Extraction of olive oil with supercritical carbon dioxide

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Background and Objectives

There is an increasing public awareness of health, safety and environmental hazards associated with the use of organic solvents in food processing and possible solvent contamination of the final product. The high cost of organic solvents together with stricter environmental regulations and higher safety standards for medical and food industries emphasise the need for the development of new and clean technologies for the processing of food products. Supercritical fluid extraction (SFE), using carbon dioxide as a solvent, may be a viable alternative to the use of chemical solvents [1].

One of the most popular and successful applications of SFE is the extraction of fats and oils with supercritical carbon dioxide (sc-CO_2). Almost all fats and oils lie within a polarity range extractable by CO_2 or CO_2 and an added modifier [2]. CO_2 is selected as solvent due to its non-reactive and non-toxic character, low cost, modest critical parameters, easy solute-solvent separation and high selectivity [3].

In this study the extraction of olive oil from dried olives with sc-CO_2 was investigated. Extraction of olive oil with sc-CO_2 has the potential advantage of obtaining high yields of oil within relatively short extraction times and a final product that is free of organic solvents and compares favourably with commercial olive oil without multiple refinements [4].

Several authors have reported solubility data for certain components of olive oil, as well as sc-CO_2 extraction of olive oil from olive husk (solid residue left after first extraction of oil) and from polishing earths used in the refining process [3-6].

SFE has been applied to the deacidification of olive oil, which has a high content of free fatty acids when extracted mechanically. The possibility of using sc-CO_2 to deacidify olive oil without modifying its triglyceride composition and nutritional quality have been suggested [7-8].

Research Objectives

The focus in this research project was on the extraction of a botanical oil which is free from solvent residues with a process that is faster, more efficient and more environmentally friendly than existing extraction methods (solvent extraction, cold pressing, centrifugal separation). The objectives of the project were to:

1. test the viability of extracting olive oil from dried olives with sc-CO_2 by employing a laboratory-scale supercritical extractor;

2. determine the composition of the extracted oil by means of a suitable analytical method;

3. optimise the yield of extract by determining the optimum process conditions (temperature, pressure, time) with a statistical experimental design and surface response analysis;

4. establish the mechanism of extraction by studying the dependence of the yield on various parameters;

5. compare the quality of extracted oil with that of commercially available oil extracted by conventional methods;

6. add value to a developing process technology while focusing on the extraction of high quality botanical substances from plants;

7. obtain a product which is free of harmful solvents and ready for human consumption without any subsequent refining.

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1

Olea europaea

1.1 *Olea europaea* as Medicinal Plant

Natural products and their derivatives represent more than 50 % of all drugs in clinical use in the world [1]. About 119 plant derived chemical compounds of known structure were identified as currently being used as drugs or biodynamic agents that affect human health, including morphine, atropine, codeine and digoxin [2].

It has been estimated that 80 % of people living in developing countries are almost completely dependent on traditional medicinal practices for their primary health care needs, which means that about 64 % of the total population of the world utilises plants as drugs [2]. Research in medicinal plants is thus of immense global importance.

Olive oil derived from the ripe fruit of *Olea europaea* is high in monounsaturated fatty acids and antioxidants, which correlates strongly with the low rates of chronic diseases and particularly coronary hart disease (CHD) seen in Mediterranean countries where the majority of fat calories consumed are derived from olive oil. Western countries in contrast, where diets are high in saturated fatty acids, have a high incidence of CHD. This suggests that olive oil, through its beneficial effects on lipid metabolism, blood pressure, diabetes and clotting mechanisms, plays a major role in health preservation [3].

1.2 History

The olive is native of Palestine. It was known in Egypt in the 17th century B.C. [4]. The oil was used for cooking as well as for burning in lamps. In the 1900s pickling and canning procedures were developed in California [5].

Today the olive is the most extensively cultivated temperate fruit in the world and is produced in 39 countries worldwide, including all the Mediterranean countries, the south western United States, southern Australia and northern Africa [5-6].

In South Africa olives has been commercially cultivated since the 20th century, residing mainly in the Western Cape [7]. More recently, cultivation extended to the drier summer rainfall regions.

1.3 Economical Significance

Worldwide 15 724 187 million tons of olives are produced on an area of over 8 million hectares. The United States industry value was $60.7 million in 2002, and has varied between $34 and $102 million over the last decade [5].

In 2004 South Africa's total olive oil production was 490 tons, compared to the total world output of about 3 million tons. The advantage South African producers have, is that oil is pressed during the European off-season, when oil is scarce and northern demand is high [8]. In addition, the local olive market shows a 10 % growth in demand each year for table olives, and 20 % annually for oil [9].

South African olive oil does very well in international competition. In 2004 Kloofenburg Estate olive oil won a prestigious award in the Italian world olive oil guide, which cited it as one of the best 15 olive oils in the world [8].

1.4 Classification and Description

1.4.1 Classical Taxonomy

Eukaryotes - nucleated cells
Kingdom: green plants
Subkingdom: Tracheobionata - vascular plants
Superdivision: Spermatophyta - seed plants
Division: Manoliophyta - flowering plants
Class: Magnoliopsida - Dicotyledons
SubClass: Asteridae
Order: Scrophulariales or Lamiales
Family: Oleaceace
Genus: Olea
Species: Europaea [10]

1.4.2 Botanical Description

The olive tree is a small evergreen tree that grows quite old but seldom exceeds ten meters in height [11]. The fruit is used in its green stage as a table olive and in its ripe stage as a black table olive and for olive oil extraction [4].

Figure 1.1 Olea *europaea*

Leaf: Opposite, simple, evergreen, lanceolate or narrow-oblong, 2 to 7 cm long, thick and leathery, blue to grey-green above and much paler beneath [12].

Flower: Clusters of small creamy white to pale yellow flowers appearing from leaf axils in spring [12].

Fruit: Oblong drupe up to 3.8 cm long, smooth, green initially but dark reddish purple (nearly black) when ripe in winter [12].

Twig: moderately stout, light grey-green and finely fuzzy when young [12].

1.5.1 Leccino

The *Leccino* cultivar originates from central Italy. In many of the new developing regions it is used as one of the major oil cultivars because of its relatively high tolerance to low temperatures and high humidity [6]. The fruit is small to medium in size (2-2.5 g) [14], and has a commercial oil content of about 17% with a somewhat fruity flavour [6].

1.5.2 Frantoio

The origin of the *Frantoio* cultivar is Toscana, Italy. It is the major cultivar of that region and has spread to many other countries [6]. The fruit is small to medium in size (2-3 g) [14] and has an oil content of 17-18% under irrigated conditions [6].

1.6 Olive Oil

Figure 1.4 Olive oil

Olive oil is the fixed oil pressed from the pericarp of the ripe fruit of *Olea europaea*. It is a clear yellow or greenish-yellow, transparent liquid **(Figure 1.4)** with a characteristic odour and bland taste [15]. When cooled, it becomes cloudy at 10 °C and a butter-like mass at about 0 °C [16]. Oil yield with conventional extraction methods amounts to between 15 and 22 %, depending on the olive cultivar as well as the degree of ripeness of the olives [17-18].

1.6.1 Characteristics of Olive Oil

Table 1.1 Characteristics of olive oil

Density or Specific Gravity:	0.9150-0.9180 g/ml @ 15.5 °C
Viscosity:	84 mPa.s (84 cP) at 20 °C
Specific Heat:	2.0 J/g.°C
Thermal Conductivity:	@ 20 degrees Celsius - 0.17
Volumetric Heat Capacity @ 20 °C :	$1.650\ 10^{6}$ J/m^3
Thermal Diffusivity @ 20 °C :	10×10^{-8} m^2/s
Boiling point:	299 °C
Energy per tablespoon olive oil:	About 28 J

1.6.2 Varieties of Olive Oil

Olive oil is offered in the market place in several grades of purity. The classification is based on the method of extraction as well as acid content. The presence of acidic fats in olive oil can change according to climate, pressing technique and length of time expired from picking to pressing [19]. Edible olive oil is divided into 3 categories:

1.6.2.1 Virgin Olive Oil

Virgin olive oil is the first oil obtained from the olive fruit. The percentage of acidic fat content, determines the classification as virgin oils [19].

- **Cold pressed virgin olive oil**

The term "cold pressed" indicates that milling temperatures were kept below 30 ºC, preventing the break-down of temperature sensitive vitamins, antioxidants and flavour components [7].

- **Extra-virgin olive oil and virgin olive oil**

Natural unrefined olive oil is classed as extra-virgin olive oil when it has a free acidity of below 1 % and as virgin olive oil when the acidity is below 2 % [7].

1.6.2.2 Refined Olive Oil

Refined olive oil has an acid content of more than 4 % and organoleptic imperfections that are refined through mechanical and chemical processes. It can also be olive oil extracted from the solid residue (pomace or husk) that remains after the extraction of virgin olive oil. Refined olive oil must be free of any trace of chemical substance and must have an acid content less than 0.5 % [19]. Refined oil, or oil that contains a portion or refined oil, is sold as pure olive oil, olive oil or light olive oil [7].

1.6.2.3 Mixed Oil

Refined oils are mixed with virgin olive oil in quantities which vary according to the desired taste and colour [19].

1.7 Extraction of Olive Oil

Olive oil is currently extracted by two major processes:

1.7.1 Pressing

Pressing is the traditional extraction process where pressure is used to separate the oily juice (a mixture of oil and olives residual water) from the olive pomace, through filtration. After being washed the olives are ground, together with their stones, and mixed into a homogeneous pulp. The olive paste is spread over olive mats. The mats are pressed hydraulically and the extracted oil is collected in containers. Traditionally, pressed olive oil is clarified by sedimentation [20].

1.7.2 Continuous Method

The olives are crushed by a hammer mill and the paste is pumped to a malaxer where it is warmed and beaten or mixed with water until the oil begins to separate. The paste is then pumped to a centrifuge where the solids are separated from the liquids. The vegetable water and oil are further separated in a final centrifugal process. The variations of this method involve adjustment of heat and addition of water [21].

1.8 Constituents of Olive Oil

Olive oil is rich in triglycerides containing unsaturated fatty acid groups. It also contains small quantities of free fatty acids, glycerol, pigments, flavour components, hydrocarbons, volatile compounds, sterols, tocopherols, phenols, unidentified resinous compounds, and others [22-23].

1.8.1 Triacylglycerols

Triacylglycerols consist of a glycerol molecule and three fatty acid molecules **(Figure 1.5).**

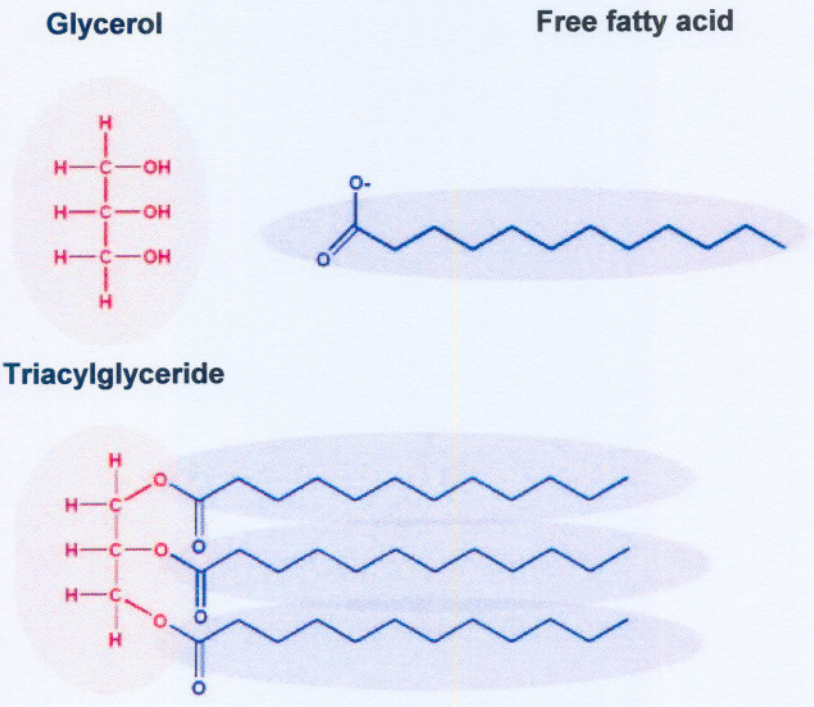


Figure 1.5 Triacylglyceride molecule composition [24]

The main fatty acid is oleic acid, with smaller amounts of linoleic, palmitic and stearic acids [25]. A typical chemical fatty acid analysis of olive oil is shown in **Table 1.2.**

Oleic acid **(Figure 1.6)** is a monounsaturated fatty acid. Olive oil contains about 80 % oleic acid, placing it at the top of the list of natural monounsaturated fats.

Table 1.2 Fatty acid analysis [26]

Fatty acids		Range
Palmitic	C16:0	5.0 - 12.0 %
Palmitoleic	C16:1	1.0 % max
Stearic	C18:0	3.5 % max
Oleic	C18:1	65.0 - 80.0 %
Linoleic	C18:2	6.0 - 25.0 %
Linolenic	C18:3	1.0 % max
Arachidic	C20:0	0.6 % max
Gadoleic	C20:1	0.5 % max
Behenic	C22:0	0.3 % max
Erucic	C22:1	0.2 % max

Linoleic acid **(Figure 1.7)** is an omega-6 fatty acid which, together with the omega-3 linolenic acid, makes olive oil a good source of essential fatty acids [24]. **Figure 1.8** compares olive oil as a source of monounsaturated fats with other oils.

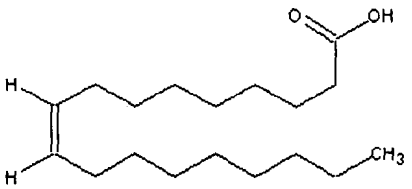


Figure 1.6
Oleic acid
(9-Octadecenoic acid)
$C_{18}H_{34}O_2$

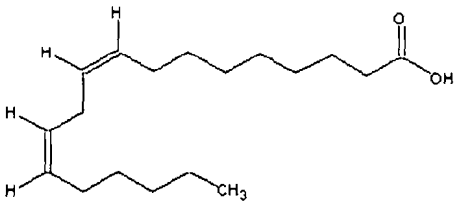


Figure 1.7
Linoleic acid
((Z)-9,12-octadecadienoic acid)
$C_{18}H_{32}O_2$

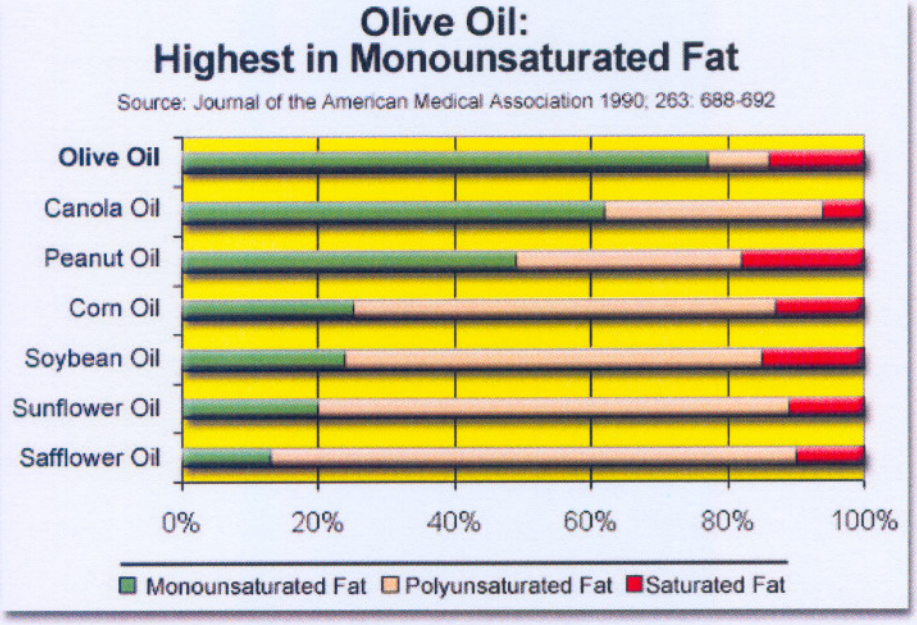


Figure 1.8 Olive oil as main source of monounsaturated fats

1.8.2 Pigments

The colour of olive oil is mainly related to the presence of chlorophyll, pheophytin and cartenoids. The presence of these pigments depends on factors such as cultivar, soil and climate, fruit maturation as well as conditions during processing [23].

