The Use of Dense Carbon Dioxide Extraction and Fractionation to Recover and Refine Natural Food Ingredients from Food Processing Wastes

A Thesis Submitted to the Centre of Bioprocessing and Food Technology in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy

by

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To my father Chengshu Shen and mother Peifen Huang

who encouraged and inspired me

ABSTRACT

This study investigated the use of dense carbon dioxide, in both liquid and supercritical states, to extract and fractionate food by-product oils dispersed in porous matrices. Two oils were studied.

Rice bran oil is a by-product whose value as a food oil is lessened by the free fatty acids present in high concentration. Certain other minor constituents add to its value significantly. Dense carbon dioxide was used to extract the oil directly from rice bran and the dissolved oil was further fractionated in a lower density carbon dioxide stage. The compositions of the oil and its fractions were determined by titration, GC-MS, GC, HPLC, and UV spectroscopy, in order to find conditions which enhanced the value of the oil. It was found that fractionation removed almost all water, reduced the free fatty acid concentration in the raffinate by up to 50% and increased the concentration of certain valuable components including oryzanol and α-tocopherol.

Orange oil is composed largely of terpenes and is a source of flavour and fragrance (oxygenated) compounds which are present in low concentrations. The terpenes are unstable to heat and light and gradually taint the flavour and aroma of the oil. In order to improve the ratio of oxygenated compounds to terpenes, orange oil was partially fractionated by adsorption of the oxygenated compounds onto porous silica gel, and then further purified by desorption into supercritical carbon dioxide. The desorption of twenty-four compounds was monitored by GC-MS and GC. Adsorption alone removed three quarters of the terpenes. Fractional extraction improved the separation further but at the expense of reduced yield. Response Surface Methodology was used in the experimental design and Regression Analysis was used to determine the effects of process variables. The most important effect was that lower temperatures improved separation. Solvent flow rate usually had no significant effect.

The conclusion from both studies was that the systems were operating close to equilibrium conditions because of the fine dispersal of the oils and the excellent mass transfer properties of dense carbon dioxide. The rice bran oil extractions could be described by the partition coefficients measured in this study.

Certificate of Originality

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to substantial extent has been accepted for the award of any other degree or diploma of a university or other institute of higher learning, except where due acknowledgment is made in the text.

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Zhiping Shen

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Abbreviation

Alc Alcohol

Ald Aldehyde

Conc Concentration

D or d Density of CO_2

Dec Decanal

F or f Flowrate of CO_2

FFA Free fatty acid

FFAs Free fatty acids

Frac fraction

GC Gas chromatography

GC - MS Gas chromatography - Mass spectrometry

HPLC High performance liquid chromatography

Lim Limonene

Lin Linalool

P or p Pressure of CO_2

SCF Supercritical fluid

SC-CO_2 Supercritical carbon dioxide

SFE Supercritical fluid extraction

T or t Temperature of CO_2

Ter Terpene

UV Ultraviolet

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Chapter 2: Pilot Scale Extraction of Rice Bran Oil Using Dense Carbon Dioxide

Chapter 3: Pilot Scale Extraction and Fractionation of Rice Bran Oil using Supercritical Carbon Dioxide

List of Publications

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Shen, Z.; Palmer, M. V.; Ting, S.S.T.; Imison, B.; Mishra, V.; Fairclough, R.J. Dense carbon dioxide extraction and fractionation of by-product food oils dispersed in a porous or cellulosic solid matrix. Presented at the 4th International Symposium on Supercritical Fluids, Sendai, Japan, 633, **1997.**

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Chapter 1

Supercritical Fluid Extraction and Applications in Food Processing- A Short Review

1.1 Introduction

Since the late nineteenth century it has been known that when gases are highly compressed (i.e. are relatively dense) they develop solvent properties and can dissolve larger quantities of volatile and relatively non-volatile materials than will dissolve in low pressure (less dense) gases. However, it is only in the last few decades that this phenomenon has been the subject of active research and development.

Figure 1.1 depicts a pressure-temperature phase diagram for a typical pure substance. When the pressure and temperature values of the substance are below the sublimation and vaporisation curves, it is in the gas state. At any temperature below the critical temperature there is a limit to the pressure at which the gas state can exist and therefore a limit to the density of the gas. However, at temperatures above the critical temperature, the pressure can be increased indefinitely without a distinct change of state of the material. The density of the gaseous material can be increased to comparatively high values and its solvent properties increased. In this region of the phase diagram the compressed gaseous matter is known as a supercritical fluid (SCF), rather than as a gas, in recognition of the fact that its properties differ markedly from those of an ideal gas.

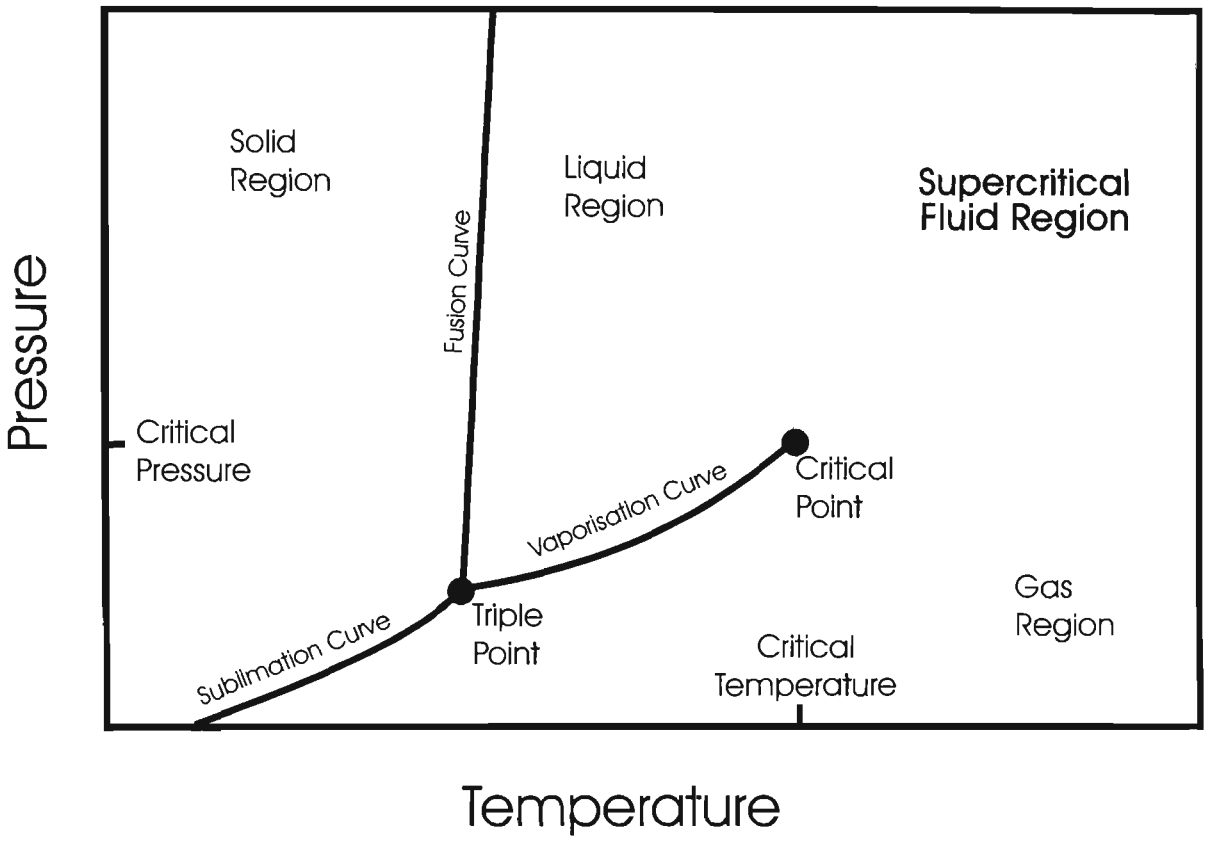


Figure 1.1 Theoretical pressure-temperature phase diagram for a pure compound

The utilisation of the solvent properties of SCF has led to the development of SCF technology which, in comparison with traditional liquid solvent processes, offers considerable flexibility for selective extraction and separation of substances. Temperature, pressure, choice of solvent, and additives (entrainers) can be manipulated as process variables.

The recent intense interest in SCF technology has resulted in a number of excellent reviews of the properties of SCF, theories of the SCF state and current applications of SCF in the chemical and food industries (Brunner and Peter, 1982; Chrastil, 1982; Stahl and Quirin, 1983; McHugh and Krukonis, 1986; Rizvi, et al., 1986 a,b; del Valle and Aguilera, 1988; Bruno and James, 1991; Kiran, and Levelt Sengers, 1994; Schneider, 1997; Reverchon, 1997). This study was undertaken to extend the application of SCF technology in the areas of extraction and fractionation of vegetable oils (specifically rice bran oil) and of essential oils (specifically orange oil). This review of the scientific literature will therefore focus on the use of SCF for extraction and fractionation of vegetable oils and essential oils. For a number of reasons to be discussed below, the SCF of choice for food applications has been dense carbon dioxide, and this review will therefore concentrate still further on this material.

1.2 Properties and advantages of SC-CO_2

SCF technology exploits the solvent power of a fluid at temperatures and pressures above its critical value. The location of the critical point of a pure compound on a pressure - temperature (P-T) phase diagram is shown in Fig.1.1. The fusion,

sublimation and vaporisation curves on the diagram indicate the border of co-existence, in equilibrium, of the 2 phases on either side of the indicated curves. The critical point lies at the end of the vaporisation curve and at this point, the gas and liquid phases become identical and merge to form a single homogeneous fluid phase. At temperatures and pressures higher than the critical point only a single fluid phase can exist. It is known as a supercritical fluid.

The special properties of a supercritical solvent have been summarised by Rizvi et al., (1986a) as follows;- "A supercritical fluid exhibits physicochemical properties intermediate between those of liquids and gases, which enhance its role as a solvent (Table 1.1). Its relatively high density gives good solvent power, while its relatively low viscosity and diffusivity values provide appreciable penetrating power into the solute matrix. These properties give rise to higher rates of mass transfer of solutes into a supercritical fluid than into a liquid. These unusual properties have been demonstrated to be useful in separating otherwise hard-to- separate constituents from their natural matrices."

One of the attractive features of a SCF is that its properties can be continuously varied from liquid-like to gas-like, and yet these properties are very sensitive to small changes in temperature and pressure in the vicinity of the critical point. The properties of the same material under sub-critical conditions are relatively unchanging while in the liquid or gas regions of the phase diagram, and undergo a step change when the vaporisation curve is crossed.

Fluid density is an important property of a SCF. Its variation with temperature and pressure is shown in Fig 1.2. Since the dissolving power of a supercritical fluid is a simple function of its density (Chrastil 1982; Schneider, 1997), and density is controlled by temperature and pressure, temperature and pressure can be used as variables to control the extraction and separation of a solute (Lucien *et. al.*, 1993). Near the critical point the solutes can be recovered from the fluid by slightly decreasing the pressure or increasing the temperature. Both these changes markedly reduce fluid density in this region of the phase diagram and allow the dissolved compound to be separated from the fluid (McHugh and Krukonis, 1986; Rizvi *et al.*, 1986a).

The effectiveness of SFE can be further appreciated when the extraction of heat-labile biological materials is considered, because all stages of a separation using SFE can be carried out at a relatively moderate temperature. Moreover, the solvent can easily be separated completely from the products at a moderate temperature by decreasing pressure to atmospheric pressure and no solvent residues remain in the products. These distinct advantages over normal solvent extraction make SFE a favoured technology to be used for processing of materials for human consumption (McHugh and Krukonis, 1986; King and Bott, 1993).

Table 1.1 Typical Physical Properties Associated with Different Fluid States (from Rizvi *et al.*, 1986a)

State of Fluid	Density (g/cm^3)	Diffusivity (cm^2/sec)	Viscosity (g/cm.sec)
Gas			
P =1 atm, T =15-30°C	$(0.6\text{-}2)\times10^{-3}$	0.1-0.4	$(1\text{-}3)\times10^{-4}$
Liquid			
P =1 atm, T =15-30°C	0.6-1.6	$(0.2\text{-}2)\times10^{-5}$	$(0.2\text{-}3)\times10^{-2}$
Supercritical			
$P = P_c$, $T = T_c$	0.2-0.5	0.7×10^{-3}	$(1\text{-}3)\times10^{-4}$
$P = 4P_c$, $T = T_c$	0.4-0.9	0.2×10^{-3}	$(3\text{-}9)\times10^{-4}$

When CO_2 was used as a solvent for hop oils, Gardner (1993) concluded that the supercritical solvent was not as selective as the liquid state solvent. However, the selectivity of SFE increases near the critical pressure of the solvent (Rizvi et al., 1986a). Further improvements to the selectivity or the solvent power of a SCF can be achieved by adding small quantities (1-5% w/w) of a cosolvent, or entrainer (Rizvi et al., 1986a; Wong and Johnston, 1986; Ramsay et al., 1991; Schmitt and Reid, 1986; Saito et al., 1991; Temelli 1992; Ting et al., 1993a, b; Dobbs, et al., 1987a,b). For example, the solubility of polar solutes in a non-polar SCF can be increased by addition of water, methanol or ethanol as cosolvents. Water and ethanol would be preferred for food applications. It is thought that the cosolvent (entrainer) provides stabilisation of the individual polar solute molecules in the supercritical phase and thus enhances the solubility.

Rizvi et al.(1986a) has listed some of the supercritical solvents used in SFE, together with their physical properties (temperature, pressure and density) at the critical point

(Table 1.2). These solvents also vary widely in their polarity and molecular weight, and hence in their solvent power for various types of solutes. Carbon dioxide has been the favoured supercritical solvent for food applications in recent years not only because of its mild critical temperature (31.1 °C) and pressure (7.38 MPa), but also because it is innocuous, noncombustible, noncorrosive, inexpensive, odourless, tasteless and readily available (Moyler, 1993).

Table 1.2 Critical property data for some supercritical solvents (From Rizvi, 1986a)

Substance	Critical temperature (°K)	Critical pressure (MPa)	Critical density (g/cm^3)
Methane	190.6	4.60	0.162
Ethylene	282.4	5.03	0.218
Carbon dioxide	304.2	7.38	0.468
Ethane	305.4	4.88	0.203
Propylene	365.0	4.62	0.233
Propane	369.8	4.24	0.217
Ammonia	405.6	11.30	0.235
Diethyl ether	467.7	3.64	0.265
n-Pentane	469.6	3.37	0.237
Acetone	508.1	4.70	0.278
Methanol	512.6	8.09	0.272
Benzene	562.1	4.89	0.302
Toluene	591.7	4.11	0.292
Pyridine	620.0	5.63	0.312
Water	647.3	22.00	0.322
Xenon	289.7	5.84	1.113

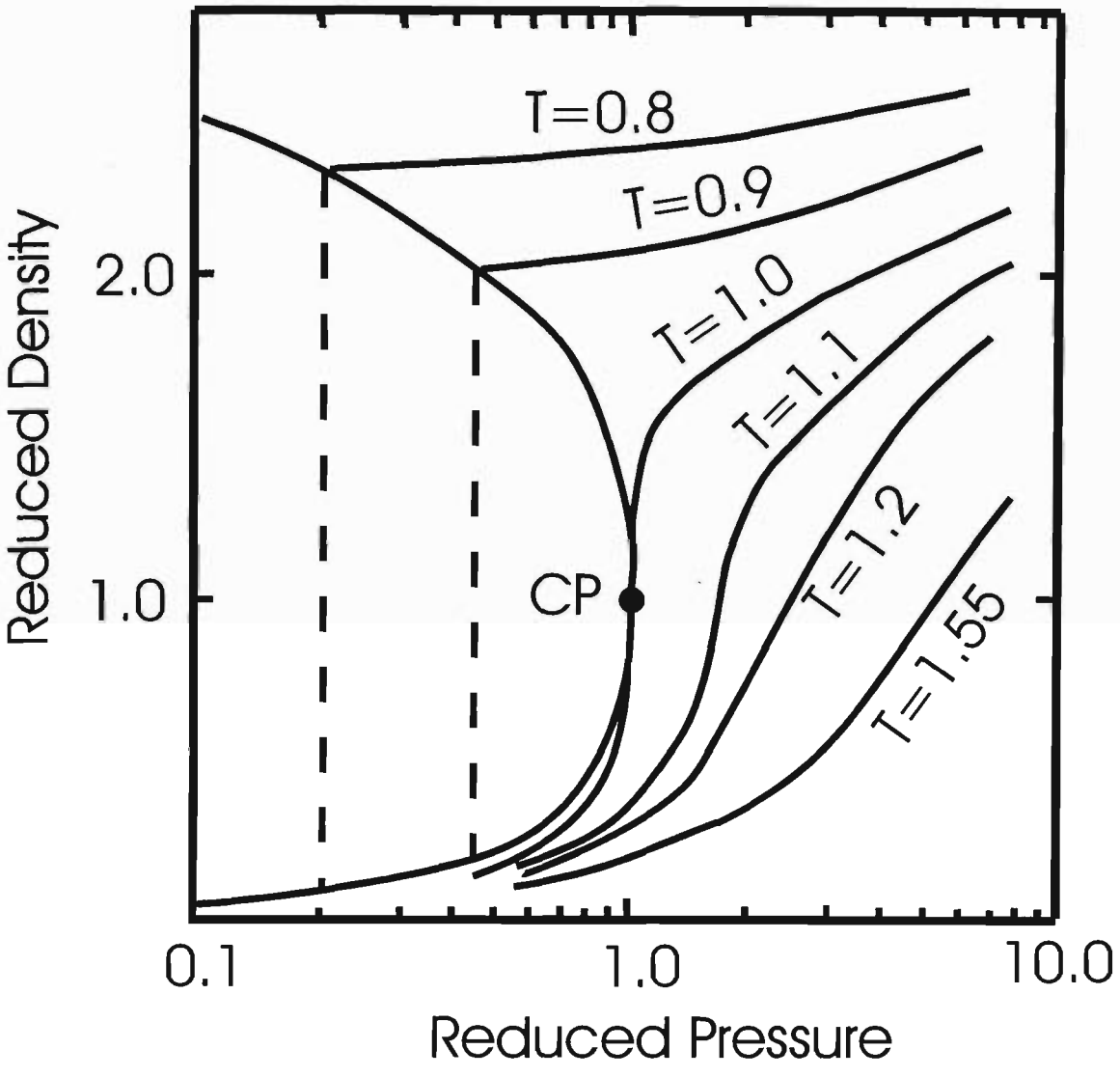


Figure 1.2
Reduced densities of carbon dioxide in the critical region at various reduced temperatures.
Extracted from Paulaitis et al. (1982)

1.3 Limitations of theories of the supercritical phase and its equilibria with solute liquids and solids.

Rizvi et al. (1986a) and McHugh and Krukonis (1986) have reviewed the theories of the supercritical phase and its equilibria with solutes. These authors showed that the theory has progressed most rapidly in relation to the supercritical phase equilibria of petrochemicals and that the behaviour of binary, ternary and higher order systems can be quite complex. The authors pointed out that using current theoretical models, the behaviour of these systems can only be predicted with some certainty if thermodynamic data are available from the critical regions of the pure solvent and the pure solutes. In a recent example the supercritical behaviour of CO_2 and butanol was measured by Hiaki et al. (1997) and modelled using the Peng-Robinson equation of state which required knowledge of the critical temperatures and pressures of both components individually. Since most substances of interest to the food industry decompose before they reach their critical temperatures, the required data cannot be measured and therefore current models are of limited use for food processing using SCF. The development of alternative models for the equations of state of supercritical solutions of biological substances is thus an important priority, but is beyond the scope of the present study.

1.4 Current and developing applications of SC-CO_2 in food processing

The increasing interest in the use of carbon dioxide in food processing must be seen in the context of other developments in the food industry and in industry in general. These other developments have rendered many existing solvents and processes less attractive.

Greater knowledge and public awareness of the potential toxic effects of many solvents has reduced the number of solvents acceptable to the food industry. Furthermore, concern about changes to the composition of the atmosphere has already resulted in certain solvents, refrigerants and propellant gases being removed from widespread industrial use. Public concern about industrial pollution of the atmosphere, coupled with fuel prices, has also led to increasing attention to energy efficiency. Consequently many conventional solvent extraction and regeneration processes are becoming unattractive and opportunities are emerging for well designed alternative processes utilising dense carbon dioxide.

In addition to the above changes, there is an increasing public demand for processed foods coupled with a demand that the foods should have organoleptic and nutritional properties similar to unprocessed foods. Since many of the effects of processing on flavour and nutritional value arise from the elevated temperatures of conventional processing, the mild processing temperatures involved in dense carbon dioxide processes make this technology more attractive. Moyler (1993) makes the point that commercial carbon dioxide is a by-product of fermentation processes, so that its use as an extraction solvent does not add to what would have been otherwise released to the atmosphere; therefore SFE has no detrimental effect on the earth's atmosphere, in contrast to the serious damage done by the release of some other solvents.

In recent decades there has been an increasing number of investigations into applications of SFE to food processing (Schultz and Randall, 1970; Stahl et al., 1984; Christopher, 1981; Reverchon et al.,1993). Table 1.3, from the review of Palmer and Ting (1995),

lists examples of food extraction systems using supercritical CO_2 or near-critical liquid CO_2. These processes have varying degrees of commercial feasibility and some of them, notably the decaffeination of coffee, the production of hop extracts, and the extraction of some natural flavours, have been put into commercial production as described below.

1.4.1 Decaffeination of coffee

Lack and Seidlitz (1993) have reviewed the development of this process. The alkaloid caffeine has various effects on human metabolism, some of which are considered beneficial in appropriate circumstances (e.g. stimulation of the nervous system). Otherwise the effects can be undesirable. Coffee drinking has become a social institution but individual choice about or sensitivity to caffeine has produced a demand for decaffeinated coffee. Decaffeination processes using organic solvents or extraction into water have been developed and have been in use since the beginning of this century. Water is a very good solvent for caffeine but also dissolves many other compounds in green coffee beans. Complex processes are required to overcome this difficulty.

Table 1.3. Examples of food extraction systems employing supercritical or near-critical carbon dioxide

Process and Food type	Reference
1. Extraction of Fats and Oils	
Fish:	Zosel (1978)
Rice Bran:	Ramsey *et al.*, (1991)
Evening primrose	Favati *et al.*, (1991)
Soybean:	List & Friedrich (1985)
Peanuts:	Goodrum & Kilgo (1987)
Sunflower:	Stahl *et al.*, (1980)
Canola:	Lee *et al.*, (1986)
Rice, Rice koji:	Taniguchi *et al.*, (1987a)
Beef:	Chao *et al.*, (1991)
Oats:	Fors & Eriksson (1990)
Potato chips:	Hannigan (1981)
Corn germ:	List *et al.*, 1984)
Cottonseed:	List *et al.*, 1984a
Egg yolk:	Froning *et al.*, (1984a)
Krill:	Yamaguchi *et al.*, (1986)
Wheat germ:	Taniguchi *et al.*, (1985)
Mustard:	Taniguchi *et al.*, (1987)
Olive:	Giovacchino *et al.*, (1989)
Cocoa:	Rossi *et al.*, (1989)
Yeast extract:	Duwe *et al.*, (1986)
Biscuit wastes:	Nakazono (1987)
2. Cholesterol Extraction	
Beef:	Chao *et al.*, (1991)
Milkfat:	Fujimoto *et al.*, (1987)
Egg yolk:	Froning *et al.*, (1990)
Fish:	Hardardottir & Kinsella (1988)
3. Fractionation of Fats and Oils	
Milkfat:	Arul *et al.*, (1987)
Sunflower oil:	Stahl *et al.*, (1980)
Rice bran oil:	Saito *et al.*, (1991)
Fish oil:	Zosel (1978)
Citrus oils:	Temelli *et al.*, (1988)
Fatty acid esters:	Liong *et al.*, (1992)
Canola oil:	Temelli (1992)
4. Refining of Fats and Oils	
Deodorisation of soybean, palm kernel and peanut oils	Zosel (1979) Ziegler & Liaw (1993)
Deacidification of olive oil:	Brunetti *et al.*, (1989)
De-oiling of soybean lecithin:	Heigel & Hueschens (1983)
Hydrogenation of vegetable oils:	Zosel (1976)
Deodorisation of rosemary extract:	Muehlnikel (1992)
De-oiling of bleaching clays:	Waldmann & Eggers (1992)
Extraction of tocopherols from soybean sludge:	Lee *et al.*, (1991)

Process and Food type	Reference
5. Flavour/Aroma extraction	
Hops:	Gardner (1993)
Ambrette, Angelica, Aniseed, Anise Basil, Capsicum, Caraway, Cardamom, Carob, Carrot, Cassia, Celery, Cinnamon, Clove, Cocoa, Coffee, Coriander, Cumin, Cubeb, Fennel, Fenugreek, Ginger, Juniper, Lovage, Mace, Marjoram, Nutmeg, Oregano, Parsley, Pepper, Pimento, Rosemary, Sandalwood, Sage, Savoury, Thyme, Turmeric, Vanilla, Vetiver:	Moyler (1993)
Onion:	Sinha *et al.*, (1992)
Dried botino (Katsuobushi):	Hamatani & Takahashi (1988)
Apple:	Bundschuh *et al.*, (1988)
Passion fruit:	Raasch & Knox (1991)
Wine:	Carnacini *et al.*, (1989)
Dried mushroom:	Valle & Aguilera (1989)
Citrus fruits:	Temelli *et al.*, (1988)
Cheese:	Gmuer *et al.*, (1986)
Elderberry:	Eberhardt & Pfannhauser (1985)
Caraway, Wormwood:	Stahl *et al.*, (1984)
Lactones from milk fat:	Rizvi *et al.*, (1993)
Peppermint oil:	Goto *et al.*, (1993)
6. Miscellaneous extractions	
Bixin pigment from annatto seeds:	Degnan *et al.*, (1991)
B-Carotene, lutein from leaf extracts:	Favati *et al.*, (1988)
B-Carotene from sweet potatoes:	Spanos *et al.*, (1993)
Pesticides from contaminated senna leaves:	Stahl *et al.*, (1984)
Antioxidants from sage extracts:	Djarmati *et al.*, (1991)
Antioxidants from *Labiatae* herbs:	Nguyen *et al.*, (1991)
Fractionation of glucose/fructose mixtures:	Bracey *et al.*, (1991)
Inactivation of orange juice pectinesterase:	Balaban *et al.*, (1991)
Off-flavours from textured vegetable proteins:	Sevenants (1987)
Debittering of citrus juices:	Kimball (1987)
Decaffeination of coffee, tea:	Lack & Seidlitz (1993)

Decaffeination of green coffee beans by SFE has been operating in Germany since 1978. With the original system, dense carbon dioxide is used to transfer caffeine from the beans to a separate water phase in a scrubbing stage (Lack and Seidlitz, 1993). The scrubbed carbon dioxide is recirculated to the extraction vessel. Rizvi et al. (1986b) reported that the process reduces caffeine from initial levels of between 0.7 and 3.0% to less than 0.02%, without affecting the characteristic flavour and aroma components of coffee. Subsequent improvements include the replacement of the scrubbing stage with adsorption of the caffeine *in situ* using pellets of activated carbon (Rizvi et al., 1986b).

1.4.2 Processing of hop extracts

The history of this process has been reviewed by Gardner (1993). Traditionally hops have been included in beer brewing to give beer its distinctive flavour and to act as a preservative. However, to improve the consistency of flavour, the modern brewer uses a hop extract rather than whole hops. Prior to the advent of carbon dioxide extraction of hops, the favoured solvents were methylene chloride, hexane, methanol or ethanol. The use of carbon dioxide to produce a hop extract began at Carlton and United Breweries in Melbourne, Australia, in 1980. This plant used liquid CO_2 for the extraction. Following the success of that operation a similar plant was built in England, while another using supercritical CO_2 was built in Germany. According to Gardner (1993), in the space of ten years carbon dioxide extraction almost entirely replaced organic solvents for the extraction of hops.

Carbon dioxide not only satisfies the environmental and human health requirements of a solvent, but has useful technical properties as a solvent for hops. The volatile oil flavour components dissolve readily in carbon dioxide while the alpha acids, as components of the bitter taste of beer, are also soluble to a useful degree. On the other hand, the solubilities in carbon dioxide of unwanted compounds with molecular weights in excess of about 400, are much lower than those of the desired compounds. The compositions of typical hop extracts obtained with liquid carbon dioxide compare favourably with compositions obtained with other extractants (Gardner, 1993).

SC-CO_2 is currently used in some hop extraction plants, but as a solvent the SCF is much less selective than liquid CO_2. This can be useful if a higher level of bitterness is required. The SCF also produces a higher apparent yield, but the yield of aroma compounds is not improved relative to liquid CO_2 extraction (Gardner 1993).

1.4.3 Extraction of flavours and fragrances

According to Moyler (1993), CO_2 as a solvent produces extracts of plant flavours and fragrances with a more natural taste and smell than achieved with any other solvent. Moyler (1993) has reviewed the commercial applications of dense CO_2 to the extraction of flavours and fragrances. Moyler (1993) concluded that most of the compounds responsible for aroma, flavour and taste of plant materials (organoleptic compounds) are soluble in liquid and near-critical carbon dioxide. These compounds are usually comparatively volatile with molecular weights below about 250. SC-CO_2, being a less selective solvent than liquid CO_2, can dissolve virtually all organoleptic compounds but

also some undesirable compounds as well. Furthermore, liquid CO_2 extractions take place at lower temperatures with less chance of changing flavour or aroma, and for these reasons liquid CO_2 extractions are favoured over the comparable supercritical extractions in many cases.

However, SC-CO_2 often has other advantages over liquid CO_2 extractions. By varying the temperature and pressure during extraction, selective extraction or fractionation of several flavour or odour components has been demonstrated. To some degree, extracts can be tailored to requirements. For example, extracting a typical plant material with supercritical CO_2 at 20.0 to 40.0 MPa and 40 to 80 °C results in an extract containing most of the plant resins and essential oils. Stepwise reduction of the pressure and/or temperature to just above or below the critical point of carbon dioxide can fractionate the extract to give an essential oil type fraction containing compounds of molecular weight up to about 400 and a resin fraction containing compounds of molecular weight greater than 400 (Moyler, 1993). The wide range of plant materials from which flavours have been extracted with carbon dioxide are included in Table 1.3.

1.5 Significance of utilising food processing wastes

Rising food production costs and environmental pressure as well as world population increase and local food shortages occurring in various parts of the world are the bases of world wide interest in reusing food industry wastes (Al-Wandawi et al., 1985; Moharram et al., 1980; Ramsay et al., 1991; Braddock et al., 1992; Hargrove, 1994; Hartman and Lago, 1976). Many so-called "wastes" are more accurately regarded as

by-products as they can be good sources of various materials such as vitamins, minerals, protein, low cholesterol oil, dietary fibre, food pigments, essential oils and complex sugars. Many are already used in animal feeds and some are processed for human food using conventional methods (Sivala et al., 1991; Al-wandawi et al., 1985; Braddock et al., 1992). However, there is still considerable scope to improve the utilisation of wastes using modern food processing technology.

1.6 Rice bran oil

1.6.1 Rice bran oil as a food resource

Rice bran is a by-product of rice milling and contains 15-20 % oil by weight, depending on the milling process and the paddy variety. The special qualities of rice bran oil, detailed below, are that it has a high level of mono- and polyunsaturated fatty acids, high natural tocopherol content, high smoke point, low flavour transference and a long frying life (quoted by Ramsay et al., 1991). It also has a high sterol content compared to most fats and oils. According to Ramsay et al. (1991) "There is a significant interest by pharmaceutical companies to find an alternative, readily available, inexpensive, renewable steroid source for the synthesis of most steroid hormones". The presence of tocopherol has been reported to result in less chemical absorption of oil by foods during cooking with rice bran oil (Sivala et al., 1991; Ramsay et al., 1991).

