10
peroxide per 1000 g sample = sample mass.

A1.3 DETERMINATION OF PHOSPHOROUS CONTENT

PREPARATION OF REAGENTS

Sodium molybdate

Add 140 mL of sulphuric acid very slowly to 300 mL distilled water. Cool it down to room temperature and add 12.5 g sodium molybdate. Fill it up to 500 mL with distilled water. Stir it very well and let it stand for 24 hours before use.

Hydrazine sulphate

Dissolve 0.150 g hydrazine sulphate in 500 mL distilled water.

Potassium hydroxide (50%)

Dissolve 50 g potassium hydroxide in 50 mL distilled water.

METHOD

1. Weigh out 3.0 - 3.3 g of the sample into a Vycor crucible.
2. Add 0,5 g zinc oxide.
3. Put the crucibles into a muffle furnace at 860°C for 4 hours. Take it out and cool it down to room temperature.
4. Add 5 mL lukewarm distilled water and 5 mL hydrochloric acid to the ash.
5. Put lids on the crucibles and heat for 5 minutes on a hotplate.
6. Filter the ash into a 50 mL volumetric flask. Wash the inside of the lids and the crucibles with 5 mL of warm distilled water. Make use of a wash-bottle with a fine spout.
7. Wash the crucibles and the filter paper with four extra portions of warm distilled water.
8. Allow it to cool down to room temperature.
9. Neutralise the samples by adding drop by drop of 50% potassium hydroxide till the samples are milky.
10. Add drop by drop of hydrochloric acid to the solvent till the zinc oxide is clear. Add an additional two drops to the solvent.
11. Fill it up to the 50 mL mark with distilled water.
12. Add 10 mL solvent into a dry and clean 50 mL flask.
13. Add 8 mL hydrazine sulphate and 2 mL sodium molybdate to the flask. It must be in that order.

14. Close it with a lid and shake gently two or three times. Take the lid off.
15. Heat it in a water bath for 10 minutes.
16. When the sample has a dark blue colour a new sample must be heated. Less sample of the solvent (5 mL or 2 mL) must be used for oils with a high phosphorous content. For oil with a low phosphorous content more sample of the solvent (40 mL) must be used. Use 2 mL of solvent for the blank.
17. Cool it down to room temperature and fill it to the 50 mL volumetric mark. Shake it well.
18. Switch the spectrophotometer on and adjust it to get a reading of 100% transmission.
19. All the samples must be read in a clean cuvette.
20. Prepare the blank (0.5 g zinc oxide) exactly like all the samples.

Phosphorous standard solution 1 mg/mL

Dissolve 1.0697 g dry potassium dihydrogen phosphate into 100 mL distilled water. Fill it up to the 250 mL volumetric mark. Shake it well.

Standard working solution

Add 5 mL of the standard stock solution into a 500 mL volumetric flask. Fill it up to the 500 mL volumetric mark and shake it well.

This solution contains 0.01 mg phosphorous per mL.

Graph settings

1. Add 0, 1, 2, 4, 6, 8 and 10 mL of the standard working solution into seven 50 mL volumetric flasks.
2. Dilute every standard with distilled water:
 - Add 10 mL distilled water to the 0 mL standard - 0.0 mg phosphorous

- Add 9 mL distilled water to the 1 mL standard - 0.01 mg phosphorous
 - Add 8 mL distilled water to the 2 mL standard - 0.02 mg phosphorous
 - Add 6 mL distilled water to the 4 mL standard - 0.04 mg phosphorous
 - Add 4 mL distilled water to the 6 mL standard - 0.06 mg phosphorous
 - Add 2 mL distilled water to the 8 mL standard - 0.08 mg phosphorous
 - Add 0 mL distilled water to the 10 mL standard - 0.10 mg phosphorous
3. Add 8.0 mL hydrazine sulphate and 2.0 mL sodium molybdate solution to the samples.
 4. Put the lids on and shake gently. Take the lids off.
 5. Heat it for 10 minutes in a water bath.
 6. Cool it down to 25°C.
 7. Fill it to the 50 mL volumetric mark with distilled water and shake it well.
 8. Adjust the spectrophotometer to give a reading of 100% transmission for distilled water.
 9. Read all the standards and plot the results on a graph (% transmission vs. phosphorous content).
 10. Combine the points on the graph.