1.8.3 Flavour Components

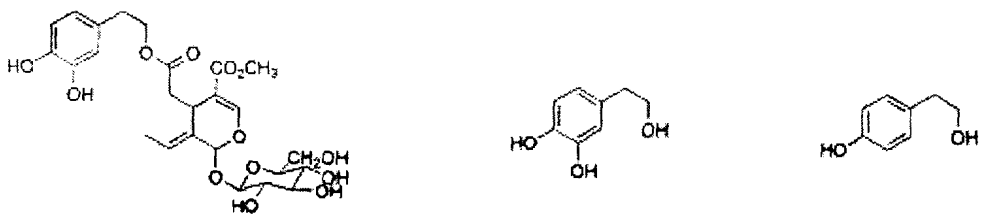
The unique and delicate flavour of olive oil is attributed to a number of components including aldehydes, alcohols, esters, hydrocarbons and ketones [24].

1.8.4 Tocopherols

Olive oil contains α-, β-, γ- and δ-tocopherols, of which α-tocopherol covers almost 88 %. The tocopherol content of olive oil does not only depend on the presence of these compounds in the olive fruit, but on several other factors involved in transportation, storage and processing of the fruit [23]. Tocopherols are extremely valuable compounds because of their activity as vitamin E and as antioxidant [27].

1.8.5 Phenols

The total phenol content of olive oil has been reported widely in literature, but there are inconsistencies with the concentrations cited. These varied from 100 to 800 mg/kg. The major phenolic compounds in olive oil are oleuropein, hydroxytyrosol and tyrosol [28]. **(Figure 1.9)** The phenolic compounds in the oil increase its oxidative stability and improve the taste. The flavonoid polyphenols in olive oil are natural antioxidants which have been shown to have beneficial effects on lowering cholesterol, blood pressure and risk of coronary heart disease [24].



oleuropeln **Hydroxytyrosol (3,4-dihydroxyphenyl ethanol** **tyrosol**

Figure 1.9 Major phenolic compounds in olive oil

1.8.6 Peroxides

Peroxides are the primary products of oxidation of olive oil. The more rancid or oxidised the oil, the more peroxides are present [24].

1.8.7 Free Fatty Acids

The acidity of olive oil is the result of the degree of breakdown of triacylglycerols due to hydrolysis to form free fatty acids. The percentage of free fatty acids is a direct measure of the quality of the olive oil [24].

1.9 Medicinal and Nutritional Value of Olive Oil

1.9.1 Nutritional Information

Table 1.3 Nutritional information of olive oil

Serving Size: 100 g

NutritionData.com		
Food Energy		
Amounts Per Selected Serving		%DV
Calories	884 (3701 kJ)	44 %
Calories from Carbohydrate	0.0 (0.0 kJ)	
Calories from Fat	884 (3701 kJ)	
Calories from Protein	0.0 (0.0 kJ)	
Fats & Fatty Acids		
Amounts Per Selected Serving		%DV
Total Fat	100 g	154 %
Saturated Fat	13.5 g	67 %
16:0	10930 mg	
18:0	1961 mg	
20:0	416 mg	
22:0	128 mg	
Monounsaturated Fat	73.9 g	
16:1 undifferentiated	1158 mg	
17:1	137 mg	
18:1 undifferentiated	72300 mg	
20:1	313 mg	
Polyunsaturated Fat	10.0 g	
18:2 undifferentiated	9212 mg	
18:3	793 mg	
Vitamins		
Amounts Per Selected Serving		%DV
Vitamin E	14.3 mg	48 %
Vitamin K	60.2 mg	75 %
Minerals		
Amounts Per Selected Serving		%DV
Calcium	1.0 mg	0 %
Iron	0.7 mg	4 %
Potassium	1.0 mg	0 %
Sodium	3.0 mg	0 %
Sterols		
Amounts Per Selected Serving		%DV
Cholesterol	0.0 mg	0 %
Phytosterols	221 mg	

1.9.2 Pharmaceutical Agent

Olive oil is classified as a pharmaceutical agent, which is used as a setting retardant for dental cements and as an ingredient in the preparation of soaps, plasters and liniments. Olive oil is also used as a demulcent, an emollient and a laxative [11]. Virgin olive oil that complies with the requirements of the British pharmacopoeia is used as an eye drop [29].

1.9.3 Medicinal Value

1.9.3.1 Heart disease and atherosclerosis

The Mediterranean diet, which is rich in olive oil, has been recommended since it reduces the incidence of coronary heart disease (CHD). The reason for this is that its low saturated and high monounsaturated fatty acid content and the natural antioxidants help to prevent lipid oxidation which leads to the formation of atherosclerotic plaques [30]. **Figure 1.10** shows the incidence of CHD deaths in Crete, the Netherlands and the United States as well as the influence of meat and olive oil consumption on these figures.

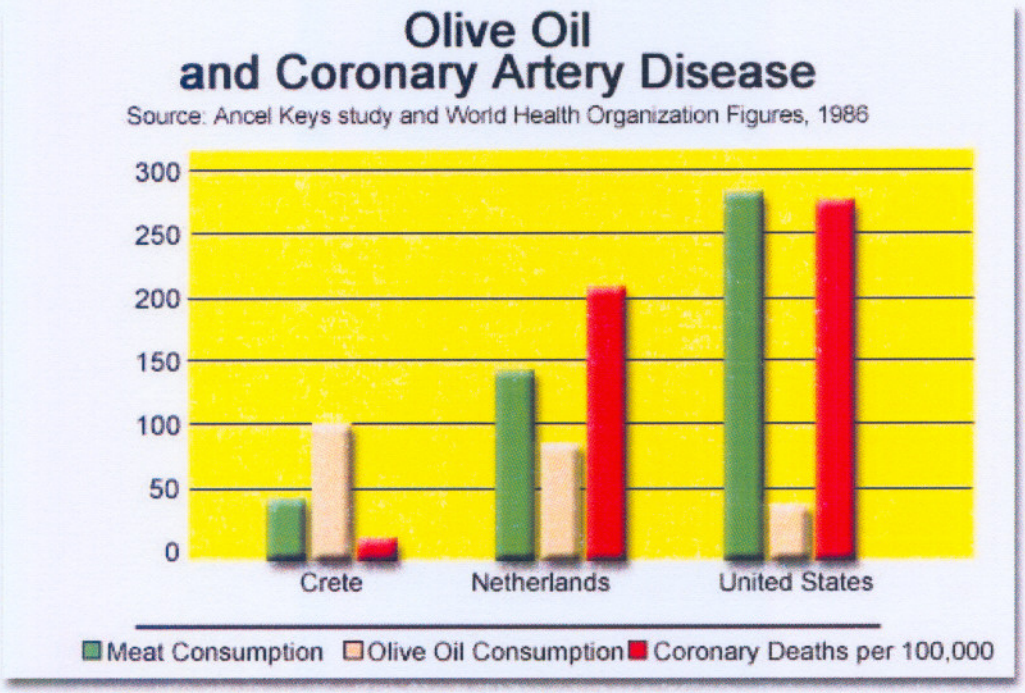


Figure 1.10 Significance of olive oil and meat consumption in the prevention of CHD

1.9.3.2 Cholesterol

Research showed that while polyunsaturated fat reduces both low-density lipoproteins (LDL) and high-density lipoproteins (HDL), monounsaturates reduce LDL while increasing HDL [31]. HDL is referred to as "good cholesterol". An increase in the levels of HDL does not only provide protection against cholesterol deposits, but also reduces cholesterol levels in the body [31].

1.9.3.3 Other health applications

There is some evidence that a diet rich in olive oil has a favourable effect on breast cancer [30], platelet aggregation [32], hypoglycemic activity [32], rheumatoid arthritis [33], and blood pressure [34].

Olive oil recently made news headlines, both locally and internationally, when the South African Minister of Health, Dr. Manto Tshabalala-Msimang, expressed her belief that olive oil may be useful in the management of HIV. On several occasions the Minister promoted the health benefits of natural products, implying that they could be effective alternatives to anti-retroviral drugs [35]. *"I think garlic is absolutely critical. Lemon is absolutely critical to boost the immune system. Olive oil is absolutely critical ... just one teaspoon, it will last the whole month."* she said in February 2004 during a parliamentary media briefing [36]. Although comments like these make the world frown upon the competence of the South African health administration, there seems to be at least some, including the Traditional Healers Organization, who shares the Minister's sentiments [37-38].

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2
Supercritical Fluid Technology

2.1 Historical Perspective

The critical point of a substance was discovered in 1822 by Baron Cagniard de la Tour during high-pressure investigations. The ability of a supercritical fluid to dissolve low-vapour-pressure solid materials was first reported in 1979 [1]. In the following decade numerous studies were published involving the solubility of inorganic as well as organic substances in supercritical fluids [2].

Supercritical carbon dioxide (sc-CO_2) attracted much attention in the later half of the nineteenth century. Extensive investigation in the mid-1800's on the phase behaviour of carbon dioxide resulted in the values 30.92 °C and 74.0 atm for the critical point of carbon dioxide, which are in close agreement to presently accepted values 31.1°C and 73.8 atm [1].

The first industrial application of supercritical fluids is considered to be the deasphalting of heavy mineral oil fractions by means of dense propane in the petrochemical industry in the late 1930's. Since the 1950's studies and development efforts have been focused on new ways of separating substances by making use of the unique properties of supercritical fluids [2]. Applying the concept of supercritical fluid extraction (SFE) to industry, scientists from the *Max Planck Institut für Kohlenforschung* studied the feasibility of using SFE in the food, petroleum, and chemical industries. Zosel and coworkers from the Max Planck Institute were the first to characterise the use of sc-CO_2 as a solvent for caffeine [3].

In 1987 the first decaffeination plant was commissioned by Hag AG in Bremen. Following this a plant for hops extraction was constructed in 1982 in Germany and later in 1985 and 1988 facilities for hops (Pfizer) and coffee (General Foods) were commissioned in the United States [2].

2.2 Fundamentals

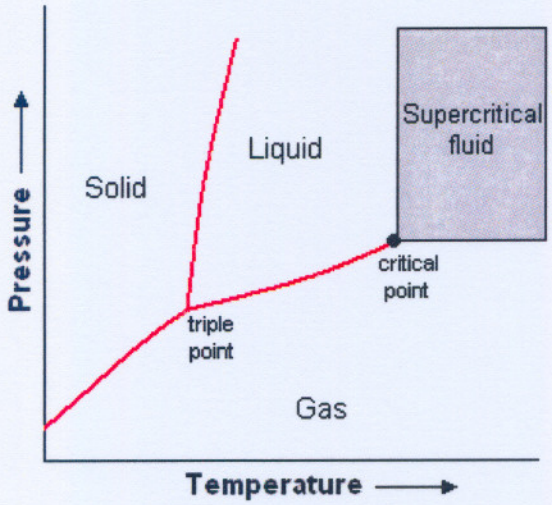

Figure 2.1 Generalised phase diagram showing critical point and supercritical domain

A substance is regarded as a supercritical fluid when it prevails at conditions above its critical point [4]. The critical point is an endpoint on the coexisting curve at which the liquid and gas phases can no longer be distinguished from each other [5]. The generalised phase diagram in **Figure 2.1** shows the supercritical region within which density, and thus solvent strength, can be adjusted by varying pressure and/or temperature. **Table 2.1** lists the characteristic critical conditions of selected substances.

Table 2.1 Critical conditions for selected substances

Fluids	Critical Temperature (°C)	Critical Pressure (atm)
H_2O	374	220
CO_2	31	73
N_2O	36	72
NH_3	132	112
CH_3OH	240	78
$CClF_3$	29	38
C_2H_6	32	48
C_2H_4	10	51

Figure 2.2 shows a phase diagram of a pure substance with qualitative indications of the density in the various regions of temperature and pressure.

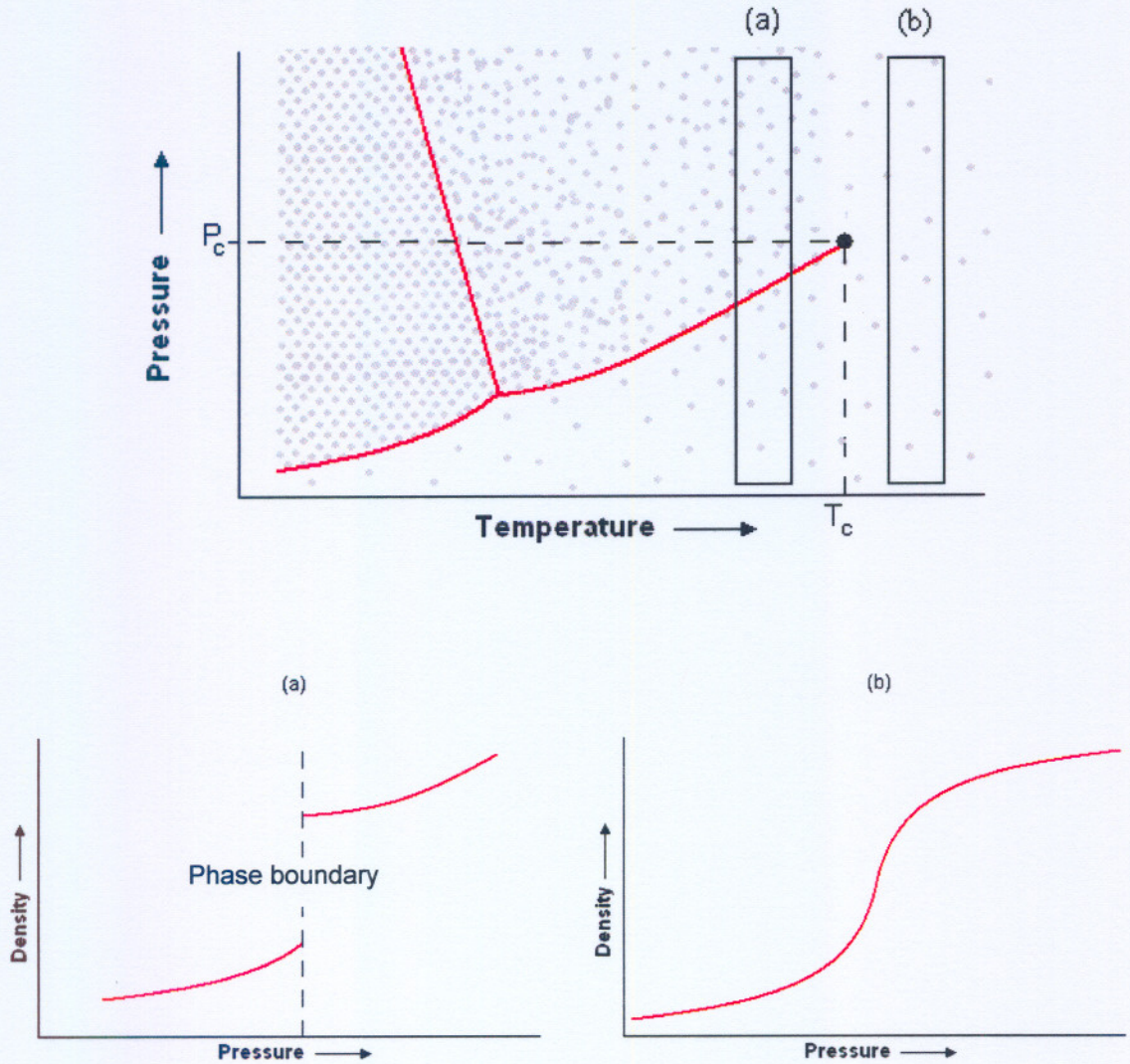

Figure 2.2 Schematic phase diagram illustrating the variable density of a supercritical fluid. Graph (a) and (b) show the contrasting effects of increasing pressure on the density of the fluid. In (a) increasing pressure leads to condensation of the gas as the phase boundary is crossed, with a corresponding step-increase in the fluid density. In the supercritical region (b), increasing pressure causes a smooth, continuous increase in the fluid density. This behaviour which allows the supercritical fluid to be exploited as an adjustable solvent [6].