However, according to Zhao et al. (1987), "its usefulness as a source of edible oil has been hampered because of its high FFA, wax content and its dark colour". In summary,

the potential for extracting high value products from rice bran for the food and pharmaceutical industries is well recognised (Hargrove et al., 1994).

1.6.2 Composition of rice bran oil

The composition of hexane-extracted rice bran oil has been reported previously (Nicolosi et al., 1994). These investigators showed that the triglyceride fraction of the oil is rich in oleic (C18:1) and linoleic (C18:2) acids, with an overall fatty acid profile between those of canola and corn oils. The major components of the unsaponifiable fraction of the oil are sterols (42 %), higher alcohols (24 %) and ferulic acid esters, also known as oryzanol, (20 %). Some ferulic acid esters are reported to have antioxidant properties. The amount of α-tocopherol present in rice bran oil is relatively large (0.1 % of the total oil or 0.02% of rice bran) compared with other vegetable oils. The FFA content of crude rice bran oil is also relatively high, due to the high level of lipase activity in the bran (Nicolosi et al.,1994; Hargrove, 1994).

According to Rogers et al. (1993), oryzanol was first isolated from rice bran and thought to be a single component, but is now known to be a mixture of ferulic acid esters of cycloartenol, 24-methylene cycloartanol, campesterol, β-sitosterol and other sterols. "Physiological effects that have been shown to be associated with oryzanol intake are decreasing plasma cholesterol, decreasing platelet aggregation, decreasing hepatic cholesterol biosynthesis, increasing fecal bile acid excretion and decreasing cholesterol absorption" - Rogers et al. (1993). The γ-oryzanol content of rice bran oil has been reported as 787 ppm (0.08%), 1.1-2.6%, 1.4% and 0.57-3.18% by Rogers et al.(1993),

Seetharamalah and Prabhakar (1986), Zhao et al. (1987) and Taniguchi, et al. (1987) respectively. The antioxidant property of oryzanol, reported above, together with these physiological effects are of considerable current interest in relation to human health. Orthoefer (1994) has reported that alkali refining of rice bran oil removes most of the oryzanol, whereas physical or steam refining leaves the oryzanol in the oil. It is concluded that the value of a rice bran oil as a nutritious food ingredient depends on the extent to which its refining process maintains its oryzanol content.

1.6.3 Conventional extraction of rice bran oil

As described by Nicolosi et al. (1994), Orthoefer (1994) and Zhao et al. (1987) the conventional process for extracting oil from an oilseed (or by-product) is to first extract a crude oil using an organic solvent. Then phospholipids, hydrolysis products and nonglyceride impurities are removed from the oil in a complicated series of processes. In rice bran oil the hydrolysis products are chiefly FFA resulting from the high lipase activity in the raw material. The FFA are generally removed by an aqueous alkaline extraction which results in large losses of oil and, as mentioned in the previous paragraph, loss of most of the oryzanol. In traditional extraction processes the unavoidable solvent residues left behind, as well as the partial degradation of some heat-labile components, are becoming of concern to some consumers, health authorities and food manufacturers.

1.6.4 Supercritical carbon dioxide as a solvent to extract rice bran oil

Several studies have reported the use of SC-CO_2 to extract high value products from rice bran.

Taniguchi et al. (1987) used a laboratory scale SC-CO_2 extraction plant to investigate the effects of pressure, temperature and time of extraction of 20 g batches of three kinds of rice bran on the recovery of oil, oryzanol, phosphorus and colour. Zhao et al. (1987) also used a small scale SC-CO_2 plant to extract oil from 20 g of rice bran. This work is detailed further in Section 1.6.5. Zhao et al. (1987) reported that, compared with hexane extracted rice bran oil, rice bran oil extracted by SC-CO_2 had poor high temperature oxidation stability which they attributed to the observed low phosphorus (phospholipid) content, by analogy with the observations of SC-CO_2 extracted soybean oil reported by List and Friedrich (1985). Zhao et al. (1987) also reported that the tocopherol and oryzanol contents were slightly lower in SC-CO_2 extracted oil than in the hexane-extracted oil.

Ramsay et al. (1991) used a larger SC-CO_2 plant than that used by Zhao et al. (1987) to extract 150 g rice bran. Under extraction conditions of 30 MPa and 35 °C and an extraction time of 5 h they were able to obtain an 89% yield of oil and a 69% yield of sterols compared to oil extracted with hexane. Use of 5% ethanol by weight as a co-solvent increased these values to 90% and 80% respectively. They proposed that the total amount of sterols obtained by SFE could be increased by increasing the time of extraction or the carbon dioxide to feed ratio and by choosing suitable cosolvents.

The lower concentration of phosphorus in the SC-CO_2 extracted oil might avoid the degumming treatment in conventional processing for edible oil, while the lighter color of SC-CO_2 oil was an important advantage over the dark color of hexane extracted oil, known to make bleaching difficult (Zhao et. al., 1987).

A further advantage of SC-CO_2 extraction of rice bran oil, claimed by Ramsay et al. (1991), is that the residual defatted rice bran is superior to that obtainable by either hexane extraction or pressing, in that it contains no solvent residues, retains its structural qualities, and needs no drying or solvent removal. Rice bran defatted by SC-CO_2 extraction is therefore suitable for inclusion in foods or animal feeds.

1.6.5 Supercritical carbon dioxide as a solvent to fractionate rice bran oil and other vegetable oils.

Partial deacidification of some other vegetable oils by SC-CO_2 has been reported previously, for example: soybean oil (Friedrich et al., 1982), palm oil (Brunner and Peter, 1982), olive oils (Brunetti et al., 1989; Bondioli et al., 1992) and peanut oil (Ziegler and Liaw, 1993). After comparing the solubility isotherms of fatty acids and vegetable oil, Maheshwari et al. (1992) suggested that separation of fatty acids from triglycerides might be possible by using SC-CO_2 at densities less than 0.7 g/mL. Chrastil (1982) measured the solubility of certain fatty acids and triglycerides in SC-CO_2 within the pressure range 8-25 MPa and the temperature range 40-80 °C. He showed that at certain temperatures and pressures, CO_2 has a higher solvent power for fatty acids than for the corresponding triglycerides. Further literature reports of the

solubilities in SC-CO_2 of pure free fatty acids and their mixtures and of vegetable oils are discussed in Section 2.1.

Zhao et al. (1987) proposed fractional extraction of rice bran with SC-CO_2 which produced oils with low acid values. These authors used a single column holding 20 g of rice bran, and carbon dioxide at pressures of 15 to 35 MPa at 40 °C. To separate the non-triglyceride impurities from oils, the extracted oil was divided into four fractions according to increasing extraction pressure. The solubility of oil in SC-CO_2 was low at the initial pressure of 15.0 MPa, whereas FFA were efficiently concentrated in fraction 1. As a result, the FFA content drastically decreased in the successive fractions and was limited to approximately 1/3 of that of hexane-extracted oil in the combined later fractions (fractions 2-4) which contained oil equal to 80% of that extractable by hexane. The later fractions also showed some other attractive characteristics such as low amounts of iron, which is a pro-oxidant strongly affecting the stability of refined oil; and low amounts of waxes. In addition, the later fraction had a significantly lighter colour than hexane-extracted oil. A maximum amount of iron of 0.2 ppm was recommended for a stable refined oil (Cleenewerck and Dijkstra, 1992; Galdi, et al., 1989).

Saito et al. (1991) performed fractional extractions of rice bran oil and methylated rice bran oil with SC-CO_2, using entrainers or a column packed with silica gel-$AgNO_3$ at 40 - 100 °C and 8.2-19.8 MPa. These workers found that the use of a column packed with silica gel-$AgNO_3$ was extremely effective for fractionating fatty acid methyl esters in the methylated rice bran oil, but had only a very small effect in separating the fatty acid components of untreated rice bran oil.

These studies suggest that there is considerable scope for improvement and optimisation of extraction conditions of rice bran by carbon dioxide to yield valuable industrial products.

1.6.6 Oxidative stability of vegetable oils extracted with SC-CO_2

List and Friedrich (1985 and 1989) have studied the oxidative stabilities of soy bean, corn and cottonseed oils extracted with SC-CO_2 and compared the results with the stabilities of oils extracted by hexane or mechanical pressing. They have also explored the effects of certain additives thought to enhance oxidative stability. SC-CO_2 extracted oils were shown to have greatly diminished oxidative stability when compared with the other oils. These authors also measured the levels of iron, phosphorus and tocopherol in the oils as these are thought to play major roles in oxidative stability or instability. Trace metals, represented by iron, are thought to promote oxidation of oils, and are probably introduced to the oils during processing (List and Friedrich, 1989). Tocopherol is a well known antioxidant which could be expected to enhance oxidative stability of oils. Phosphorus, principally present as phospholipid, was thought to inhibit oxidation either through metal inactivation, through a synergistic antioxidant effect, or by forming a barrier to oxygen at the air/oil interface.

List and Friedrich (1985 and 1989) showed conclusively that the addition of soy lecithin (a source of phospholipid), or blending with hexane extracted soybean oil, markedly increased oxidative stability of SC-CO_2 extracted oils. However the

mechanism of this action remains unclear. SC-CO_2 extracted corn oil was the only oil tested which had no detectable iron content and yet it showed poor oxidative stability (List and Friedrich, 1989). Soybean oils with high levels of tocopherol also showed lowered oxidative stability whereas addition of tocopherol to lard improved its oxidative stability (List and Friedrich, 1989). However these authors showed that phosphatidylcholine and phosphatidylethanolamine added to lard diminished its oxidative stability, but showed a strong synergistic antioxidant effect in the presence of tocopherol, indicating that the action of the phosphatidyl moiety depends very much on the chemical environment.

The field of oxidative stability of SC-CO_2 processed vegetable oils is complex and beyond the scope of the experiments reported in this thesis. However, oxidative stability is central to the studies of citrus oil which are introduced in Section 1.7. In the case of citrus oils the emphasis is on the removal of oxidisable precursors from the oil, but there could be potential for knowledge of oxidation of vegetable oils to be extended to citrus oils.

1.7 Citrus oil

1.7.1 Citrus oil as a food and cosmetic resource

When citrus fruit or citrus fruit peels are cold-pressed, oils are produced which are valuable as a beverage and food flavouring and as ingredients for perfumes and fragrances (Kirchner and Miller, 1952; Crandall et al, 1983; Vora et al., 1983; Ferrer

and Matthews, 1987; Matthews and Braddock, 1987; Temelli et al., 1988; Braddock and Cadwallader, 1992; Barth et al., 1994). Citrus essential oils are present in small glands contained in the flavedo, which is the coloured portion of the peel of the citrus fruit (Matthews and Braddock, 1987; Braddock and Cadwallader, 1992). More than 200 compounds have been characterised (Temelli et al.,1990; Dugo et al., 1995; Sato et al.,1995), falling into the following classes:-terpenes, sesquiterpenes, aldehydes, alcohols, other oxygenated compounds, waxes, pigments, resins and gums.

The compositions of cold-pressed oils have been shown to be dependent on variety, stage of fruit maturity and geographical location of the trees, as well as the process method and year to year variations (Matthews et al., 1991; Dugo G., 1994). However, the oil from mature fruit is always principally composed of a terpene fraction acting more as a carrier of flavour than an actual flavour contributor (Kimball 1991). The terpenes have two organoleptic effects:-

(i) excess *d*-limonene, such as occurs in pure orange oil, irritates skin, eyes and mucous membranes (Kimball 1991);

(ii) terpenes as a group are unstable to heat, light and/or oxygen and change the flavour or aroma profile during ageing or processing (Temelli et al., 1988a, b & 1990; Fleisher, 1994; Tateo, 1981; Dugo et al., 1995; Barth et al., 1994; Chouchi et al., 1995; Sato et al., 1994). The changes are generally regarded as undesirable (Temelli et al., 1988) and described as unpleasant flavours (Ferrer and Matthews, 1987; Kirchner and Miller, 1952), off-flavours (Yaw et al., 1986; Dugo et al., 1995; Yamauchi and Saito, 1990; Sato et al., 1994) or "off-notes" (Barth et al., 1994; Temelli et al., 1988b).

The oxygenated compounds (aldehydes and alcohols) are mainly responsible for flavour (Temelli et al., 1988a, b &1990; Ferrer and Matthews, 1987; Yaw et al., 1986; Braddock and Kesterson, 1976; Kirchner and Miller, 1952; Braverman and Solomiansky, 1957; Tateo, 1981; Dugo et al., 1995; Yamauchi and Saito, 1990). Among the cold-pressed oils from various citrus species, the oil from oranges has the lowest concentrations of flavour and fragrance compounds and the highest concentrations of terpenes, with levels of up to 98% (Sato et al., 1995; Ferrer and Matthews, 1987; Tzamtzis et al., 1990; Yaw et al., 1986; Tateo, 1990; Dugo et al., 1995). Current technology for the use of cold-pressed orange oil has been described by Fleisher (1994). The terpene fraction is less soluble in water than the oxygenated fraction. When whole orange oil is added to an aqueous beverage in sufficient amount to produce flavour, a "ring" of terpenes forms at the top (Temelli et al., 1988).

Processes of deterpenation have been developed (See section 1.7.3) and so-called "terpeneless" extracts are marketed (Kirchner and Miller, 1952; Tzamtzis et al., 1990). A terpeneless extract is one which has had at least 50% of the original terpenes removed. The major advantages are the improved solubility and stability of the flavour (Lund and Coleman, 1977; Dugo et al., 1995).

There are various reports about the amount of non-volatile residues in citrus oil, which include hydrocarbons, fatty acids, sterols, carotenoids, waxes, coumarins, psoralens and flavanoids, some of which are phototoxic. They constitute from 1% (Temelli et al., 1988b) to 10% (Dugo et al., 1995) of the oil.

1.7.2 Analysis of components of citrus oil

The various methods used for the analysis of citrus oils have undergone major changes over the last few decades. The earliest analytical methods relied on wet chemistry and simple physical measurements to characterise citrus oils. The equipment required to carry out these analyses is relatively inexpensive and still widely used in the industry. Many of these methods have been described in detail by Kimbal (1991). As *d*-limonene is the major component of most citrus oils, the amount of oil in a sample is readily estimated by titration of the double bonds in *d*-limonene with bromine. Further quality control of the oil is achieved by measurement of its optical rotation, refractive index, and specific gravity, again largely due to *d*-limonene. Aldehydes, as a group, can be measured by colorimetric methods and expressed as the equivalent decanal content (Kimbal, 1991 and Shaw, 1979).

Since the 1960s there has been increasing use of chromatography, mostly gas-liquid chromatography (GLC), to characterise citrus oils. The various methods and results have been thoroughly reviewed by Shaw (1979). GLC instruments have allowed identification and quantitation of a much larger number of citrus oil components. By 1971 there were known to be more than 200 different compounds in citrus oil, of which more than 100 had been identified (Wolford et al., 1971). Difficulties in analysis have included the effects of sample pretreatments and the various response factors of the constituents of citrus oils. Because of these difficulties, and variations in procedures between laboratories, many of the early GLC results are difficult to use quantitatively, but are nevertheless very valuable in a semi-quantitative way.

Aldehydes have also been quantified by thin-layer chromatography of their dinitrophenylhydrazone derivatives (Shaw, 1979).

In more recent times, combined gas chromatography - mass spectrometry (GC-MS) has been used to analyse citrus oils (Shaw and Wilson, 1976; Shaw 1979; Shaw and Moshonas, 1985; Chamblee et al., 1985; Hawthorne et al., 1988; Chouchi and Barth, 1994). Computerised GC-MS incorporating a mass spectrum library has now enabled unambiguous identification of compounds comprising up to 99.7% of the volatiles of lemon peel oil (Chamblee et al. 1991).

1.7.3 Conventional methods to refine citrus oil

The main processes for refining citrus oil are vacuum distillation, steam distillation and aqueous ethanol and alkane liquid/liquid extraction (Fleisher, 1994; Van Dijck and Ruys, 1937; Tateo, 1981; Barth et al., 1994). Other documented processes are rapid distillation (Tateo, 1990) and adsorption/desorption (Ferrer and Matthews, 1987; Tzamtzis et al., 1990; Kirchner and Miller, 1952; Dugo et al., 1994; Barth et al., 1994).

The drawbacks of the various present methods are well described (Temelli et al., 1988; Ferrer and Matthews, 1987; Van Dijck and Ruys, 1937; Kirchner and Miller, 1952; Dugo et al., 1994) and include:- loss of some oxygenated compounds with the terpenes; damage to the flavour profile from the effects of heat and/or hydrolysis by water; contamination of the concentrate with solvent residues.

Changing attitudes towards "natural" ingredients and regulations enforcing lower levels of chemical solvent residues are providing an impetus to the exploration of novel methods. Added to this pressure is the inexorable rise in energy costs relative to other process inputs, so that process cost structures continually change (Temelli et al., 1988a,b). Opportunities therefore exist for development and implementation of new processes for refining citrus oil.

1.7.4 Supercritical carbon dioxide as a solvent to refine citrus oil

This area of SC-CO_2 technology has been the subject of a recent comprehensive review (Reverchon, 1997). A number of studies (Temelli et al., 1988a,b; Dugo et al., 1995; Yamauchi and Saito, 1990; Shin et al., 1992; Sato et al., 1994, 1995, 1996a, 1997) have concluded that simple batch SC-CO_2 refining of citrus oils is not attractive because of the incompatibility of conditions for high solubility and for high selectivity. The equilibrium between cold-pressed citrus oil and SC-CO_2 has been studied (Temelli et al., 1988b) using gas chromatographic analysis of the citrus oil liquid and the SC-CO_2 phase in equilibrium at 8.3 MPa and 70 °C. The terpenes, which are non-polar, of low molecular weight and high vapour pressure, were more soluble in the SC-CO_2 than were the oxygenated compounds. It is therefore possible to concentrate the flavour fraction of cold-pressed citrus oil with supercritical fluid technology. Conditions that give the lowest amount of flavour compounds (the oxygenated compounds) in the extract are 70 °C and 8.3 MPa. However, this is the region where the amount of extracted terpenes is also low, which means that larger amounts of carbon dioxide are necessary.

More complicated SC-CO_2 methods have therefore been investigated. The use of countercurrent flow of SC-CO_2 and oil to improve separation has been demonstrated (Perre et al., 1994; Sato et al., 1994), as has the use of solute reflux driven by temperature difference in the fractionation column (Sato et al., 1994,1995, 1996b).

1.7.5 Use of adsorbents to refine citrus oil

Another approach for fractionating citrus oils has been the introduction of selective adsorbents, including the most successful adsorbent, silica gel.

The use of selective adsorbents for orange oil refining has a long history predating the use of SC-CO_2. Kirchner and Miller (1952) demonstrated that they could produce a high degree of deterpenation of citrus oil using silica gel in a glass column. After loading 100 g of cold-pressed orange oil onto a bed of 136 g of silicic acid packed in hexane, the hydrocarbons (terpenes and sesquiterpenes) were eluted into a further 625 mL of hexane. Ethyl acetate (700 mL) was then used to elute the non-terpene fraction. Both oil fractions were then recovered by vacuum distillation. This procedure achieved virtually complete separation of the two fractions.

Braverman and Solomiansky (1957) pointed out that the method of Kirchner and Miller (1952) did not make full use of the adsorptive capacity of silica gel for oxygenated compounds, and demonstrated the ability of silica gel to strip oxygenated compounds from a much larger volume of orange oil. To achieve this high adsorptive capacity it was necessary to pack the silica gel dry. To avoid chanelling, the packing

was done "at a uniform pressure". Apart from the packing of adsorbent and loading of oil, the method was the same as described by Kirchner and Miller (1952). The result was a great saving in silica gel and solvent used. Braverman and Solomiansky (1957) also investigated alternative inorganic adsorbents and column geometries, and concluded that silica gel was the best adsorbent. Essentially the same method was used by Tzamtzis et al.(1990). Ferrer and Matthews (1987) modified the process of Braverman and Solomiansky (1957) by replacing hexane and ethyl acetate with the potable solvent, 95% aqueous ethanol. Finally, Meireles and Nikolov (1994) used a process similar to that of Ferrer and Matthews (1987), with liquid CO_2 as the solvent, to refine a by-product orange oil from the orange juice concentration process.

Alternative adsorbents to silica gel have been investigated by Lund and Coleman (1977) who studied Florisil (a magnesia silica gel), egg albumin, porous glass, cellulose acetate, polystyrene derivative, polyamide, rice starch, corn starch, dextran, and polyacrylic ester. Braverman and Solomiansky (1957) compared the adsorption characteristics of silica gel with magnesia, magnesia levis-kieselguhr, alumina, and alumina kieselguhr and Dugo et al. (1994) compared chromatographic sand, anhydrous $MgSO_4$, Celite (diatomaceous earth), anhydrous $CaSO_4$, and silica gel. Only in this last case was SC-CO_2 used as the desorbing solvent, but it is assumed that the relative capacities and selectivities of the adsorbents for the components of orange oil is similar in the presence of any lipophilic solvent.

A different approach was taken by Tateo (1981), working with lemon oil, who found that an acrylic/styrene copolymer preferentially adsorbed the terpene compounds and allowed the oxygenated compounds to be eluted with 70% ethanol (aqueous).

Section 4.1 contains a more detailed technical discussion of the use of adsorbents for refining citrus oils, including a review of loading ratios of oil to adsorbent used by various researchers.

1.7.6 Use of supercritical carbon dioxide and adsorbents to refine citrus oil

Parallel to the above developments has been the use of silica gel chromatographic methods with SC-CO_2 as the solvent. A number of authors (Dugo et al., 1995; Chouchi et al., 1994; Chouchi et al., 1995; Yamauchi and Saito, 1990;Barth et al., 1994; Sato et al., 1996a, 1997) have described semi-preparative fractionation of citrus oils. In all the above quoted studies the adsorptive capacity of silica gel for oxygenated compounds was not fully utilised. Studies involving an excess of silica gel can approach analytical chromatographic conditions and produce very high levels of deterpenation, but at the cost of inefficient use of the adsorbent and excess consumption of SC-CO_2.

However, in the work of Chouchi et al. (1995) loadings of bergamot oil (39% oxygenated compounds) were increased to the point of full utilisation of adsorptive capacity. Using the terminology and theory of column adsorption as summarised by Perry and Chilton (1973), it can be stated that full utilisation of the adsorbent capacity

of a column for oxygenated compounds is achieved when the column is loaded to the point at which the concentration of oxygenated compounds emerging from the column is equal to the concentration in the feed. If the loading rate is slow enough relative to the adsorption process this point is reached very soon after the "breakthrough" point for oxygenated compounds, or the point at which they first appear in the effluent.

1.8. Response Surface Methodology for process optimisation

Extraction of high value products from natural materials using SC-CO_2 involves the choice of values for a number of process variables which have the potential to affect the profitability of the process. These variables could include the temperature and pressure of CO_2 and its flow rate, as well as the method of packing the raw material into the extraction cell and any pretreatments of the material. Among such a range of variables there is likely to be some interaction. Without a systematic approach to investigation of the effects of the variables a large amount of experimental time could be wasted in the search for optimum conditions. Response Surface Methodology was developed in the 1960s as a coherent set of statistical methods for designing multifactorial experiments and analysing their results to obtain an estimate of the optimum combination of values for the process variables or a range of acceptable combinations (Myers and Montgomery, 1995).

The methodology includes principles for designing experimental programs with the maximum production of useful experimental data from a limited amount of experimental time. The Response Surface is a curved surface in a multi-dimensional

space which describes the dependence of the chosen response variable on all the process variables. Provided the optimum combination of process variables is within the range of values of the variables studied, the optimum point corresponds to the highest point on the curved surface. Reponse Surface Methodology involves measuring the value of the response variable at enough locations on the Response Surface to enable estimates to be made of the "location" and "height" of the optimum. This estimation is made by assuming that in the region of the optimum the curvature of the Response Surface can be approximated by a function of the process variables and their squares together with second order cross-products of the process variables.

The equation : $Y_1 = \beta_o + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} \beta_{ij} X_i X_j$ (Adasoglu et al., 1994) is the general form of a Response Surface of the response variable, Y_1, as a function of the process variables, X_i, The response variable could be a yield or concentration of a specific component or group of components in fractions recovered. Response and independent variables can be specifically chosen for a particular study, based on preliminary experiments and the quality parameters required in the products. Adasoglu et al. (1994) made extensive use of Response Surface Methodology in their study of SCE of essential oil from lavender flowers.

1.9 Conclusion

Supercritical Fluid Science and Technology is a rapidly developing field. It particularly suits the extraction and fractionation of natural materials. Nevertheless, as mentioned in this chapter, one of the major problems faced in using dense carbon dioxide to

concentrate required compounds and separate them from compounds which can cause off-flavour in vegetable oils and essential oils is that often both classes of compounds have similar solubilities in carbon dioxide of a density which produces reasonably high solvent power. Therefore, close examination of conditions for dense carbon dioxide extraction, and development of better separation methods is important.

Chapter 2

Pilot Scale Extraction of Rice Bran Oil Using Dense Carbon Dioxide

2.1 Introduction

As reviewed in Section 1.6.1, there is great potential to extract high value products from rice bran for the food and pharmaceutical industries. However, knowledge of the solubilities of the various oil constituents in SC-CO_2 is essential for the design and development of such extraction processes. An overview of the current literature concerning rice bran oil extraction and purification with SC-CO_2 is given in Sections 1.6.4 and 1.6.5. Solubilities of some pure FFAs and triglycerides and their synthetic mixtures in SC-CO_2 have been determined and modelled by a number of investigators (Chrastil, 1982; Bamberger et al., 1988; Ikushima et al., 1988; Brunetti et al., 1989; Nilsson et al., 1991; Maheshwari et al., 1992). Other studies have reported the solubilities of various vegetable oils including canola, soybean and wheat germ oils in CO_2 (Friedrich and List, 1982; Bulley et al., 1984; Taniguchi et al., 1985; del Valle and Aguilera, 1988; Fattori et al., 1988; Temelli, 1992; Maheshwari et al., 1992). A detailed, pilot-scale study of the recovery of oil, FFA, α-tocopherol, sterols and oryzanol of rice bran in CO_2 with varying temperatures and pressures, and the extraction trends of oil and individual components with time has not been reported previously.

The aims of the present experimental program were (i) to investigate effects of pressure, temperature and time on the extraction yields of oil, FFA, α-tocopherol, sterols and oryzanol in rice bran with sub- and supercritical CO_2, using a pilot scale extraction plant; (ii) to gather data for a feasibility study of suitable conditions for refining and fractionation of rice bran oil.

2.2 Materials

Rice bran was provided by the Ricegrowers' Co-operative Limited, Leeton, Australia. Moisture content, total hexane extractable oil in the bran and FFA content of the hexane-extractable oil were 10.1%, 18.7% and 6.8%, respectively. Food grade liquid CO_2 (99.8 % purity) was supplied by CIG, Melbourne and hexane (95% analytical grade) was supplied by Ajax Chemicals, Australia. The particle size distribution of the rice bran material, as determined by sieving, is listed in Table 2.1.

Table 2.1. Physical Properties of Rice Bran Used in Present Work

Particle size	>600 μm	600-500 μm	500-300 μm	300-250 μm	250-180 μm	<180 μm
(% by weight)	15.6	5.7	21.2	10.9	40.0	6.6

2.3 Experimental methods

2.3.1 Layout and operation of supercritical pilot plant

The term "dense" rather than "supercritical" CO_2 has been used in the general description of this study, since two of the six extraction conditions used CO_2 below its critical temperature of 31.1 °C. A schematic diagram of the pilot plant extraction unit (Distillers MG Ltd., UK) is shown in Figure.2.1. Food grade liquid CO_2 was cooled and pressurised by a piston pump to 17, 24 or 31 MPa, which was regulated and checked by a variable pressure indicator controller. The pressurised CO_2 passed through a heater to adjust the temperature to 0, 20, 40 or 60 °C and then flowed up through the vertically mounted extraction cell equipped with a water jacket to maintain the extraction temperature. The extraction cell (internal diameter, 38 mm; total length, 1428 mm; loading length, 747 mm) was loaded with 300 g of rice bran and each end was plugged with stainless steel mesh. The oil-laden CO_2 from the extractor passed through a separation vessel with a glass window, where it was depressurised and vented through a packed tailing column and vaporiser, leaving the extracted oil in the separation vessel. The CO_2 flow rate was manually adjusted, by changing the pump stroke length, to average 2.5 kg/h. Extractions were continued for 6 h and performed in duplicate. After each hour the CO_2 flow was stopped and the rice bran oil sample was collected. After collection the samples were stored at -17 °C until analysis to minimize sample deterioration.

For comparison and standardisation purposes, rice bran was extracted with hexane by Soxhlet extraction for 7 h at 70 °C (water bath temperature).

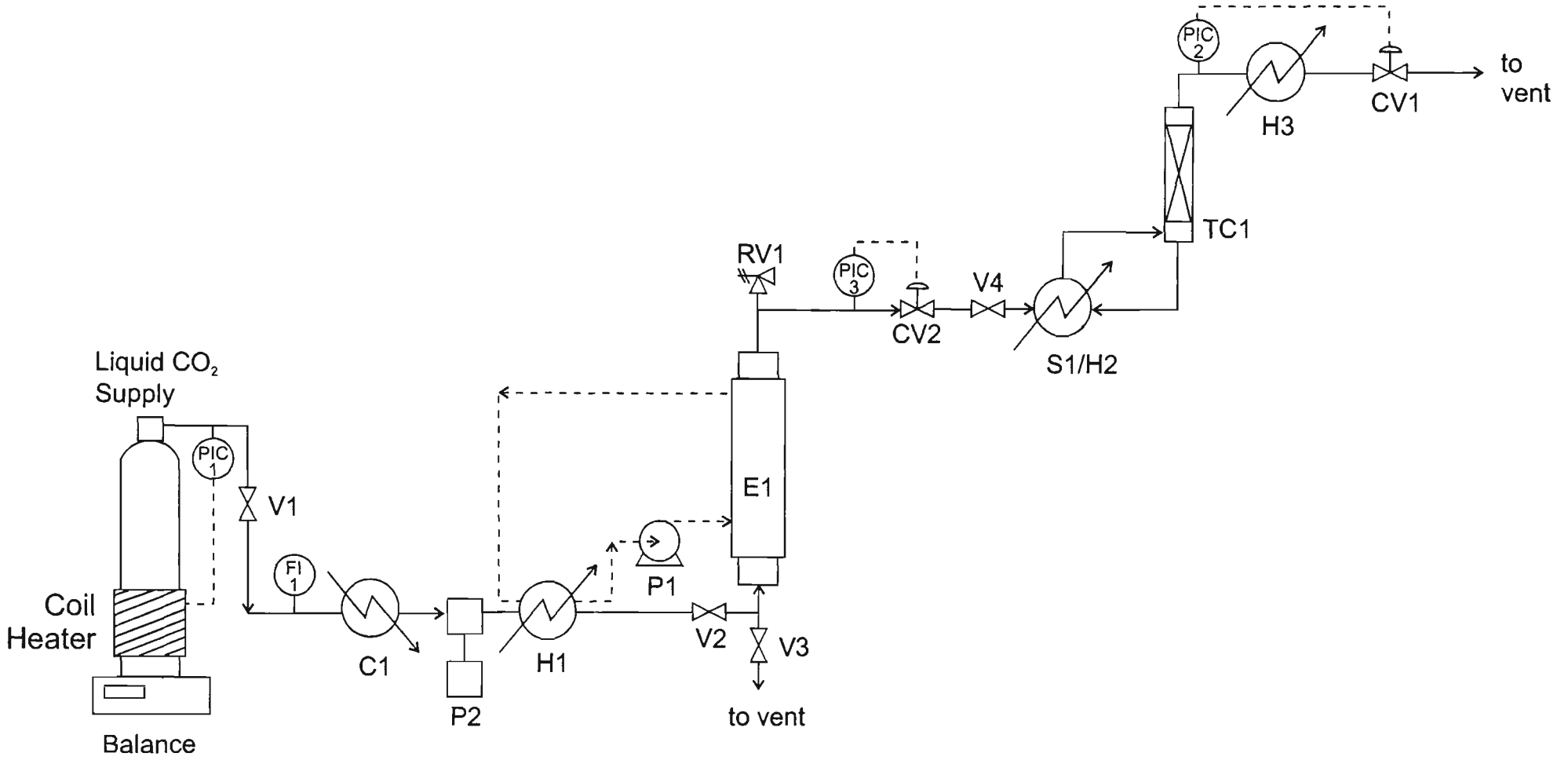


Figure 2.1 Schematic diagram of the pilot scale CO_2 extraction plant used in this study: variable pressure indicator controllers (PIC), heaters (H), cooler (C), piston pumps (P), separation vessel (S), tailing column (TC), valves (V), relief valves (RV) and control valves (CV).