CALCULATION

Reading of sample (plotted on graph) - blank = A

A + mass of sample = B

$B \times 0.5 \times (10 + \text{mL sample}) = \% \text{ phosphorous}$

A1.4 DETERMINATION OF SOAP IN OIL

PREPARATION OF REAGENTS

Acetone

Add 2% distilled water into a 1000 mL volumetric flask. Fill it to the mark with acetone. Add 2.5 mL bromophenol blue to the acetone. Neutralise the acetone solution by adding dropwise 0.01 N hydrochloric acid until the solution turns yellow.

Hydrochloric acid

Add 0.9 mL concentrated hydrochloric acid to 500 mL distilled water. Fill it up to the 1000 mL mark.

Bromophenol blue

Weigh out 0.4 g of bromophenol blue. Dissolve it in 50 mL distilled water. Fill it up to the 100 mL mark.

METHOD

1. Weigh out 30.5 g of oil in an Erlenmeyer flask.
2. Add 40 mL of the acetone solution to the flask.
3. Do a titration with the 0.01 N hydrochloric acid until the sample turns yellow.
4. Report as 0.0 ppm if the sample is already yellow before titration.

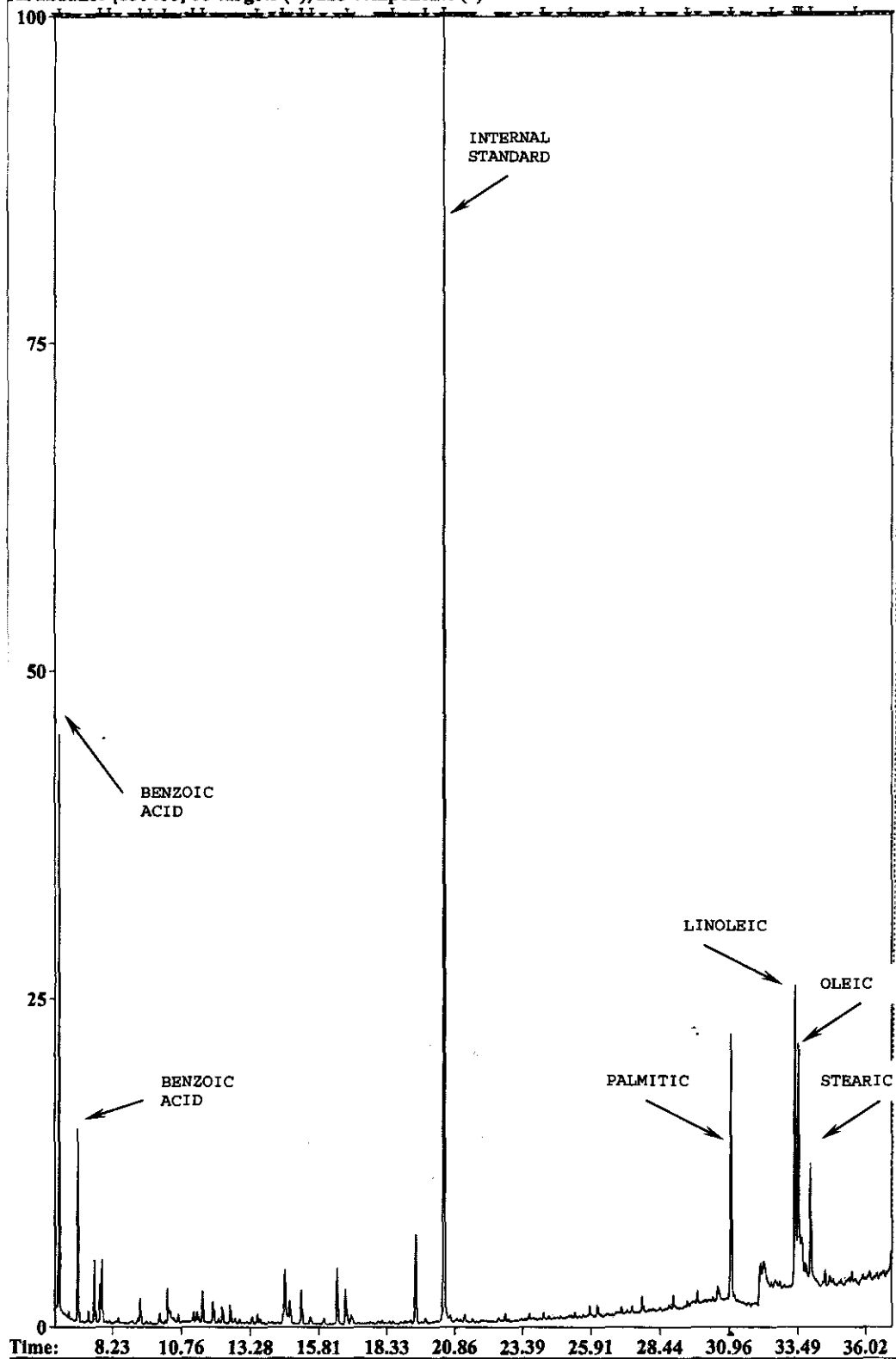
CALCULATION

1. If 10 g sample is used (soap as sodium oleate), then
$$\text{ppm} = \text{mL HCl} \times 304.4$$
2. If 30.4 g sample is used:
$$\text{titration} \times 100 = \text{soap ppm}$$

A2 GC/MS ANALYSIS OF EPKO REFINED SUNFLOWER OIL

GC/MS Analysis - Data:C:\AMDIS32\DATA\EPKO

Abundance [150406] 33 targets (-), 225 components (-)