The compressibility of a substance increases indefinitely as the critical point is approached, and a dramatic change in the density and therefore solvent strength is observed as the pressure is increased. The variable solvent strengths of supercritical fluids make them suitable for a variety of solubility related applications [7].

Apart from the unique solvent strengths, supercritical fluids also possess other properties which make them viable solvents for a number of applications. Diffusivity is typically one to two orders of magnitude higher, and viscosity an order of magnitude lower than that of organic liquids, even at high pressure (300-400 atm) [1]. Negligible surface tension allows supercritical fluids to penetrate easily into microporous substances, or into amorphous polymer matrices. The diffusion and mass transfer properties of supercritical fluids can be compared with those of gases, and the density and solvent strength with those of liquids. These characteristics, together with low viscosity and negligible surface tension, make supercritical fluids ideal solvents for a number of applications. In particular, supercritical fluids are capable of either desorbing physically bound substances from surfaces and micropores due to their excellent diffusion, mass transfer and mobility characteristics, or dissolving chemically bound compounds from matrices in view of their adjustable solvent strengths [7].

2.3 Instrumentation

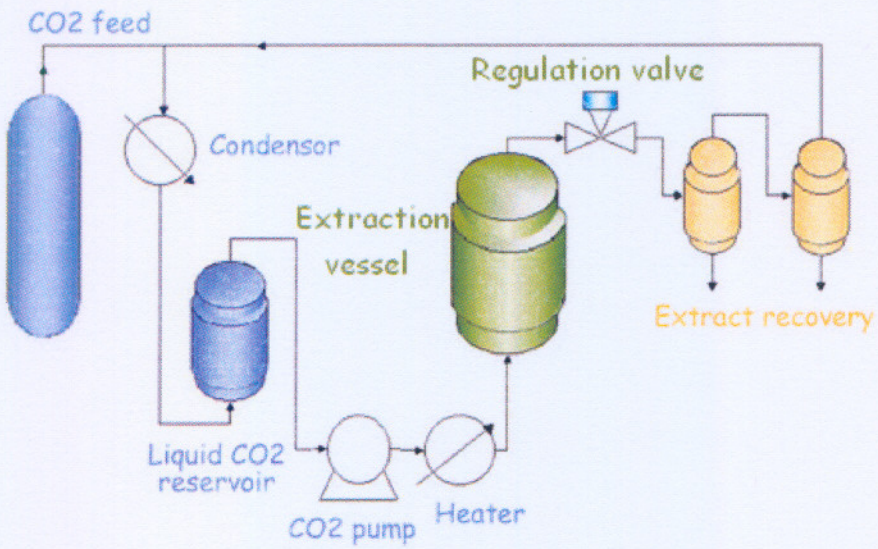

Figure 2.3 Schematic diagram of a SFE system

The instrumentation used in SFE is relatively simple. **Figure 2.3** shows a schematic diagram of a basic SFE system. Extractions can be performed in static or dynamic mode or a combination of the two. During static extraction, the cell is filled with the supercritical fluid, pressurised and allowed to equilibrate. In the dynamic mode, the fluid is run continuously

through the cell. Liquid CO_2 is pumped from a reservoir and then heated and pressurised to reach supercritical conditions. sc-CO_2 enters the extraction vessel where contact with the matrix occurs. After the required contact time the sc-CO_2 is relaxed to atmospheric conditions via a regulation valve or restrictor and the extract is precipitated in a collection vial. Gas is recycled by condensation before returning to the liquid reservoir [8].

2.4 sc-CO_2 as Solvent

A variety of organic liquids and a selection of inorganic substances, noble gases and water may be used as supercritical fluids. The choice of supercritical fluid is determined by the polarity of the substance to be extracted and the technical feasibility of the conditions required to exist as a supercritical fluid. Corrosive, environmentally hazardous, flammable and explosive substances are typically unsuitable as supercritical fluids. The relatively mild critical conditions, abundance, low cost, non-toxicity and unreactive nature of CO_2 make it a suitable supercritical fluid for a variety of processes [3].

CO_2 can be used for the removal or extraction of non-polar and weakly polar compounds like alkenes, terpenes, aldehydes, esters, alcohols and fats. Variation of temperature and/or pressure, or density, allows the solvent strength of *sc*-CO_2 to be adjusted to dissolve specific substances better than common organic solvents. The addition of small amounts of cosolvent allows *sc*-CO_2 to dissolve more polar compounds. Highly polar compounds are insoluble in CO_2, though water is soluble up to 0.3 mass % in CO_2 at 250 atm and 50°C [9]. Two classes of polymers are notable exceptions, viz. amorphous flouropolymers and silicones. These materials, which have been found to be CO_2-phillic, serve as essential building blocks for surfactants designed for application in near-critical and *sc*-CO_2 [10].

2.5 Role of Modifiers in sc-CO_2

The solubility of both polar and nonpolar solids in a supercritical fluid may be enhanced through the use of a modifier. Modifiers are added to the fluid in low concentrations (5% or less on a v/v basis) and are either polar (acetone, methanol) or nonpolar (propane, octane). CO_2 has a small polarisability and no dipole moment, making it possible for additives to increase the polarisability of the substance. Modifiers have been shown to increase the solubility of a solute in the sc-CO_2 by an order of magnitude. While it is known that the

polarisability of CO_2 is affected by the modifier, its interaction with the solute is still a matter of investigation. Methanol and acetone have been the modifiers most often studied. Methanol can act as either a Lewis acid or a Lewis base. During SFE the methanol may interact with functional groups on the solute or it may only be involved in solvent sphere formation. Solvent sphere formation seems to be more a function of methanol concentration rather than its ability to gain or lose electron density. Acid-base interactions between *sc*-CO_2 and an aqueous system cause pH to have a definite influence on any process occurring in such a medium. The fact that water is present in a large variety of extraction matrices stresses the importance of acid-base interactions within supercritical fluids [3].

2.6 Applications

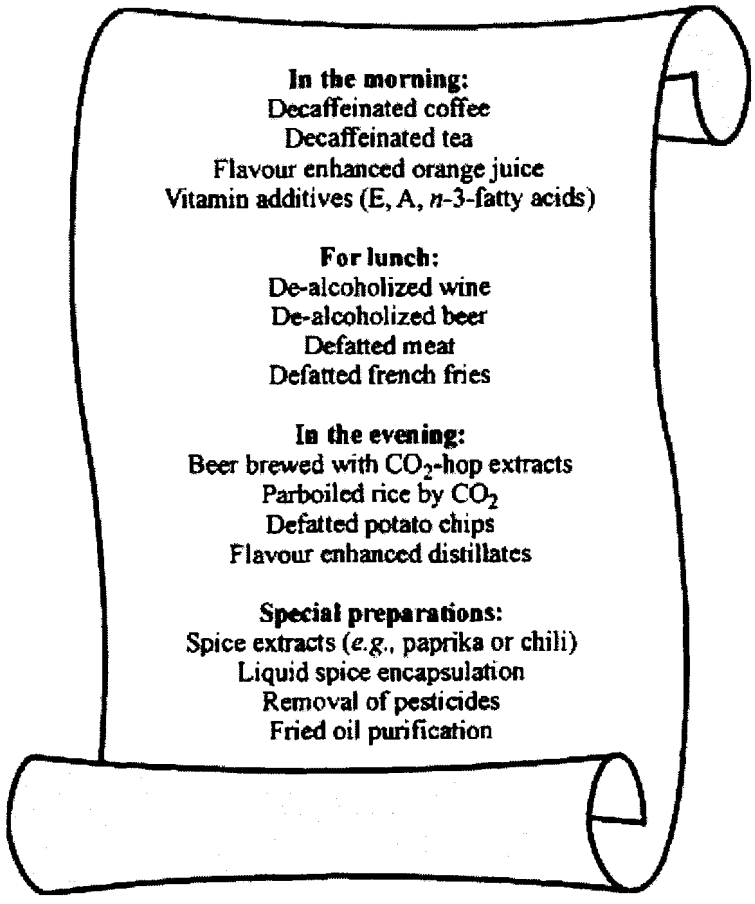

Figure 2.4 Supercritical fluid technology applied to everyday's table [11].

2.6.1 Extractions

Supercritical fluid extraction has been traditionally applied in the food and pharmaceutical areas. Recent advancements in supercritical fluid technology are nutraceutical extracts (natural extracts from plants or natural products that exhibit physiological or health benefits) [12]. One of the most popular and successful applications of SFE is the extraction of fats and oils. Besides the ecological benefits, lipids have very high diffusion coefficients in supercritical fluids, much larger than in conventional liquid solvents. Thus, the extraction rates are enhanced and less degradation of solutes occurs [13].

2.6.2 Reactions

The main motivation for carrying out chemical and biochemical reactions in supercritical media are that

- reactions can be carried out in a homogeneous phase by manipulating the pressure and temperature to control phase behaviour;
- reaction rates can be increased by 1 to 3 orders of magnitude because of increased diffusivities in supercritical fluids;
- preferred reaction mechanisms can be optimised by manipulating the pressure and temperature;
- easy separation products from solvents and reactants can be achieved.

Examples of reactions in supercritical media are polymerization reactions, oxidation in supercritical water of highly toxic wastes, enzymatic reactions, synthesis of aroma and stereoselective and enantioselective separation or synthesis [12].

2.6.3 Supercritical Fluid Chromatography (SFC)

As the demand for purity in pharmaceutical products and for rapid development of products in the clinical trial stage increases, so does the need for chromatographic separation. Conventional HPLC generates a large amount of solvents, which results in increased regulatory and disposal costs. Since most of the solvent in SFC is carbon dioxide, the hydrocarbon disposal and its associated high cost is relatively lower. Applications where analytical SFC is utilised include polymers, aromatic content of petroleum samples, oleochemicals and more recently pharmaceutical products, mainly for analysing chiral purity SFC is similar to normal-phase HPLC, but much faster due to high mass transfer coefficients. Besides the reduction in solvent usage, the advantages of SFC are higher selectivity, purity and throughput, and elimination of costly eluent-product separation procedures [12].

2.6.4 Particle Formation

One of the major areas in research and development of supercritical fluids is particle design. RESS (Rapid Expansion of Supercritical Solution) generates amorphous fine powders from organic and inorganic materials that are insoluble in conventional solvents. The solid is initially dissolved in a solvent at elevated temperature and pressure. It is then heated under supercritical conditions and allowed to expand rapidly through a short nozzle into a region of low pressure and temperature which results in the dissolved material precipitating rapidly as a fine powder or thin film with narrow size distribution [14]. SAS (Supercritical Anti-Solvent) uses supercritical fluids as antisolvents to precipitate materials from conventional solvents since substances are insoluble in supercritical fluids. The key advantage of SAS is that the size and morphology of the particles can be manipulated by adjusting the parameters (flow rates, pressure, temperature, etc.) [15].

2.6.5 Impregnation

Impregnation (opposite of extraction) utilises the high diffusivity property of sc-CO_2 to transport a suitable agent into a porous matrix. The supercritical fluid is a vector for the product to be impregnated [8].

2.6.6 Degreasing/Decontamination

Several supercritical fluid extraction processes have been proposed for removing toxic and intractable organic compounds from a range of contaminated solids. These include soil remediation and the regeneration of adsorbents used to treat waste water streams such as granular activated carbon [16].

2.7 Advantages and Disadvantages of SFE

Although a SFE system may have some disadvantages, including high cost equipment and high energy consumption levels, the development of extractive separation techniques with supercritical fluids is motivated by

- low temperatures and mild conditions;
- residue-free extracts due to simple and complete separation of the solvent;
- substitution of problematic traditional solvents as a result of regulations regarding solvent residues in food and more restrictive environmental standards [2].

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3
Experimental

This study was aimed at extraction of olive oil with sc-CO_2 from dried olive fruit and analysis of the obtained oil, as well as at a comparison of the quality of the obtained oil with that of commercial olive oil. The equipment, materials, methods and procedures utilised to achieve this, are highlighted in this chapter.

3.1 Instrumentation

Figure 3.1 Leco TFE 2000 supercritical fluid extractor

The Leco TFE 2000 supercritical extractor **(Figure 3.1)** used in this study offers considerable advantages over previous extractors. It allows three extraction runs to be performed simultaneously. Each channel has an automated heated variable restrictor (HVR) which controls the flow of CO_2. Flow rates from 0-5 L/min (previously mL/min) enable shorter extraction times. The unit accommodates three 10 mL extraction thimbles and operates up to 680 atm and 150 °C. The wrench-free extraction thimbles has high-pressure seals on the

end-caps and a valve-less flow path to the restrictors which reduce the possibility of losing analyte through end-cap seals or valve failure. An optional M 2000 modifier addition system is connected to the TFE 2000 system to provide a constant flow of cosolvent to the CO_2 stream for improved extraction efficiency [1-3]. **Figure 3.2** shows a schematic flow diagram of the operation of the extractor [4].