2.3.2 Analytical methods

Sterol analysis

The unsaponifiables were extracted with hexane after an internal standard, 5α-cholestane (98% purity, Sigma, St. Louis, USA) was added to a saponified solution of rice bran oil. The unsaponifiables were analyzed as free sterols (Ramsay et al., 1991). A Varian GC 3400 was used for the analysis of sterols. Chromatographic separations were performed using a J & W Scientific DB-17HT coated 15 m x 0.25 mm capillary column, with a temperature programme from 220 °C-270 °C and a split injection system.

The gas chromatograph was directly coupled to the input of a Varian Saturn GC-MS and mass spectra were produced using electron impact ionisation. Reference factors with 5α -cholestane as the internal standard were used to quantify campesterol, stigmasterol and β-sitosterol. These sterols were identified by the GC-MS spectrum library (NIST90) and by comparing their retention times with authentic standards of campesterol (65% purity), stigmasterol (96% purity) and β-sitosterol (98.3% purity). All of these sterols were obtained from Sigma, St Louis, USA.

Other analyses

Water was separated from crude extracts of rice bran by centrifugation using a Sorvall Superspeed Centrifuge (SS-3 Automatic) at 16000 rpm for 30 min. at room temperature. The amounts of total extract and oil were determined gravimetrically. The total amount of FFA in each sample was determined by titration, according to AOAC Method 940.28 (1990). Oryzanol content of the extracts was determined by ultra-violet spectroscopy

(UVS) at 315 nm, according to the method of Seetharamaiah and Prabhakar (1986). The analytical reference standard of oryzanol was obtained from Tokyo Chemical Industry Co. Ltd., Japan.

Tocopherols were analysed by high performance liquid chromatography according to the method of Speek et al. (1985), using the modified solvent system of hexane / 2 - propanol (99.5% / 0.5%, v/v) as recommended by Pocklington and Dieffenbacher (1988). The tocopherols were separated using a Merck Lichrosorb Si 60 (5 mm) column (250 x 4 mm) and a solvent flow rate of 1.5 mL / min. Tocopherols were identified by fluorescence detection at 296 nm (excitation) and 320 nm (emission). Standard compounds were obtained from the Sigma CO., St. Louis, USA. The purity of these reference standards was checked by UV using the procedure outlined by Pocklington and Dieffenbacher (1988). All analyses were performed in duplicate except for sterol analysis.

2.4 Results and discussion

2.4.1 Oil extraction conditions

The effect of temperature and pressure on oil yield is shown in Figure 2.2 as a function of the amount of CO_2 used. Oil yield is reported as a percentage of the amount extractable by hexane. Oil was extracted linearly up to 80% of the hexane-extractable amount at 24 MPa / 20 °C and 40 °C and 31 MPa / 40 °C or remained linear throughout the whole extraction experiment at 24 MPa / 0 °C and 60 °C and 17 MPa / 40 °C. The

extractions at 31 MPa / 40 °C gave the highest oil yield which was 96.8% of hexane extractable oil. There are several possible explanations for the extraction trends shown in Figure 2. 2. Firstly, the flattening of the extraction profiles could be due to the heterogeneous nature of the rice bran with respect to particle size and differences in oil accessibility to solvent in various types of particle which result from brown rice grain polishing and bran milling processes (Zhao et al., 1987; Silvala et al., 1991). Oil contained in intact aleurone cells and within larger bran particles would be extracted slower than oil in small particles and free oil contained in and on the surface of broken aleurone cells. Thus, under high solubility conditions (24 MPa / 20, 40 °C and 31 MPa / 40 °C), it appears that free oil is extracted faster and diffusion-controlled extraction is reached. Under lower solubility conditions (24 MPa / 0, 60 °C and 17 MPa / 40 °C) not all of the free oil is extracted even after 6 h (Figure.2 2).

Secondly, CO_2 passing from the base of the extractor column through the 74.7 cm long bed of rice bran (300.0 g), would extract rice bran oil from the lower part of the bed first. Towards the end of the run, when the oil mainly existed in the higher part of the bed, the CO_2 may not be in contact with the oil sufficiently long enough for saturation to occur.

Thirdly, the slower extraction of components of lower solubility (e.g. higher molecular weight triglycerides, oryzanol and some sterols) may also account for the extraction profile.

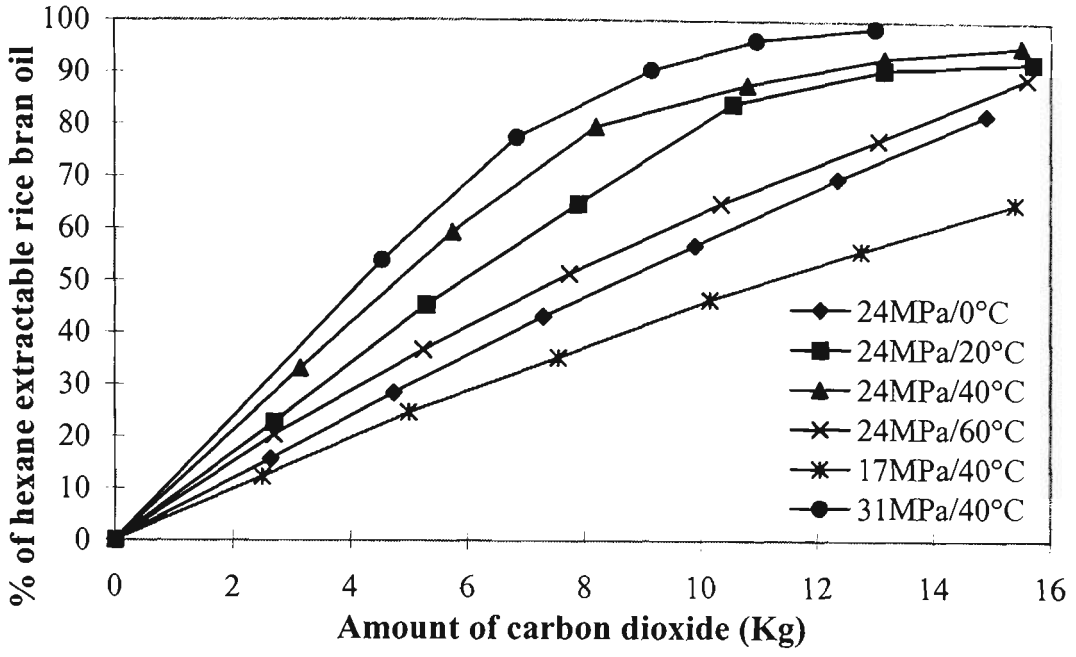


Figure 2.2. Extraction of oil from rice bran by dense CO_2 at different pressures and temperatures (CO_2 flow rate, average of 2.5 Kg/h). Single points represent the mean values of data from duplicate or triplicate experiments.

Table 2.2. Apparent solubility of rice bran and vegetable oils in CO_2

CO_2 density g/ml	Temperature ° C	Pressure MPa	Apparent solubility g of oil/kg CO_2	
			vegetable oil (published data)	rice bran oil (this study)
1.0359	0	24	*	3.16
0.9587	20	24	4.8[b]	4.58
0.8087	40	17	2.2[a]	2.50
0.8732	40	24	3[a]	5.52
0.9159	40	31	6.5[a]	6.93
0.7767	60	24	3.2[a]	3.52

[a]: Maheshwari et al., 1992 (measured from Figure 4)

[b]: del Valle and Aguilera, 1988 (measured from Figure 3)

*: not available

2.4.2 Solubility of rice bran oil in SC-CO_2

It is notable that the apparent solubilities of rice bran oil in CO_2, as determined from the initial linear portions of the extraction profiles, were similar to the modelled solubilities of vegetable oil under similar conditions (del Valle and Aguilera, 1988; Maheshwari et al., 1992) (Table 2. 2). Fattori et al. (1988) measured the solubility of canola oil from 7 g of flaked seed in CO_2 at 24 MPa / 40 °C with a flow rate of 0.7 g/min of CO_2, and found a value of about 4.4 g oil / kg CO_2. Temelli (1992) measured the solubility of canola oil from 50 g of canola flakes or press cake in CO_2 at 34.5 MPa / 40 °C with a flow rate of 1.3 g/min of CO_2 and obtained a value of about 7 g oil/kg CO_2. In the present study we found a value of 5.52 g/kg CO_2 for the apparent solubility of rice bran oil at 24 MPa / 40 °C and 6.93 g/kg CO_2 at 31 MPa / 40 °C.

A series of experiments was performed to show that the CO_2 flow rate was sufficiently low to ensure saturation of CO_2 with rice bran oil. At 24 MPa and 40 °C, the apparent solubility of rice bran oil was measured at 2.5 kg/h, 3.25 kg/h and 3.65 kg/h of CO_2 and found to be 5.52 g/kg CO_2, 5.60 g /kg CO_2 and 5.56 g/kg CO_2, respectively.

As expected, the apparent solubility of oil in CO_2 increased with pressure at 40 °C due to an increase in CO_2 density which increases its solvent power. At 24 MPa, solubility increased with temperature up to 40 °C due to an increase in the tendency of oil molecules to leave the oil phase, as reflected in the increase of oil vapour pressure with temperature (Fattori, 1988). A further increase in temperature resulted in a decrease in CO_2 density which reduced its solvent power sufficiently to overcome the increasing oil

vapour pressure, as discussed by Fattori (1988) in relation to canola oil. Similar behaviour has been reported for other vegetable oils (Friedrich, 1982).

2.4.3 Extraction of water

The effects of temperature, pressure and the amount of CO_2 used on the extraction of water from 300 g of rice bran are shown in Figure 2. 3. The extraction yield of water from the rice bran matrix with CO_2 increased with extraction temperature at 24 MPa, due to an increase in the vapour pressure of water. At 60 °C, about 65% of the water in the feed material was extracted in this study.

At 50 °C and pressures from 20-60 MPa, the solubility of pure water in CO_2 is approximately 0.3 wt% (Evelein et al., 1976). The relatively small effect of pressure (at 40 °C) in the present study is consistent with the finding of Evelein et al. (1976) that the solubility of pure water in CO_2 is almost independent of pressure above 20 MPa. Taniguchi et al. (1987) reported that the water solubility in CO_2 from rice bran was 0.17 wt% at 40 °C and 30 MPa with a CO_2 flow rate of 8.5 kg/h. The difference between Taniguchi's value of 0.17% and the value of 0.08% determined in this experiment at 40 °C and 24 MPa may have arisen from the different type of rice bran used, different initial moisture contents and different methods used to measure the water content. Taniguchi et al. (1987) measured water content by drying the extract at 135 °C for 3 h whereas centrifugation of the crude extract was used to separate water in the present study.

2.4.4 Extraction of free fatty acids

The effects of temperature, pressure and the amount of CO_2 used on the extraction of FFAs from 300 g of rice bran are shown in Figure 2. 4. The results obtained in this study indicated that there was a higher percentage (39.6% to 64.0%) of hexane extractable FFAs extracted in the first hour, compared to the lower percentage (16.0% to 31.4%) found for the total hexane extractable rice bran oil. Thus, the FFA recovery yields are about twice that of rice bran oil, which is mainly composed of triglycerides. From the fatty acid profile of hexane extractable and CO_2 extracted rice bran oil (Table 2. 3), oleic and linoleic acid constituted more than 75% of the total fatty acid. The data shown in Table 2. 3 indicate that there were no significant differences in fatty acid profile between hexane extractable and CO_2 extracted rice bran oil, which is consistent with the results of Zhao et al. (1987).

The relative differences between the recovery yield of FFA and rice bran oil extracted from rice bran may be explained by differences in solubility between pure oleic acid and triolein, as reported by Brunetti (1989) and the solubility differences between pure oleic acid, linoleic acid and vegetable oil predicted by Maheshwari (1992). At 20 MPa / 40 °C the solubility of oleic acid is 4.1 times that of triolein and at 30 MPa / 40 °C the solubility of oleic acid is 3.64 times that of triolein (Brunetti 1989). Maheshwari (1992) predicted a higher solubility of oleic and linoleic acid than that of vegetable oil in CO_2 at 40, 50, and 60 °C for CO_2 densities from 0.5 to 1.0 g/mL. The FFA have lower molecular weights than their respective triglycerides, which explains why in the present work they are selectively extracted in the initial stages of the extraction process.

In the present study the FFA yield increased with increasing pressure at constant temperature and with increasing temperature at constant pressure (Figure 2.4). At 24 MPa, extracted free fatty acid yield slightly increased with temperature even though the density of CO_2 decreased with increasing temperature. This is another illustration of the opposing effects of increased temperature on the distribution of an oil component between the oil phase and the CO_2 phase, discussed in section 2.4.2 in relation to solubility of the whole oil.

Maheshwari (1992) has previously reported that at 20.7 MPa, when the temperature increased from 313 K to 323 K, the solubility of oleic acid remained unchanged. Alternatively, at 27.6 MPa, when the temperature increased from 313 K to 323 K, the solubility of oleic acid increased by 9.5%, but when the temperature increased from 313 K to 333 K, the solubility of oleic acid decreased by 4.8%. Brunetti (1989) reported that when total free fatty acids were extracted at 20 MPa the solubility decreased when temperature increased at constant pressure. Total FFAs in rice bran oil are in a different chemical environment than are pure fatty acids, so that the same extraction trends may not occur.

Zhao et al. (1987) extracted 20 g of rice bran with SC-CO_2 at pressures from 15 to 35 MPa, a temperature of 40 °C and a flow rate of 0.7-1.2 kg/h and obtained similar results to those found in present study since the FFAs were concentrated in the first of 4 fractions.

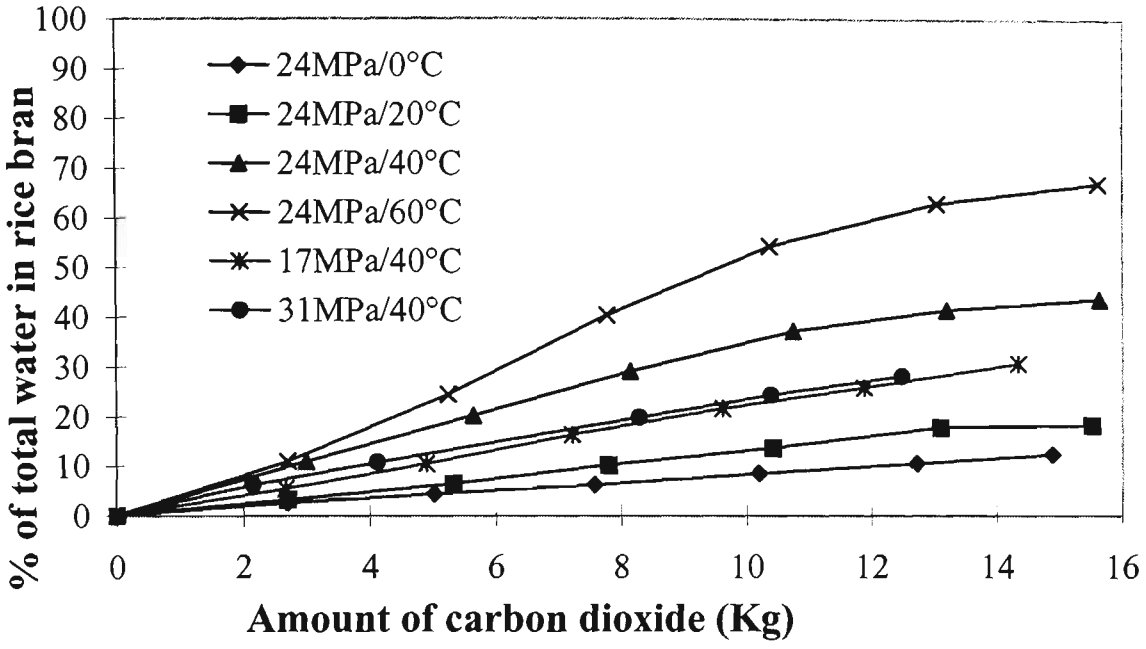


Figure 2.3. Extraction of water from rice bran by dense CO_2 at different temperatures and pressures (CO_2 flow rate, average of 2.5 Kg/h). Single points represent the mean values of data from duplicate or triplicate experiments.

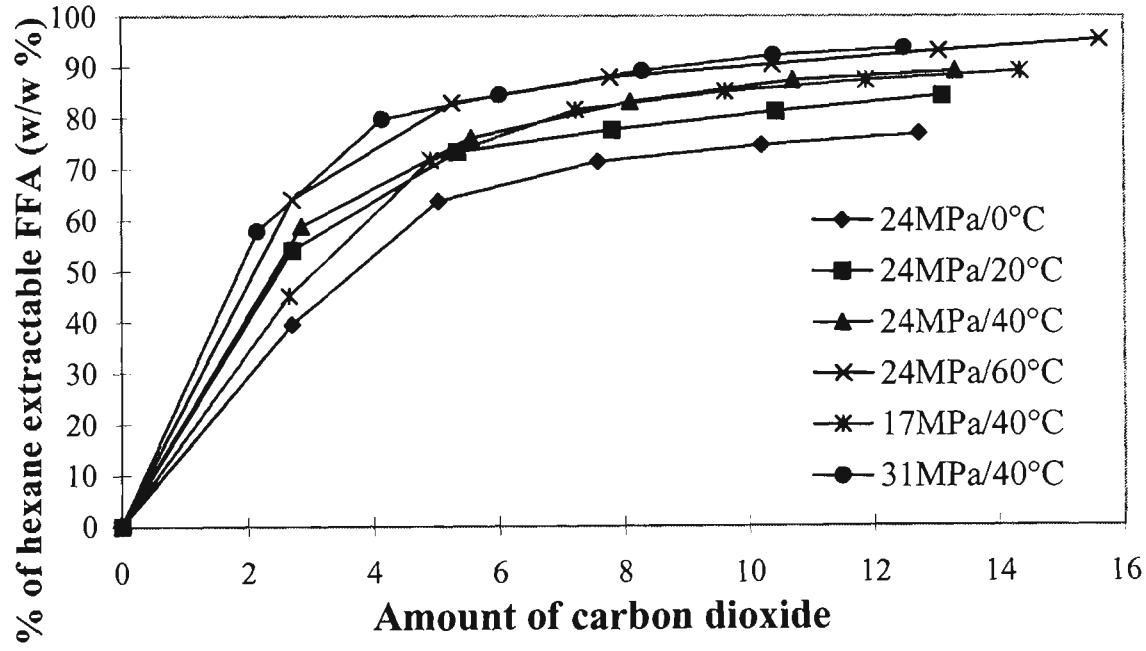


Figure 2.4. Extraction of total FFA from rice bran by dense CO_2 at different temperatures and pressures (CO_2 flow rate, average of 2.5 Kg/h). Single points represent the mean values of data from duplicate or triplicate experiments.

2.4.5 Extraction of α-tocopherol.

The effects of temperature, pressure and the amount of CO_2 used on the extraction yield of α-tocopherol are shown in Figure 2.5. About 90% (wt%) of total extracted α -tocopherol was recovered after 3 h with an average CO_2 flow rate of 2.5 kg/h at 40 and 60 °C / 24 MPa. The extraction of α -tocopherol occurred almost linearly over 3-4 h at 0 °C / 24 MPa and at 40 °C / 17 MPa. The concentration of α -tocopherol in the CO_2 extracts and hexane extracts were comparable with the findings of Zhao et al. (1987). The reason for the early flattening of the extraction profile at 31 MPa / 40 °C and 24 MPa / 20 °C is not clear.

2.4.6 Extraction of oryzanol.

The effects of pressure, temperature and the amount of CO_2 used on the amount of oryzanol extracted with SC-CO_2 are shown in Figure 2.6. Approximately 4.0 - 14.4% of hexane extractable oryzanol was extracted in the first hour during all runs. In contrast to the extraction curves found for FFA and α -tocopherol, those of oryzanol either became steeper as extraction progressed (at 40°C / 24 MPa and 31 MPa), or remained almost constant for all other treatments. In contrast with the extraction profile of α-tocopherol, FFAs and the triglycerides, oryzanol was more difficult to extract from rice bran. The molecular weight of oryzanol is approximately 270 Daltons lower than triolein. However its recovery yield was much lower than that of total rice bran oil, which could be attributed to its more rigid and voluminous polycyclic structure or linkage with other components of the rice bran matrix. The oryzanol concentration of the rice bran oil

extracted at 40 °C and 24 MPa was 1.5% which is close to the value of 1.1% reported by Zhao et al.(1987).

2.4.7 Extraction of sterols.

The effects of temperature and pressure on the extraction of the three sterols, campesterol, stigmasterol and beta-sitosterol are shown in Figures 2.7, 2.8 and 2.9 respectively. Overall, these extraction curves are similar to that of the rice bran oil itself, which is composed mainly of triglycerides. The reason for the incomplete recovery of hexane -extractable campesterol from rice bran (Figure 2.7) is unknown. On the basis of all the sterol results it seems that sterol concentration in the remaining rice bran oil (oil bodies) is relatively constant , and therefore the extraction rate would also stay constant until the sterols neared exhaustion.

Ramsay et al. (1991) extracted 150 g of rice bran using SC-CO_2 at 30 MPa and 35 °C for 5 hours and reported values which are comparable with those found in this study. The comparable results for the study of Ramsay et al (1991) and this study are: campesterol (1.85 and 1.65 g/kg oil), beta-sitosterol (4.05 and 5.11 g/kg oil) and stigmasterol (1.35 and 1.02 g/kg oil) respectively.

Table 2.3 Main Fatty Acid Profile of Rice Bran Oil (%)

extraction conditions	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$
hexane extractable	16.7	1.3	40.6	37.4	1.5	0.5	0.6
24 MPa/0 °C	17.3	1.1	38.8	40.4	1.7	0.3	0.4
24 MPa/20 °C	17.2	1.4	41.1	37.8	1.5	0.5	0.5
24 M Pa/60 °C	17.9	1.4	40.4	37.9	1.5	0.4	0.5
17 MPa/40 °C	17.7	1.2	40.1	38.6	1.5	0.4	0.5
31 MPa/40 °C	16.5	1.4	41.4	38	1.5	0.6	0.6

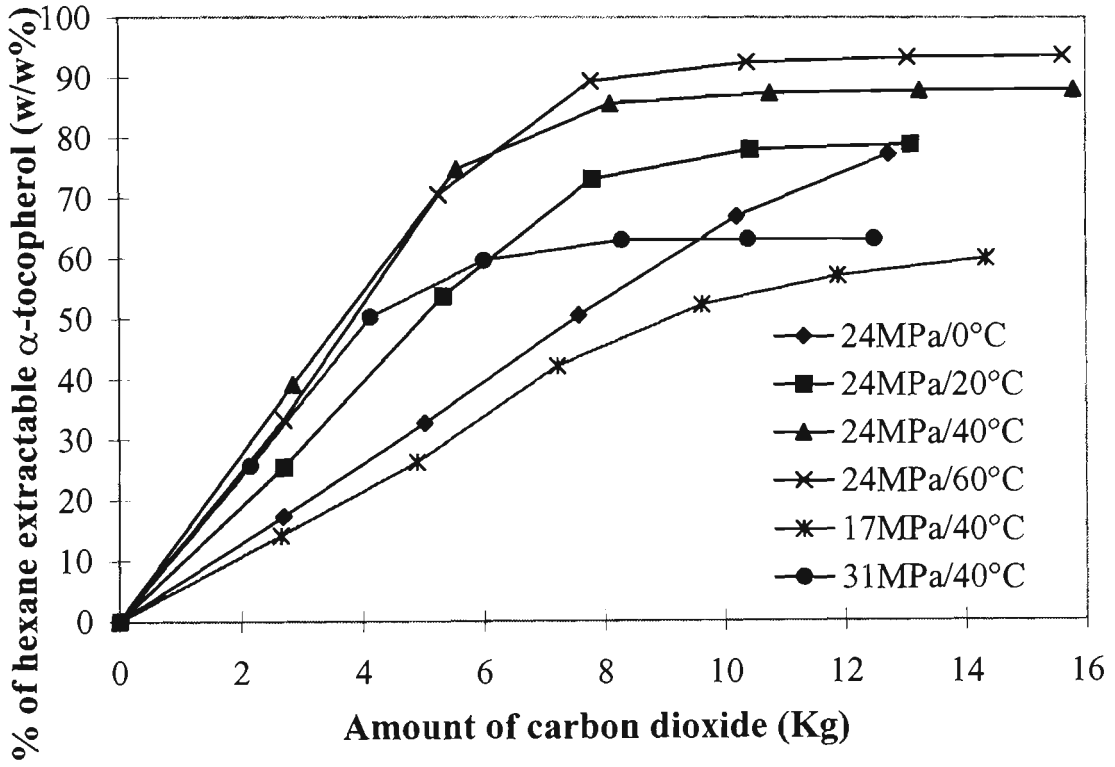


Figure 2.5. Extraction of α-tocopherol from rice bran by dense CO_2 at different temperatures and pressures (CO_2 flow rate, average of 2.5 Kg/h). Single points represent the mean values of data from duplicate or triplicate experiments.

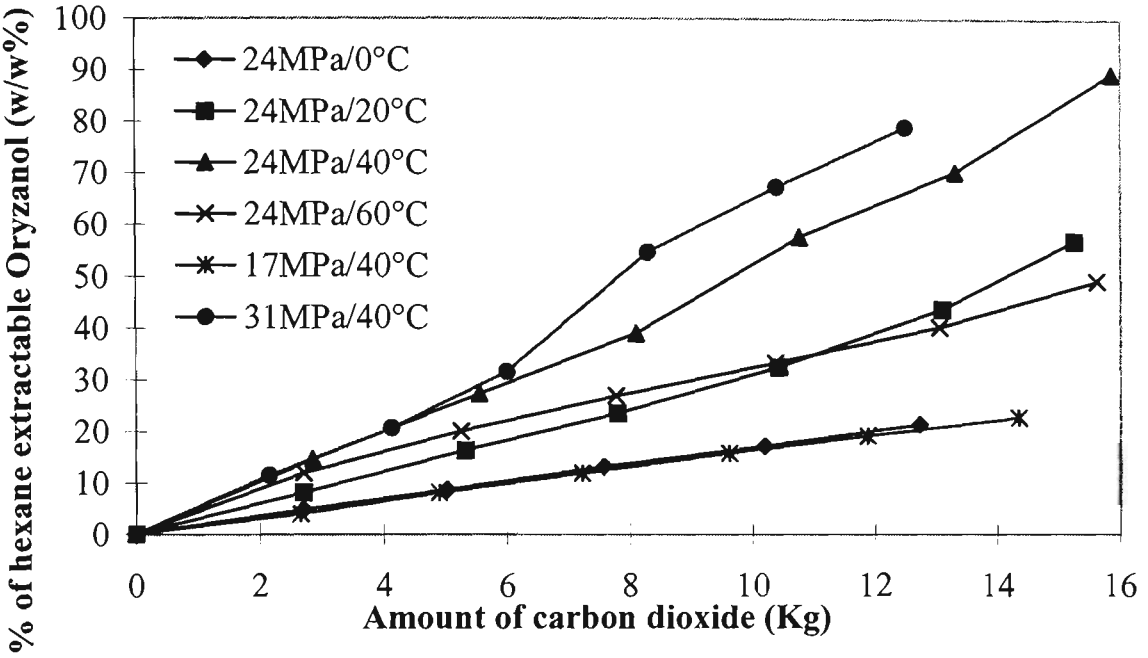


Figure 2.6. Extraction of oryzanol from rice bran by dense CO_2 at different temperatures and pressures (CO_2 flow rate, average of 2.5 Kg/h). Single points represent the mean values of data from duplicate or triplicate experiments.

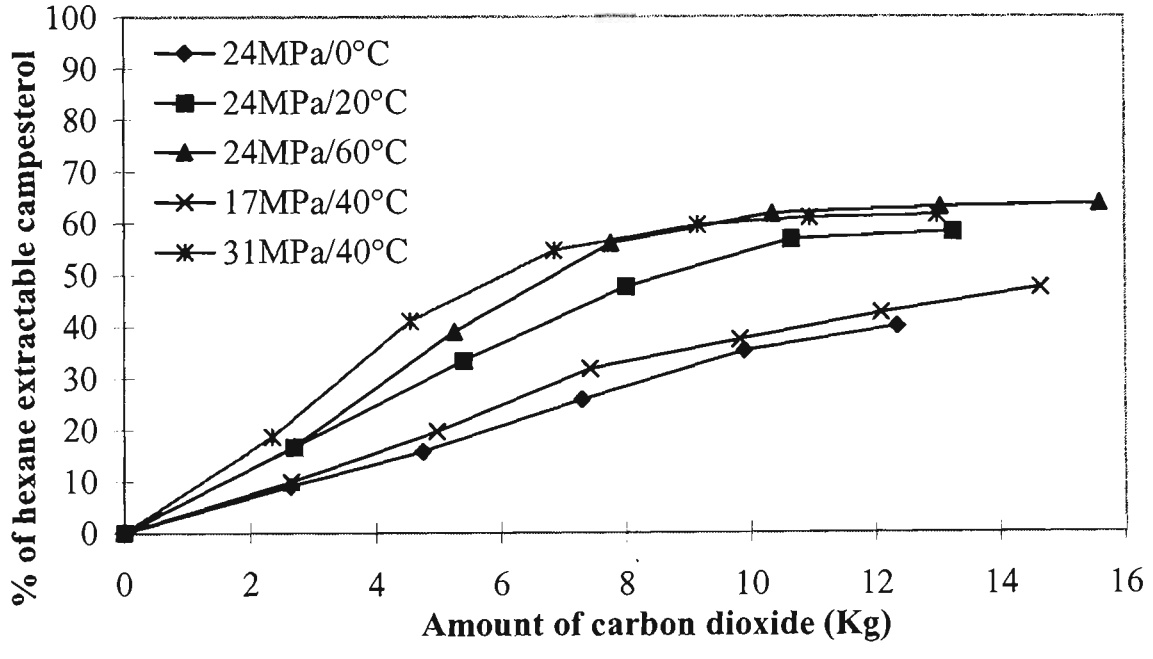


Figure 2.7. Extraction of campesterol from rice bran by dense CO_2 at different temperatures and pressures (CO_2 flow rate, average of 2.5 Kg/h).