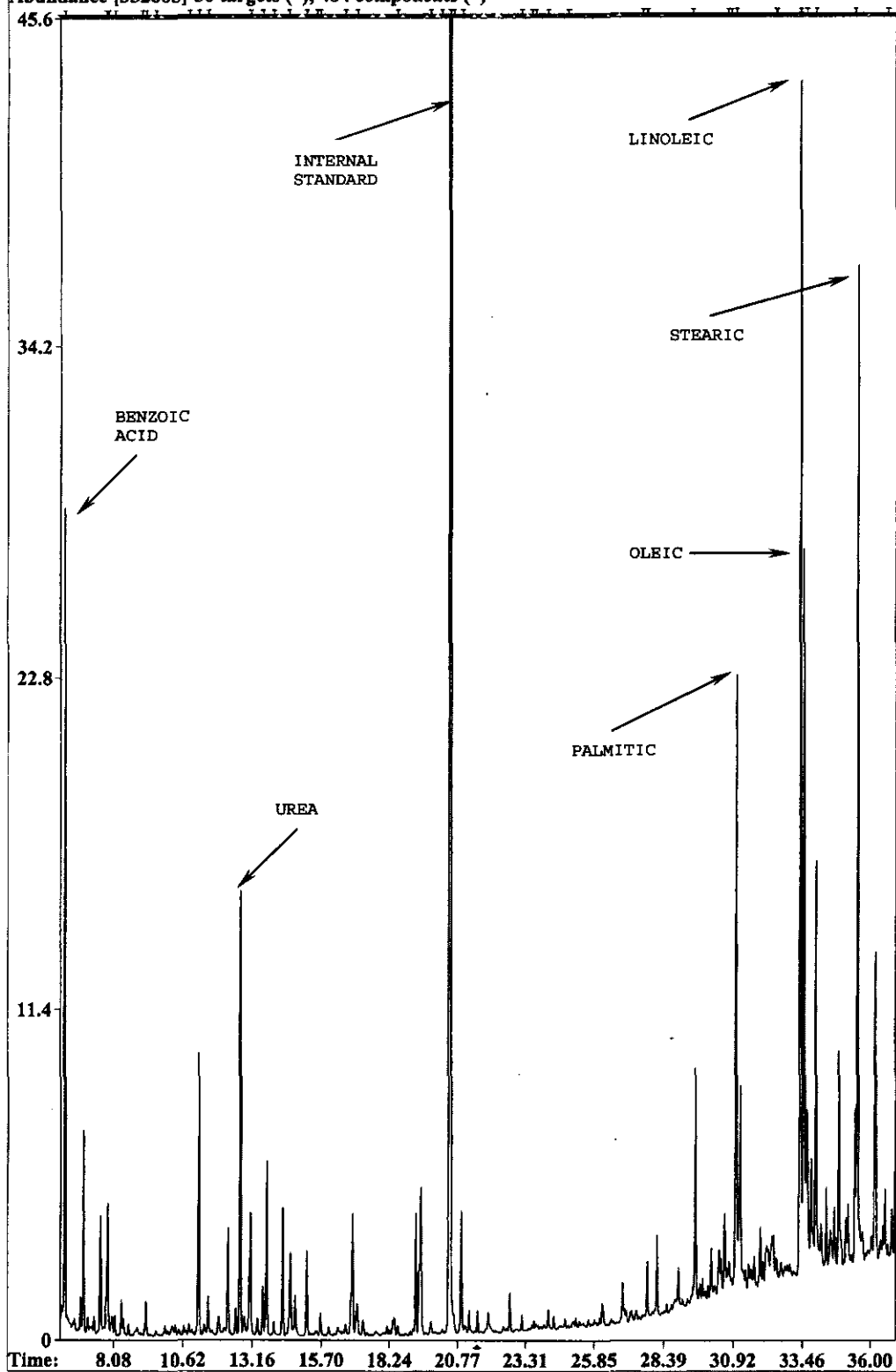


AMDIS32 NAME	EPKO R.T.	Scan	Tot.signal	Concentration
BENZOIC ACID (3-METHYL-2-HYDROXY)	6.264	48	2408439	35.89
BENZOIC ACID (4-METHYL-2-HYDROXY)	6.267	48	2511034	37.42
LACTIC -DITMS2	7.781	471	112797	1.68
GLYCOLIC-DITMS	8.114	564	9531	0.14
cystationineuria	9.271	887	56676	0.84
MALONIC-DITMS	9.635	989	8273	0.12
FUMARIC-DITMS	10.162	1123	15321	0.23
BENZOIC ACID (2,6-DIHYDROXY)	11.197	1346	26930	0.4
MANDELIC-ACID (4-HYDROXY)	11.548	1421	84946	1.27
MANDELIC-ACID (3-HYDROXY)	11.548	1421	81352	1.21
THIODIGLYCOL-DITMS	13.545	1851	30153	0.45
2,3-DIHYDROXYBUTANE-TMS2	13.552	1853	28284	0.42
PHOSPHORIC-DITMS	14.484	2053	42461	0.63
GLYCERIN-DITMS	15.176	2202	54869	0.82
SUCCINIC-DITMS	15.547	2282	8388	0.13
lorazepam (bis TMS)	16.878	2568	11684	0.17