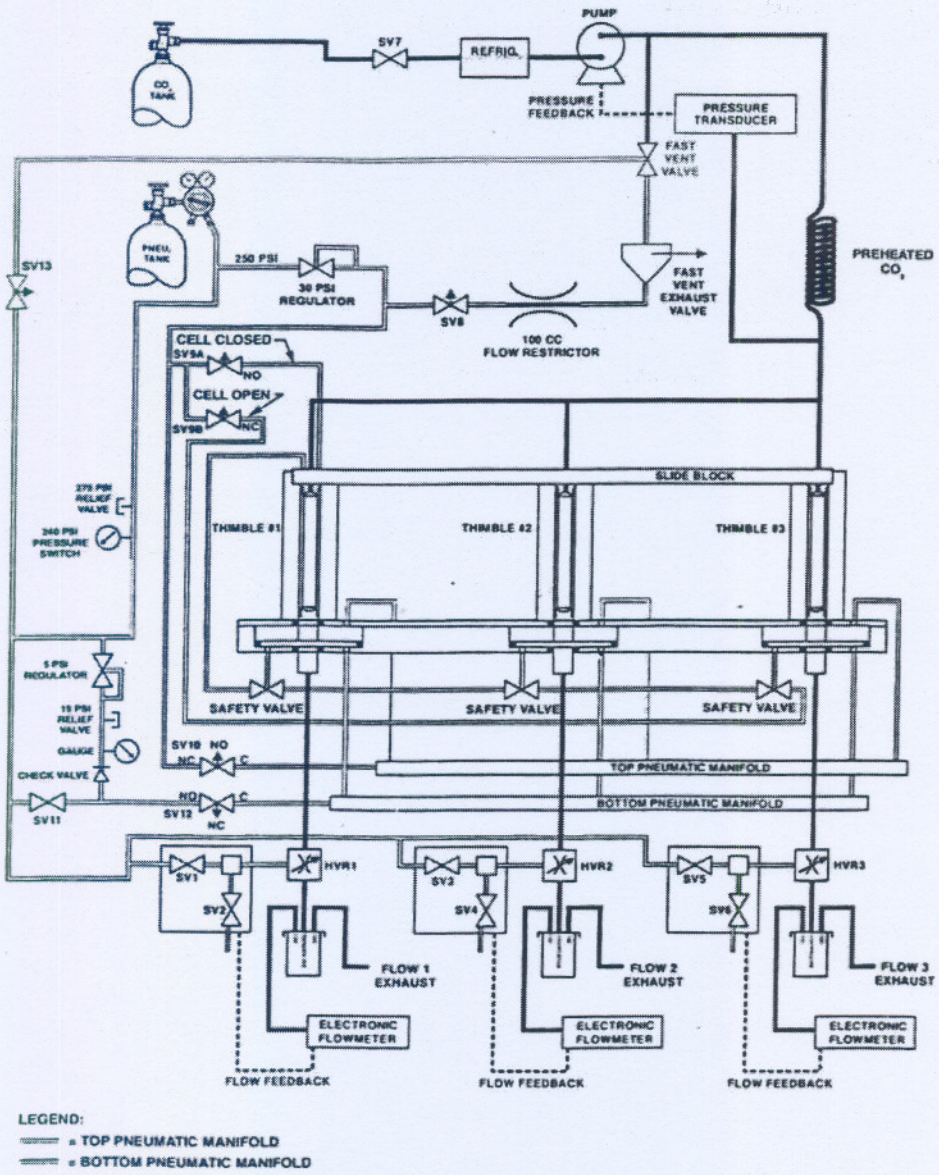


Figure 3.2 Leco TFE 2000 flow diagram

3.2 Materials

Olives of two different cultivars were used. Leccino olives (2004 harvesting year) were obtained from the Laingsburg district, Western Cape. The Frantoio cultivar (2005 harvesting year) was supplied by Olives South Africa, situated at Hartswater in the Northern Cape.

CO_2 from Afrox was used for extraction. GC-GC/TOF-MS analysis of the extracted oil was performed by a product specialist employed by Leco Africa (Pty) Ltd. on an instrument situated at the CSIR, and chemical analysis of the oil was done by J. Muller Laboratories (Pty) Ltd. The chemicals used in this accredited laboratory are listed in the description of standard methods for the analysis of olive oil in the Appendix.

3.3 Sample Preparation and Extraction Procedures

Although drying of olive fruit affects the quality of extracted oil, sc-CO_2 extraction of fresh olives yielded a sticky mixture of oil and plant juice which blocked the flow lines of the extractor.

Olives were ground together with the stone in a food grinder and oven-dried at 60 ºC for 2 hours. Samples of between 4 and 5 g were used for extraction. After installing the lower end-cap on a thimble and inserting a small square tissue into the thimble, an accurately weighed mass of plant material was inserted into the thimble and the upper end-cap was put into place **(Figure 3.3)**. The sample containing thimbles were loaded into the sample chambers of the extractor and the collection vials, each weighed beforehand, were put into place as shown in **Figure 3.4**.

Figure 3.3 Loading of thimbles

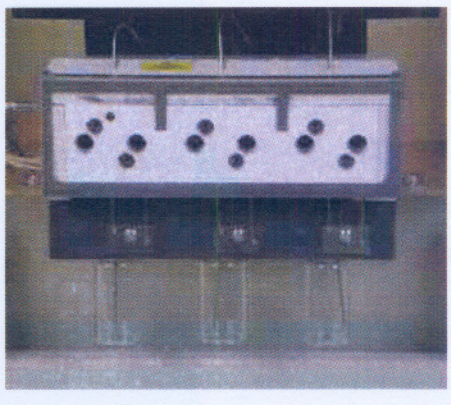


Figure 3.4 Collection vials

The extraction parameters were set and the extraction performed for a selected period of time. After an extraction run, the collection vials were weighed to determine the mass of extract by subtracting the original mass from that of the collection vial and its contents. The remainder of the plant material in the thimble was also weighed to compare the loss in plant material to the yield of extracted oil.

Extractions with the Leco TFE 2000 can be performed in static, dynamic or combined static/dynamic modes.

During static extraction the material in the thimble is exposed to a given aliquot of sc-CO_2 for the entire duration of the extraction run while being maintained at the selected pressure and temperature, i.e. no through-flow of fluid occurs during extraction and interaction is limited to that between the sample matrix and the captured amount of fluid.

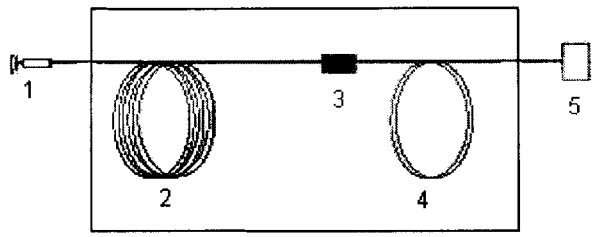
In dynamic mode, sc-CO_2 at the selected conditions is continuously pumped through the thimble and its contents, i.e. fresh aliquots of the fluid passes through the sample matrix for the entire duration of the extraction run.

It is often argued that a combination of static and dynamic extraction should be employed to allow for desorption/dissolution of the desired substance by sc-CO_2 during the static step and for removal of the extracted substance from the thimble during the subsequent dynamic step. It was important to know the effect that different modes of extraction would have on the yield of extract. In a few previous studies it was impossible to accumulate all of the extracted material, as part of it was retained in the flow lines of the extractor. The amount of oil obtained by employing different modes of extraction was therefore investigated in this study.

3.4 Analysis

3.4.1 Instrumental Analysis

The sc-CO_2 extracted olive oil was analysed using a Leco Pegasus 4D two-dimensional gas chromatograph in order to determine its composition. A sample of commercial olive oil was also analysed in order to compare the composition of the two differently obtained oils.



1 First-dimension inlet;
2 First-dimension column;
3 Modulator;
4 Second-dimension column;
5 Detector

Figure 3.3 Basic GC-GC system

The two-dimensional (GC-GC) system employs two separation mechanisms to vastly improve the separation of a complicated array of overlapping chromatographic peaks. Typically, the first column in the system is non-polar and effects a boiling point based separation. The second column is much shorter and facilitates a polarity based separation [5]. The basic configuration of such a system is illustrated in **Figure 3.3.**

The increased chromatographic resolution linked to the automated peak find and spectral deconvolution algorithms of the instrument made it possible to identify several components in the olive oil samples.

The instrument conditions for the analysis of the olive oil samples are listed in **Table 3.1**. An SPB-1 and a DB wax column were used as the boiling point and polarity based separation columns, respectively.

Table 3.1: GC-GC/TOF-MS conditions for sample analysis

Instrument:	Leco Pegasus 4D Time-of-Flight Mass Spectrometer
Acquisition Rate:	100 spectra/s
Stored Mass Range:	35 to 450 u
Transfer Line Temperature:	240 °C
Source Temperature:	200 °C
Detector Voltage:	-1700 V
GC:	Modified Hewlett Packard 6890N*
Column 1:	SPB-1, 30 m x 0.25 mm ID, 0.25 µm film thickness
Column 2:	DB Wax, 2 m x 0.1 mm ID, 0.1 µm film thickness
Column 1 Oven:	50 °C for 1 min, to 250 °C at 10 °C/min., hold for 11 min.
Column 2 Oven:	55 °C for 1 min, to 255 °C at 10 °C/min., hold for 11 min.
Second Dimension Separation Time:	6 s
Inlet:	Split at 240 °C; split ratio 5:1
Injection:	1 µL
Carrier Gas:	Helium, 1.0 mL/min constant flow

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

3.4.2 Chemical Analysis

Chemical analysis for quality assessment of the oil was performed by J. Muller Laboratories (Pty) Ltd. (Reg. No. 1980/004037/07), Paarden Eiland. This analysis included determination of free fatty acid content, peroxide value, iodine value and fatty acid methyl ester profile according to the Codex Alimentarius. The analytical procedures are listed in the Appendix.

3.5 Statistical Design

An experimental design based on statistical methods was used to determine the influence of certain variables on the sc-CO_2 extraction of olive oil. The principal feature of such an approach is to obtain a maximum amount of information from a minimum number of experimental runs. Statistica for Windows® was used to create an experimental design to determine the effect of temperature and pressure combinations on the yield of extract by means of surface response analysis. Extraction conditions were chosen between 35 °C and 65 °C and between 100 atm and 460 atm based on literature information that 50 °C and 280 atm are ideal conditions for good solubility of olive oil in sc-CO_2 without compromising the quality of the oil [6].

Table 3.2 Experimental design

2**(2) central composite, nc=4 ns=4 n0=4 Runs=12		
	Temperature	**Pressure**
1	40	100
2	40	400
3	60	100
4	60	400
5	35	250
6	65	250
7	50	100
8	50	460
9	50	250
10	50	250
11	50	250
12	50	250

In **Table 3.2** the runs suggested by a central composite design are listed. Runs 1 to 4 represent a 2 x 2 orthogonal design, and runs 5 to 8 are star points which were added to the design to calculate the quadratic components of the relationship between the variables without sacrificing the requirement of orthogonality and rotatability [7]. Runs 9 to 12 are the central point runs (repeated four times) which test for a linear or polynomial model. Additional runs were performed in order to draw reliable conclusions regarding the mechanism underlying the sc-CO_2 extraction of olive oil. These runs were not utilised for the surface response analysis since they did not comply with the statistical design requirements.

3.6 Process Analysis

The nature of the extraction process could be explored by considering the relationship between the yield of extract and different variables (pressure, temperature and density). A strong dependence on the density of the fluid, for instance, could be indicative of chemical dissolution as the solvent strength of the fluid is related to its density. An independence of density, to the contrary, could point to a physical rather than a chemical extraction mechanism. The individual contribution of temperature and pressure to the extraction yield could provide further information on the extraction process since these two variables opposingly affect the density of the fluid.

The density of sc-CO_2 at different combinations of temperature and pressure (such as those in **Table 3.2**) was determined by implementing SF-Solver™, a utility software program distributed by Isco Inc. which creates a PDT (pressure, density and temperature) curve for supercritical fluids.

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

The Arrhenius equation [8]

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

with E_a the activation energy, R = 8.314 J K^{-1} mol^{-1} the universal gas constant, T the temperature in kelvin and A the frequency factor was used in the logarithmic form

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

to determine E_a from the slope of the straight line obtained by plotting ln k against 1/T. The rate constant k could be substituted by the yield of extract without changing the magnitude of the slope and thus the value of E_a.

The empirical equation [9]

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

could likewise be employed to determine the volume of activation $\Delta^{\neq}V$ 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 could, once again, be substituted by the yield of extract without changing the magnitude of the slope of the resulting straight line.

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4

Optimisation and Process Mechanism

The yield of olive oil obtained by cool pressing of the fruit is typically 15-22 % [1-2]. It was interesting to compare this figure to the yield obtainable by sc-CO_2 extraction of dried olives and to compare the quality of the olive oil obtained by the two methods. A comparison of the yields required a maximum of oil to be extracted by sc-CO_2 and, therefore, optimum extraction conditions needed to be established. This was done by surface response analysis based on a statistical design as outlined in the previous chapter. The relationship between yield and a few significant variables was determined in order to draw a response surface from which a combination of conditions for maximum yield could be derived. The optimisation of process conditions also allowed trends to be observed in the effect of different variables on the process. The extraction mechanism could be explored by considering the effect of temperature, pressure and density on the extraction yield. Finally, the quality of the sc-CO_2 extracted oil with regard to appearance, composition and required purification for domestic use was investigated and compared to commercial olive oil. The results obtained for all these aspects of sc-CO_2 extraction of olive oil are presented in this chapter.

4.1 Mode of Extraction

Extraction was performed by a combination of static and dynamic runs of different duration as outlined in **Table 4.1.** These include a long static run followed by a short dynamic run, a shorter static run followed by a longer dynamic run, and an entire dynamic run. The total duration for all runs was 60 minutes. **Figure 4.1** shows the influence of the mode of extraction on the yield as well as on the difference between the loss in sample mass and the gain in oil mass (expressed as m/m percentage).

Table 4.1 Results for different combinations of static and dynamic runs

	Time (min)		% (m/m)	
	Static	**Dynamic**	**Oil Gain**	**Plant Material Loss**
1	55	5	5.66	12.27
2	40	20	10.09	14.66
3	0	60	14.91	22.67

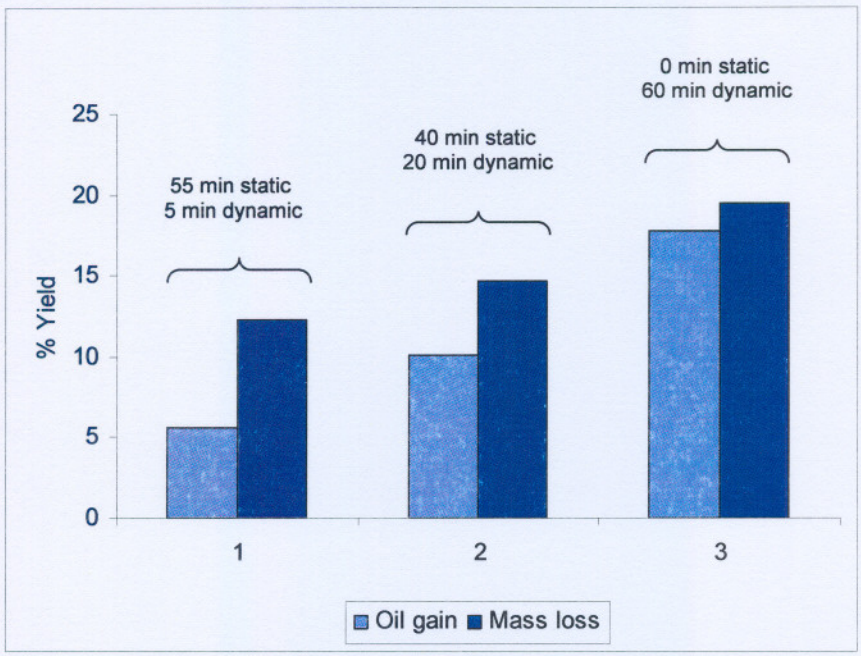

Figure 4.1 Influence of extraction mode

It can be concluded from the figure that it is beneficial to perform dynamic rather than static extraction runs for two reasons, viz. (1) a higher yield of extract is obtained in dynamic mode since there is a continuous flow of "fresh" solvent through the thimble and (2) a better correlation is obtained between the loss in mass of plant material and the yield of oil since the oil is more efficiently removed from the flow lines of the extractor by a constant throughput of fluid.