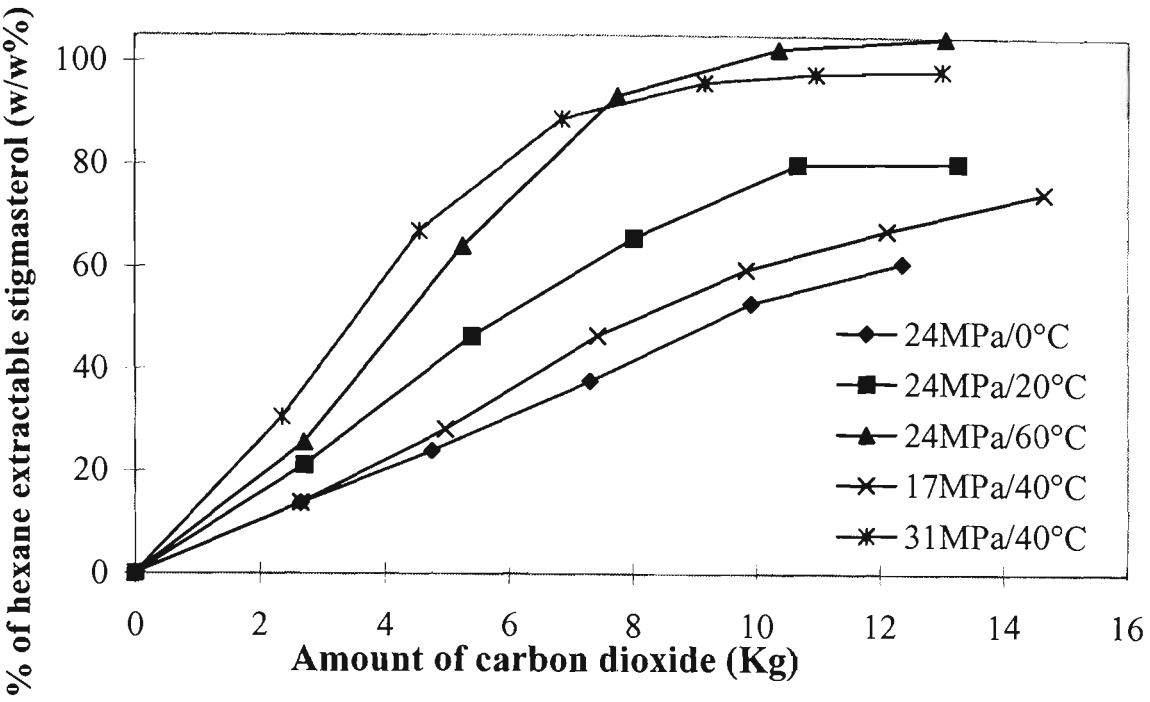


Figure 2.8. Extraction of stigmasterol from rice bran by dense CO_2 at different temperatures and pressures (CO_2 flow rate, average of 2.5 Kg/h).

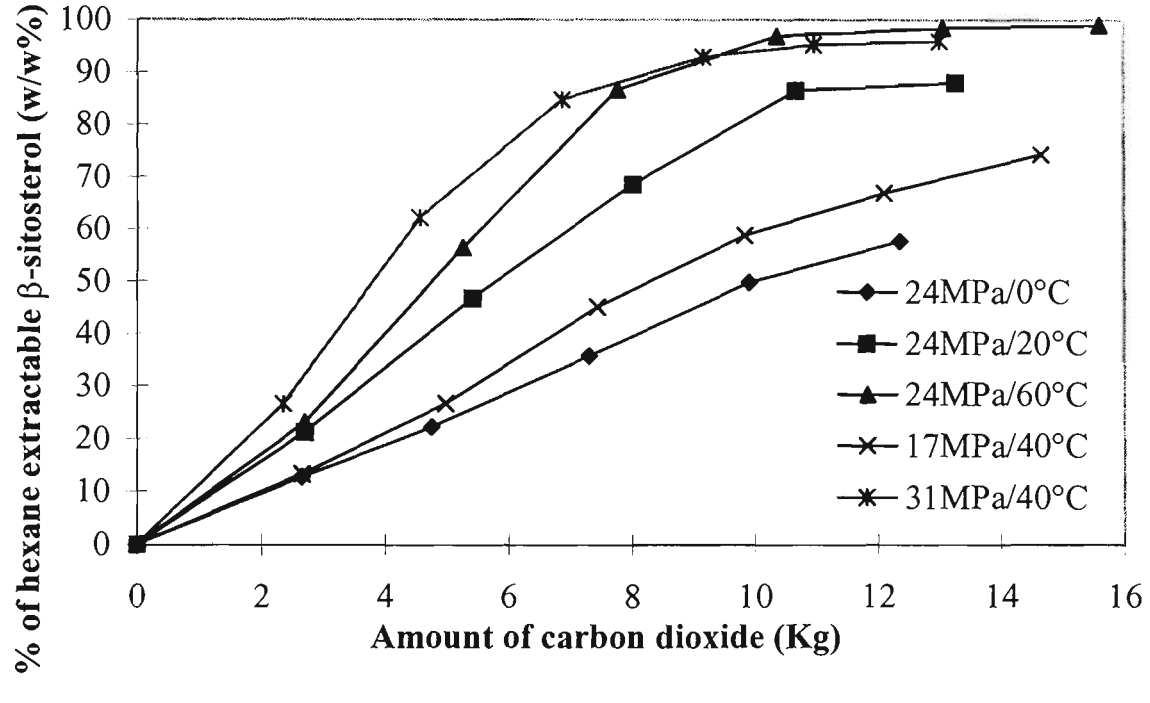


Figure 2.9. Extraction of beta-sitosterol from rice bran by dense CO_2 at different temperatures and pressures (CO_2 flow rate, average of 2.5 Kg/h).

2.4.8 Apparent partition coefficients of rice bran oil components.

Bamberger et al. (1988) investigated the solubilities of pure fatty acids, pure triglycerides, and mixtures of triglycerides in SC-CO_2 and suggested that the intermolecular interactions in the liquid phase would affect the solubilities in the supercritical phase. A correlating variable was chosen to be the partition coefficient, K_i, defined as ($K_i=Y_i/X_i$) where Y_i is the measured mole fraction of component (*i*) in the supercritical phase and X_i is the calculated mole fraction of the same component in the liquid phase, neglecting the concentration of CO_2 dissolved in the liquid.

Nilsson et al. (1991) followed the usual procedure of defining the partition coefficient to interpret quaternary system (monoolein-diolein-triolein-CO_2) data to understand better the optimum conditions of pressure and temperature for selective removal of the lower acylglycerols from such mixtures. In Nilsson's work, X_i was calculated from material balance considerations and Y_i was measured gravimetrically.

Rice bran oil is a mixture of many components, therefore similar coefficients can be useful in obtaining a better understanding of the optimum pressure and temperature conditions needed to remove FFA and concentrate other high value products like oryzanol, α-tocopherol and sterols from rice bran oil. In the present study, the calculations of apparent partition coefficients of components of rice bran oil, over the first hour of CO_2 extraction, were based on the saturation of these components in carbon dioxide over this period. The assumption was made that the composition of the oil bodies in rice bran at the beginning of each extraction was the same as the composition

of the hexane extract of rice bran. The apparent partition coefficients, calculated by dividing the concentrations measured in the CO_2 phase by the concentrations of the components of the hexane extract of rice bran on a w/w basis are presented in Table 2.4. The concentration of each component of the oil was expressed in units of g/kg oil, and the concentration in CO_2 in units of g/kg CO_2 in this work.

The apparent partition coefficients of all the components of rice bran, whether major or trace components, are almost of the same order of magnitude because of the chemical similarities of all the components which exist together in oil globule of rice bran. The earlier extracting components, FFA and α-tocopherol, have larger apparent partition coefficients, while the late extracting component, oryzanol, has a smaller apparent partition coefficient.

If two components have the same partition coefficient under given system conditions, they will be extracted by CO_2 in the same ratio as exists in the rice bran oil body. The end result is that no selectivity can be achieved. On the other hand, if the partition coefficients differ greatly it should be possible to selectively extract the various components. For example, at 17 MPa / 40°C, the partition coefficient of FFA is 3.78 times that of triglycerides , 3.19 times that of α-tocopherol and 11.52 times that of oryzanol, which are the greatest differences observed among all our extraction conditions. The present calculations are similar to trends observed by Maheshwari et al. (1992) who showed that the lower the density of CO_2, the more efficiently FFAs are separated from triglycerides.

The work in Chapter 3 describes the addition of a second flash separator operating at different pressure and temperature to better exploit the differences of partition coefficients between the components removed and concentrated. The use of such partition coefficients should assist in the selection of the most suitable conditions for the extraction of high value oils in a commercial plant.

Table 2.4. Apparent Partition Coefficients on a w/w basis(x10^3) for Components of Rice Bran Oil

Component	24 MPa / 0 °C	24 MPa / 20 °C	24 MPa / 40 °C	24 MPa / 60°C	17 MPa / 40°C	31 MPa / 40°C
Triglycerides	3.16	4.58	5.52	3.52	2.50	6.93
FFA	8.18	11.17	11.51	13.23	9.45	15.01
α-Tocopherol	3.58	5.26	7.66	6.87	2.96	6.65
Oryzanol	1.00	1.69	2.86	2.46	0.82	2.97
Campesterol	1.91	3.45	4.29	4.04	2.20	4.43
Stigmasterol	2.87	4.80	6.00	6.71	3.16	7.25
β-Sitosterol	2.68	4.82	5.87	6.24	2.98	6.31

Note: The partition coefficients are defined as the concentration (w/w) in the CO_2 phase divided by the concentration (w/w) in the oil phase.

2.5 Conclusion

Extraction of rice bran was almost complete in 6 h, and rates of extraction were consistent with saturation of the CO_2 with rice bran oil throughout most of the process. Extraction of the oil components was described by apparent partition coefficients between the oil and CO_2 phases. The observed differences in partition coefficients provide a basis for refining and fractionation of rice bran oil. Addition of a second separator to the extraction unit to collect water separately from the lipid extract would be beneficial in any future studies.

Chapter 3

Pilot Scale Extraction and Fractionation of Rice Bran Oil using Supercritical Carbon Dioxide

3.1 Introduction

As reviewed in Section 1.6.5, rice bran oil has been fractionated by SC-CO_2 in laboratory scale equipment to reduce the FFA level to 1/3 of that of hexane extracted oil. Partial deacidification of some other vegetable oils by SC-CO_2 has also been demonstrated. However, use of a SC-CO_2 pilot plant to remove FFA of rice bran oil has not been reported. In Chapter 2 the feasibility of extraction of oil from rice bran using SC-CO_2 at various temperatures and pressures in a single stage pilot plant has been shown and apparent partition coefficients for triglycerides, FFA, α-tocopherol, sterols and oryzanol in SC-CO_2 have been calculated.

There may be potential to develop a fractionating extraction system through exploiting all this information. In Chapter 2 the time courses of extraction of rice bran oil and its components using dense CO_2 at various temperatures and pressures have been explored. While the partition coefficients measured in that study are of general utility in design of oil extraction and refining procedures, the time course data are directly applicable only to design of batch separations. This chapter is an extension of that research and was conducted to determine the feasibility of using SC-CO_2 to

simultaneously extract and fractionate oil from rice bran, with particular reference to deacidification, by means of a second stage on-line solvent density reduction step.

More specifically the aim of the present study was to continuously produce a rice bran oil of enhanced composition using a second stage expansion column after primary SC-CO_2 extraction. In addition, the data from the expansion column was to be used to calculate the solubility of rice bran oil, and the partition coefficients and the selectivities of its components, as functions of temperature, pressure and density under these lower density conditions.

3.2 Materials

Rice bran was provided by the Ricegrowers' Co-operative Limited, Leeton, Australia. Moisture content, total hexane extractable oil in the bran and FFA content of the hexane-extractable oil were 8.5 %, 17.6 % and 9.8 % respectively. Food grade liquid CO_2 (99.8 % purity) was supplied by CIG, Melbourne and hexane (95% analytical grade) was supplied by Ajax Chemicals, Australia.

3.3 Experimental methods

3.3.1 Layout and operation of supercritical pilot plant

A schematic diagram of the pilot plant extraction and fractionation unit (Distillers MG Ltd., UK) is shown in Fig 3.1. Food grade pure liquid carbon dioxide (99.8% purity;

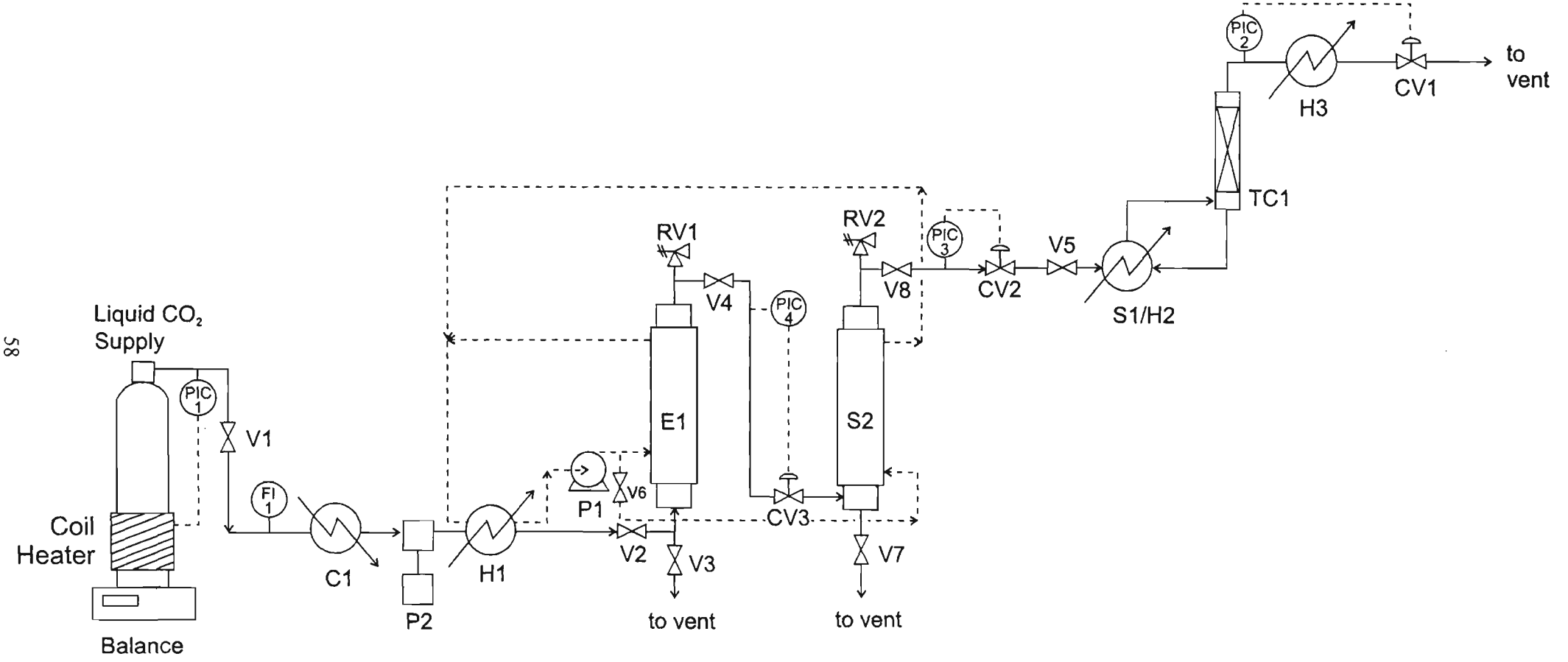


Figure 3.1 Schematic diagram of the pilot scale CO_2 extraction plant used in this study: variable pressure indicator controllers (PIC), heaters (H), cooler (C), piston pumps (P), separation vessel (S), tailing column (TC), valves (V), relief valves (RV) and control valves (CV).

CIG, Melbourne) was cooled and pressurised by a piston pump to a pressure of 24.1 MPa which was regulated and checked by a variable pressure indicator controller. The pressurised CO_2 passed through a heater and flowed up through a vertically mounted 1 L extractor equipped with a water jacket to maintain the set temperature of 40°C. The extractor was loaded with 300.0 g rice bran in each case and each end was plugged with stainless steel mesh. The oil-laden CO_2 at 24.1 MPa/40 °C from the extractor passed through the valve into the flash separator, in which the pressure and temperature of the oil-laden CO_2 was held at the desired values, 8.6 MPa/40 °C, 8.6 MPa/45 °C, 8.6 MPa/50 °C, 9.9 MPa/40 °C and 11.2 MPa/40 °C, by a back pressure regulator and a water jacket in which water was circulating through a water bath heater.

On pressure reduction the CO_2 stream separated into an oil-rich phase and a CO_2 -rich phase, with the oil-rich phase precipitating to the bottom of the flash separator. The CO_2-rich phase from the flash separator flowed through a separation vessel with a glass window, where SC-CO_2 was depressurised and vented through a packed tailing column and vaporiser, leaving the extract in the separation vessel. The raffinate was collected from the bottom of the flash separator. The CO_2 flow rate was manually adjusted to an average of 3.5 kg/h. Under the above conditions, extraction and fractionation were continued for 4 h simultaneously.

3.3.2 Analysis Methods

The amounts of total extract and raffinate were determined gravimetrically. Moisture in the extract and raffinate was measured by the vacuum oven method (AOAC, 1990). The methods of analysis for FFA, oryzanol, α-tocopherols and sterols were as described in Chapter 2 except the sterol analyses were performed in duplicate.

3.4 Results and discussion

3.4.1 Isothermal fractionation at 40°C

The effect of the pressure in the flash separator on the distribution of the major components of rice bran oil between the raffinate and extract is shown in Table 3.1. As expected, the increased CO_2 density, resulting from increased pressure at constant temperature of 40 °C, increased the solvent power of the SC-CO_2 for all components except water, and thus increased the mass of the extract at the expense of the raffinate. In the case of water, because only a small fraction remained in the raffinate under all conditions studied, it was not possible to detect any trends in the distribution of water between oil and CO_2 phases. The second stage fractionation step left only about 0.1% water in the raffinate. This value is well below the specified maximum of 0.3% water for many vegetable oils of similar composition (ANZFA, 1997), and is a very useful result because the removal of water from rice bran oil increases its microbiological stability and commercial value. This improvement on the single-stage CO_2 extraction

Table 3.1 Distribution of Major Components of Rice Bran Oil, Extracted at 24.1 MPa/40 °C and Fractionated at various Temperatures and Pressures[a]

Used CO_2 (Kg)	Pressure (MPa)	Temperature °C	CO_2 density (g/ml)	amount of raffinate g	amount of extract g	raffinate FFA %[b]	extract FFA%[b]	conc of water in raffinate (%)	amount of water extracted (g)
13.1	8.6	50	0.2477	42.56	1.51	9.21	18.15	0.10	9.72
13.8	8.6	45	0.2804	41.37	2.03	7.39	68.9	0.08	10.02
14.0	8.6	40	0.3668	39.67	4.51	6.10	50.44	0.13	10.72
14.6	9.9	40	0.6242	35.31	9.13	5.34	33.61	0.14	10.34
13.9	11.2	40	0.6941	31.81	12.15	4.78	27.96	0.11	9.40

[a] FFA % in hexane extractable oil is 9.79%. [b] as oleic acid %.

Table 3.2 Distribution of Minor Components of Rice Bran Oil, Extracted at 24.1 MPa/40 °C and Fractionated at Various Temperatures and Pressures

P (MPa)	Temp. °C	oryzanol mg/g oil		campesterol mg/g oil		stigmasterol mg/g oil		β-sitosterol mg/g oil		α-tocopherol mg/g oil	
		raffinate	extract	raffinate	extract	raffinate	extract	raffinate	extract	raffinate	extract
8.6	50	10.85	ne	1.80	ne	1.60	ne	10.40	ne	0.25	ne
8.6	45	12.06	5.20	1.75	1.49	1.55	1.29	9.89	7.10	0.24	nd
8.6	40	11.20	6.43	1.65	3.40	1.50	3.10	9.30	18.50	0.22	0.00
9.9	40	12.15	6.36	1.45	2.70	1.25	2.45	7.76	15.09	0.19	0.10
11.2	40	12.65	5.43	1.45	2.35	1.20	2.15	6.90	13.48	0.19	0.23

nd: not detected. **ne**: not enough sample to detect

at 24 MPa/40 °C reduced water content from 20% of CO_2 extract in the previous chapter to 0.1% in raffinate in the present work.

Bondioli et al. (1992) have refined lampante olive oil in a SC-CO_2 extraction plant operating in continuous countercurrent mode and reported the influence of pressure on FFA/triglyceride separation with pressures of 8.0, 9.0, and 11.0 MPa at 40 °C. They obtained decreased refined oil yield, decreased FFA concentration in refined oil and a decreasing and then increasing trend for FFA concentration in the extract, as pressure was increased. Our fractionation trends are similar to those reported by Bondoli et al. (1992) except for FFA concentrations in the extracts (Table 3.1).

Zhao et al. (1987) fractionally extracted rice bran oil from 20 g of rice bran, consuming 3.5 kg CO_2 at pressures in steps from 15 to 35 MPa/40 °C. By combining the fractions collected after the first pressure step, an oil low in FFA was obtained. The oil low in FFA contained 84.5% of the rice bran oil, 50.0% of the FFA, 81.8% of the oryzanol and 84.5% of the tocopherols in the total extract. This result can be compared with the present results of fractionation at 11.2 MPa/40 °C, for which the respective recoveries were 72.3%, 31.0%, 85.9% and 68.0%. The present method has achieved a greater reduction in FFA than by the method of Zhao et al.(1987). Furthermore, the consumption of CO_2 per gram rice bran in the present method was only 26% of the consumption reported in the work of Zhao et al.(1987).

In the present study, after 13.9 kg of CO_2 had been used only 83.39% of hexane extractable oil was recovered, whereas in Chapter 2, 92.97% of hexane extractable oil

was recovered after 13.2 kg of CO_2 was consumed. In the work described in this chapter, a higher average flow rate of 3.5 kg/h of SC-CO_2 was used rather than the 2.5 kg/h of SC-CO_2 used in Chapter 2. One possible explanation for these different results is that after about 75% of hexane extractable oil has been recovered, mass transfer difficulties became important and the yield of oil decreased with increased CO_2 flow rate. In other words, at the higher CO_2 flow rate the later stages of extraction deviated more from equilibrium since the contact time between the solvent and the oil was reduced.

The distribution of other oil components between the raffinate and extract is shown in Table 3.2. These mostly followed the trends in the partition coefficients between oil and SC-CO_2 phases reported in Chapter 2. The sterols (campesterol, stigmasterol, and β-sitosterol) were concentrated in the extract. Oryzanol was preferentially distributed to the raffinate, which is consistent with the respective partition coefficients reported in Chapter 2. However, it appears that in the present study, because of the lower densities of CO_2 at 8.6 MPa and 9.9 MPa, α-tocopherol was concentrated in the raffinate and not preferentially extracted as occurred with CO_2 at higher densities (Chapter 2).

3.4.2 Isobaric fractionation at 8.6 MPa

Table 3.1 shows the effect of temperature in the flash separator on the distribution of the major components of rice bran oil between the raffinate and extract at 8.6 MPa. By increasing the separator temperature from 40 °C to 45 °C and then to 50 °C the SC-

CO_2 density and hence its solvent power was progressively decreased. This resulted in a progressively increasing yield of raffinate and corresponding decreasing yield of extract. The FFA concentration in the raffinate increased with increasing temperature while the FFA concentration in extract initially increased and then decreased. In SC-CO_2 fractionation of olive oil, Bondioli et al. (1992) reported that with temperatures of 40 and 60 °C at a pressure of 13 MPa there was an increase in raffinate yield and FFA concentration in both the raffinate and extract at the higher temperature. The trends in the present fractionation are in accord with this report except for the reduced FFA concentration in the rice bran oil extract at higher temperatures.

Table 3.2 shows the effect of the temperature in the flash separator on the distribution of rice bran oil minor components between the refined oil fraction and extract. When the temperature increased from 40 °C to 45 °C and then to 50°C at constant pressure, resulting in decreased density of CO_2, the concentration of all minor components increased in the refined oil with the exception of oryzanol. The reason for this behaviour of oryzanol is still uncertain. The densities of CO_2 at 50 °C/8.6 MPa were too low to produce sufficient extract material for analysis of any extract component other than FFA.

3.4.3 Selectivities and partition coefficients

In the previous report on the extraction of rice bran oil in dense CO_2, the results were presented as partition coefficients calculated on a mass fraction basis. In the present chapter, ratios of the partition coefficients are used to derive "selectivity" (S), which

provides a useful basis for comparison with respect to the fractionation process. S values were calculated for different extraction conditions according to the following equation: (Brunetti, 1989):

$$S = \frac{W^{I}{}_{E} / W^{I}{}_{R}}{W^{T}{}_{E} / W^{T}{}_{R}}$$

Where $W^{I}{}_{E}$ and $W^{I}{}_{R}$ are the weight fraction of the component (I) in the extract and refined oil, and $W^{T}{}_{E}$ and $W^{T}{}_{R}$ the weight fraction of triglycerides in the extract and refined oil. The "separation factors" calculated by Arul et al. (1994) are actually ratios of partition coefficients calculated on a mole fraction basis. Brunetti (1989) had earlier used the term "distribution coefficient or solvent selectivity" to refer to a ratio of partition coefficients calculated on a mass fraction basis. Nilsson et al. (1991 and 1992) have consistently used partition coefficients calculated on a mass fraction basis and have defined the term "selectivity" as the ratio of such partition coefficients.

Table 3.3 shows the selectivities for some minor rice bran oil components isothermally at 40 °C and isobarically at 8.6 MPa, respectively. In every case, FFA had the highest selectivities, meaning that the FFA were preferentially enriched in the extract under all CO_2 conditions used, compared with the other components. The selectivities of oryzanol decreased with increasing CO_2 density meaning that the best separation of oryzanol from triglycerides was obtained at 11.2 MPa/ 40 °C among the conditions used. α-tocopherol was preferentially retained in the refined oil (raffinate) under most conditions.

Figure 3.2 plots the partition coefficients versus CO_2 density, for triglycerides, FFA, sterols and oryzanol. Data from Chapter 2 are included with data from the present work.

The former data were determined at high pressure at which the assumed equilibrium condition is approached by passing CO_2 over dispersed oil droplets in rice bran. In the present study data were determined at lower pressures where the assumed equilibrium is approached by condensing oil from solution in CO_2. The two sets of data are approximately contiguous, indicating that the assumption of equilibrium is justified in both sets of experiments.

Table 3.3 Selectivities for Some Minor Components of CO_2 Extracted Rice Bran Oil, relative to triglycerides.

pres. (MPa)	temp. °C	FFA	oryzanol	campesterol	stigmasterol	β-sitosterol	α-tocopherol
8.6	50	2.23	n.e.	n.e.	n.e.	n.e.	n.e.
8.6	45	27.82	1.29	2.55	2.49	2.14	< 0.06
8.6	40	15.64	1.08	3.90	3.91	3.76	0.02
9.9	40	9.00	0.75	2.66	2.80	2.78	0.75
11.2	40	7.73	0.57	2.14	2.36	2.58	1.62

The selectivities are ratios of partition coefficients and determine the maximum possible degree of separation of any pair of components under given conditions in a simple batch or co-current process. For optimal separation the selectivity must be maximised (or minimised, depending on how it is defined). This condition is best shown by maximum distance between the respective partition coefficient curves when plotted on a logarithmic scale. Figure 3.2 has been plotted on a logarithmic scale to illustrate the possibilities and limitations of CO_2 as a fractionating solvent for rice bran oil.

The vertical distances between the curves clearly show that FFA are best separated from tryglycerides at low CO_2 densities, (less than 0.7 g/mL), which is in agreement with the data of Maheshwari et al. (1992). The curves for sterols are all very close to each other, indicating the poor ability of CO_2 alone to separate the sterols from each other at 40 °C

in the CO_2 density range tested. This could be expected because of the similarities of the molecular structures of these sterols. Since the sterol curves are located between the FFA and triglyceride curves at CO_2 densities less than 0.8, it is inevitable that any simple process to separate FFA from triglycerides at 40° C and a CO_2 density of less than 0.8 will also partially remove sterols from the triglycerides. In other words, at 40 °C, in order to preserve the sterol content of rice bran oil, it is necessary to conduct the de-acidification at densities higher than optimal. It is possible that another temperature could be more favourable, or that the use of entrainers or adsorbents could overcome this difficulty.

Oryzanol had a partition coefficient which was less than or equal to the partition coefficient of triglycerides under all CO_2 conditions at 40° C. At CO_2 densities over 0.6 it would be possible to separate rice bran oil into a high oryzanol fraction and a low oryzanol fraction. The high oryzanol fraction would also inevitably have a reduced FFA content compared with the unfractionated oil.

In the present study, partition coefficients were also measured at temperatures of 45 and 50° C and a CO_2 pressure of 8.6 MPa. These conditions produced extremely low partition coefficients which are outside the useful range. However, the earlier work (as seen in Chapter 2) included some measurements at 60, 20 and 0° C using higher CO_2 densities, which show the direction and magnitude of temperature effects. These measurements are included with the triglyceride and FFA partition coefficient isotherms of the present study in Figures 3.3 and 3.4 respectively.

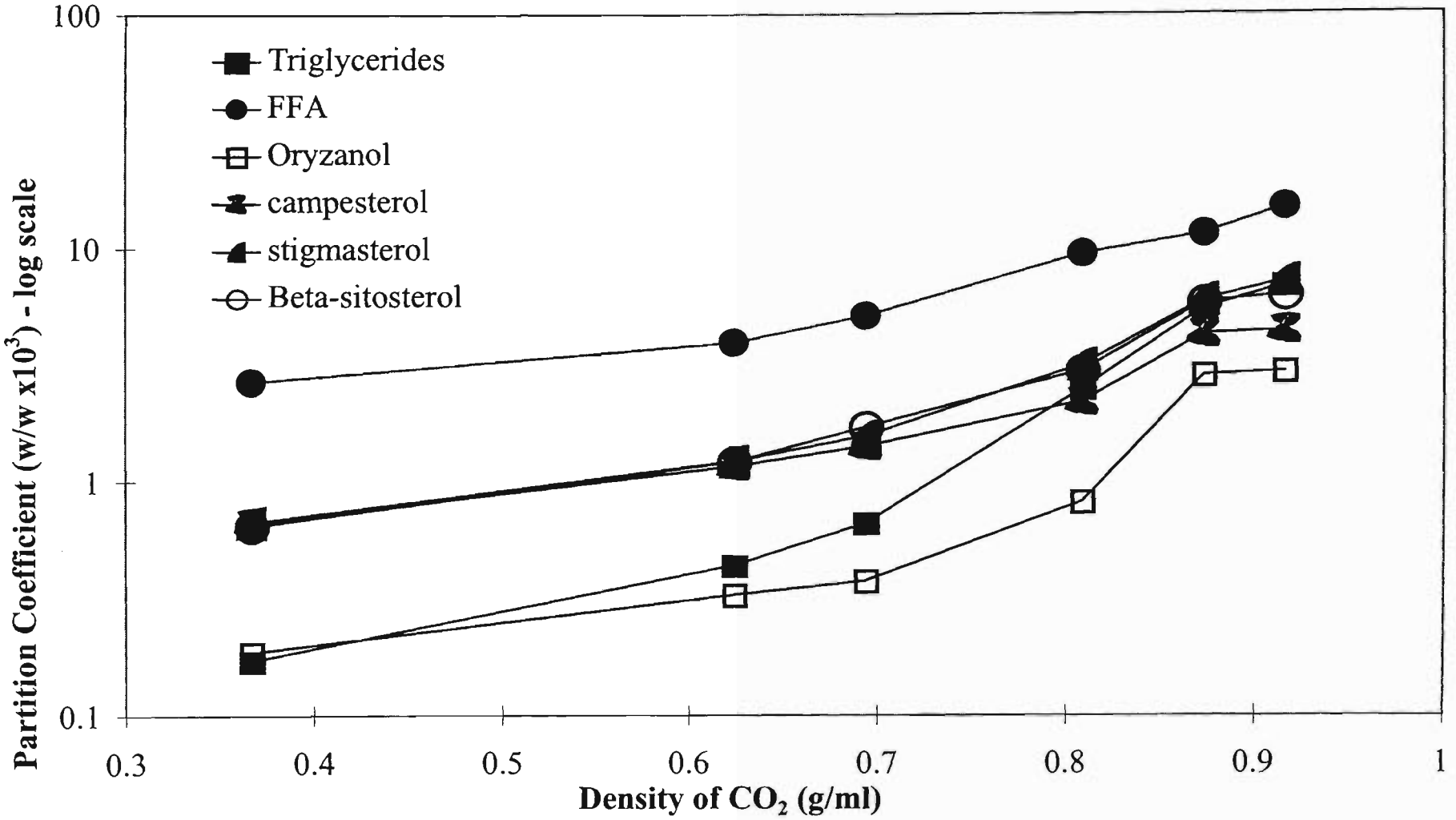


Figure 3.2. Partition coefficients for some components of rice bran oil at 40 °C plotted on a logarithmic scale. Single points represent the mean values of data from duplicated experiments.