BENZOIC ACID (2,3,4-TRIHYDROXY)	18.561	2906	3441	0.05
3,4-DIHYDROXYBUTYRIC-DITMS	19.761	3116	8792	0.13
DECANOIC-TMS	19.795	3122	4969	0.07
LAURIC-DITMS	24.163	3886	14996	0.22
HEXACOSANE	25.187	4065	8108	0.12
MYRISTIC-TMS	273803	4523	33312	0.5
PENTADECANOIC-TMS	29.456	4812	9869	0.15
PALMITIC-TMS	31.032	5088	597116	8.9
HEPTADECANOIC-TMS	32.519	5350	9166	0.14
LINOLEIC-TMS	33.377	5498	275942	4.11
OLEIC-TMS	33.516	5522	266830	3.98
T-9-OCTADECENOIC-TMS	33.605	5538	19524	0.29
STEARIC-TMS	33.959	5600	259115	3.86
BENZOIC ACID (2,3-DIHYDROXY)	35.617	5890	4613	0.07
STD MALONIC ACID (IS)	9.635	989	8273	0.12
STD Internal Standard	20.483	3242	3529312	52.6

A3 GC/MS-ANALYSIS OF sc-CO₂ DERIVED SUNFLOWER OIL

GC/MS Analysis - Data: C:\AMDIS32\DATA\LECO

Abundance [352665] 50 targets (-), 484 components (-)