4.2 Extraction Time

The required duration of an extraction run with sc-CO_2 for maximum yield was determined beforehand in order to reduce the number of parameters considered for a statistical design. To determine the time dependence of the extraction, several runs of different duration (0 to 90 minutes) were performed and the resulting yield was plotted against extraction time as shown in **Figure 4.2.** For these runs, a pressure of 280 atm was chosen as a value approximately in the center of the operational pressure range of the extractor, whereas a relatively low temperature of 50 °C was chosen in view of the sensitivity of plant material to high temperatures. The selected values also correlated well with those used by other authors [3].

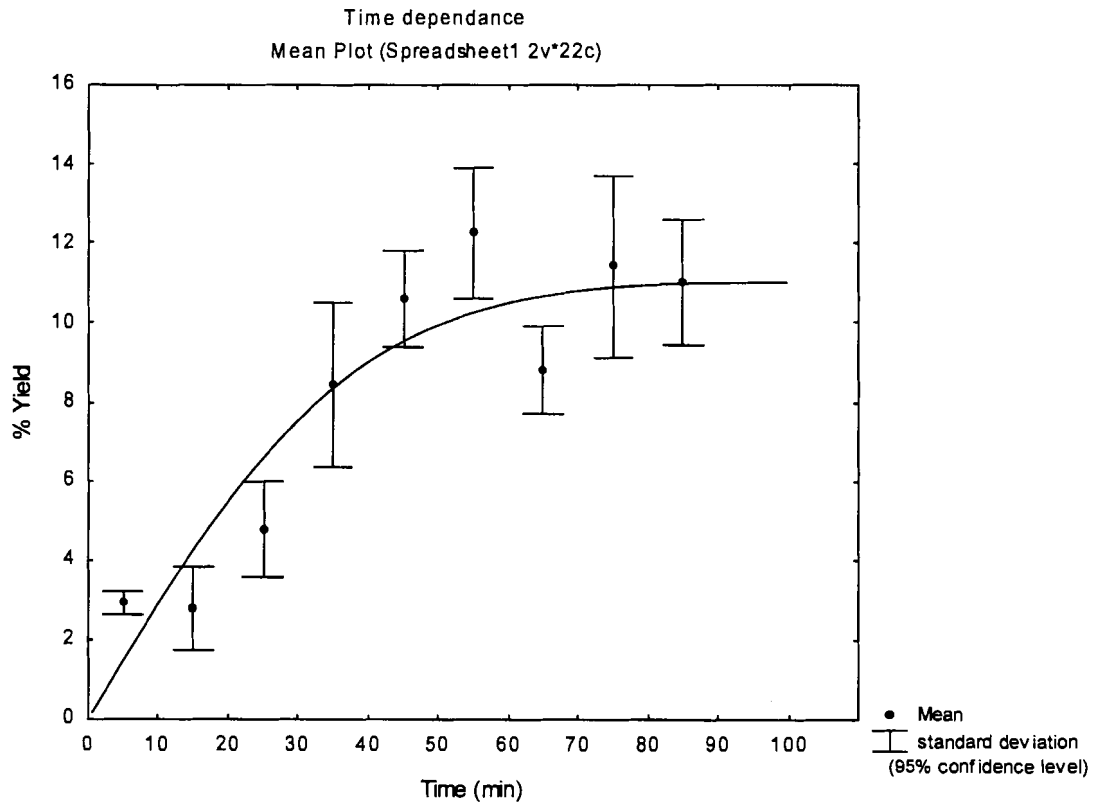

Figure 4.2 Time dependence of olive oil extraction

The scatter in the data presented in **Figure 4.2** indicates that repeatability could not always be achieved. This can be explained by the retention of oil in the flow-lines or by minor

fluctuations in extraction conditions (temperature, pressure, flow rates might not always be maintained perfectly). A smooth curve through the average of the data for the three different flow lines, with an indication of the standard deviation, shows that at the conditions under consideration a maximum yield is obtained at an extraction time of about 60 minutes as the plateau reached after this time period related to the maximum amount of oil that could be extracted.

4.3 Process Optimisation

The results for process optimisation, which focused mainly on the effect of temperature and pressure, are listed in **Table 4.2** for Leccino olives and in **Table 4.3** for Frantoio olives. For both types the results are presented in terms of the amount of oil obtained as a percentage of the total plant material used, as well as in terms of the loss in mass of plant material as a percentage of the original plant material. The reason for this is to establish by virtue of a mass balance how successful the extracted oil could be recovered from the sample vessel, flow lines and restrictor of the supercritical fluid extractor. Several previous investigations showed a fairly large discrepancy between the loss in mass of plant material and the yield of extract as a result of extract either staying behind in the extractor (e.g. waxy compounds) or escaping at the restrictor (e.g. volatile compounds).

For Leccino olives **(Table 4.2)** the runs suggested by statistical design **(Table 3.2)** were performed in triplicate by using the three flow lines of the extractor simultaneously. For Frantoio olives **(Table 4.2)** several runs have been added to those based on the statistical design for the purpose of more closely investigating the process mechanism.

Table 4.2 Optimisation results for Leccino olives

	Temperature (°C)	Pressure (atm)	% Yield (oil)	% Plant material lost
1	40	100	1.333	3.819
2	40	100	2.571	10.642
3	40	100	0.432	6.627
4	40	400	11.834	14.827
5	40	400	6.442	14.046
6	40	400	11.823	15.270
7	60	100	0.512	6.870
8	60	100	0.537	7.904
9	60	100	0.631	5.176
10	60	400	13.107	13.964
11	60	400	10.209	12.452
12	60	400	12.585	13.403
13	35	250	7.669	12.987
14	35	250	10.618	12.650
15	35	250	6.458	11.705
16	65	250	6.100	16.261
17	65	250	11.615	21.446
18	65	250	8.577	17.872
19	50	100	1.662	10.729
20	50	100	1.718	6.358
21	50	100	1.513	5.345
22	50	460	16.570	17.283
23	50	460	10.740	18.837
24	50	460	23.774	25.166
25	50	250	5.539	5.671
26	50	250	2.837	9.148
27	50	250	7.374	13.317
28	50	250	4.406	6.933
29	50	250	4.225	8.761
30	50	250	8.246	13.360
31	50	250	4.580	11.231
32	50	250	3.622	11.298
33	50	250	12.015	19.252
34	50	250	8.161	13.969
35	50	250	5.382	11.253
36	50	250	8.147	14.658

Table 4.3 Optimisation results for Frantoio olives

	Temperature (°C)	Pressure (atm)	% Yield (oil)	% Plant material lost
1	35	150	2.7	8.22
2	35	200	1.56	4.67
3	35	250	3.92	6.25
4	35	450	11.31	8.78
5	40	100	1.12	2.38
6	40	300	6.25	5.93
7	40	400	8.82	13.65
8	40	450	11.77	15.7
9	45	400	17.77	19.54
10	50	100	2.4	2.17
11	50	250	3.69	7.09
12	50	400	17.69	18.64
13	50	460	10.18	11.93
14	55	400	19.63	21.75
15	60	100	1.49	3.12
16	60	400	19.35	20.90
17	65	250	4.269	7.06
18	65	400	17.94	21.01
19	70	200	1.81	3.61
20	70	400	18.48	20.66
21	70	500	13.44	24.53
22	75	400	18.15	21.41
23	80	400	19.59	22.15
24	85	400	21.37	24.56

The results show effective extraction of oil from dried olives for various combinations of temperature and pressure. Yields of up to 21 % of oil, as a percentage of the total mass of dried olives, were obtained. The figures in **Table 4.2** for triplicate runs show that fairly good repeatability could generally be achieved with the three independent flow lines of the supercritical fluid extractor.

According to literature [1] both Leccino and Frantoio olives contain between 17 and 18% oil. Conventional pressing yields between 15-22 % as a percentage of the mass of ground fresh olives. The yield of 21 % obtained from dried olives with sc-CO_2 extraction can be regarded to be equivalent to approximately 14 % if the calculation is based on the corresponding mass of fresh olives, though such a conversion does not take into account the potential loss of substances from the fruit during the drying process.

A three-dimensional surface response graph was obtained by plotting the yield of olive oil as a function of temperature and pressure as shown in **Figure 4.3.** The influence of temperature and pressure on the yield, as well as the tendency with which the yield varies with the density of the fluid, could be derived from the surface response graph as outlined in the next paragraphs.

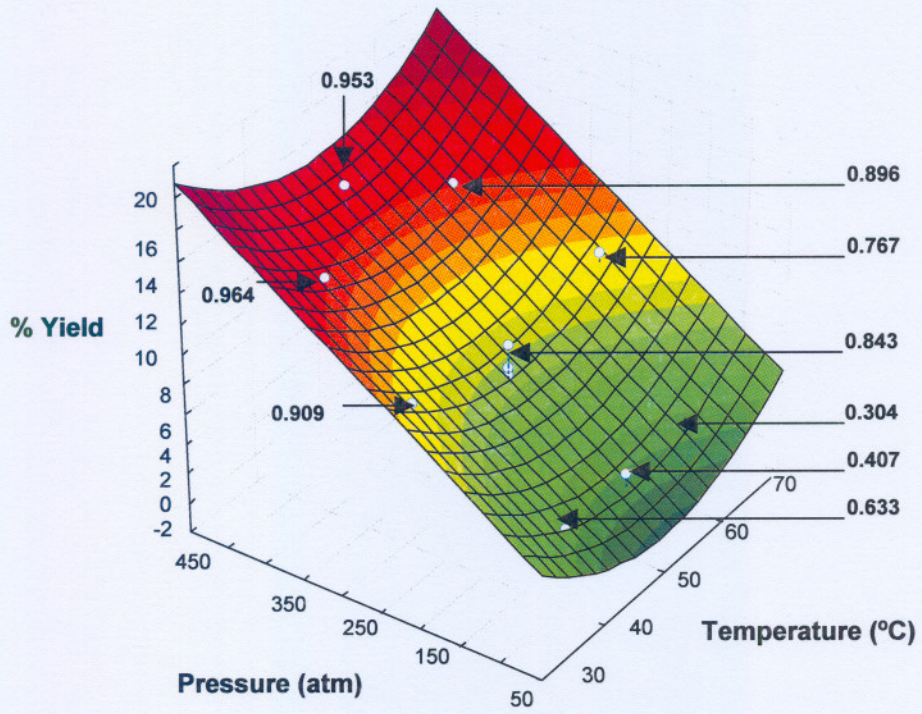

Figure 4.3 Surface response graph

The yield increases markedly with pressure, suggesting that a resulting increase in fluid density accounts for better solvent strength and thus for a larger amount of oil dissolved. Temperature seems to be a less significant variable. In a certain temperature range the yield decreases with increasing temperature, whereas in another range an increase in yield for an increase in temperature is observed. This could be explained in terms of two opposing effects, namely that increasing temperature on the one hand lowers the activation energy of extraction and thus improves the yield, and on the other hand lowers the density of the fluid and thus deteriorates the solvent strength of the fluid and thus the amount of oil dissolved.

Optimum extraction conditions based on yield were obtained from the response graph and turned out to be 50 °C and 460 atm within the range of conditions investigated. The percentage yield of extracted oil increases as density increases from 0.407 to 0.633 to 0.843 to 0.896 g/mL along a diagonal from the lowest to the highest yield. This tendency was also seen in another presentation of the data in a later paragraph.

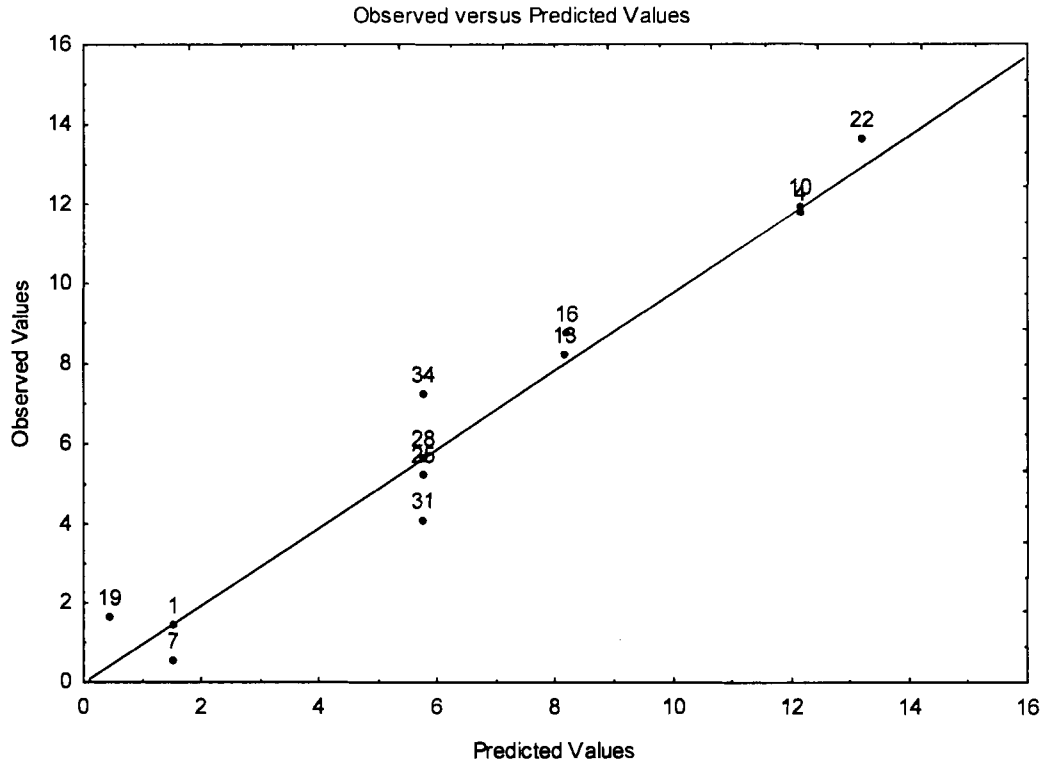

Figure 4.4 Observed vs predicted values

The mathematical model underlying the surface response graph was tested by examining the extent to which the calculated values (predicted) correspond to the experimental (observed) values. The 95 % correlation obtained between the observed and the predicted values **(Figure 4.4)** made the established optimum conditions acceptable.

4.4 Process Characteristics

4.4.1 Influence of Density

The significant role of density, which determines the solvent strength of a supercritical fluid and thus its capability to dissolve chemical substances, was already mentioned in the previous section. **Figure 4.5** illustrates the exponential increase in yield with an increase in density in the region where this variable changes form gas-like to liquid-like values ($0.7 < \rho < 1.0$ g/mL). This indicates that the extraction of olive oil is based on chemical dissolution in sc-CO_2. A single curve could be drawn through the data obtained by simultaneous extraction via the three independent flow systems of the supercritical fluid extractor, illustrating again the reasonably good repeatability which could be obtained. The practically zero yield at gas-like densities ($0.0 < \rho < 0.6$ g/mL) proves that chemical dissolution is the only process by which olive oil is extracted from the fruit by sc-CO_2. The influence of density shown in **Figure 4.5** for the Leccino cultivar is echoed by corresponding data for the Frantoio cultivar.