The use of density rather than pressure as the independent variable simplifies the understanding of the temperature effect. This was approached in a similar way by Schneider et al. (1997).

This approach has also been taken by del Valle and Aguilera (1988) who measured and compiled the solubility data of vegetable oil in SC-CO_2 over a range of temperatures from 20 °C to 80 °C and plotted them on a logarithmic scale against CO_2 density to show a family of parallel straight lines. The solubility isotherms of canola oil in CO_2 as a function of CO_2 density at temperatures from 25 °C to 70 °C measured by Fattori et al. (1988) displayed 4 parallel curves. Maheshwari et al. (1992) reported the predicted solubilities of 5 free fatty acids as a function of temperature and density of SC-CO_2. When plotted on a logarithmic scale these appeared as a group of parallel straight lines in all cases. The data plotted in Figures 3.3 and 3.4 of the present study are consistent with the partition coefficient isotherms being families of parallel curves. For comparison, Fattori et al. (1988) also plotted their data using pressure as the independent variable and showed that the isotherms exhibited crossover points. Yun et al. (1991) reported that solubility curves of cholesterol in CO_2 plotted on a logarithmic scale versus CO_2 density showed a parallel linear trend of data at temperatures from 40 °C to 60 °C. Examination of the data points of Figure 3.3 of the present study indicates a complex relationship between isotherms if they were plotted against pressure. The use of density as the independent variable is therefore recommended for studies of isotherms in supercritical fluids.

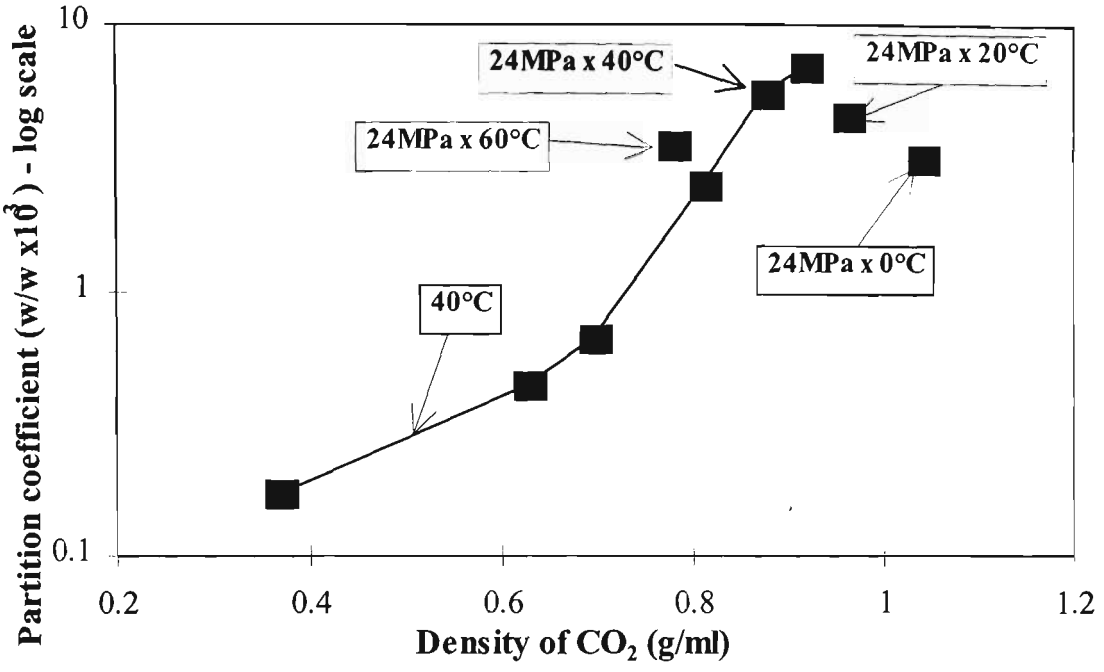


Fig. 3.3. Partition coefficients of triglycerides in rice bran oil plotted on a logarithmic scale. Single points represent the mean values of data from duplicated experiments.

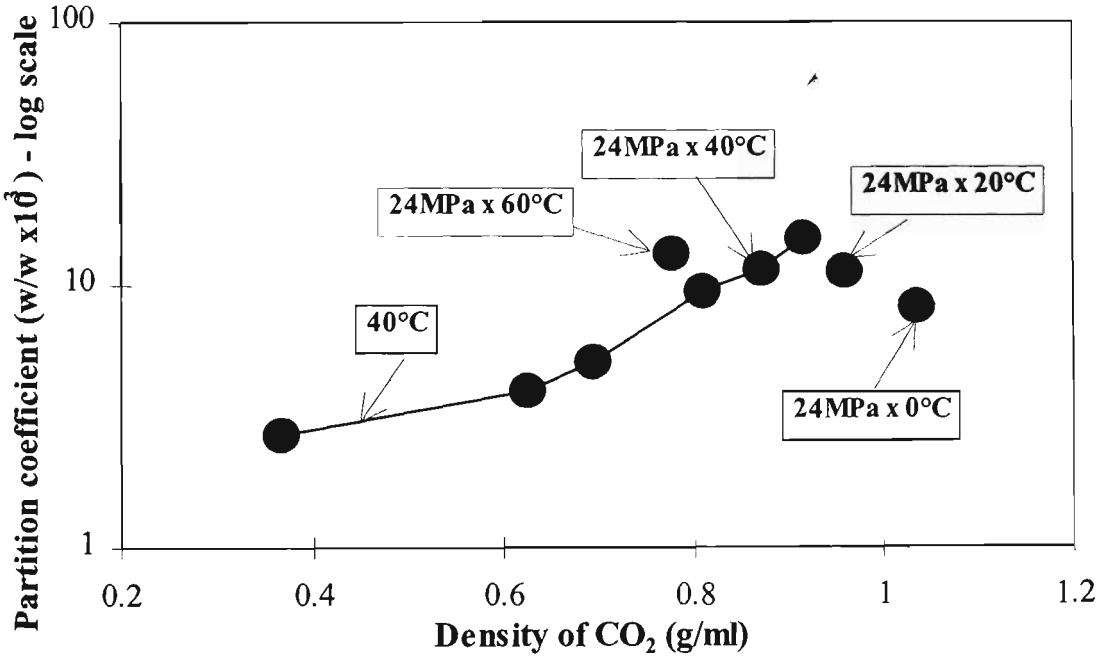


Figure 3.4. Partition coefficients of FFA in rice bran oil plotted on a logarithmic scale. Single points represent the mean values of data from duplicated experiments.

3.4.4 Partition coefficients and solubilities.

Any data on total solubility of a relatively homogeneous oil mixture carries the assumption that the measured solute is present in the oil phase at 1000 g/kg. Thus, if solubility data are expressed in g solute /kg CO_2, they can be converted to partition coefficients for comparison with the present results by dividing by 1000. A similar treatment of the connection between measured solubilities and partition coefficients was described by Bamberger et al. (1988) using partition coefficients based on mole fractions. Table 3.4 compares the present results with those from the literature on vegetable oil solubilities. The partition coefficients for triglycerides measured in this chapter and previous chapter can thus be directly related to the extensive literature on the solubility of vegetable oils (mixtures of triglycerides) in dense CO_2. (Brunetti et al., 1989; Eggers et al., 1985; Lee et al., 1986; Stahl et al., 1980).

Table3.4 Comparison of Partition Coefficients of Triglycerides with Solubility of Vegetable Oil

CO_2 density (g/ml)	0.6941	0.7767	0.8087	0.8406	0.8732	0.9159
temperature (°C)	40	60	40	40	40	40
pressure (MPa)	11.2	24.1	17.2	20.0	24.1	31.0
partition coefficient of triglycerides ($x10^3$)	0.66[a]	3.52[a](3.1[e])	2.50[a]		5.52[a]	6.93[a]
solubility of vegetable oil and triolein (g/kg CO_2)	0.6[b]	3.1[e]	2.0[b]	2.9[c](3.1[d])	5.0[b]	7.2[b]

[a] This work.

[b] Fattori et al., 1988 (Figure 5 for canola oil).

[c, d] Stahl et al., 1980 (Figure 4 for sunflower oil and Figure 5 for soybean oil).

[e] Nilsson et al., 1991, solubility and partition coefficient of triolein.

3.5 Conclusion

Fractionation removed almost all water and reduced the FFA concentration in raffinate by up to 50 % compared to the original oil. Under conditions favouring FFA removal, α-tocopherol concentration in the raffinate was not reduced by fractionation, but the sterol concentration was reduced. Oryzanol concentration was increased under these same conditions. Under the flow rate conditions studied (3.5 kg CO_2/h), the fractionations could be described by equilibria between oil and CO_2 phases.

Chapter 4

Assessment of Adsorbents for Refining Orange Oil

4.1 Introduction

As mentioned in Section 1.7.5, investigations into the use of adsorbents for refining citrus oil have been carried out for about half a century and various preferred adsorbents have been reported. Most research has utilised adsorbents which selectively adsorb the polar compounds. Rice starch was reported to be the best edible adsorbent of a range of tested edible adsorbents (Lund and Coleman, 1977). The adsorbed oil was equivalent to 27 - fold concentrated orange oil, but the capacity of the adsorbent for polar orange oil components was only 34% of the capacity of the inorganic adsorbent Florisil.

An alternative approach has been to adsorb the non-polar compounds. A styrenic co-polymer, Kastel S 112 (a nonpolar co-polymer) was selected to adsorb terpene compounds from essential lemon oil and allow the oxygenated compounds to be eluted first with 70% aqueous ethanol (Tateo, 1981). Terpene compounds were subsequently eluted with 95% aqueous ethanol. However, this approach is at an economic disadvantage relative to the former because of the relatively large proportion of non-polar compounds (more than 96%) in the essential lemon oil used. This factor alone would result in the use of twenty-four times more adsorbent than would be required to adsorb the polar compounds.

Silica gel has been demonstrated to refine citrus oil by adsorbing oxygenated compounds. In these procedures the terpene compounds were removed with the aid of a non-polar solvent and concentrated oxygenated compounds were later eluted by more polar organic solvents or supercritical or liquid carbon dioxide (Kirchner and Miller, 1952; Braverman and Solomiansky, 1957; Ferrer and Matthews, 1987; Tzamtzis, et al., 1990; Yamauchi and Saito, 1990; Chouchi, et al., 1994; Meireles and Nikolov, 1994; Barth, et al., 1994; Dugo, et al., 1995; Chouchi, et al., 1995; Sato, et al., 1995). Many different loading ratios of oil to adsorbent have been reported, including 0.003% (Dugo, et al., 1995), 10% (Chouchi, et al., 1995), 20% (Yamauchi and Saito, 1990; Chouchi, et al., 1994), 20-50% (Barth, et al., 1994), 73.5% (Kirchner and Miller, 1952), 2.7 times, or 270%, (Braverman and Solomiansky), 4.4 times (Ferrer and Matthews, 1987) and 6-8 times (Tzamtzis, et al., 1990).

The adsorptive capacity of silica gel for orange oil has been compared with other adsorbents (Braverman and Solomiansky, 1957). A mixture of alumina and kieselguhr had the same adsorptive capacity as silica gel, based on the ratio of volume of oil adsorbed to total volume of oil stripped of oxygenated compounds. For both adsorbents the ratio was 1:6 (Braverman and Solomiansky, 1957). On a weight basis, the adsorptive capacity of silica gel was 3.2 g oil/ g adsorbent. Ferrer and Matthews (1987) found that the adsorptive capacity of Florisil was 2.2 g oil/ g adsorbent, calculated as the maximum amount of oil added onto a column of the adsorbent that gave a negative test for aldehydes in the eluate. For silica gel a loading ratio of oil to silica gel of 4.4 times was deemed the maximum adsorptive capacity. Tzamtzis et al. (1990) reported that 1.4 kg of silica gel could strip oxygenated compounds from 11.0

kg of orange oil, which corresponded to a loading ratio (by weight) of 7.9. Yamauchi and Saito (1990) showed that an advantage of using a lower oil loading ratio such as. 20%, was the ability to separate components in lemon peel oil into different groups. In their work, a column filled with silica gel (50 mm bed height x 7.2 mm bed diameter) was eluted semi - preparatively with supercritical fluid to obtain four fractions from lemon peel oil as follows:-

fraction 1 was characterised as only terpene compounds

fraction 2 was composed largely (89%) of neryl acetate and geranyl acetate

fraction 3 was composed almost entirely (98%) of alcohols and aldehydes and

fraction 4 contained mostly non - volatile compounds.

Indeed, the loading ratio of oil to adsorbent to give a good separation is not a well defined amount. Because the optimum loading ratio is principally determined by the capacity of the adsorbent for oxygenated compounds, the optimum loading ratio can be expected to be inversely proportional to the content of oxygenated compounds in the feed oil. According to one report the ratio of column length to diameter also affects the optimum loading ratio (Braverman and Solomiansky, 1957) whereas Tzamtzis et al. (1990) reported that the separation achieved at a fixed loading ratio was not affected by the ratio of length to diameter in the range studied. Furthermore the solvents used to subsequently elute the adsorbate can affect the degree of separation achieved by the process as a whole, and therefore affect the optimum loading ratio. Finally, the optimum loading ratio will depend on the level of separation required, which is determined by commercial considerations.

In summary, it appears that there is a need for more detailed comparisons of adsorptive capacities of adsorbents for individual components of orange oil, and for more detailed monitoring of the breakthrough curves of the various components. "Breakthrough" of a compound is the first appearance of that compound in the effluent from an adsorbent bed or column. The breakthrough curve, in its simplest form, is a plot of the concentration of the compound in the effluent from a column versus time, from the initial concentration of zero until the effluent concentration is eventually the same as the concentration of the feed material. Breakthrough data can also be plotted in other forms which allow comparison of runs using different amounts of adsorbent, as was done in the present work. The breakthrough curves presented in this work plot the accumulated recovery of compounds of interest versus the amount of oil passed, normalised for the amount of adsorbent used. This method of plotting the breakthrough curves is discussed further in Section 4.4.4.

Physico-chemical characteristics required of adsorbents for orange oil fractionation were considered to be:-

1 Insoluble in orange oil or dense CO_2

2 Available as a granular solid suitable for column packing

3 Able to adsorb the oxygenated compounds of orange oil in preference to the terpene compounds

4 Of negligible catalytic activity towards the oxygenated compounds of orange oil.

The aims of the present work were:-

(I) to briefly compare some materials satisfying criteria 1 and 2 for their adsorptive capacity for the more valuable components of orange oil;

(II) to measure the breakthrough curves of adsorbents to identify those with the most potential for use in further work.

In the present work, fourteen materials were screened for significant adsorptive capacity for orange oil. The four adsorbents which showed promise were then further assessed by measuring batch adsorption and packed bed (column) breakthrough curves.

4.2 Materials

The sources and purities of the various materials screened are given in Table 4.1. Table 4.2 lists the purity, particle size and moisture of the four adsorbents subjected to a more detailed quantitative assessment. Cold-pressed navel orange oil ("Auroma" brand, batch Ausil 21062) was from *Citrus sinensis*. The oil was 100% essential oil and had a specific gravity of 0.8124 g/mL.

Table 4.1 Source and purity of materials screened*

Substances	Source	Purity
Celite (a form of diatomaceous earth)	Aldrich	Filter agent
Charcoal	Ajax	Activated, granular, Technical
Activated carbon	Aldrich	G- 60, 100 mesh, powder
Activated celite diatomite	Filter Aid Celite Corporation	Sorbo - cel R
β- cyclodextrin	Sigma	Tissue culture media and reagent
Cellulose powder	Sigma	High purity for partition chromatography
Maltose	Serva	Research grade
Sodium alginate	Ajax	Laboratory chemical
casein	Sigma	High protein from bovine milk
casein sodium salt	Sigma	From bovine milk

* The other 4 screened adsorbents are described in table 4.2, below.

Table 4.2 Particle size and purity of tested adsorbents

	Silica Gel 60 (Merck)	Silica Gel 60 (Merck)	Aluminium Oxide 90, activity I, neutral (Merck)	Florisil (Merck) - a magnesia silica gel
particle size	35-70 mesh (0.200 - 0.500 mm)	70-230 mesh (0.063 - 0.200 mm)	70 - 230 mesh (0.063 - 0.200 mm)	60-100 mesh (0.150-0.250 mm)
purity	for column chromatography	for column chromatography	for column chromatography	for column chromatography
moisture	3.48% (103°C - o'night) 3.98% (150°C - o'night)	2.99 % (103°C - o'night) 3.44% (150°C - o'night)	not measurable with oven method	0.73% (103°C - o'night) 0.86% (150°C - o'night)
surface area (approx.)	490 m^2/g *	490 m^2/g *	120 m^2/g	300 m^2/g

* Note that the surface area of all these adsorbents is almost entirely associated with internal pores. The external surface area of the particles makes no measureable contribution to the total surface area and consequently the specific surface areas are the same for these two preparations of silica gel with different particle sizes.

4.3 Experimental methods

4.3.1 Preliminary screening of adsorbents by "breakthrough" of flavour compounds

Cold-pressed orange oil was filtered before experimentation. Each adsorbent material (1.0 g) was placed into a vertically mounted Pasteur pipette to form a small column, and orange oil was manually added drop-wise to the top of the column. The first few drops of oil emerging from the bottom of the pipette were collected and analysed by

GC. The gas chromatogram was compared with that of the feed oil, and if the concentration of the major flavour components - decanal and linalool - in the collected orange oil was not reduced significantly, it was concluded that the material either had a low affinity for these components or that the adsorption process was very slow. In either case the material was considered to have little potential as an adsorbent and was not investigated further. When no decanal or linalool was detected in the collected oil, it was concluded that the material under test had a high affinity for those compounds and a rapid rate of adsorption. In these cases the material had good potential as an adsorbent and was further assessed. Four materials were eventually chosen for further assessment:- Silica Gel 60 (35-70 mesh), Silica Gel 60 (70-230 mesh), Florisil and Aluminium Oxide 90.

4.3.2 Characterisation of selected adsorbents

The four adsorbents were used without drying in order to keep them in a less catalytically active state. This is most important for silica gel (Ferrer and Matthews, 1987). The abilities of Silica Gel 60 (35-70 mesh), Silica Gel 60 (70-230 mesh), Aluminium Oxide 90 and Florisil to adsorb flavour components from orange oil were tested with batch and packed bed (chromatography) methods.

4.3.2.1. Batch adsorption.

Each adsorbent (about 5 g weighed to 2 decimal places in duplicate) was placed in an Erlenmeyer flask (125 mL) with stopper. Navel oil (20 mL, weighed in grams to 2 decimal places) was added to the flask. The flask was stoppered and shaken for two

minutes every half hour for 3 hours at approximately 24°C. The oil was then decanted from the flask, and weighed. The feed navel oil and the collected oil from each flask were analysed by GC.

4.3.2.2. Packed bed adsorption and breakthrough of oil constituents.

a. Drop-wise feed of oil (preliminary experiments)

Silica Gel 60, 35-70 mesh (6.39 g, bed height 7.2 cm) was loaded into a chromatography column [17 mm (i.d.) x 20 cm height] by gravity and navel orange oil was added drop-wise to the top of the column by burette. The stripped oil emerging from the bottom of the column was collected into fractions and analysed by GC. The procedure was repeated for Silica Gel 60, 35 -70 mesh (6.59g, bed height 7.7 cm), as a duplicate of the first experiment, and for Silica Gel 60, 70 - 230 mesh (5.20 g, bed height 5.0 cm), Aluminium Oxide 90 (5.39 g, bed height 2.8 cm), and Florisil (5.64 g, bed height 5.5 cm, and 5.33 g, bed height 5.3 cm). The number of fractions collected in each experiment varied from 11 to 16.

b. Pumped oil feed

The drop-wise oil feed described above could not be carried out reproducibly but provided sufficient information to reduce the number of promising adsorbents to two; aluminium oxide and Silica Gel 60 (70-230 mesh). A series of experiments similar to

the drop-wise feed experiments was conducted with a known pumped oil feed rate of 0.68 and 0.96 g oil/ min.

In order to further reduce experimental error, larger quantities of adsorbent and oil were used. Silica Gel 60, 70-230 mesh (14.95 g, bed height 14.5 cm, and 14.82 g, bed height 14.0 cm) or Aluminium Oxide 90 (14.70 g, bed height 7.8 cm, and 14.84 g, bed height 7.4 cm) was packed into a chromatography column [17 mm (i.d.) x 20 cm] by gravity. Navel orange oil was pumped by HPLC pump (BIO-RAD ECONO PUMP) onto the top of the column at a fixed rate. The stripped oil emerging from the bottom of the column was collected as fractions, between 11 and 15 in number. These fractions and the feed were analysed by GC - MS and GC or by GC only.

4.3.3 Analysis methods (GC-MS and GC analysis)

4.3.3.1. Gas Chromatography

GC analyses were performed in a Shimadzu GC- 17A Gas Chromatograph equipped with a flame ionisation detector and AOC - 17 Auto injector. Separations were achieved on a J & W DB-5 capillary column (30 m x 0.32 mm I.D.). The helium flow was 1.5 mL/min at 60 °C and 50 KPa gauge pressure. The injector and detector temperatures were 220 °C and 270 °C respectively. Oven temperature programme was 60 °C to 240 °C at 3 °C/min., injection volume used was 0.2 μL (5% orange oil as a chloroform solution), and the split ratio was 60:1. A sufficient number of chromatographic analyses were run in duplicate to verify that the variation between

duplicates was less than 5%. The results were calculated on the basis of a normalisation method (GC peak area percentage) according to Wilson and Shaw (1980), Baaliouamer et al.(1988), Chouchi and Barth (1994) and Reverchon and Senatore (1994).

4.3.3.2. Gas Chromatography - Mass Spectrometry

A Varian GC 3400 with a split injection system was directly coupled to the input of a Varian Saturn GC-MS and mass spectra were produced using electron impact ionisation. A J & W DB-5 capillary column (30 m x 0.25 mm I.D) was used with a helium flow rate of 1 mL/min; injection volume of 0.1 μL (5% soln.). The GC oven temperature was increased from 60 °C to 240 °C at 3 °C/min (Adams, R. P., 1989). The other temperature settings used were injector 220 °C, manifold 220 °C and transfer line 255 °C. Mass spectrometer operating parameters were as shown in Table 4.3.

Components were identified by the GC-MS spectrum library (NIST90), the Terpene Library, and the elution order of terpene compounds on DB - 5 (Adams, R. P., 1989), and by comparing their retention times with some authentic standards (see Table 4.4 for standard detail).

Table 4.3. Mass spectrometer operating parameters .

Tune Parameter	Segment 1	Segment 2	Segment 3	Segment 4
AGC Scale Factor (%)	100	100	100	100
AGC RF Level (dac steps)	125	125	125	125
Fixed Ion Time (%)	100	100	100	100
Fixed RF level (dac steps)	50	60	60	60
Other Parameter				
Multiplier set voltage (volt)	2200			
Emission set current (u amps)	20			
A/M amplitude set voltage (volt)	4.5			
Target value	18000			
Mass scan range (amu)	35 - 300			
scan rate (second)	1			
Threshold	2			
Filament delay (second)	120			
Mass defect (mmu/100amu)	0			
Background mass (amu)	34			
Calibration gas	FC - 43			

Table 4.4. Source and purity of analysis standards used

Compound	Source	Purity
γ-terpinene	Sigma	—
β- myrcene	Sigma	90%
decanal	Sigma	99%
citral	Sigma	—
n-octanol	Sigma	99%
linalool	Sigma	95-97%
β-caryophyllene	Sigma	—
n- octanal	Sigma	—
α-terpinene	Sigma	89%
citronellal	Sigma	85-90%
α-terpineol	Sigma	95%
limonene	Sigma	—
α-pinene	Sigma	—

4.4 Results and discussion

4.4.1 Major components of cold pressed Navel orange oil

The major components of "Auroma" navel orange oil identified in the present work are listed in Table 4.5 and the gas chromatogram of the oil is shown in Fig 4.1a.

Terpenes accounted for more than 97.65% (peak area percent) of the oil constituents and the major terpene, d-limonene, and the second major terpene, β-myrcene, comprised 96.40 % of the oil constituents. Linalool and decanal were the major alcohol and aldehyde respectively, which is in agreement with other reports (Shaw and Coleman, 1974; Shaw, 1979; Vora, 1983; Temelli, 1988; Pino, 1992). Six of the identified substances are classified as oxidised limonene based on a number of reports (Proctor and Kenyon, 1949; Buckholz and Daun, 1978; Shaw, 1979; Temelli, 1988). Although α-terpineol is considered as a degradation product of d-limonene (Shaw, 1979; Temelli et al., 1988; Tseng et al., 1991), it is still listed as an alcohol in this table.

Table 4.5 Amount (area%) of major components of cold pressed Auroma navel orange oil and fractions from silica gel, as determined in the present work (variation between duplicate injections was less than 5%)

Peak number	Compound	Cold - pressed oil	fraction 1	fraction 12
	Monoterpenes	***97.52***	***99.52***	***96.90***
1	α - Thujene	<0.01	nd	nd
2	α - Pinene	0.46	0.59	0.46
3	Sabinene	0.46	0.31	0.10
4	β - Pinene	0.03	0.03	0.03
5	β - Myrcene	1.66	1.71	1.70
7	α - Phellandrene	0.03	0.03	0.03
8	Carene -3	0.09	0.10	0.09
9	α - Terpenene	<0.01	nd	nd
10	p - Cymene	<0.01	nd	nd
11	Limonene	94.74	96.69	94.43
12	Ocimene (trans)	0.03	0.03	0.03
13	γ-terpinene	<0.01	0.02	0.02
15	Terpinolene	0.02	0.02	0.02
	Aliphatic aldehydes	***0.75***	***nd***	***0.07***
6	n - Octanal	0.22	nd	0.02
17	n - Nonanal	0.04	nd	0.01
22	Decanal	0.38	nd	0.04
34	Dodecanal	0.11	nd	nd
	Terpene aldehydes	***0.23***	***nd***	***0.01***
20	Citronellal	0.05	nd	0.01
26	Neral	0.02	nd	nd
28	Geranial	0.03	nd	nd
29	Perillaldehyde	0.06	nd	nd
41	β– Sinensal	0.03	nd	nd
42	α- Sinensal	0.04	nd	nd
	Alcohols	***0.58***	***nd***	***nd***
14	n- Octanol	0.12	nd	nd
16	Linalool	0.40	nd	nd
21	α - Terpineol	0.06	nd	nd
	Oxidized limonene compounds	***0.33***	***nd***	***0.02***
18	Limonene oxide (cis)	0.08	nd	nd
19	Limonene oxide (trans)	0.05	nd	nd
23	Dehydro carveol (iso)	0.01	nd	0.02
24	Carveol (trans)	0.05	nd	nd
25	Carveol (cis)	0.05	nd	nd
27	Carvone	0.08	nd	nd
	Esters	***0.02***	nd	***0.02***
30	Neryl acetate	0.01	nd	0.02
32	Geranyl acetate	0.01	nd	nd
	Sesquiterpenes	***0.13***	***0.18***	***0.12***
31	α - Copaene	0.01	0.02	nd
33	β - Cubebene	0.01	0.01	nd
35	β - Caryophyllene	0.01	0.02	0.01
36	β - Farnesene	0.02	0.02	0.02
37	Germacrene -D	0.01	0.01	0.01
38	Valencene	0.03	0.05	0.04
39	α -Farnesene	0.02	0.02	0.02
40	δ - Cadinene	0.02	0.03	0.02

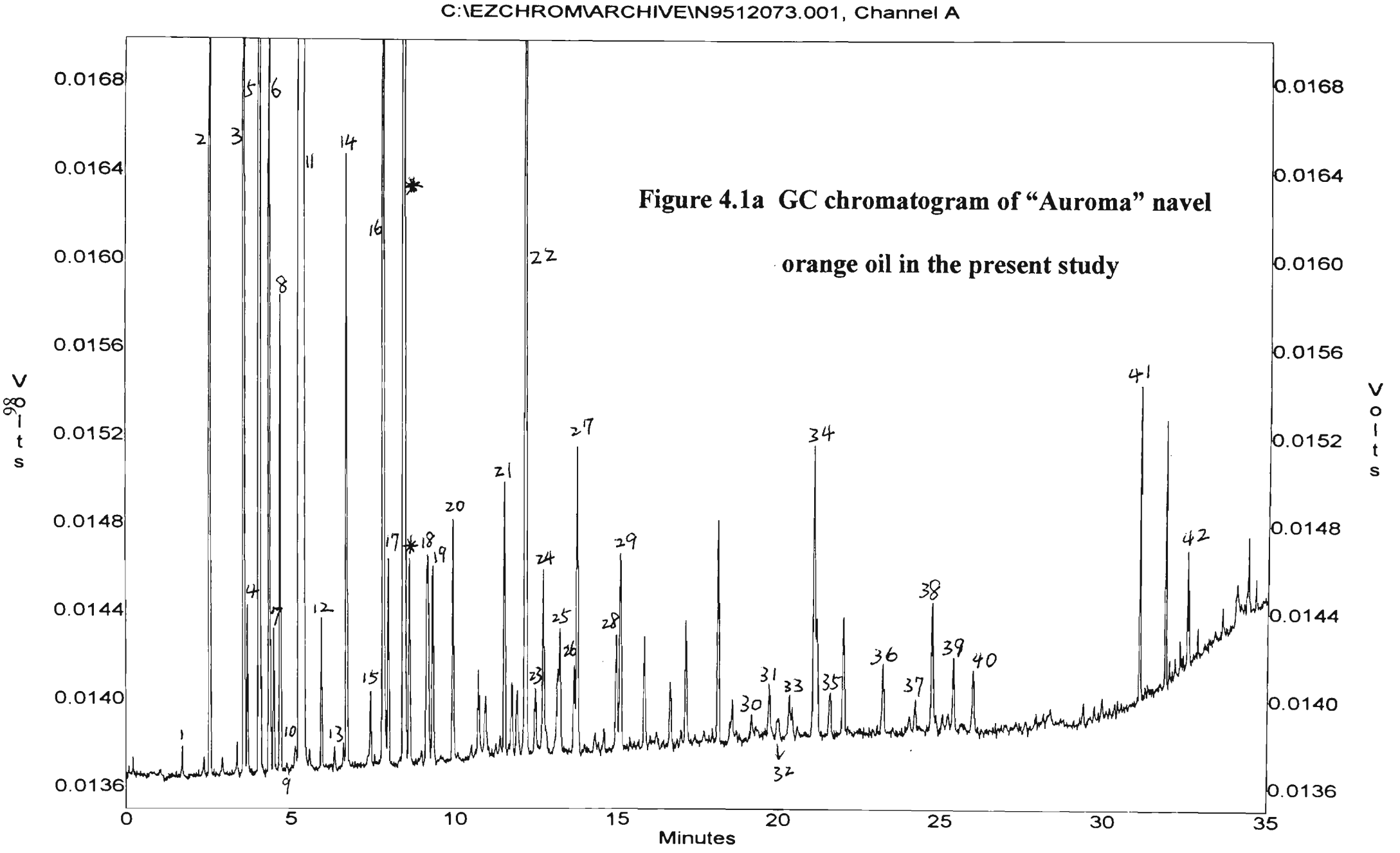


Figure 4.1a GC chromatogram of "Auroma" navel orange oil in the present study

4.4.2 Relative affinities of adsorbent materials for oxygenated components

Decanal and linalool were found in the first few drops of orange oil passed through celite, charcoal, activated carbon, activated celite diatomite, β-cyclodextrin, CF 11 cellulose powder, maltose, sodium alginate, casein and casein salt. Therefore, these materials were not further investigated. Celite was reported to adsorb aldehydes much more strongly than monoterpenes when CO_2 was used as the solvent to desorb lemon essential oil (Dugo, 1995), but in this brief screening experiment celite did not show any ability to bind aldehydes in the presence of orange oil.