AMDIS32	LECO			
NAME	R.T.	Scan	Tot.signal	Concentration
BENZOIC ACID (4-METHYL-2-HYDROXY)	6.279	51	3553646	29.66
LACTIC-DITMS	7.812	479	112141	0.94
CAPROIC-TMS	7.891	501	78492	0.66
GLYCOLIC-DITMS	7.143	571	28315	0.24
LEVOLINIC-TMS	9.14	850	5309	0.04
cyctationineuria	9.284	890	74252	0.7
OXALIC-DITMS	9.646	991	18737	0.16
HEPTATONIC-TMS	10.862	1274	17285	0.14
BENZOIC ACID (2,6-DIHYDROXY)	11.212	1349	837192	6.99
MANDELIC ACID (3-HYDROXY)	11.559	1424	102808	0.86
MANDELIC ACID (4-HYDROXY)	11.56	1424	112348	0.94
UREA-DITMS	13.111	1757	416076	3.47
2,3-DIHYDROXYBUTANE-DITMS	13.557	1853	163188	1.36
OCTANOIC-TMS	13.956	1940	33610	0.28
PHOSPHORIC-TRITMS	14.513	2059	69386	0.58
GLYCERIN-TRITMS	15.177	2202	152953	1.28
SUCCINIC-DITMS	15.552	2283	16943	0.14
ACETOPHENONE (3-HYDROXY)	15.684	2311	51188	0.43
p-ETHYLPHENOL-TMS	16.611	2510	21498	0.18
NONANOIC-TMS	17.05	2605	52320	0.44
BENZOIC ACID (2,3,4-TRIHYDROXY)	18.553	2904	16288	0.14
DECANOIC-TMS	19.787	3121	24991	0.21
DECANOIC-TMS	19.787	3121	25178	0.21
BENZOIC ACID (3-METHOXY)	20.203	3193	10571	0.09
benzocaine (TMS)	20.518	3248	662305	5.53
PHENYLPROPENOIC ACID	20.642	3270	58392	0.49
PIPECOLIC-DITMS	20.98	3329	6589	0.05
HEXACOSANE	23.181	3714	41749	0.35
BENZOIC ACID (4-HYDROXY)	23.557	3780	11974	0.1
PHENYLACETIC ACID (4-HYDROXY)	23.697	3805	12569	0.1
LAURIC-DITMS	24.159	3885	40401	0.34
BENZOIC ACID (5-METHYL-2-HYDROXY)	24.895	4014	7306	0.06
THYMOL-TMS	27.631	4493	9195	0.08
CITRIC-TETRATMS	27.77	4517	11648	0.1
MYRISTIC-TMS	27.797	4522	133943	1.12
PENTADECANOIC-TMS	29.451	4811	21489	0.18
BENZOIC ACID (2HYDROXY-5-METHYL)	30.724	5034	17577	0.15
1,9-DIMETHYLZANTHINE-DITMS	30.85	5056	33318	0.28
PALMITIC-TMS	31.034	5088	1449599	12.1

7-METHYLZANTHINE-DITMS	32.484	5342	33326	0.28
MARGARIC-TMS	32.52	5348	32072	0.27
HEPTADECANOIC-TMS	32.525	5349	35418	0.3
LINOLEIC-TMS	33.392	5501	1145351	9.56
OLEIC-TMS	33.629	5542	161722	1.35
STEARIC-TMS	33.967	5601	917635	7.66
2-KETOADIPIC-TRITMS	35.426	5857	429883	3.59
EICOSAMNOIC-TMS	36.659	6072	19766	0.16
STD MALONIC ACID (IS)	9.652	993	16129	0.13
STD Internal Standard	20.518	3248	6301520	52.6

SUMMARY

The principal objective of this study was to extract sunflower (*Helianthus annuus*) oil from seed with supercritical carbon dioxide (sc-CO₂) as an alternative to the cold-press method and subsequent 3-stage refining of the acquired crude oil.

Extractions were performed on laboratory scale with a commercially available supercritical fluid extractor of the latest design.

A minimum number of extraction runs based on a statistical design was performed to establish the conditions (time, pressure, temperature) required for a maximum yield of oil by virtue of surface response analysis and kinetic yield-versus-time data fitting.

Several additional extraction runs were performed at randomly selected conditions to properly reveal the temperature, pressure and density dependence of the extraction process and thereby elucidate its mechanism.

The physical appearance, chemical quality and composition of the sc-CO₂ derived sunflower oil were evaluated against the crude and refined oil obtained by the cold-press method as well as a standard specification for commercial sunflower oil. This evaluation was done by chemical analysis performed in the quality control laboratory of a commercial sunflower oil supplier and by GC and GC-MS

analysis of samples of both sc-CO₂ and cold-press obtained sunflower oil. It was concluded that sunflower oil of a superior quality can be obtained by sc-CO₂ extraction, especially when de-hulled seed is used to minimise the wax content of the extract.