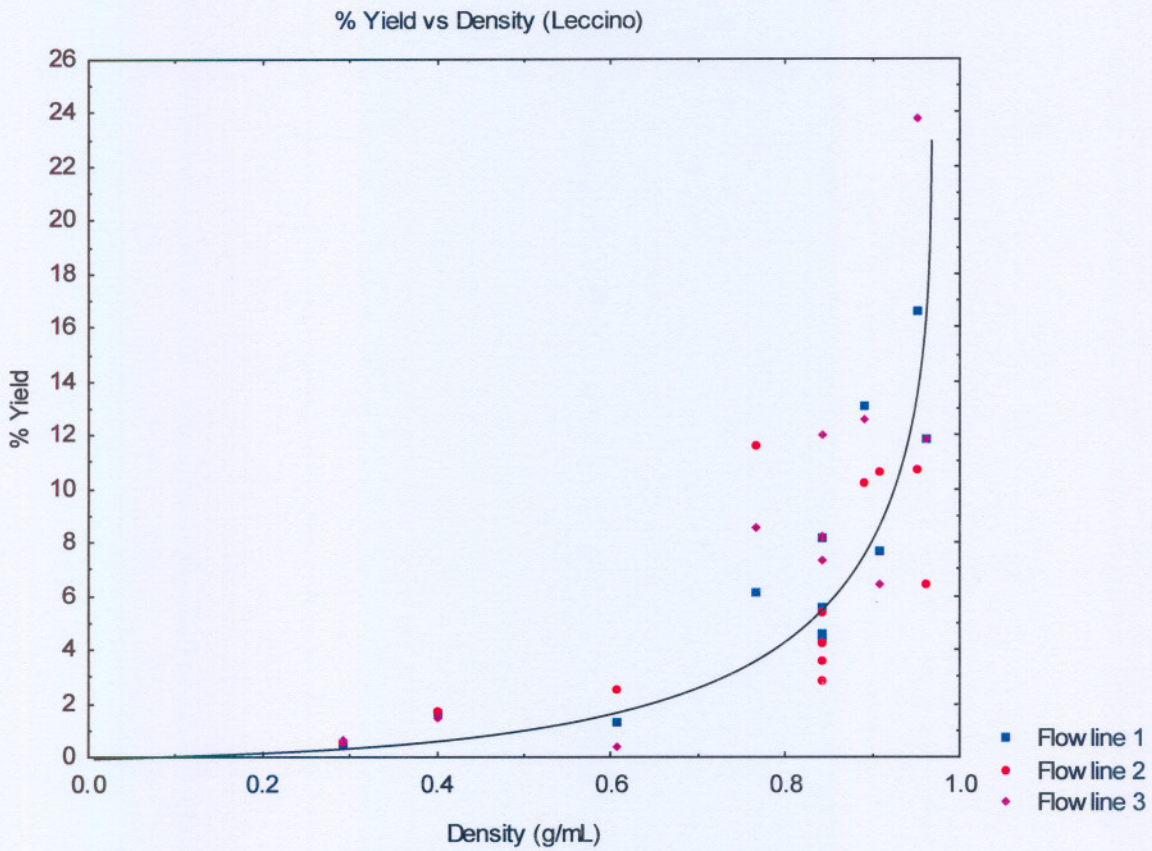

Figure 4.5 Influence of density

4.4.2 Influence of Temperature

The effect of temperature on sc-CO_2 extraction of olive oil is insignificant as the plot of % yield against temperature at a constant pressure (400 atm) in **Figure 4.6** illustrates. This can be attributed to the two opposing effects of temperature on the process as outlined previously (Paragraph 4.3). An increase in temperature results in a decrease in the activation energy barrier, but at the same time it decreases the density and thus the solvent strength of the fluid. A decrease in the activation energy barrier would affect the yield positively, while a decrease in density would have a negative effect on the yield. When equal in magnitude, the two opposing effects cancel each other, hence leading to the observation that temperature has no marked influence. The very slight tendency of increasing yield with increasing temperature in **Figure 4.6** is too small to allow for a calculation of the energy of activation as outlined in Paragraph 3.6.

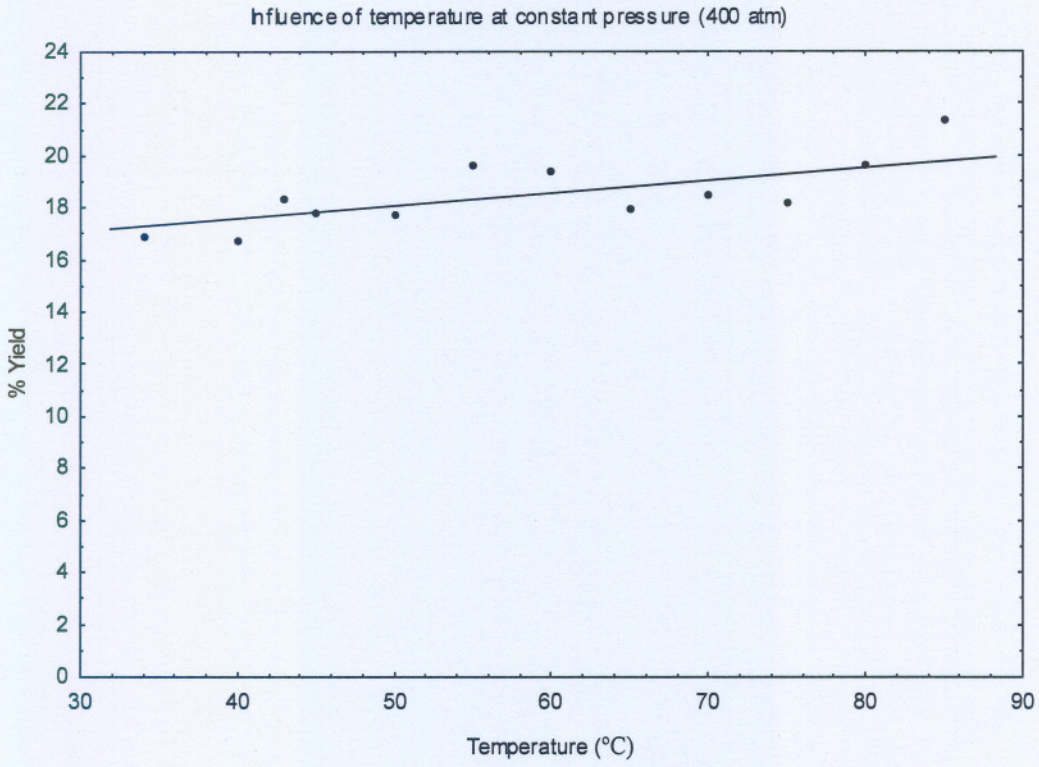

Figure 4.6 Influence of temperature at constant pressure

4.4.3 Influence of Pressure

As discussed earlier (Paragraph 4.3), pressure has a noticeable effect on the yield of extracted oil. By plotting ln {yield} versus pressure (p) within a limited range of temperatures ($35 < T < 50$ °C), a straight line is obtained as shown in **Figure 4.7.** The slope of this line is

$$4.5 \times 10^{-3} = -\frac{\Delta^{\neq}V}{RT}$$

from which the volume of activation [4] can be calculated as $\Delta^{\neq} V = -116$ mL/mol. The large negative value is in agreement with the large decrease in volume in the transition state when the oil dissolves in the highly compressed supercritical fluid.

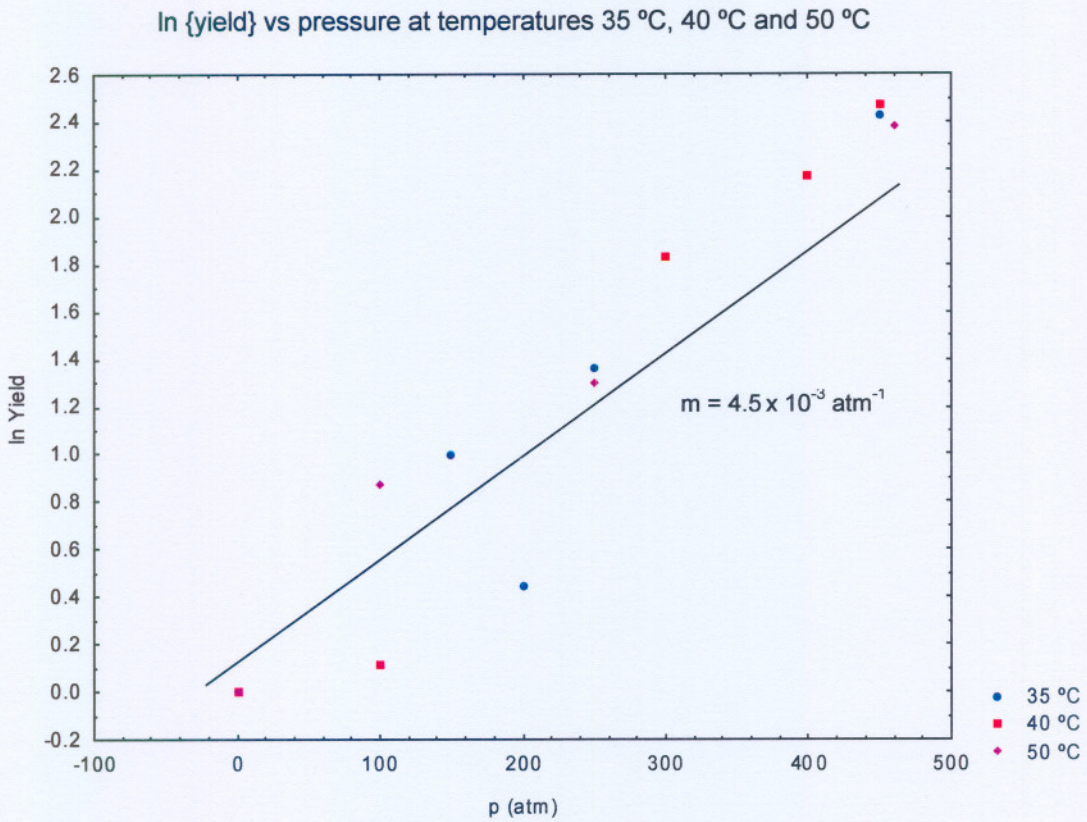

Figure 4.7 ln {yield} against pressure within limited range of temperatures

References

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5
Analysis

The analysis of sc-CO_2 derived olive oil was divided into two parts. Firstly, instrumental analysis of the oil was performed by GC-GC/TOF-MS in order to establish the composition of the oil by identification of individual components present in the oil. Secondly, chemical analysis was done by an accredited laboratory according to standard specifications in order to assess the quality of the sc-CO_2 derived oil in comparison to commercially approved olive oil.

5.1 GC-GC/TOF-MS Analysis

The primary objectives with the GC-GC analysis were (1) to establish the composition of the sc-CO_2 extracted oil by identifying as many of the observed peaks as possible, (2) to compare the composition of this oil to that of commercial olive oil and (3) to use this information together with the results of the chemical analysis to evaluate the extraction methods employed.

The analysis of a sc-CO_2 extracted sample of olive oil (Frantoio cultivar) resulted in 27 located peaks.

The contour and surface plots showing the Total Ion Chromatograms (TIC) for the analysis are shown in **Figure 5.1** and **Figure 5.2.** In **Figure 5.1** the x-axis represents the separation on the SPB-1 column, while the y-axis shows the separation on the DB wax column. In **Figure 5.2** the angle from which the plot is observed (which is fully rotatable), displays the low level peaks as well as the major components.

For peak finding a second dimension column peak width of 0.1 seconds and a signal-to-noise cut-off of 2000:1 were used. Only those peaks meeting these criteria were added to the peak table **(Table 5.1)** [1].

The data was processed with automated peak finding and spectral deconvolution software, followed by a search of the NIST (National Institutes of Standards and Technology) library.

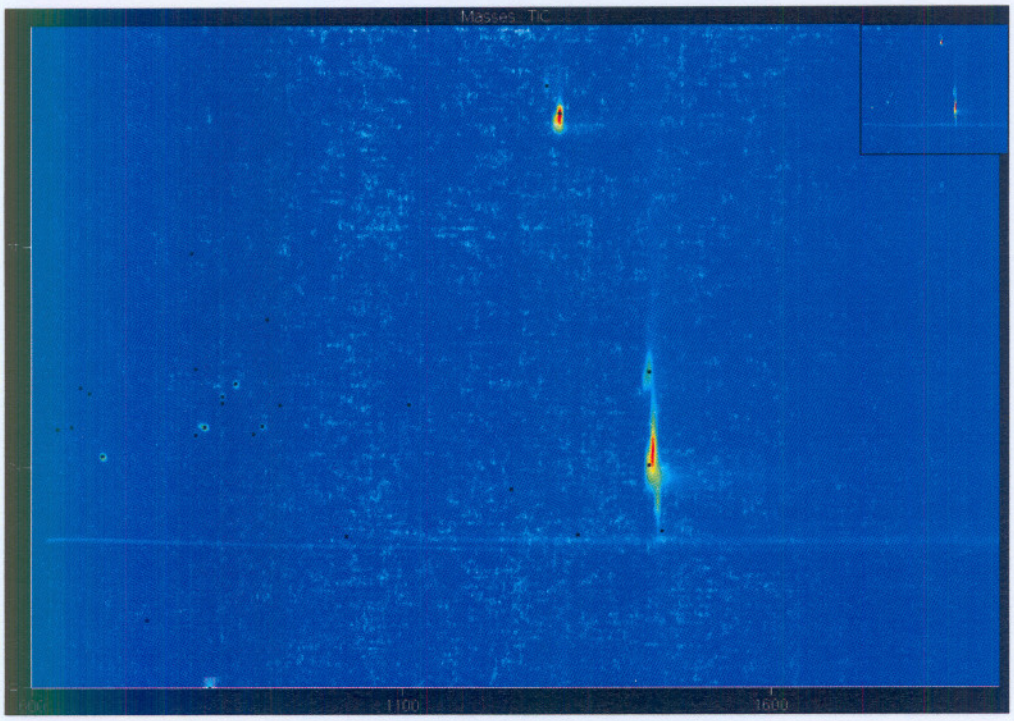

Figure 5.1 Contour plot showing Total Ion Chromatogram (TIC) of sc-CO_2 extracted olive oil

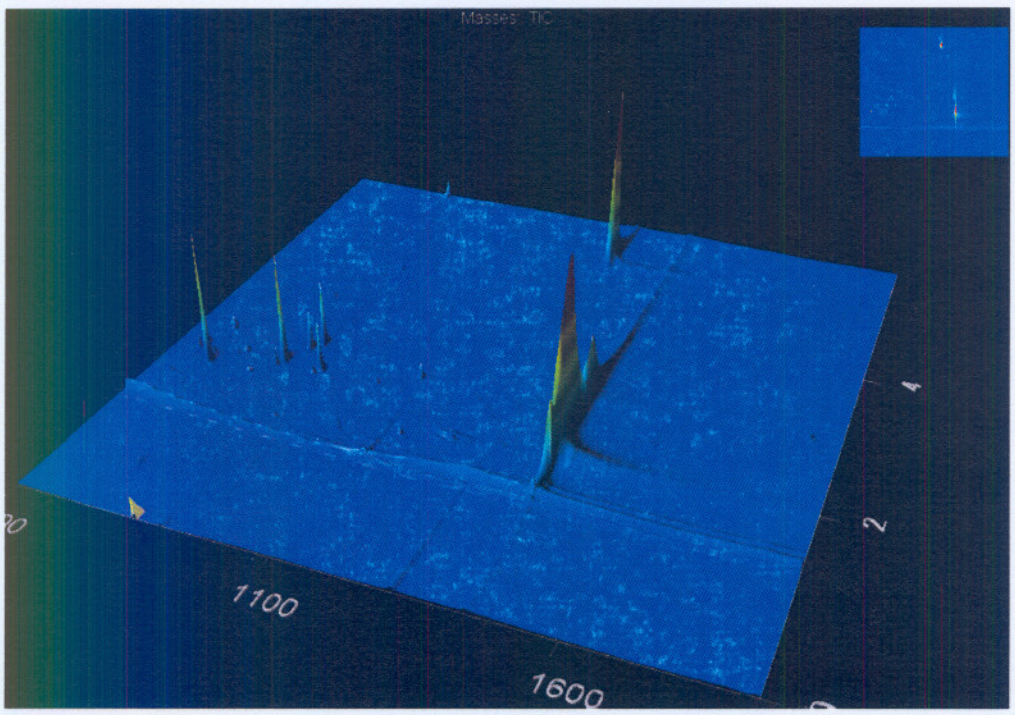

Figure 5.2 Surface plot showing Total Ion Chromatogram (TIC) of sc-CO_2 extracted olive oil

Table 5.1 Components present in sc-CO_2 extracted olive oil.