Four of the materials, Silica Gel 60 (35-70 mesh), Silica Gel 60 (70-230 mesh), Florisil, and Aluminium Oxide 90, stripped decanal and linalool from oil passing through them and were selected for further investigation.

4.4.3 Batch adsorption

Table 4.6 shows the adsorptive capacity of the four adsorbents measured by the batch method and expressed in five different units, each calculated as the amount of adsorbed component divided by the amount of adsorbent. Each value is the mean of duplicate measurements. The capacity for adsorption of decanal and linalool has not only been expressed as mg/g of adsorbent but also as gram of feed oil stripped per gram of adsorbent. The four adsorbents had similar apparent capacities to adsorb decanal, but for linalool there were differences, with Florisil having the lowest capacity while the two forms of silica gel had highest capacity.

Batch equilibration of oil with adsorbent does not necessarily demonstrate the maximum adsorptive capacity of the adsorbent, but merely shows one value on the adsorption isotherm, where adsorbed oxygenated compounds are in equilibrium with partially stripped oil. This is illustrated schematically in Figure 4.2 showing two adsorption isotherms described by the equation:

$q = \frac{ac}{1+bc}$, where q is the amount of compound adsorbed, c is its concentration in the oil phase, and a and b are constants (Wankat, 1987). For the purpose of the example the feed oil is assumed to have a concentration of oxygenated compounds of 1.0 and the partially stripped oil a concentration of 0.5. If the adsorbent has an adsorption isotherm like Type 1 (a = 1, b = 1), then batch measurement of adsorptive capacity greatly underestimates the maximum adsorptive capacity of the adsorbent for the feed oil. On the other hand, if the isotherm is like Type 2 (a = 3, b = 12), then batch measurement provides a good estimate of maximum adsorptive capacity.

By passing feed oil through a bed of adsorbent until the point of breakthrough of oxygenated compounds, most of the adsorbent is equilibrated with oil of initial composition, and the adsorption capacity of the bed is close to the maximum possible with that feed oil.

Table 4.6 Adsorptive capacity of the adsorbents

	Silica Gel 60 (35-70 mesh)	Silica Gel 60 (70-230 mesh)	Florisil	Aluminium Oxide 90
oil holding (**g oil/ g** adsorbent)	1.37	1.32	1.21	0.71
capacity of ads. for linalool (**mg/g** adsorbent)	8.60	9.03	5.57	7.06
capacity of ads. for decanal (**mg/g** adsorbent)	9.03	9.44	9.45	9.33
capacity of ads. for linalool (**g oil/g** adsorbent)	2.45	2.57	1.59	2.01
capacity of ads for decanal (**g oil/g** adsorbent)	2.74	2.86	2.87	2.83

Ads. Adsorbent

4.4.4 Breakthrough curves of four adsorbents

Figure 4.3 to Figure 4.8 show the breakthrough curves for the four adsorbents, as determined with the drop-wise feed of orange oil. As outlined in Section 4.1, the breakthrough curves are expressed with the x-axis representing the loading ratio of oil to adsorbent (weight basis). This is different from the practice of Chouchi (1995) and Sato (1995) in which the x-axis represented desorption time, but is similar to the practice of Tseng et al.(1991) in which the x-axis represented the volumetric loading ratio. The reason for using the loading ratio as the unit of the x-axis is to clearly describe adsorptive capacity more directly than using time, which is affected by several factors including the amount of adsorbent and oil, bed length, and diameter of bed.

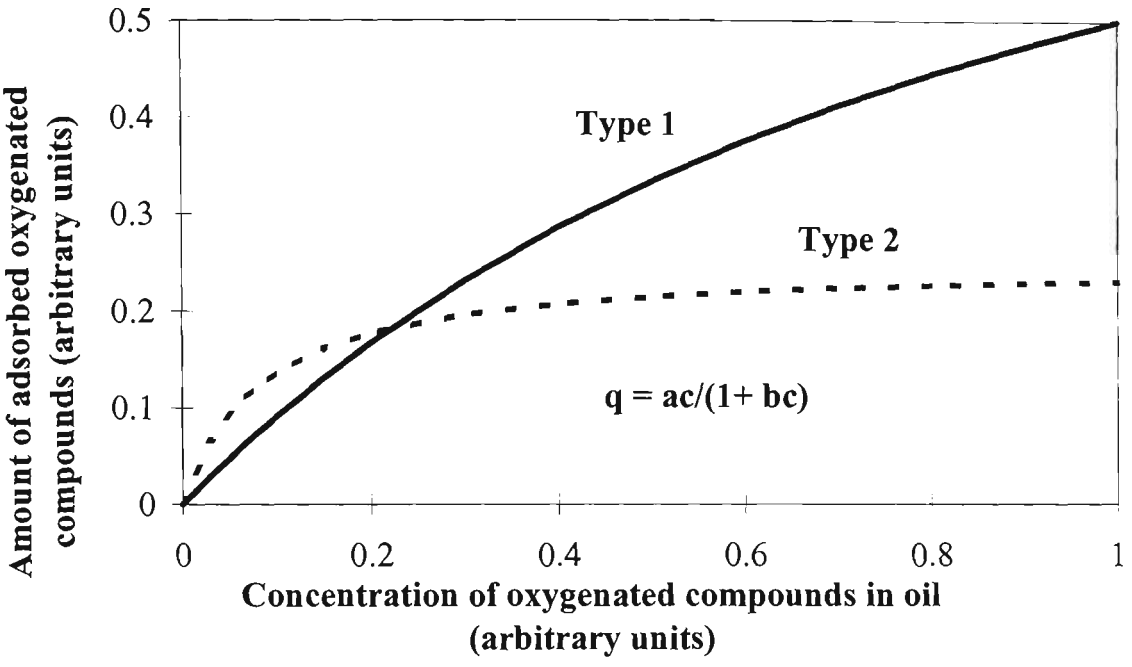


Fig. 4.2. Typical adsorption isotherms, q is the amount of compound adsorbed, c is its concentration in the oil phase; a and b are constants (Wankat, 1987).

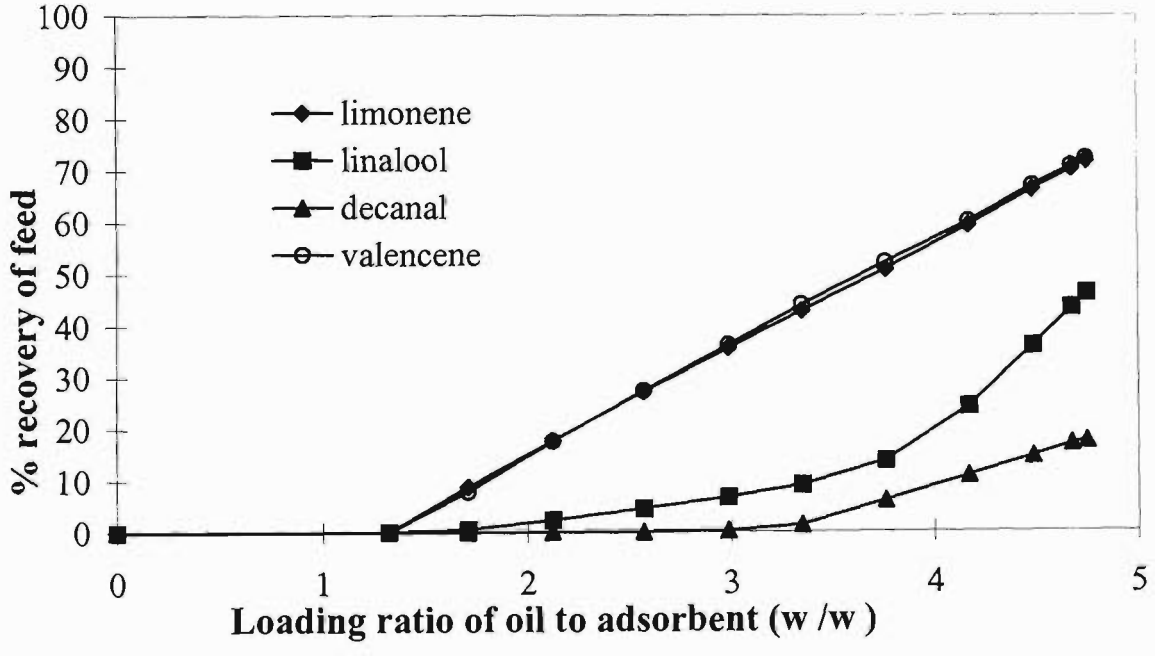


Fig. 4.3. Breakthrough curve of orange oil (25.33 g) on Florisil (5.33 g).

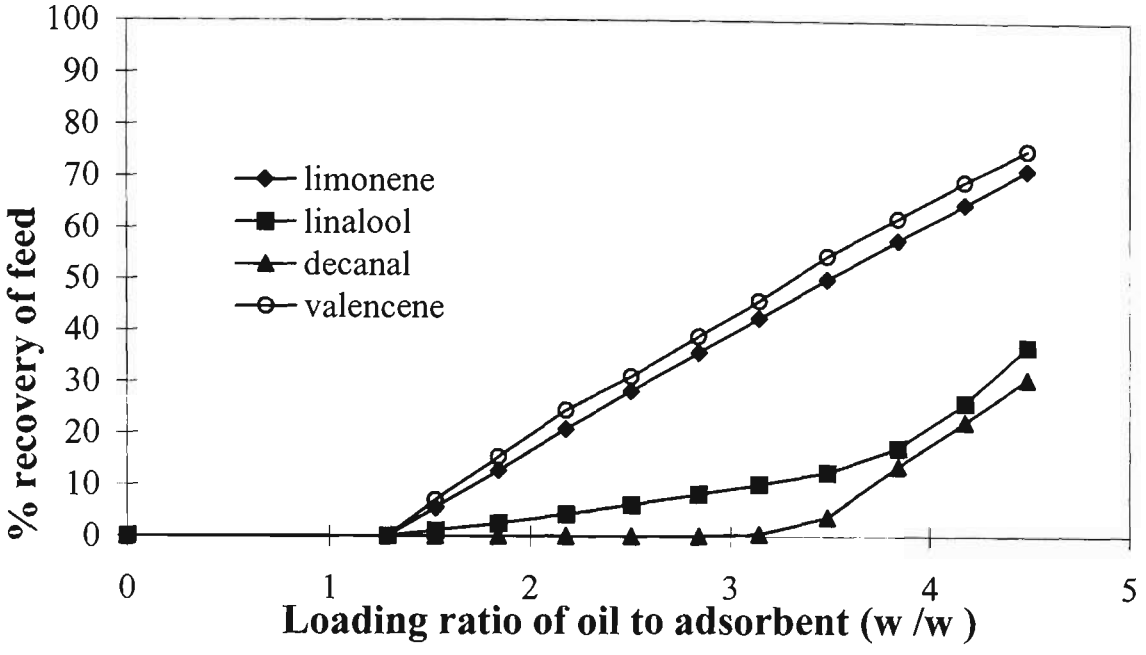


Fig. 4.4. Breakthrough curve of orange oil (25.33 g) on Florisil (5.64 g).

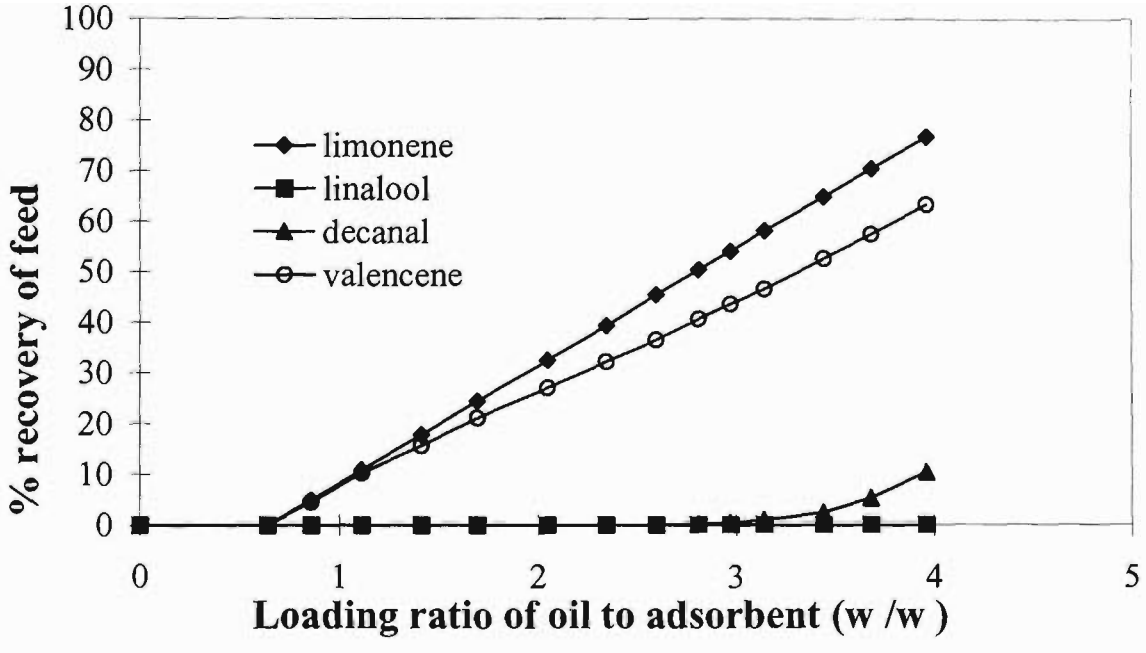


Fig. 4.5. Breakthrough curve of orange oil (25.33 g) on Silica gel (35-70 mesh, 6.39 g).

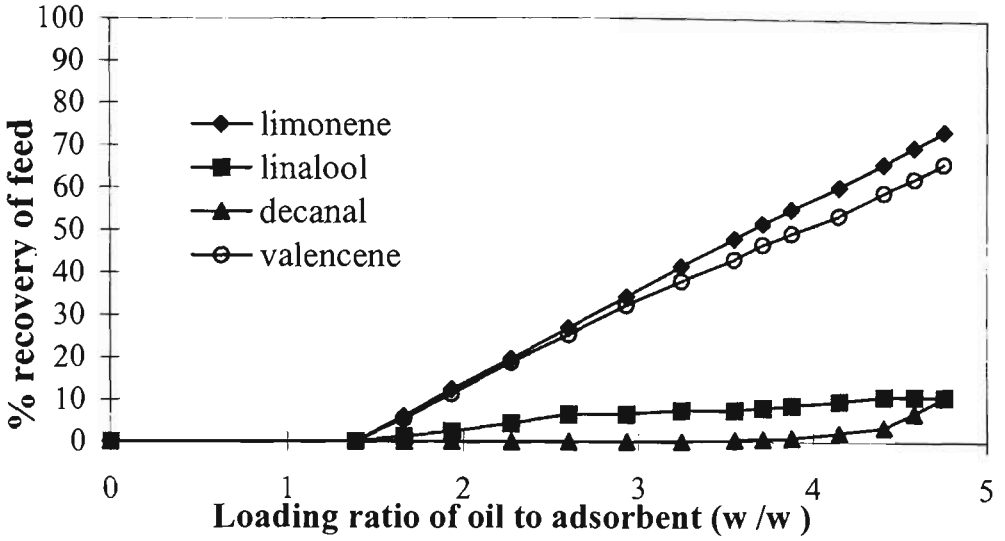


Fig. 4.6. Breakthrough curve of orange oil (31.32 g) on Silica gel (35-70 mesh, 6.59 g).

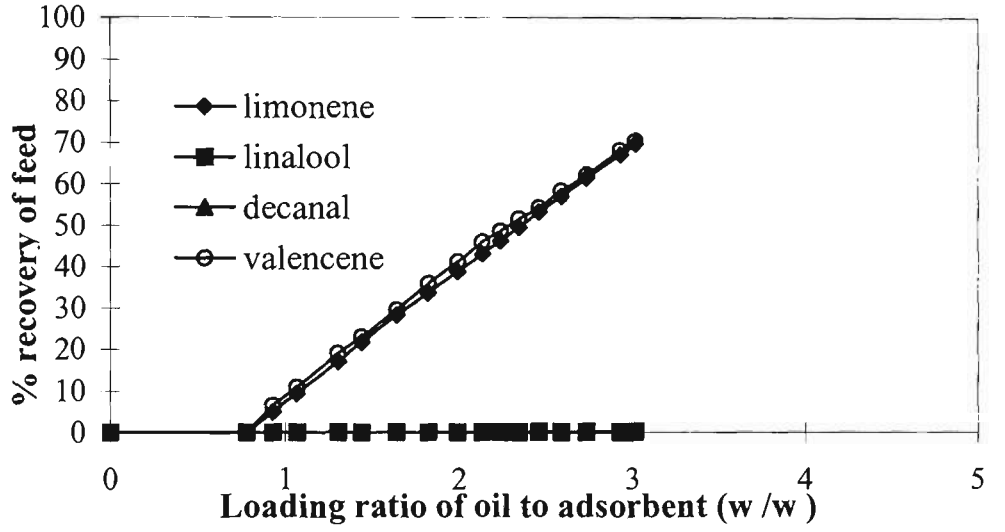


Fig. 4.7. Breakthrough curve of orange oil (16.25 g) on Aluminium Oxide (5.39 g). The two breakthrough curves of decanal and linalool overlap each other.

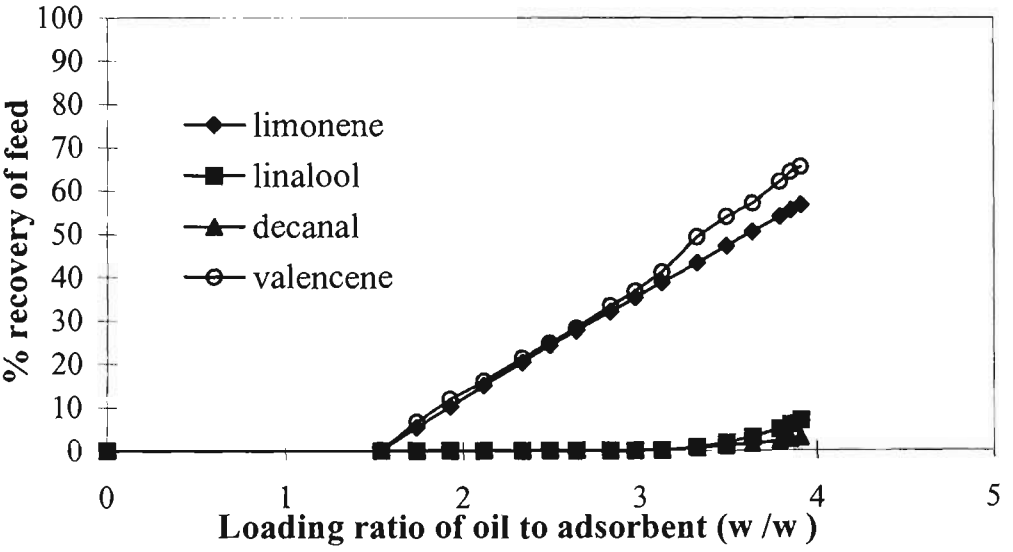


Fig. 4.8. Breakthrough curve of orange oil (20.31g) on Silica gel 60 (70-230 mesh, 5.20 g).

Under similar conditions, linalool broke through the Florisil bed earlier than it broke through either the Aluminium Oxide 90 or both forms of silica gel beds. In most cases decanal broke through the packed bed of adsorbent shortly after the linalool breakthrough. The exception was in one of the experiments with silica gel (Figure 4.6). The reason for this behaviour is unclear, but it would seem to indicate channelling in the bed together with a much slower rate of equilibration for linalool than for decanal. It is important to load the adsorbent into the column at a constant pressure; irregular filling can cause channelling in the column which reduces the chromatographic efficiency (Braverman et al.1957).

The present method of measuring the adsorptive capacity was different from the method reported by Ferrer and Matthews (1987) who passed different amounts of oil through the same weight of adsorbent and tested the effluent for total aldehyde by Schiff's test. However, both methods showed that the adsorptive capacity of silica gel was higher than that of Florisil. According to Ferrer and Matthews (1987), Florisil is made by co - precipitating silica and magnesia. This results in fewer free hydroxyl groups (active adsorbing sites) than occur on silica gel.

From the above experiments it was concluded that Silica Gel 60 and Aluminium Oxide 90 showed more promise than Florisil. There was no obvious difference between the two forms of silica gel in adsorption of oxygenated components, taking into account experimental error. Silica Gel 60 (70 - 230 mesh) was chosen for further study because its particle size was similar to adsorbents used in other studies (Kirchner and Miller, 1952; Ferrer and Matthews, 1987; Tzamtzis, et al., 1990).

4.4.5 Breakthrough curves of two adsorbents, with improved precision

In these tests a pump was used to load oil at a constant and reproducible speed and larger amounts of adsorbents and oil were used to reduce experimental error. The results are summarised in Figures 4.9-4.12. Under these conditions, it was shown that decanal and linalool broke through the aluminium oxide column much earlier than through the silica gel column.

Figure 4.13 is an enlargement of the graphical data of Figure 4.12, clearly showing the beginning of the breakthrough of decanal on silica gel at a loading of 5 g navel oil/ g adsorbent. The same data are shown in Table 4.5 where the fractions 1 and 12 represent the first and twelfth fractions emerging from the bottom of the Silica Gel at loading ratios of oil to adsorbent of about 1.3 and 5 respectively. The first fraction contained 99.7% terpenes and no oxygenated compounds. Fraction 12 contained 0.1% oxygenated compounds, whereas the feed navel oil contained 1.58% oxygenated compounds. Therefore fraction 12 marks the very beginning of breakthrough of oxygenated compounds at a loading of 5 g navel oil/ g adsorbent. Figure 4.1b and Figure 4.1c show the chromatograms for the fraction 1 and 12 respectively.

From these data the adsorptive capacity of Silica Gel 60 (70 - 230 mesh) was found to be approximately 5 g navel oil/ g adsorbent in the present work, which is similar to the value of 4.4 g oil/g silica gel reported by Ferrer and Matthews (1987). The difference could have arisen from the different measurement method, different feed oil composition, different ratio of column length to diameter, and different moisture

content of silica gel, or different batch of silica gel. In Figures 4.9 and 4.10, there is an apparent recovery of the sesquiterpene - valencene of greater than 100% of the feed, which is probably experimental error.

4.5 Conclusion

Fourteen materials were screened and the breakthrough curves of the four best adsorbents were measured. It was found that the adsorptive capacity of the materials was not very reproducible. This can be attributed to the effect of the speed of loading oil and/or the quality of packing of each column.

Silica Gel 60 (70-230 mesh) showed the greatest adsorptive capacity for oxygenated compounds in orange oil, 10% more than that reported by Ferrer and Matthews (1987). Consequently, Silica gel 60 (70 - 230 mesh) was chosen for the experiments of Chapter 5 which involved the desorption of oxygenated compounds with supercritical fluid carbon dioxide.

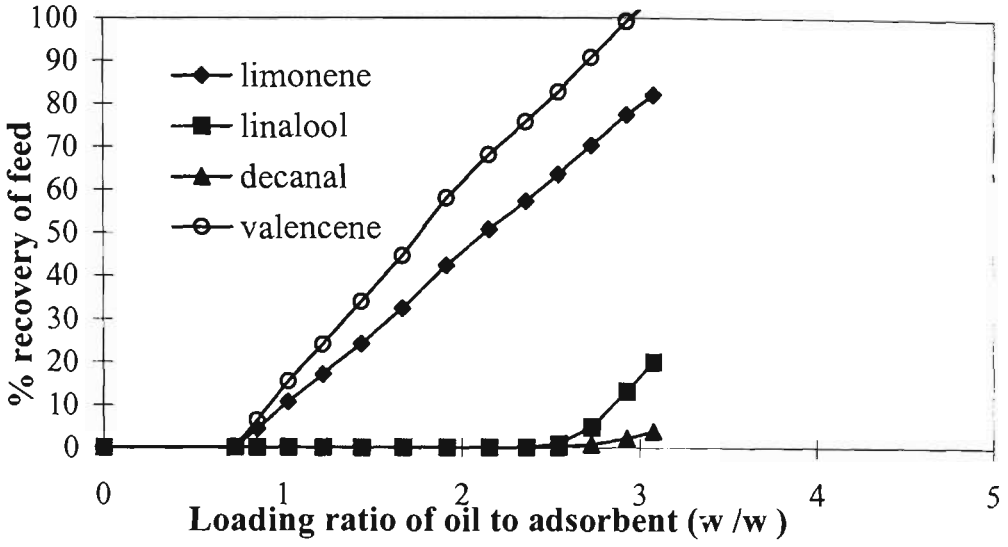


Fig 4.9. Breakthrough curve of orange oil (pumping - 0.96 g oil/min) on Aluminium Oxide (14.70 g).

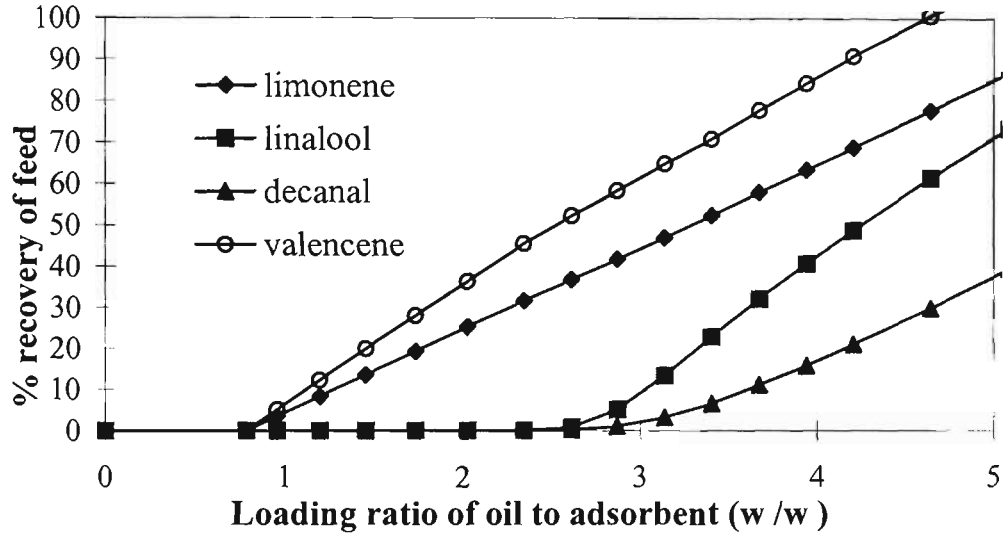


Fig. 4.10 Breakthrough curve of orange oil (pumping - 0.70g oil/min) on Aluminium Oxide (14.84g).

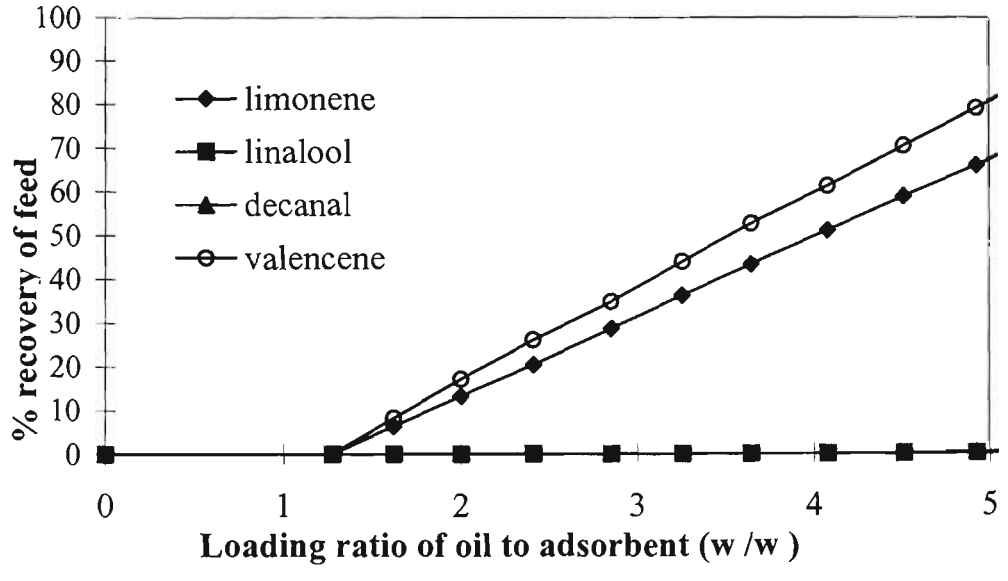


Fig. 4.11. Breakthrough curve of orange oil (pumping- 0.65 g/min) on Silica gel (70-230 mesh, 14.82 g). The two breakthrough curves of decanal and linalool overlap each other.

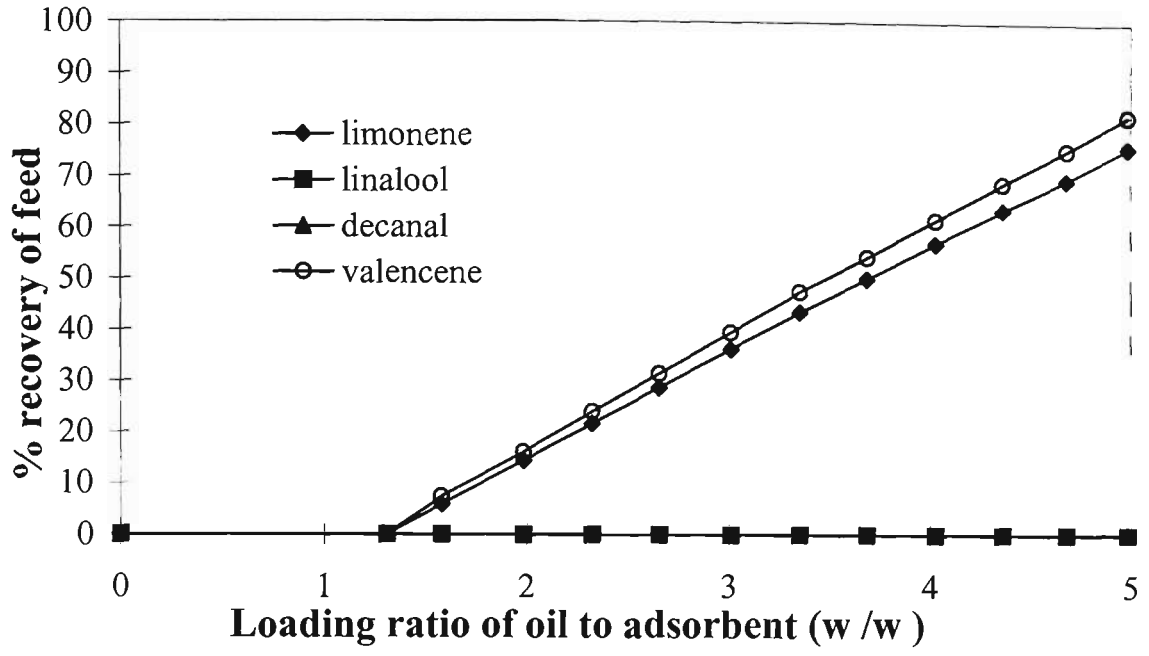


Fig. 4.12. Breakthrough curve of orange oil (pumping- 0.85g oil/min) on Silica gel (70-230 mesh, 14.95 g). The two curves of decanal and linalool overlap each other.