Extraction runs were performed on seed with natural moisture content as well as on oven-dried, freeze-dried and sun-dried seed in order to establish the crucial role played by moisture content as a yield-determining factor.

Preliminary results of upscaling on a supercritical pilot-plant suggest that sc-CO₂ extraction of sunflower oil could be performed on industrial scale should an extruder based continuous feed mechanism and a viable process of dehulling of seed be implemented.

OPSOMMING

Die primêre doelstelling van hierdie studie was om sonneblomolie (*Helianthus annuus*) uit saad met superkritieke koolstofdioksied (sc-CO_2) te ekstraheer as alternatief vir die koue-pers-metode en daaropvolgende 3-stap raffinering van die verkreë ru-olie.

Ekstraksies is op laboratoriumskaal uitgevoer met 'n superkritieke-fluïed-ekstraktor van die jongste ontwerp wat in die handel beskikbaar is.

'n Minimum getal ekstraksielopies gebaseer op 'n statistiese ontwerp is uitgevoer om die vereiste kondisies (tyd, druk, temperatuur) vir 'n maksimum olieopbrengs met behulp van oppervlakresponsanalise en kinetiese opbrengs-versus-tyd datapassing te bepaal.

Heelwat bykomende ekstraksielopies is by ewekansig geselekteerde kondisies uitgevoer ten einde die temperatuur-, druk- en digtheidafhanklikheid van die ekstraksieproses bloot te lê en sodoende die proses-meganisme op te klaar.

Die fisiese voorkoms, chemiese gehalte en samestelling van die sc-CO_2 verkreë sonneblomolie is geëvalueer deur vergelyking met ru- en geraffineerde olie wat met die koue-pers-metode verkry is asook aan die hand van 'n standaard spesifikasie vir kommersiële sonneblomolie. Hierdie evaluering is gedoen deur chemiese analise in die

gehaltebeheerlaboratorium van 'n kommersiële sonneblom-olieverskaffer en deur GC- en GC-MS-analise van sonneblomoliemonsters wat met sowel sc-CO₂ as die kouepers-metode verkry is. Die gevolgtrekking was dat sonneblomolie van 'n voortreflike gehalte met sc-CO₂ ekstraksie verkry kan word, veral indien gebruik gemaak word van ontdopte saad wat die wasinhoud van die ekstrak beperk.

Ekstraksielopies is uitgevoer op saad met natuurlike voggehalte asook op oondgedroogde, gevriesdroogde en songedroogde saad ten einde die kritieke rol wat voggehalte as 'n opbrengs bepalende faktor speel, vas te stel.

Die voorlopige resultate vir opskalering met 'n superkritieke loodsaanleg suggereer dat die ekstraksie van sonneblomolie met sc-CO₂ op nywerheidskaal uitgevoer sou kon word indien 'n ekstrusie gebaseerde kontinue toevoermeganisme en 'n werkbare saadontdoppingsproses geïmplimenteer word.

ACKNOWLEDGEMENTS

As author, I give all honour and praise for the completion of this dissertation to my Saviour and Provider, my Lord Jesus Christ.

I would furthermore like to sincerely thank the following people for their help and contributions:

- **Pharmaceutical Chemistry at NWU Potchefstroom:** for the opportunity to do my M.Sc.
- **Professor E.L.J. Breet:** for guiding me in this study with insight and wisdom, the provision of a bursary and for setting an example in striving for the best and as a Christian man.
- **Professor J.C. Breytenbach:** as co-supervisor, helping me on my research goal-setting and for his insight, advice and support from the start unto completion of this project.
- **Doctor André Nel:** for supplying me with the sunflower seed I needed as well as the most recent literature on sunflower.
- **Leon Koekemoer:** for his help with the statistical planning of this project.
- **Daniël van Niekerk and Biochemistry at NWU Potchefstroom:** for helping me with the GC-MS analysis.
- **Pieter van Zyl:** for his help in handling the instruments.
- **My mother and father:** for their support and encouragement through ongoing incomprehensible love.
- **Clive Tubes and THRIP:** for their financial support.