Peak #	Name	R.T. (s)	Similarity
1	3-Octen-2-one	636 , 2.350	924
2	*2-Octenal, (E)-	654 , 2.370	942
3	1-Octanol	666 , 2.730	934
4	Furan, 2-butyltetrahydro-	678 , 2.670	857
5	*Nonanal	696 , 2.110	965
6	Octanoic Acid	756 , 0.620	945
7	2(3H)-Furanone, 5-butyldihydro-	816 , 3.940	942
8	*2-Decenal, (E)-	822 , 2.300	852
9	Cycloheptane, bromo-	822 , 2.900	758
10	*2-Decenal, (Z)-	834 , 2.370	932
11	Nonanoic acid	840 , 0.000	942
12	trans-2-Methyl-4-hexen-3-ol	858 , 2.580	808
13	*2,4-Decadienal, (E,E)-	858 , 2.650	940
14	2,4-Decadienal	876 , 2.760	921
15	*2-Undecenal	900 , 2.310	865
16	2-Undecenal	912 , 2.380	928
17	2,3-Nonadiene	918 , 3.340	733
18	2-Hepten-3-ol, 4,5-dimethyl-	936 , 2.570	798
19	Isopropyl Alcohol	1026 , 1.380	797
20	Tributyl phosphate	1110 , 2.570	926
21	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1248 , 1.810	882
22	Hexadecenoic acid, Z-11-	1296 , 5.450	938
23	*n-Hexadecanoic acid	1314 , 5.200	899
24	*1-Cyclopropene-1-pentanol,2-tetramethyl-3-(1-methylethenyl)-	1338 , 1.390	629
25	*Oleic Acid	1434 , 2.020	937
26	9,12-Octadecadienoic acid (Z,Z)-	1434 , 2.860	913
27	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	1452 , 1.420	718

* Components also present in commercial olive oil

Table 5.1 lists the components present in the sc-CO_2 extracted olive oil. The two major components present in the sample are hexadecanoic acid and oleic acid. Both these substances are accompanied by smaller amounts of unsaturated analogues, hexadecenoic acid and octadecadienoic acid, to the left and above the main components. The components marked with * corresponds with those present in commercial olive oil, a sample of which was also analysed by GC-GC/TOF-MS. The commercial olive oil sample seemed to have a more elaborate composition since 37 peaks could be detected. Moreover, only 9 of the components correspond with those present in the sc-CO_2 derived olive oil. The difference in composition of the two olive oil samples may be attributed to several factors. The higher temperatures of sc-CO_2 extraction (35 °C minimum) as opposed to those of cool pressing (below 20 °C) could cause components to decompose in, for instance, fatty acids which are quite abundant in the sample concerned. sc-CO_2 possibly also extracted components such as waxes and moisture due to the high pressures involved. Certain components could be eliminated or destroyed during the drying process.

5.2 Quality assessment

5.2.1 Organoleptic Properties

The sc-CO_2 derived olive oil has a yellow/green colour which is in accordance with the colour of other olive oils. It was noted that a few oil samples were cloudy and generally had a viscosity higher than typical olive oil. This may be attributed to the presence of moisture and gums, which may also lead to rapid acid build-up [2]. The appearance of such oil could be improved by sedimentation and filtration.

The sc-CO_2 extracted oil has a characteristic olive oil odour but tastes slightly bitter. The bitterness in olive oil is caused by polyphenols (especially oleuropein) [3] but it is generally regarded as a preferred characteristic since it is an indication of the antioxidant content of the oil [4].

5.2.2 Chemical Analysis

Analysis of the oil was performed by J. Muller Laboratories, which performed all standard quality control tests according to specifications of the Codex Alimentarius listed in the Appendix.

Table 5.3 Analysis of sc-CO_2 derived olive oil measured against standard specifications for virgin olive oil and lampante olive oil

Analysis	sc-CO_2	Standard spesification	
		Virgin Olive Oil	Lampante Olive Oil
Free fatty acid % m/m (as oleic)	10.0	≤ 2.0	> 3.3
Peroxide Value ($mcqO_2/kg$)	96.4	≤ 20	> 20
Iodine Value	86.6	75 - 94	-
Fatty Acid Methyl Esters			
Mysteric Acid %	< 0.1	≤ 0.5	
Palmitic Acid %	13.38	7.5 - 20	
Palmitic Acid %	1.33	0.3 - 3.5	
Margaric Acid %	< 0.1		
Stearic Acid %	1.17	0.5 - 3.5	
Oleic Acid %	64.02	56 - 83	
Linoleic Acid %	17.16	3.5 - 20	
Linolenic Acid %	1.64	< 1.5	
Arachidic Acid %	0.35	Trace	
Eicosenoic Acid %	0.36	Trace	
Behenic Acid %	< 0.1	Trace	
Erucic Acid %	< 0.1	-	
Lignoceric Acid %	< 0.1	Trace	

The results of the analysis in **Table 5.3** show that the sc-CO_2 derived oil complies with standard specifications for virgin olive oil (Codex Alimentarius) with respect to iodine value and fatty acid methyl ester composition, but that it does not meet the requirements applicable to free fatty acid and peroxide content. According to the International Olive Oil Council (IOOC), the oil can be classified as Lampante olive oil, which means that it is not suitable for consumption as is. Lampante olive oil can either be refined or used for industrial purposes [2].

Refining can be done by treating the oil with sodium hydroxide to reduce the free fatty acid content to less than 0.3 %. It is important to note that the glyceride structure of the oil must be retained. If refining is satisfactory, the oil may be classified as "refined olive oil". It may also be blended with virgin olive oil and classified as "olive oil" [2].

The industrial uses for lampante olive oil include manufacture of soap, textile oils and sulphonated oils.

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6

Conclusion and Future Perspective

Several aspects of supercritical carbon dioxide (sc-CO_2) extraction of olive oil were investigated. These included a viability study using a laboratory-scale supercritical extractor, optimisation of the extraction conditions, analysis of sc-CO_2 derived olive oil using GC-GC/TOF-MS, elucidation of the extraction mechanism and comparison of the quality of the obtained oil with that of commercial olive oil. Satisfactory results were obtained in a few of the investigated areas, but several shortcomings were identified. The successes and shortcomings are briefly discussed below. A few suggestions for further work are also proposed.

6.1 Successes and Shortcomings

A fairly reasonable yield of olive oil could be obtained from dried olive fruit, although not as high as with conventional methods. The time, pressure, temperature and density dependence of the extraction could be established and a few important process parameters could be calculated. The principal variable was found to be the density of the extracting fluid, which rendered the degree of dissolution of the oil in sc-CO_2 to be the limiting mechanism of extraction. The extraction conditions for maximum yield were determined to be 50 °C and 460 atm (density of 0.953 g/mL) for a run of 60 minutes duration.

The quality assessment of the sc-CO_2 derived oil was done with a standard specification as benchmark. It was not possible to extract olive oil of a quality suitable for human consumption. The reason for this is that oil was extracted from dried olives instead of freshly picked olives and the time lag, from picking to pressing, changed the free fatty acid content of the oil and thus its overall quality. The elevated temperatures used for sc-CO_2 extraction probably also played a role in the breakdown of olive oil components, which led to high free fatty acid and peroxide values. It seems that with sc-CO_2 some undesirable substances were extracted with the oil, resulting in moisture, gums and waxes in the extract.

GC-GC/TOF-MS analysis of the sc-CO_2 extracted oil resulted in the identification of 27 components of which at least 9 are also present in commercial olive oil. The technique seemed to be extremely suitable for the analysis of component rich sc-CO_2 derived botanical extracts.

The sc-CO_2 derived olive oil was chemically analysed by an accredited laboratory. A few aspects of the extracted oil complied with criteria for virgin olive oil, but since high levels of free fatty acids and peroxides were detected, the oil was classified as lampante olive oil according to criteria of the International Olive Oil Council (IOOC). Lampante olive oil can either be refined or used for industrial purposes.

6.2 Future Perspective

In view of increasing interest in sc-CO_2 extraction of edible oils and successes of the technique already achieved with regard to sunflower, palm kernel, black pepper, hazelnut and brazil nut oil [1-5], it can be envisaged that many similar investigations will be undertaken in future and that expansion of knowledge and expertise in this field will take place.

The problem of the inferior quality of the oil may be addressed by developing a method for the extraction of oil from fresh olive fruit, without obstructing the flow lines of the extractor, and a method for subsequent separation of oil and plant juice.

Filtration and sedimentation of turbid oil may lead to reduced levels of moisture and waxes, which in turn may cause less acid build-up. Oxidation may be prevented by passing an inert gas, N_2 or Ar, through the extract directly after extraction.

Future prospects for sc-CO_2 extraction include the implementation of a continuous instead of batch mode currently used. Extruder designs could possibly solve the problem of continuous transport of material into, through and out of a high-pressure reactor and thereby warrant sufficiently large yields to operate on an industrial scale [6].

References

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Appendix

Standard Methods for the Analysis of Olive Oil

(Codex Alimentarius)

IA

Determination of Free Acidity

According to IUPAC 2.201 or (EEC) No 2568/91.

1. Foreword

Acidity is a conventional expression, frequently used in contract for the percentage of free fatty acids present. According to the nature of the fat it can be expressed as:

Nature of fat	*Expressed as*	*Mol mass (g/mol)*
Coconut, palm kernel and similar oils	Lauric acid	200
Palm oil	Palmitic acid	256
All other fats	Oleic acid	282

When the result required is "acidity" not further defined, the acidity is conventionally expressed as oleic acid.

2. Reagents

0.5 N or 0.1 N ethanolic solution of potassium hydroxide (standardized).

This solution is stored in a brown or yellow glass bottle furnished with a rubber stopper and then decanted for use. The solution should be colourless or straw yellow. A stable colourless solution can be prepared in the following manner: reflux 1000 mL of ethanol with 8 g of potassium hydroxide and 5 g of aluminium pellets for one hour, distil immediately. Dissolve the required amount of potassium hydroxide in the distillate. Allow to stand for several days and decant off the clear supernatant liquid from the deposited potassium carbonate.

The solution can also be prepared without distillation in the following manner: add 4 mL of aluminium butylate to 1000 mL ethanol and allow the mixture to stand for several days. Decant the supernatant liquid and dissolve therein the necessary amount of potassium hydroxide. This solution is ready for use.

1 % w/v solution of phenolphthalein in 95 % v/v ethanol;

95 % v/v ethanol containing 5 drops of phenolphthalein solution in 100 mL. Neutralise immediately before use by means of 0.1 N ethanolic KOH solution.

3. Procedure

Weigh 5 to 10 g of fat to within 0.01 g into a 250 mL Erlenmeyer flask. Dissolve in 50-150 mL of a 1:1 v/v mixture of ethanol and diethyl ether neutralised to phenolphthalein.

Titrate, while shaking, with the 0.5 ethanolic KOH solution (or 0.1 N for acidities less than 2 % by mass) until the colour of the indicator changes.

4. Calculation

$$\text{Percentage acidity} = \frac{aMN}{10p}$$

a = number of mL of the ethanolic KOH solution used
N = exact normality of the ethanolic KOH solution used
M = mol mass adopted
P = mass of sample in g

In the presence of mineral acids it may be necessary to apply special procedures.

IB

Determination of Peroxide Value

According to IUPAC 2.501, ISO 3960: 1998 or (EEC) NO 2568/91.

1. Foreword

The peroxide value is a measure of the active oxygen present in the oil or fat. Owing to the confusion regarding the meaning of the term "peroxide value" which is defined in different countries and even in the same country in different ways, the term "peroxide value" hereafter is defined as the mass in micrograms of active oxygen contained in one gram of fat.

2. Apparatus

3 mL glass scoop for weighing the fat;
250 mL glass stoppered bottles cleaned with great care, dried and filled with a pure, dry, inert gas (carbon dioxide or nitrogen).

3. Reagents

Pure chloroform and glacial acetic acid rendered free from dissolve oxygen by bubbling a current of a pure, dry inert gas through them;

saturated aqueous solutions of pure potassium iodide free from iodine and iodates;

0.01 N or 0.002 N aqueous solutions of sodium thiosulphate (standardised).

4. Procedure

Weigh the test sample to within 0.001 g in the glass scoop, selecting an amount of oil according to the table below:

Expected value	*Test sample (g)*
0 to 150	2 to 1.2
150 to 250	1.2 to 0.8
250 to 400	0.8 to 0.5
400 to 700	0.5 to 0.3

Unstopper a bottle and introduce the scoop containing the fat. Add 10 mL of chloroform. Dissolve the fat rapidly by shaking. Add 15 mL of glacial acetic acid, then 1 mL of potassium iodide solution.

Insert the stopper quickly. Shake for 1 minute and leave in the dark for 5 minutes.

Add 75 mL of distilled water. Titrate the liberated iodine with the thiosulphate solution shaking vigorously, using starch paste as an indicator.

Use the 0.002 N solution for values less than 100, and the 0.01 N solution for values above 100.

Carry out simultaneously a blank test without the fat. This should not liberate any trace of free iodine.

5. Calculation

$$\text{Peroxide value} = 8000 \frac{aN}{p}$$

a = number of mL of aqueous solution of sodium thiosulphate used for the test with the oil

N = exact normality of this solution

p = mass of test sample in g

6. Remarks

It may be requested that the result is expressed in millimolecules of oxygen per kg of fat; to obtain this, divide the peroxide value by 16. The result expressed in milliequivalents of oxygen per kg of fat is obtained by dividing the peroxide value by 8.

IC

Determination of Iodine Value

According to IUPAC 2.205/1, Wijs method, ISO 3961: 1996 or (EEC) NO 2568/91.

1. Definition

The iodine value of a fat is a measure of its degree of insaturation. In practice, it is determined by the amount of halogen absorbed and is conventionally expressed as the mass of iodine absorbed by 100 parts by mass of the fat.

2. Apparatus

The apparatus must by scrupulously clean and perfectly dry.
Glass weighing scoops, of 2 to 3 ml capacity;
wide-necked glass bottles with ground glass stoppers, capacity about 300 mL;
50 mL burette.

3. Reagents

10 % w/v aqueous potassium iodide solution; this must contain no free iodine or iodate;
0.1 N aqueous sodium thiosulphate solution (standardised);
pure glacial acetic acid, free from ethanol and oxidisable matter;
pure carbon tetrachloride, free from oxidisable matter.

The absence of oxidisable matter in both the above reagents is checked by shaking 10 mL of the reagent with 1 mL of saturated aqueous potassium dichromate solution and 2 mL of concentrated sulphuric acid. No green colouration should appear.

Pure iodine, re-sublimed;
iodine trichloride, ICl_3, or iodine monochloride, ICl.

4. Preparation of the Wijs reagent

4.1 With ICl_3

Weigh 9 g of iodine trichloride into a brown glass bottle; dissolve in 1000 mL of a mixture composed of 700 mL glacial acetic acid and 300 mL of carbon tetrachloride.