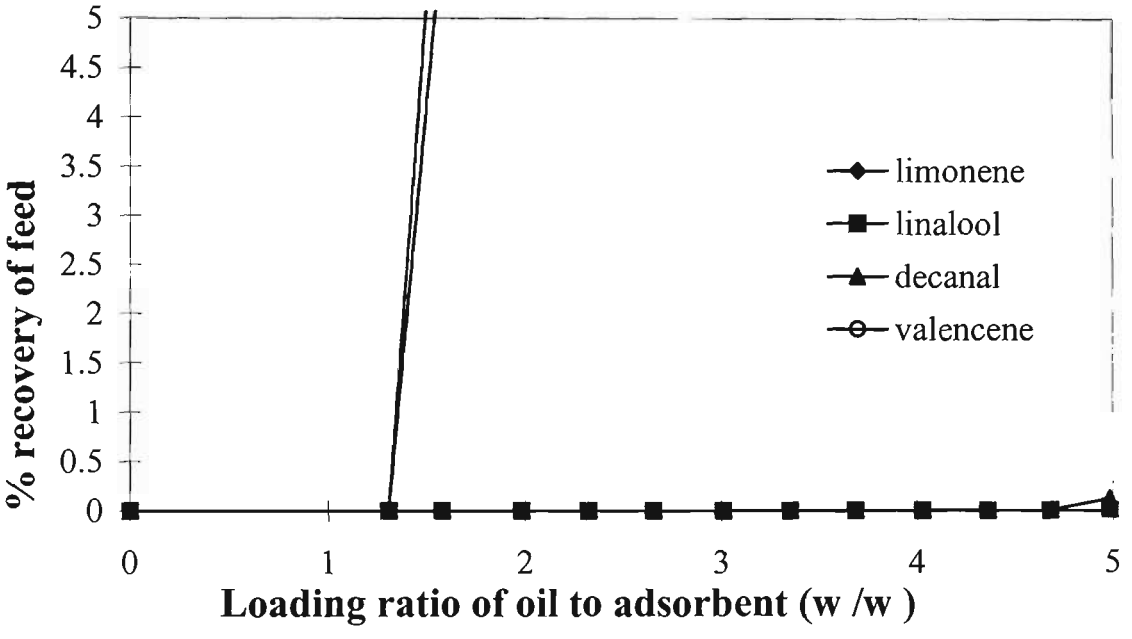


Fig.4.13. Breakthrough curve of orange oil (pumping- 0.85g oil/min) on Silica gel (70-230 mesh, 14.95 g), magnified, the two steeply rising lines represent breakthrough curves of limonene and valencene.

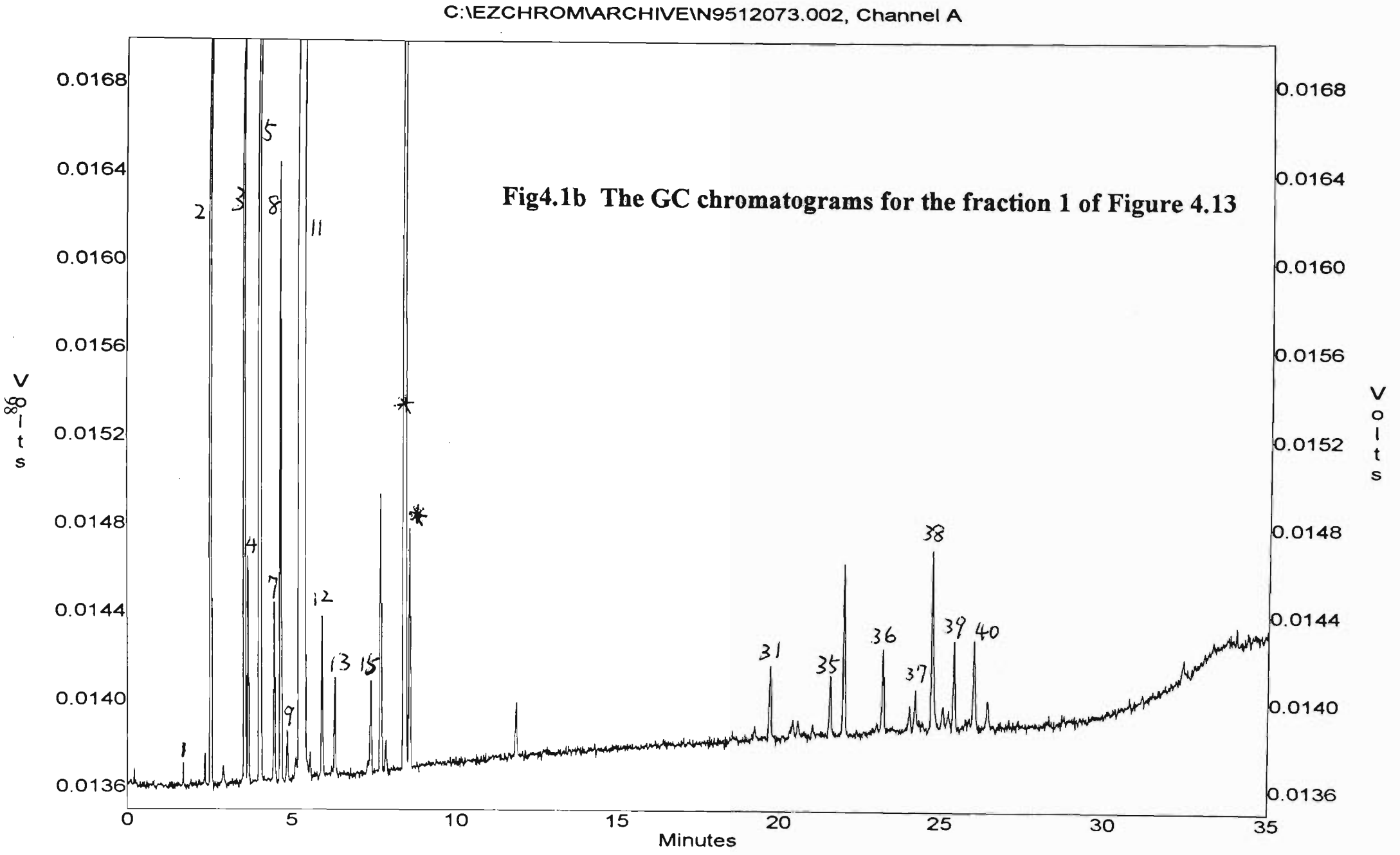


Fig4.1b The GC chromatograms for the fraction 1 of Figure 4.13

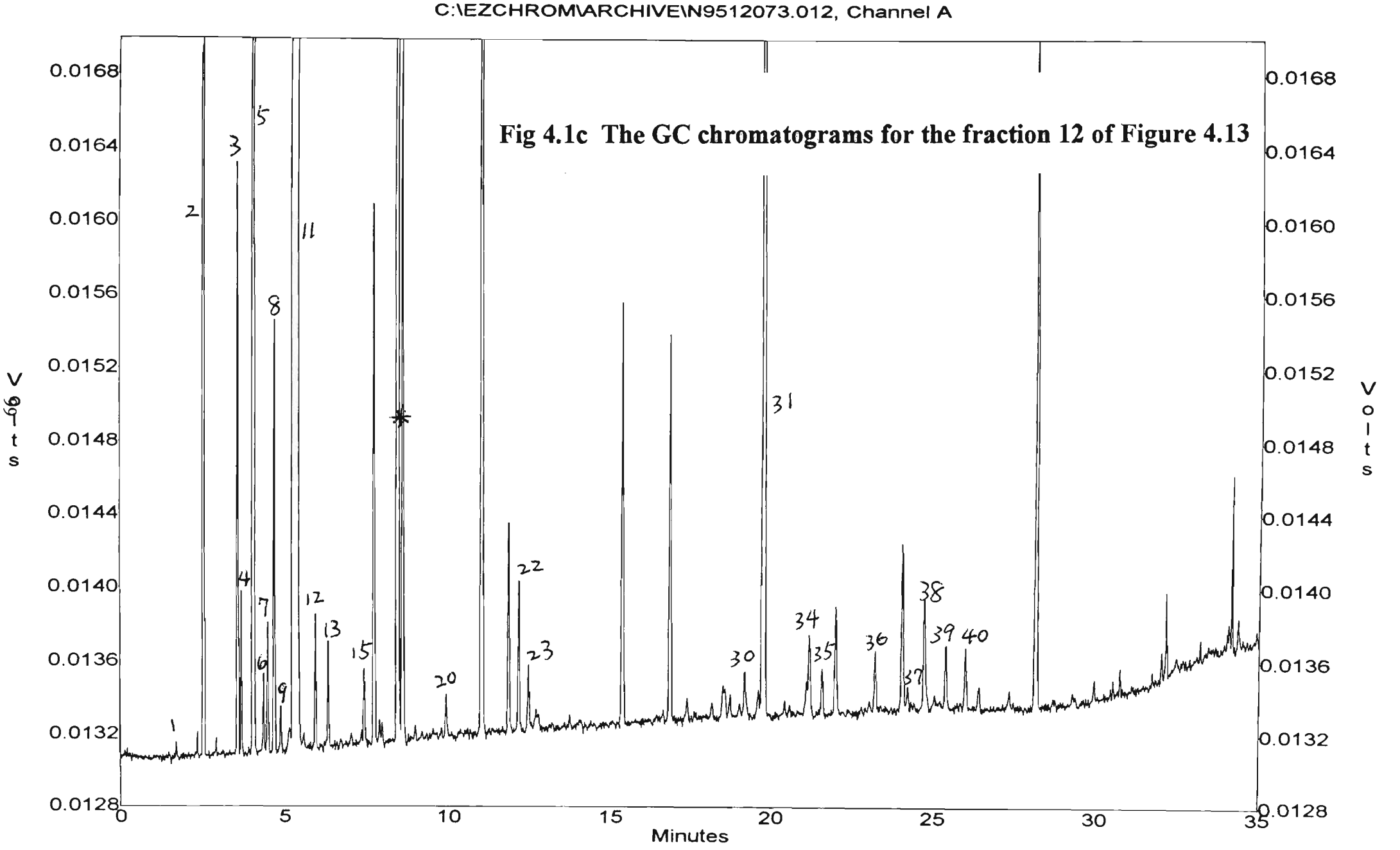


Fig 4.1c The GC chromatograms for the fraction 12 of Figure 4.13

Chapter 5

Refining Orange oil with Supercritical Carbon Dioxide and Silica gel

5.1 Introduction

In this chapter the application of SC-CO_2 to the refining of cold-pressed orange oil is explored.

As discussed in Section 1.7.6, there have been some reports of the results of refining citrus oil with SC-CO_2 and adsorbents, but as pointed out in Section 1.7.6, these reported processes did not make full use of the adsorbent capacity. Chouchi, et al. (1996) quoted the work of Vega-Bancel and Subra (1995) who examined the desorption breakthrough curves of a model mixture containing six hydrocarbon terpenes and six oxygenated terpenes through silica gel and showed that at CO_2 densities around 0.75 g/cm^3 (37 °C, 130 bar) all breakthrough curves overlapped and no selectivity was observed. At a lower CO_2 density of around 0.50 g/cm^3 (47 °C, 100 bar) two different curve families were observed for hydrocarbons and oxygenated terpenes respectively. The conclusion was drawn that the lower the density of CO_2, the better the selectivity that can be obtained. However, at low density, SC-CO_2 has less solvent power to extract desired compounds.

Most of the authors who used dense CO_2 with an adsorbent to refine citrus oil in a batch run used similar procedures: loading citrus oil onto the adsorbent with a small ratio of oil to adsorbent of between 0.003% and 20% (Dugo, et al., 1995; Barth, et al., 1994; Chouchi, et al., 1995; Chouchi, et al., 1996) as the first step (see Section 4.1), and then desorbing the bound citrus oil from the adsorbent with dense CO_2. As mentioned in Section 4.1, the aim of the preliminary tests of adsorbent capacity was to use the adsorbent more economically and efficiently to concentrate the oxygenated compounds.

The aim of the present work was to conduct a detailed study of the fractionation of orange oil by adsorption with full utilisation of the adsorbent capacity, followed by desorption into SC-CO_2. The adsorption was carried out essentially as described by Ferrer and Matthew (1987), making full use of the adsorptive capacity of the adsorbent. The desorption into SC-CO_2 was conducted under conditions of reasonable oil solubility and moderate temperature so that the process was quick and did not damage heat labile compounds. The study was conducted in the SC-CO_2 pilot plant described in Section 2.3.1, using a factorial design to determine the effects of process variables on quantity and quality of refined orange oil.

5.2 Materials (Orange oil, adsorbents and standard chemicals)

Cold-pressed Valencia orange oil, with a specific gravity of 0.842 at 20°C, was generously supplied by Keith Harris & Co. Ltd. (Thornleigh, N.S.W., Australia). Silica Gel 60 (Merck, 70-230 mesh) was used as the only adsorbent for the

experiments in this Chapter, based on the comparisons of alternative adsorbents detailed in Chapter 4. The properties of Silica Gel 60 are detailed in Section 4.2. Standard chemicals were the same as those used in Chapter 4, Section 4.3.3.2.

5.3 Experimental Design and Methods

5.3.1 Layout and operation of supercritical pilot plant

The pilot plant was designed for continuous extraction. Liquid CO_2 was pumped through a water bath to bring it to the desired supercritical pressure and temperature. The rate of CO_2 flow was adjusted by varying the pumping stroke and measured by the weight loss of the CO_2 cylinders. Silica Gel 60 (70-230 mesh, 100 g) was dry packed into the extractor column by gravity onto a glass wool plug supported on a coiled length of stainless steel chain resting on the bottom cap of the column. Valencia orange oil was pumped by HPLC pump (BIO-RAD ECONO PUMP) onto the top of the silica gel at a pumping speed of 2.8 g oil/min.

After 3 hours, 500g (± 3%) of Valencia orange oil had been pumped through the silica gel bed and the oil fraction passing through the silica gel had been collected from the bottom of the column. After the oil fraction had stopped draining from the column a glass wool plug was put on the top of silica gel, followed by a coil of stainless steel chain and finally the column was sealed. The column was slowly pressurised with CO_2 to 3.45 MPa at room temperature (21-22 °C) and slowly depressurised to atmospheric pressure to expel a further oil fraction held in the void

space of the silica gel bed. The CO_2 pressure of 3.45 MPa at room temperature corresponds to a density of only 0.0793 g/ml at which density the solubility of orange oil components is negligible. This was also the pressure at which the separation cell was maintained during collection of purified fractions in the present study of orange oil as well as in the studies of rice bran oil extraction detailed in Chapters 2 and 3. CO_2 was then charged into the column at the required pressure and temperature. Under the set conditions, desorption was continued for 2.5 h and samples were collected at the following times; 5, 10, 20, 30, 50, 70, 100 and 150 min. All fractions were weighed and aliquots subjected to GC analysis and/or analysis by GC - MS.

5.3.2 Analysis methods (GC-MS and GC analysis)

5.3.2.1 Instrument conditions

All samples collected were flushed with N_2 and either were stored at -17 °C for later analysis or analysed immediately. All extracts were analysed by GC - MS and /or GC. GC - MS conditions were the same as described in Section 4.3.3.2. The GC conditions had some significant differences from those described in Chapter 4 including: Quantification was achieved by using an internal standard, durene (Dugo, et al., 1995) instead of the peak area normalisation method. Response factors with durene as the internal standard were used to quantify all major components. Details of these standards are presented in Table 4.4. Some chromatographic analyses were run in duplicate for statistical purposes and the results were calculated on the basis of an internal standard method using durene as the internal standard.

5.3.2.2 Quantification and semiquantification of compounds in orange oil

Structures of some major compounds in orange oil are shown in table 5.1. In most literature reports and in Chapter 4 of this thesis, quantification and semiquantification of compounds in orange oil have been achieved by calculating the area percentage of individual peaks from a gas chromatogram (Temelli et al., 1988; Goto et. al., 1995; Barth and Chouchi, 1994; Chouchi, et al., 1995; Chouchi, et al., 1996). However, there are two reasons why this simple approach can produce substantial errors.

Firstly, some orange oils contain a significant content of high boiling temperature compounds which are retained in the injection port during analysis and do not contribute to the chromatogram. Therefore quantification based on the percentage areas will result in an overestimation of the concentrations of volatile compounds. This could be especially important for refined fractions rich in high boiling point compounds. In addition, any contamination or bleeding from the column can also contribute to the total area of the chromatogram, leading to underestimates of the sample concentrations. Therefore an internal standard was routinely used in the present investigation. This procedure was also used by Dugo (1995), Wilson and Shaw (1980), and Chamblee et al. (1991).

Secondly, flame ionisation detectors as used in all the above studies show different responses to the different classes of compounds in orange oil. Therefore the area

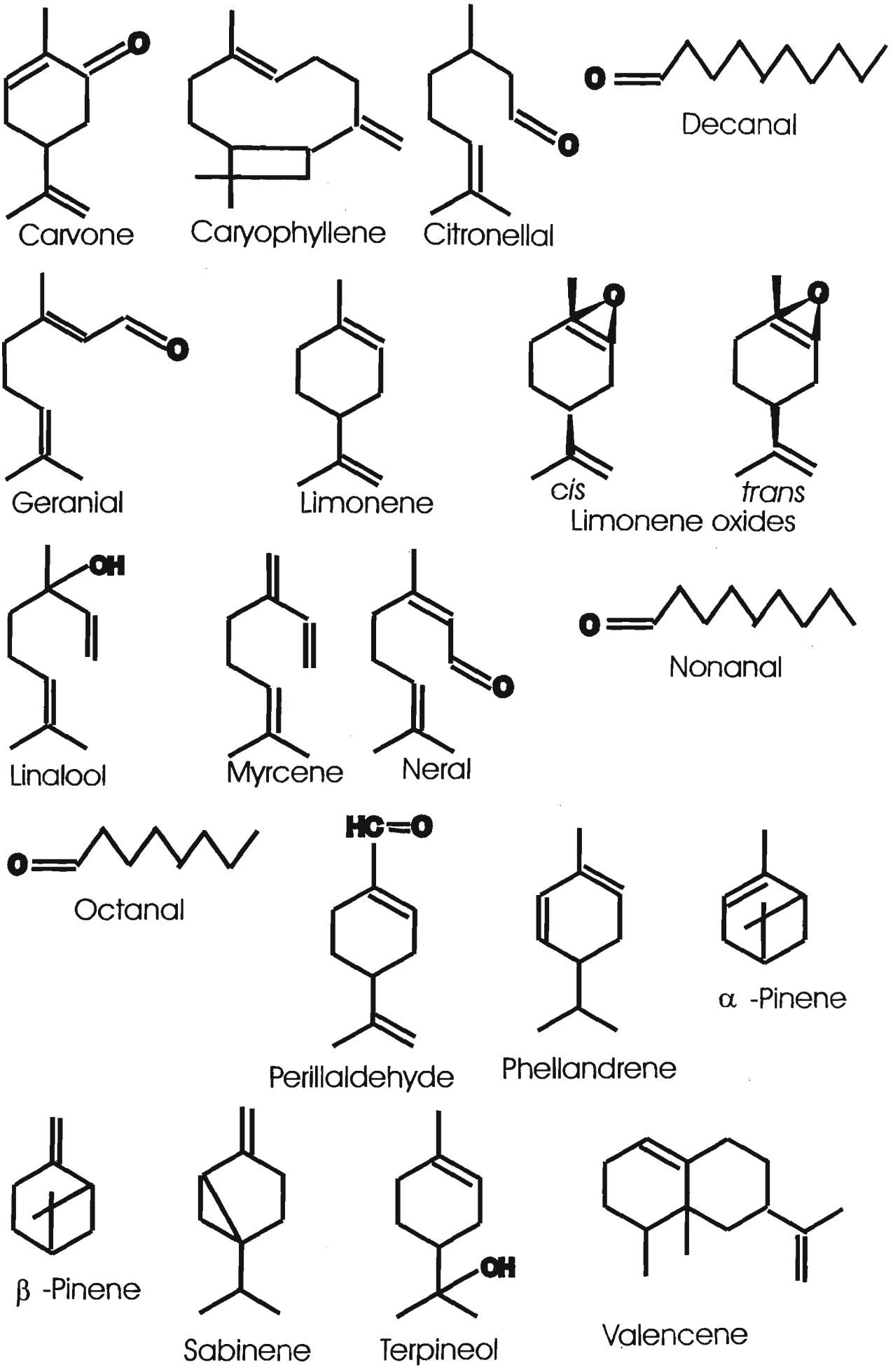


Table 5.1 Molecular Structures of some Components of Orange Oil, arranged alphabetically.

percentage does not truly indicate the weight percentage. The GC response factor of a given compound is defined by $R = \frac{A_s W_i}{A_i W_s}$ (where A_s and A_i are the measured peak areas of the internal standard and the compound standard ; W_s and W_i are the weights of the internal standard and the compound standard, respectively). In the present investigation the response factor of limonene was 1.02 and the response factor for decanal was 1.21. The former figure was taken to be the response factor for all terpene compounds, while the latter was used as the response factor for all oxygenated compounds in this work. The concentration of a compound in orange oil can then be calculated as $C_i = \frac{A_i W_s}{A_s W_o} * R$ (where A_s and A_i are the measured peak areas of the internal standard and the compound measured; W_s is the weight of the internal standard; R is the response factor for the measured compound, and W_o is the weight of oil). Durene ($C_{10}H_{14}$) does not exist in citrus oil and has a molecular structure similar to terpene compounds.

A similar approach was taken by Chamblee et al. (1991) in their analysis of lemon peel oil. Chamblee et al. (1991) used tetradecane as the internal standard and measured or estimated response factors for 56 compounds in the lemon peel oil. The response factors ranged from 0.901 for *p*-cymene to 1.410 for undecyl acetate. Chamblee et al.(1991) reported that using this method over a number of years had produced very accurate and reproducible results.

Standard solutions in present work were made by dissolving approximately 1g of durene, weighed to 0.1mg accuracy, in 100g of chloroform to produce an accurately

known concentration close to 10 mg/g. All chemicals and solvents were measured by weight in the present work.

As the limonene content of orange oil is more than 90% and the concentrations of all other compounds are almost 2 orders of magnitude lower, it is not possible to analyse all compounds accurately with one injection when an internal standard is used. For an accurate analysis of limonene, the concentration of the internal standard, durene, should be close to that of limonene, and therefore all the other compounds cannot be quantified precisely. Consequently all samples were analysed twice: once with a high added concentration of durene to allow quantification of limonene, and once with a low added concentration of durene to allow quantification of other compounds. Furthermore, the injection conditions were different for the two analyses based on the earlier eluting and larger amount of limonene.(see Table 5.2).

Table 5.2 GC analysis conditions

	Limonene	oxygenated compounds
Column temperature	$60°C \xrightarrow{3°C/min} 108°C \xrightarrow{20°C/min} 240°C$	$60°C \xrightarrow{3°C/min} 240°C$
Split ratio	60 : 1	20 : 1
Sample injection	0.1 µl	1 µl
Sample preparation	50 µl oil +1000 µl durene solution	100 µl oil +50 µl durene+ 900 µl $CHCl_3$

5.3.3 Full factorial experiment design

Some preliminary experiments for refining of Valencia oil with CO_2 and silica gel in the supercritical pilot plant were carried out to assess the ability of CO_2 to extract adsorbed components. The solvent power of CO_2 is determined in part by its density, which was controlled by setting the pressure and temperature. At higher densities (above 0.67 g/ml) good solubility was observed but there was a lack of selectivity. At densities lower than 0.3 g/ml, the solubility was too low and the rate of extraction too slow (see appendix 1). The factorial experiment was designed on the basis of these observations.

As a first step towards optimisation of the process, the study was designed according to the principles of Response Surface Methodology. "Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing process. It also has important applications in the design, development, and formulation of new products, as well as in the improvement of existing product designs" (Myers and Montgomery, 1995).

Pressure, temperature and flow rate of carbon dioxide are independent variables which could all affect the results of refining citrus oil. For technical reasons we used fixed values of pressure and temperature which were chosen carefully to confine the design to regions where the fluid density was reasonable. The final design consisted of 18 treatment combinations (3 temperatures x 3 pressures x 2 flow rates) which were trialed in 22 runs. Four of the 18 treatment combinations were replicated so that an

estimate of the "pure error" (i.e. between run) variance could be calculated, thereby enabling tests for the adequacy of fitted models. The process (primary) variables and the chosen levels are listed in Table 5.3.

Table 5.3. The primary variables and the levels used for full factorial experiment design

P		T		flowrate	
MPa	level code	°C	level code	kg/h	level code
9.66	-1	35	-1	2	-1
13.10	-0.5238	45	0	4	1
24.14	1	55	1		

The reason why the central pressure value was not selected half way between the lowest and the highest pressures was to ensure an even distribution of densities of CO_2 between the highest and lowest values, In this way the role of density as a process variable could also be explored.

According to Draper and Smith (1981) and the special design requirements of this experiment, the relation between the coded and the original levels of the primary variables is defined as follows:

$$\text{p (coded)} = \frac{pressure - 16.8966}{7.2414}$$

[16.8966 =(highest pressure + lowest pressure)/2; 7.2414=(highest pressure-lowest pressure)/2];

$$\text{t (coded)} = \frac{temperature - 45}{10}$$ (45 is the value of the middle temperature; 10 is the interval of temperature variables)

f (coded) = f - 3 (3 is the average value of the 2 flowrates)

5.3.4 Choice of response variables

The choice of response variables to characterise the process for the Response Surface Methodology (Myers and Montgomery, 1995) is not a simple matter. Clearly the response variable should be related to the value of the oil to the end-user, and the potential uses of orange oil concentrates are very diverse. The highest value uses are, of course, as flavours and fragrances, and it is generally agreed that the oxygenated compounds contribute most to this use.

Temelli et al. (1988) have stated that one of the major classes of oxygenated compounds, total aldehydes can be used as an indicator of quality and that decanal, being usually the most abundant aldehyde, can be used as a measure of concentration of flavour. Braverman and Solomiansky (1957) also characterised their product by the decanal concentration. In another study, Temelli et al. (1990) argued that since linalool is the most difficult of the flavour compounds to separate from the terpenes, the concentration of linalool can be indicative of the recovery of all flavour compounds.

Other authors have drawn attention to the role of terpenes in producing off-flavours in storage or processing and concluded that a lack of terpenes is of value in itself (Ferrer and Matthews, 1987; Tateo, 1981). Tzamtzis et al.(1990) used the recovery of oxygenated compounds and, separately, the removal of total terpenes to characterise

their products. Lund and Coleman (1977), using adsorbed concentrates, and Sato et al. (1995) using SC-CO_2 extracts, characterised their products by the total polar (oxygenated) compounds. Temelli et al. (1988), Tateo (1981) and Dugo et al. (1995) characterised their extracts as the ratio of the total oxygenated compounds to the total terpenes.

Commercially, orange oil concentrates are characterised by their "fold" or degree of concentration. They are marketed, for example, as "5-fold", "10-fold", or "25-fold" oils (Temelli et al., 1988; Dugo et al., 1995). This term is not well defined. At its least precise, it can be defined by the weight reduction achieved by a distillation process (Temelli et al., 1988). More rigorously, it can be defined as the ratio of the respective decanal concentrations in the extract and feed oils (Keith Harris and Co.Ltd, Australia). Lund and Coleman (1977) calculated "fold" from the octanal/limonene ratio, following a report that octanal is the most important contributor to orange flavour. Including the terpene content in the denominator in the definition of the "fold" seems most satisfactory since it incorporates not only the present value of the oil as a flavour or fragrance, but also its future stability.

For the present study, a range of response variables have been chosen to accommodate the various practices in the flavour and fragrance industry, and the practices and recommendations of other researchers. These are:- major aldehydes, major alcohols, decanal and linalool, expressed as proportions of total oil, or as ratios to total terpenes or limonene. In addition, after it became clear that the whole adsorbate produced during this experiment was already sufficiently concentrated to have commercial

value, the mass of adsorbate recoverable in a chosen amount (5 kg) of CO_2 was also used as a response variable. The time courses of extraction of all the main identifiable compounds in the orange oil have been measured and are recorded in an appendix 2, but only the chosen response variables have been statistically analysed.

Lund and Coleman (1977) have detailed organoleptic tests of the taste and longevity of their adsorbed concentrates. For the present study it was not considered practical to use organoleptic testing as a response variable, but selected samples were partially tested to confirm that the refined concentrates were of marketable quality.

5.3.5 Data processing and expression

All extraction curves exhibited the same general shape consisting of rapid initial extraction followed by a progressive reduction in rate of extraction which tended towards zero. Figures 5.4 - 5.9 illustrate the typical curve shape. For comparison of yields between curves it was necessary to choose an arbitrary amount of CO_2 used which was taken to be the end of the extraction. The value of 5 kg CO_2 was chosen because, under all conditions studied, the extraction was almost complete at that value.

For measurement of fractional extraction the quantity of interest was the remaining adsorbate extractable in 5 kg CO_2 since the adsorbate always contained the highest concentration of oxygenated compounds, and eventually became the refined oil fraction. Consequently, the raw extraction data were first transformed to show the

quantity of each component remaining on the column at each stage of the extraction, relative to the amount present after 5 kg CO_2 had been used. That is, any adsorbate remaining on the column at 5 kg CO_2 was considered to be not practically recoverable and the amount of adsorbate on the column at 5 kg CO_2 was defined as the zero point for calculations of amounts of adsorbate.

Therefore, the basic calculations for the major components of Valencia oil refined by SC-CO_2 were:

remaining adsorbed amounts of key terpene and key oxygenated components, remaining concentration of key oxygenated components in adsorbate,

ratio of concentrations of a group of oxygenated components to terpene components in adsorbate, and ratios of single key oxygenated components to limonene (the major terpene component) in the adsorbate, after every fraction was collected.

The percentage recovery of components using 5 kg CO_2 was determined by dividing the total amount of components recovered in 5 kg CO_2 by the total initial adsorbate of the same components and multiplying by 100. No fractions were actually collected at exactly the time at which 5 kg of CO_2 had been used. In most cases fractions were collected both before and after 5 kg CO_2 had been used and the amount of oil collected in both fractions was very small as a proportion of the total recovery, indicating that a linear interpolation at 5 kg CO_2 would accurately estimate the true amount. For two experimental runs the experiment was terminated before 5 kg CO_2 had been used and a linear extrapolation to 5 kg CO_2 was used.

The percentage of components recoverable as 10 or 15 times concentrate was calculated differently because both the concentration and yield values of the adsorbate sometimes showed considerable curvature in the vicinity of 5 kg CO_2. However, plots of the logarithm of concentration or yield versus used CO_2 were generally almost linear (see appendix 3) and so a logarithmic interpolation (or extrapolation where necessary) to 5 kg CO_2 was used. This logarithmic transformation of yield and concentration data was repeated for all the concentration expressions including the ratio of oxygenated component concentration to terpene concentration.

5.3.6 Statistical analysis method

All statistical analyses were carried out using the Genstat 5 program, Release 3.2, copyright Lawes Agricultural Trust (Rothamsted Experimental Station). About 4% of experimental values were known to have a higher degree of uncertainty because of experimental difficulties such as spillage. The effect of leaving these data out of the statistical analyses was determined for a small number of response variables and found to produce no consistent improvement in the subsequent regression analyses. Consequently the whole set of data has been used throughout for the generation of results.

5.3.7 Estimation of response surfaces by regression analysis

The Genstat 5 program was used to fit full models comprised of regression coefficients and first order and second order functions of the three process variables as

described in Section 1.8. In general the three process variables were temperature, pressure and flow rate, but the substitution of density or a function of density for one of the process variables was sometimes found to improve the fit of models. (Temperature, pressure and density of CO_2 are not simultaneously independent variables. They are related by an equation of state such that only two can be varied independently). All the models are termed Linear Regression Models despite the fact that the Response Surfaces often contained second order functions of the process variables (Myers and Montgomery, 1995).