Determine the halogen content by the following method:

Take 5 mL of the solution and add 5 mL of the aqueous solution of potassium iodide and 30 mL of water. Titrate with 0.1 N sodium thiosulphate solution in the presence of a few drops of starch solution as indicator.

Add 10 g of powerded iodine to the bulk of the reagent and dissolve by shaking.

Determine the halogen content as above. The titre should now equal one and a half times that of the first determination. If this is not the case, add a small quantity of iodine until the content slightly exceeds the limit of one and a half times. It is important that no trace of iodine trichloride should remain as it would cause secondary reactions.

Let the solution stand, then decant the clear liquid into a yellow or brown bottle. If stored in a well-stoppered bottle away from light, the solution can be used for several months.

4.2 With ICl

Dissolve 19 g of iodine monochloride in 1000 mL of a mixture of 700 mL acetic acid and 300 mL carbon tertrachloride. After the addition of a few milligrams of pure iodine, determine the content of free halogen as described in 4.1. Dilute, if necessary, with the specified mixture of solvents until 5 mL of the solution are equivalent to about 10 mL of the 0.1 N thiosulphate solution.

5. Procedure

The mass of fat to be taken varies according to its expected iodine value in the following way:

Iodine value expected	*Mass to be taken for test (g)*
< 5	3.00
5 to 20	1.00
21 to 50	0.60
51 to 100	0.30
101 to 150	0.20
151 to 200	0.15

Weigh the appropriate quantity of fat to within 0.001 g in the glass weighing scoop. Place it in the 300 mL stoppered bottle. Add 15 mL of carbon tetrachloride to dissolve the fat. Add exactly 25 mL of the reagent, insert the stopper, shake gently and place the bottle in the dark.

For fats having an iodine value below 150 and for polymerised or oxidised fats, leave for two hours.

At the end of this time, add 20 mL of the potassium iodide solution and 150 mL of water.

Carry out a blank test simultaneously without the fat under the same conditions.

6. Calculation

The iodine value is given by the expression

$$\frac{12.69\ c\ (V_1 - V_2)}{m}$$

c = numerical value of the exact concentration, in moles per litre, of the standard volumetric sodium thiosulphate solution used

V_1 = volume, in milliliters, of sodium thiosulphate solution used for the blank test

V_2 = volume, in milliliters, of the standard sodium thiosulphate solution used for the determination

ID

Determination of Fatty Acid Composition

According to IUPAC 2.303, 2.302 and 2.304, ISO 5508: 1990/5509: 1999 or (EEC) NO 72/77.

1. Foreword

The choice of process will be dictated by the acid composition and acidity of the fatty substance under examination and the gas chromatograph analysis to be carried out.

More specifically:

— only sealed phial processes or processes using dimethyl sulphate can be used for fatty substances containing fatty acids with fewer than 12 carbon atoms,
— only the methanol-hydrochloric acid or methyl sulphate processes can be used for fatty substances with acidity of over 3 %,
— only processes using sodium methylate or dimethyl sulphate can be used for gas chromatography measurements of *trans*-Isomers,
— the methanol-hexane-sulphuric acid process must be used for the preparation of the methyl esters of small quantities of fatty substances from separation by thin layer chromatography.

The presence of unsaponifiables can be discounted, provided it does not exceed 3 %, otherwise the methyl esters will have to be prepared from the fatty acids.

2. Scope and field of application

A description is given of five processes (Process A - E) for the preparation of methyl esters from fatty substances:

(a) with sodium methylate;
(b) with sodium methylate in a sealed phial;
(c) with methanol-hydrochloric acid in a sealed phial;
(d) with dimethyl sulphate;
(e) with methanol-hexane-sulphuric acid.

Process A

PRINCIPLE

The fatty substance which is being subjected to analysis is heated under reflux with methyl alcohol and sodium methylate. The methyl esters obtained are extracted with ethyl ether.

APPARATUS

100 mL flask with a reflux condenser with a soda lime tube fitted to the top, with ground glass joints;
50 mL measuring glasses;
5 mL measuring pipette marked off in 0.1 mL;
250 mL separating funnels;
200 mL flask.

REAGENTS

Anhydrous methanol.
solution of approximately 1 % sodium methylate in methanol; this is prepared by dissolving 0,34 g of metallic sodium in 100 mL of anhydrous methanol;
ethyl ether;
10 % sodium chloride solution;
40 to 60 °C petroleum ether.

PROCEDURE

Place in the 100 mL flask 2 g of the fatty substance which has previously been dried out on sodium sulphate and filtered. Add 35 mL of methanol, fit the condenser and boil under reflux a few minutes.

Stop the heating process, remove the condenser and add rapidly 3.5 mL of sodium methylate solution; refit the condenser and boil under reflux for at least 3 hours. Methylation

is complete when all the fatty substance has liquefied and the reagent mixture is perfectly clear at room temperature.

Cool and pour the reagent mixture into a 250 mL separating funnel, add 35 to 40 mL of ethyl ether, 100 mL of water and 5 to 6 mL of 10 % sodium chloride solution. Shake and allow the layers to separate. Transfer the aqueous phase to a second separating funnel and extract once again with 25 mL of ethyl ether.

Add 50 mL of 40 to 60 °C petroleum ether to the combined ether extracts. Water will be separated and can be eliminated. Wash the ether phase three times with 10 to 15 mL of water, dry on sodium sulphate and filter through paper, collecting the filtrate in the 200 mL flask.

Evaporate the solvent to 20 mL, completing the process over a water bath in a current of pure nitrogen.

Process B

PRINCIPLE

The fatty substance which is being subjected to analysis is treated with sodium methylate in a methanol solution, in a sealed phial, at 85 to 90 °C.

APPARATUS

Strong glass phial with a capacity of approximately 5 mL (height 40 to 45 mm, diameter 14 to 16 mm).

1 ml measuring pipette marked off in 0.1 mL.

REAGENTS

Solution of approximately 1,5 % sodium methylate in methanol prepared by dissolving 0,50 g of metallic sodium in 100 mL of anhydrous methanol.

PROCEDURE

Place in the glass phial 2 g of the fatty substance, which has previously been dried out on sodium sulphate and filtered. Add 0.3 g (approximately 0,4 mL) of sodium methylate solution and heat seal the phial.

Immerse the phial for 2 hours at 85 °C to 90 °C, shaking from time to time. The esterification process is complete when the contents of the phial are clear after sedimentation of the glycerine and the residue of the reagents.

Cool at room temperature. Open the phial when the methyl esters are to be used. These do not require any further treatment before being placed in the gas chromatograph.

Process C

PRINCIPLE

The fatty substance which is being subjected to analysis is treated with methanol-hydrochloric acid,in a sealed phial at 100 °C.

APPARATUS

Strong glass phial with a capacity of approximately 5 mL (height 40 to 45 mm, diameter 14 to 16 mm);
2 mL calibrated pipettes.

REAGENTS

Solution of hydrochloric acid in 2 % methanol prepared from gaseous hydrochloric acid and anhydrous methanol (Note 1).

Hexane for gaschromatography.

PROCEDURE

Place in the glass phial 0.2 g of the fatty substance, which has previously been dried out on sodium sulphate and filtered, and 2 mL of hydrochloric acid-methanol solution. Heat seal the phial.

Immerse the phial at 100 °C for 40 minutes.

Cool the phial under running water, open and add 2 mL of distilled water and 1 mL of hexane. Centrifuge and remove the hexane phase which is ready for use.

Process D

PRINCIPLE

The fatty substance which is being subjected to analysis is saponified with a methyl alcohol solution of potassium hydroxide, and then treated with dimethyl sulphate. When hydrochloric acid is added, the methyl esters which have formed are automatically separated. Very pure methyl esters are obtained by subsequent treatment with alumina.

APPARATUS

Strong test tube with a capacity of approximately 20 ml, with 10/19 ground glass stopper and safety clips.

Reflux condensers, with 10/19 ground glass attachment.

Glass filters with sintered disc, G 2 size, 20 mm diameter.

Glass test tubes with a capacity of approximately 10 ml and a conical base.

1 mL and 5 mL syringes.

REAGENTS

Potassium hydroxide, 10 % solution in methyl alcohol for gas chromatography.

Green bromocresol indicator: 0.05 % solution in methyl alcohol.

Dimethyl sulphate (ρ = 1.335g/mol at 15 °C).

Concentrated hydrochloric acid (ρ = 1.19 g/mol) diluted in equal parts with methyl alcohol for gaschromatography.

Brockmann aluminium oxide for adsorption chromatography.

PROCEDURE

Place in the 20 mL test-tube 2.2 mL of the fatty substance, which has previously been dried out on sodium sulphate and filtered. Add 5 mL of the potassium hydroxide solution and a few quartz granules to control boiling. Attach the reflux condenser and heat over a low flame for five minutes, shaking. Saponification will be complete when the solution is clear. Finally, cool with running water and remove the condenser.

Add two drops of the indicator and, using a syringe, 1 mL of dimethyl sulphate, slowly. Seal the test tube hermetically and shake for two to three minutes, immersing the bottom of the test tube in a boiling water bath at frequent intervals. The reaction is complete when the indicator changes from blue to yellow. Finally, cool the test tube under running water, then open and add 5 mL of the hydrochloric acid methanol solution.

After shaking for a few seconds, lay the test tube at an angle and then tap it lightly. This will help the methyl esters to rise to the surface in the form of an oily mass (Note A).

Remove the methyl esters with a syringe, place in a test tube with a conical base, add a volume of alumina equal to approximately ¼ of the volume of the methyl esters, shake and filter with filter paper.

Note A: If the methyl esters do not separate spontaneously, add 5 mL of water to the test tube and shake.

Process E

PRINCIPLE

The fatty substance which is being subjected to analysis is heated under reflux with methanol-hexane-sulphuric acid. The methyl esters obtained are extracted with petroleum ether.

APPARATUS

Test tube of a capacity of approximately 20 mL, fitted with an air reflux condenser approximately 1 m in length, with ground glass joints.

5 mL calibrated pipette;
50 mL separating funnel;
10 mL and 25 mL measuring glasses;
15 mL test tube with conical base.

REAGENTS

Methylation reagent: anhydrous methanol-hexane sulphuric acid (ρ = 1.84 g/mol) in the ratio 75:25:1 (v/v/v).

40 to 60 °C petroleum ether;

anhydrous sodium sulphate.

PROCEDURE

Place the matter taken from the plate in the 20 mL test tube and add 5 mL of methylation reagent. Fit the reflux condenser and heat for 30 minutes in a boiling water bath (Note 2).

Transfer quantitatively the mixture into a 50 mL separating funnel, with the aid of 10 mL distilled water and 10 mL petroleum ether. Shake vigorously, and allow the phases to separate, remove the aqueous phase and wash the ether layer twice with 20 mL distilled water. Add to the separating funnel a small quantity of anhydrous sodium sulphate, shake, allow to settle for a few minutes and filter, collecting the filtrate in a 15 mL test tube with a conical base.

Evaporate the solvent over a water bath in a current of nitrogen.

Note 1: Small amounts of gaseous hydrochloric acid can easily be prepared in the laboratory by simple displacement from the commercial solution (ρ = 1.18 g/mol) by dripping concentrated sulphuric acid (ρ = 1.84 g/mol). The liberated gas is easily dried by bubbling through conc. sulphuric acid. Since hydrochloric acid is very rapidly absorbed by methanol, it is advisable to take the usual precautions in dissolving it, e.g. introduce the gas through a small inverted funnel with the rim just touching the surface of the liquid. Large quantities of methanolic hydrochloric acid solution can be prepared in advance, as it keeps perfectly in glass-stoppered bottles stored in the dark.

Note 2: To control the boiling point insert a glass rod into the test tube and limit the temperature of the water bath to 90 °C.

Summary

The principal objective of this study was to extract olive oil from the fruit of *Olea europaea* by means of supercritical carbon dioxide (sc-CO_2) as an alternative to traditional methods.

Extractions were performed on a laboratory scale supercritical fluid extractor of the latest design, featuring three mutually independent flow systems and extremely high flow rates.

A number of extraction runs based on a statistical design was performed to establish the conditions (time, pressure, temperature) for a maximum yield of extracted olive oil. These conditions turned out to be 50 °C and 460 atm (corresponding density of 0.953 g/mL) for a 60 minute extraction run.

The influence of different variables on the yield of extracted oil was investigated by means of computer-assisted surface response analysis. Additional extraction runs were performed at selected conditions to make reliable conclusions regarding the temperature, pressure and density dependencies of the extraction process and the nature of the mechanism of extraction. The density of the extracting fluid was found to be the key variable which limits the extraction by the extent to which olive oil dissolves in sc-CO_2.

The composition of the sc-CO_2 derived oil was determined by GC-GC/TOF-MS analysis and compared to that of commercial olive oil. A total of 27 peaks were detected in the chromatogram of the sc-CO_2 derived oil as opposed to 37 peaks observed for commercial olive oil. These peaks could all be identified, and the two major components in the sc-CO_2 derived olive oil are hexadecanoic and oleic acid.

The quality and composition of the oil were evaluated against standard specifications of the International Olive Oil Council and classified on the basis of these criteria as lampante olive oil. This oil is suitable for industrial use but not for human consumption.

Opsomming

Die hoofdoel van die studie was om olyfolie uit die vrug *Olea europaea* met behulp van superkritieke koolstofdioksied (sc-CO_2) as 'n alternatief tot tradisionele metodes te ekstraheer.

Ekstraksies is met 'n laboratoriumgrootte superkritieke-fluïed-ekstraktor van die jongste ontwerp uitgevoer. Dit beskik oor drie onderling onafhanklike vloeistelsels en lewer buitengewoon hoë vloeitempo's.

'n Aantal ekstraksielopies gebaseer op 'n statistiese ontwerp is uitgevoer ten einde die kondisies (tyd, druk, temperatuur) vir 'n maksimum opbrengs geëkstraheerde olyfolie te bepaal. Hierdie kondisies is vasgestel as 50 °C en 460 atm (ooreenstemmende digtheid van 0.953 g/mL) vir 'n ekstraksielopie van 60 minute.

Die invloed van verskillende veranderlikes op die opbrengs van geëkstraheerde olie is met behulp van rekenaargesteunde oppervlakresponsanalise ondersoek. Bykomende ekstraksielopies is by geselekteerde kondisies uitgevoer om betroubare afleidings oor die temperatuur-, druk- en digtheidsafhanklikhede van die ekstraksieproses en die aard van die ekstraksiemeganisme te maak. Die digtheid van die fluïed het geblyk die sleutelveranderlike te wees wat die omvang van die ekstraksie bepaal in terme van die hoeveelheid olyfolie wat in sc-CO_2 oplos.

Die samestelling van die sc-CO_2 verkrygde olie is met GC-GC/TOF-MS analise bepaal en met dié van kommersiële olyfolie vergelyk. 'n Totaal van 27 pieke is in die chromatogram van die sc-CO_2 geëkstraheerde olie verkry, in teenstelling met die 37 pieke wat vir kommersiële olyfolie waargeneem is. Hierdie pieke kon almal geïdentifiseer word, en die twee belangrikste komponente in die sc-CO_2 verkrygde olie is heksadekanoësuur en oleïensuur.

Die gehalte en samestelling van die olie is aan die hand van standaardspesifikasies van die International Olive Oil Council geëvalueer en op grond van hierdie kriteria as lampante olyfolie geklassifiseer. Hierdie olie is geskik vir gebruik in die nywerheid maar nie vir menslike gebruik nie.

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