For each term in the regression equation Genstat was programmed to calculate the probability that the term was not contributing to the observed variance. Terms with high probabilities were then eliminated from the model to produce reduced models and the best fit regression coefficients were again calculated. These models were then converted from scaled variables to the measured variables according to the relationships detailed in Table 5.3. The goodness of fit of the models to the measured data was determined in two ways:-

(1) Using the Genstat program, the probability that the model did not fit the data was calculated by comparing the variance associated with lack-of-fit with the variance associated with pure error. This calculation is termed the test of lack-of-fit. The pure error variance was calculated from the reproducibility of the four duplicate runs in the experimental design.

(2) The Genstat program was also used to calculate the percentage of the total variance observed which could be accounted for by the model.

In all cases the outcome of the statistical test for lack-of-fit was that the model described the data adequately, but this could be seen to be the result of the somewhat poor reproducibility of the duplicate runs. The lack-of-fit test did not discriminate between models. Consequently the percentage of variance which could be accounted for by a model was used as a measure of the goodness of fit. Models which could account for less than 50% of the variance were not considered useful approximations to the Response Surface in this study.

5.4 Results and Discussion:

5.4.1 Selective adsorption by silica gel

Forty-two components in feed Valencia oil were identified by GC-MS and numbered as presented in Table 4.5 (see Fig 5.1) and twenty-four components were quantified in Table 5.4. As detailed in 5.3.1, orange oil was slowly pumped onto the top of the packed silica gel column. As the oil passed down the column it was stripped of its oxygenated compounds and the oil which drained from the bottom of the column contained nothing but terpenes. The relative amounts of adsorbent and oil were calculated to prevent any measurable loss of oxygenated compounds from the column. That is, the amount of loaded oil was calculated to reach the breakthrough point of the adsorbent, with respect to oxygenated compounds, as this approximated to full utilisation of the column capacity and minimised product loss in the effluent. See Chapter 1 for further discussion.

The free-draining step was followed by pressurisation and depressurisation to remove further oil from the void space. This depressurised fraction comprised approximately 10% of the feed terpenes and contained between 0 and 4% of the amount of feed aldehydes and no alcohols at all (the composition of this fraction is recorded in most of the tables of Appendix 2). When combined with the stripped oil collected by free drainage, the total fraction stripped of oxygenated compounds contained approximately 75% of the amount of feed terpenes. This combined fraction was therefore called the terpene fraction. For this calculation, the total of the amounts of limonene, α-pinene, sabinene, β-pinene, β-myrcene, α-phellandrene, carene, valencene and δ-cadinene was used as the total yield of terpene compounds. Since 75% of the terpenes had thus been separated from the adsorbed compounds, the adsorbate was then about 4 times as concentrated as the feed oil with respect to oxygenated compounds.

Figure 5.2 and Figure 5.3 are GC chromatograms of terpene fraction and the 7th collected fraction desorbed by SC-CO_2 at 13.10 MPa, 35 °C and 2 kg/h of CO_2, showing that there are no detectable oxygenated compounds in the terpene fraction. The adsorption step itself therefore achieved a useful degree of concentration of oxygenated flavour compounds. Therefore one possible development of the process would be to ignore the possibilities of further concentration by fractional desorption and measure only the amount of adsorbate recoverable in a reasonable amount of SC-CO_2.

Table 5.4. Amount (mg/ g oil) of 24 major components of cold pressed Valencia orange oil as determined in the present work by GC.

Peak number	Compound	w/w (mg/g oil)
2	α - Pinene	3.80
3	Sabinene	1.92
4	β - Pinene	0.14
5	β - Myrcene	15.70
7	α - Phellandrene	0.20
8	Carene -3	0.82
11	Limonene	911.32
6	n - Octanal	0.99
17	n - Nonanal	0.29
22	Decanal	2.67
20	Citronellal	0.35
26	Neral	0.29
28	Geranial	0.57
29	Perillaldehyde	0.64
14	n- Octanol	1.39
16	Linalool	3.71
21	α - Terpineol	0.85
18	Limonene oxide (cis)	0.71
19	Limonene oxide (trans)	0.38
24	Carveol (trans)	0.35
25	Carveol (cis)	0.28
27	Carvone	0.49
38	Valencene	0.70
40	δ - Cadinene	0.15

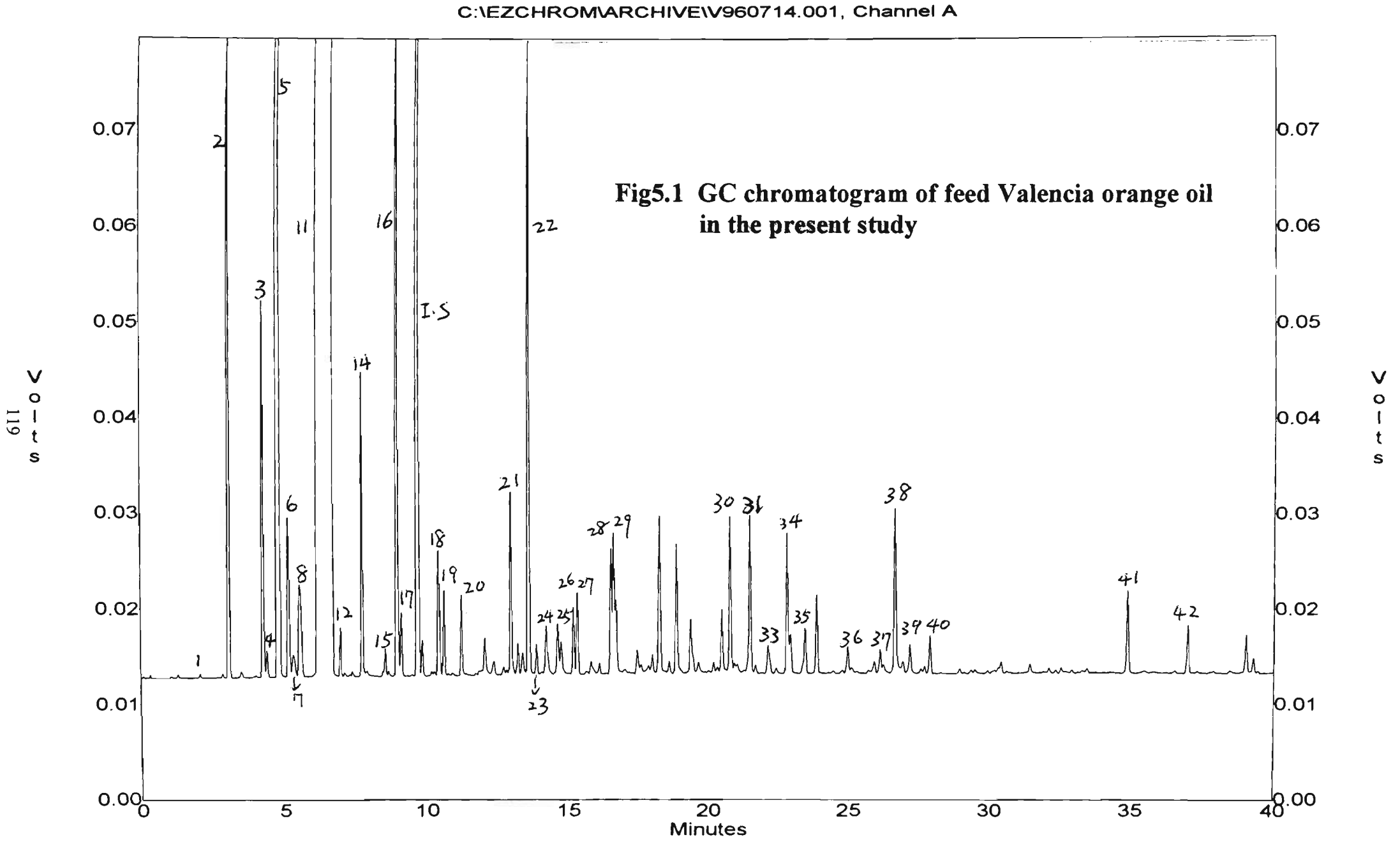


Fig5.1 GC chromatogram of feed Valencia orange oil in the present study

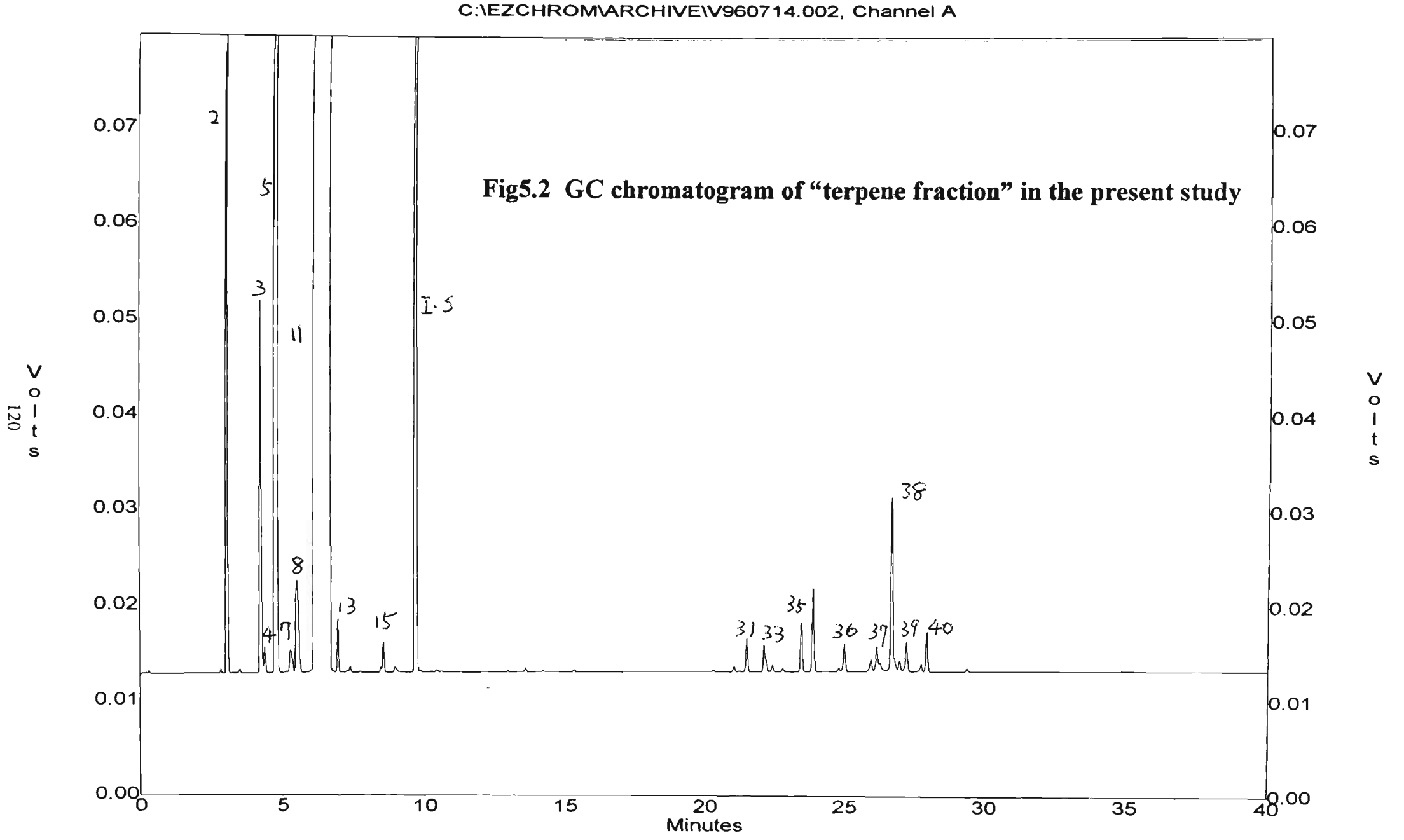


Fig5.2 GC chromatogram of "terpene fraction" in the present study

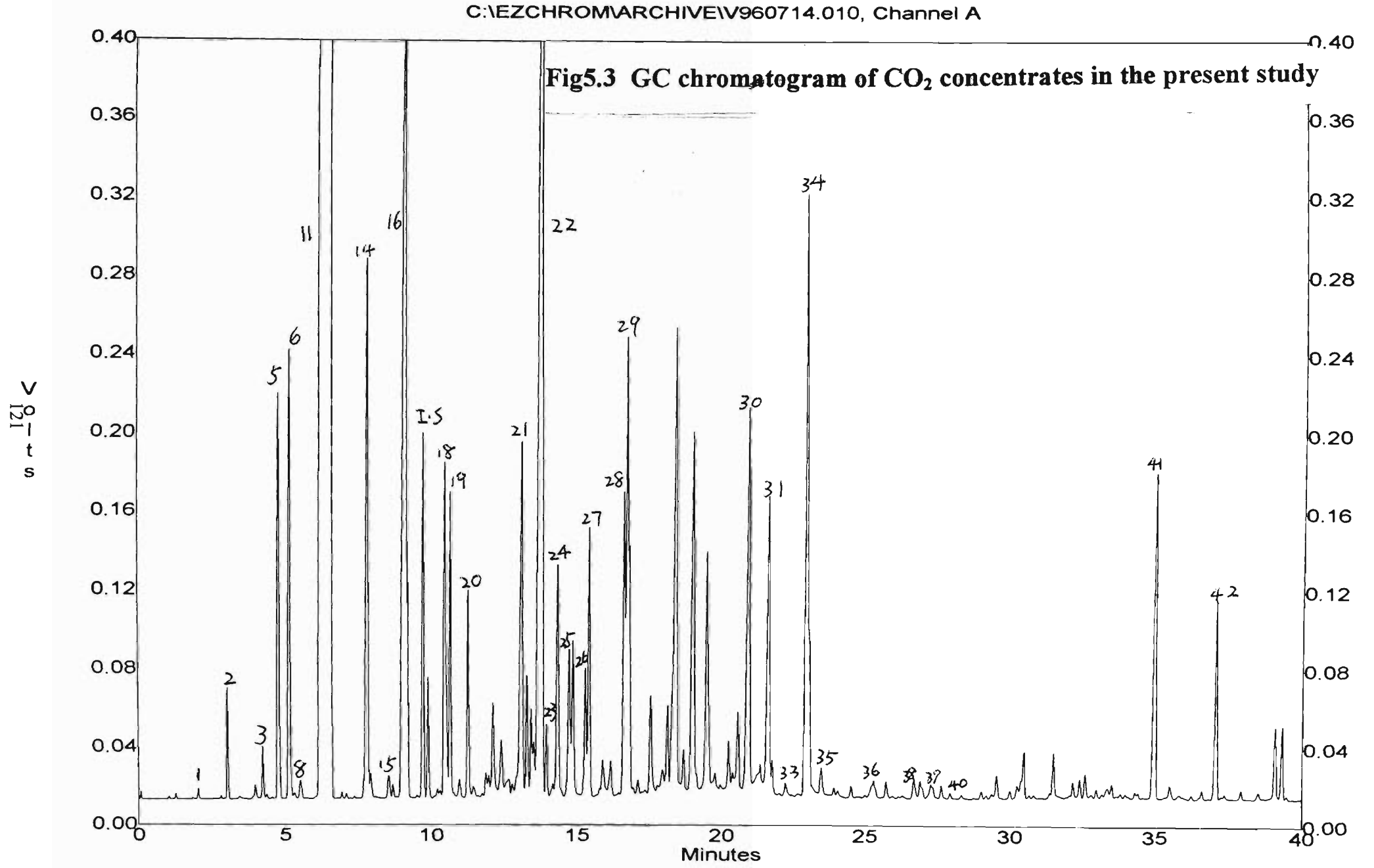


Fig5.3 GC chromatogram of CO_2 concentrates in the present study

5.4.2 Full extraction of adsorbed (concentrated) orange oil by CO_2

5.4.2.1 Total adsorbed oil extraction

Figures 5.4 to 5.9 show the recovery of total adsorbed orange oil as a function of used CO_2 under the various temperature, pressure and flow rate conditions. The curves have been grouped to show the effects of the three process variables on the recovery of adsorbed orange oil. Full recovery (100%) was defined as the mass of feed oil less the mass of stripped oil (terpene fraction) collected during loading of the column and initial depressurisation. In all cases the recovery of adsorbed orange oil virtually ceased after 5 kg of CO_2 had been used and the amount ultimately recoverable varied from 70% to 80% of adsorbed oil. The percentage recovery at 5 kg CO_2 was calculated for 20 of the 22 desorption curves by linear interpolation between the points immediately above and below 5 kg of CO_2. The other two desorption curves were stopped before 5 kg of CO_2 had been used and therefore a linear extrapolation was used to estimate the percentage recovery at 5 kg CO_2. The values for recovery at 5 kg CO_2 were subjected to regression analysis, see Appendix 4a (Genstat file oil_a3.out), to determine the effects of the process variables. The effects were then summarised as Response Surfaces described by linear regression equations. Using temperature, pressure and flow rate of CO_2 as the process variables, the following regression equation could account for 59.6% of the observed variance (Appendix 4a, oil_a3.out, reduced model: $Y=76.85 + 1.88T + 2.42P + 1.17T^2 - 2.18P^2$).

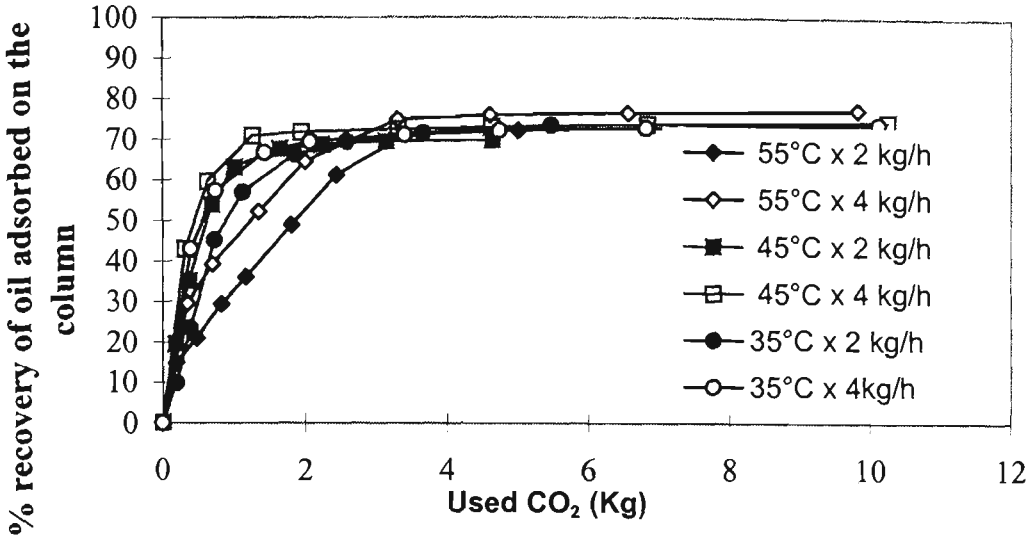


Fig 5.4. Effect of temperature and flowrate on the total orange oil recovery of CO_2 desorption at 9.66 MPa.

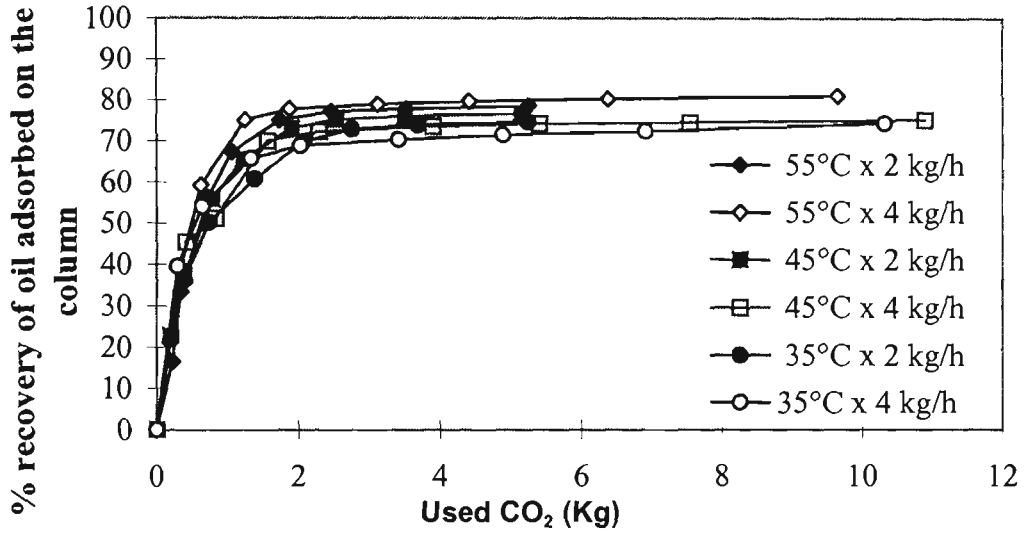


Fig 5.5 Effect of temperature and flowrate on the total orange oil recovery of CO_2 desorption at 13.10 MPa.

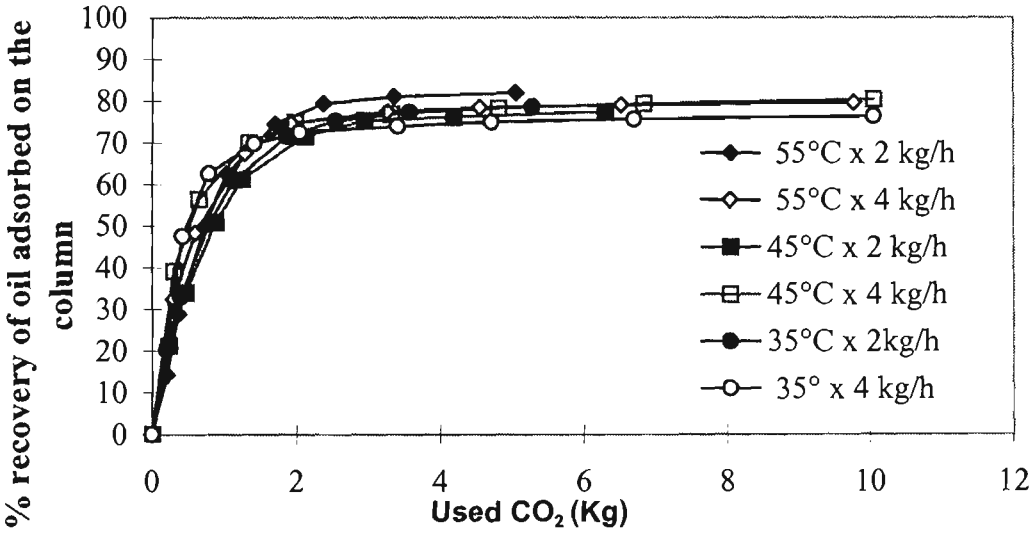


Fig 5.6 Effect of temperature and flowrate on the total orange oil recovery of CO_2 desorption at 24.14MPa.

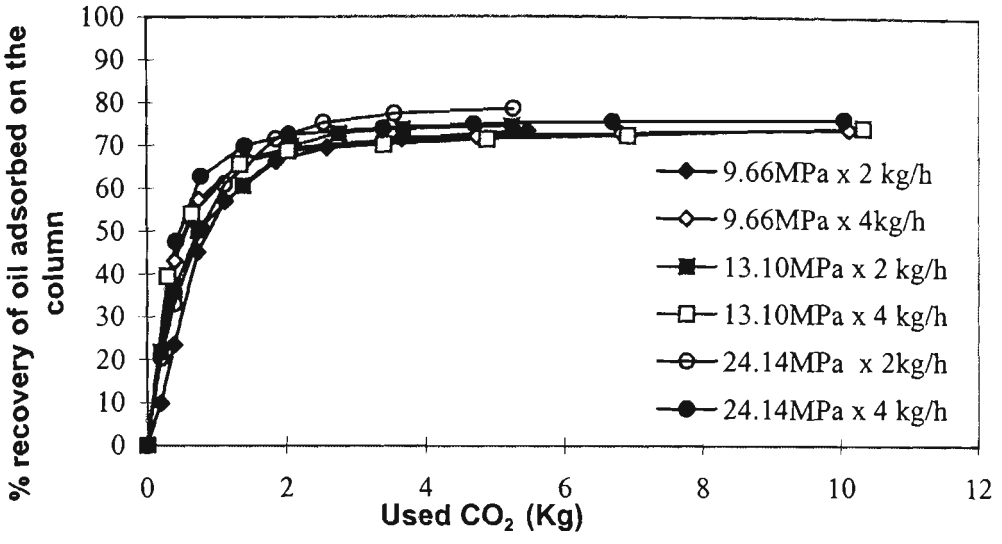


Fig 5.7. Effect of pressure and flowrate on the total orange oil recovery of CO_2 desorption at 35°C.

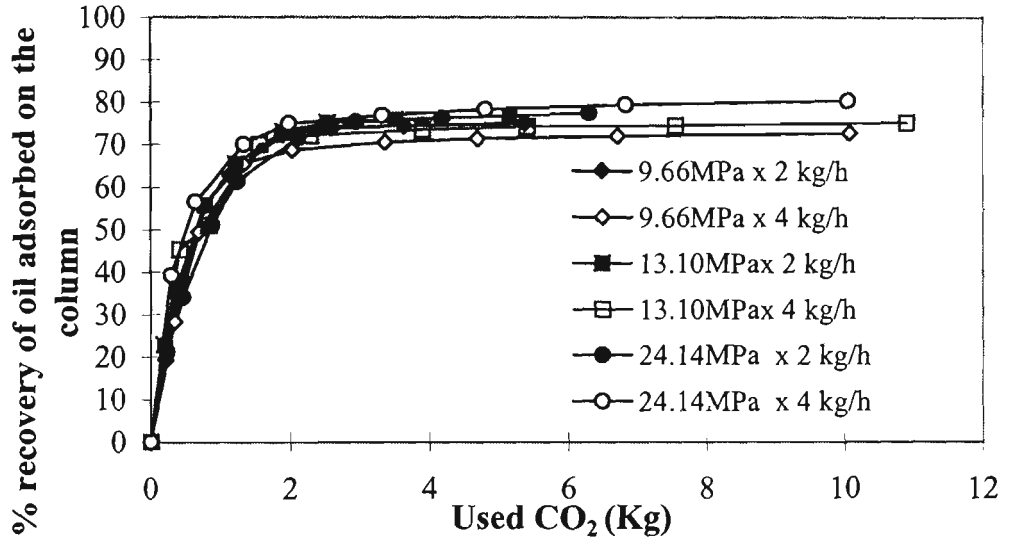


Fig 5.8 Effect of pressure and flowrate on the total orange oil recovery of CO_2 desorption at 45°C

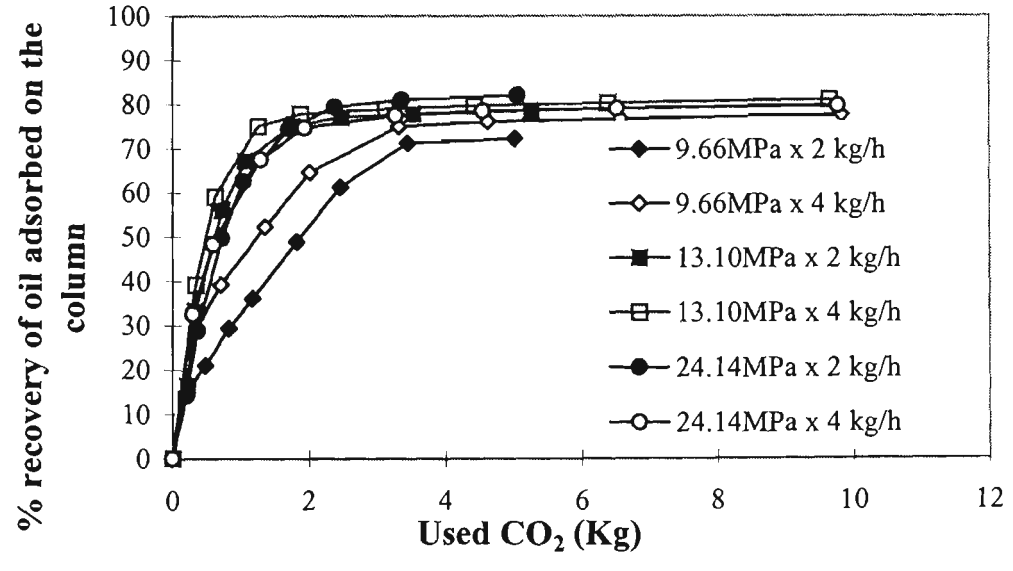


Fig 5.9. Effect of pressure and flowrate on the total orange oil recovery of CO_2 desorption at 55°C.

Using temperature, density and flow rate of CO_2 as the process variables, the best fit regression equation was:- $Y= 66.46 + 3.33T + 13.0D - 0.52F + 0.97T^2 - 0.6D^2 + 0.74TF$ (in Appendix 4b- oil_ad2.out). This equation could account for 63.3% of the variance. The coefficients of the various terms in the equations indicate the relative importance of the terms. By far the major effect on the yield was the CO_2 density, as expected from the reported relationship between supercritical solvent density and solute solubility (see Chapter 2, 3, 4). Temperature had a smaller positive effect on the oil yield. Flow rate of solvent had a very small negative effect on oil yield, which was of doubtful significance. Since the yields of oil extractable in 5 kg of CO_2 are close to asymptotic values, i.e. they are values determined by the equilibria of desorption of oil components, it is expected that CO_2 flow rate would have no effect. Any small negative effect of flow rate on yield is likely to result from increased losses of product in the separation cell at higher CO_2 flow rates.

The 20% to 30% of the adsorbed oil (corresponding to between 5% and 8% of the feed oil) which was not recoverable can be attributed to poorly soluble components such as waxes and pigments being 'irreversibly' bound to the silica gel when CO_2 was used as the desorption solvent, as well as to losses during processing.

From examination of the data from the early stages of desorption, it can be seen that, in general, differences in the rates of desorption were small, but at the highest temperature and lowest pressure the differences became noticeable (Figures 5.4 and 5.9). These are the conditions of lowest density, lowest solvent power, and lowest residence time of solvent in the column for a given rate of mass flow of solvent.

Figure 5.10 contrasts the recovery curves of Valencia orange oil from silica gel by SC-CO_2 at the highest and lowest densities used. At the higher density the desorption rate was higher than that at the lower density. After 1.1 kg CO_2 was consumed, 77.5% of feed oil was recovered at the higher density compared with 49.9% at the lower density. The initial common part of both curves could possibly be an artefact of the starting procedure of the experiments. At the commencement of each desorption experiment, cold liquid CO_2 was pumped through a coiled tube in a water bath at the set temperature of the experiment and charged into the extractor. The temperature of the extractor was maintained at the set point of the experiment by means of a water jacket. The water jacket and water bath adequately maintained a constant process temperature in the low CO_2 flow rates used for extraction, but were unable to maintain the set temperature during the rapid initial pressurisation of the extractor. Consequently, during initial pressurisation, the CO_2 temperature in the extractor was between 10 °C and 15 °C lower than the set point and therefore its density was higher than during the actual extraction. It follows that the solubility of the orange oil in CO_2 was higher before the commencement of extraction than it was during the isothermal extraction run.

The extra dissolved orange oil should have precipitated as liquid oil in the extraction column as the CO_2 density decreased, but it is possible that some was able to enter the first desorption sample because not enough time was allowed for equilibration of CO_2 in the column before extraction commenced. The interpretation of this phenomenon as well as the experimentally observed effects of flow rate is complicated by the lack of

agreed values for the solubility of orange oil (Reverchon, 1997), or of its principal constituent, limonene in supercritical CO_2.

It is interesting to compare the rate of orange oil extraction observed at 9.66 MPa and 55 °C with the solubility of orange oil reported by Temelli et al. (1988). The linear portion of the curve (Fig 5.10) up to 2.5 kg CO_2 has a slope corresponding to 33 g of oil per kg CO_2. Under similar temperature and pressure conditions, including both higher and lower values, Temelli et al. (1988) measured the solubility of orange oil to be approximately 22 mg per litre of CO_2, where the CO_2 volume was measured at 100 KPa and 25 °C. After conversion to units consistent with the present work, the solubility of orange oil reported by Temelli et al. (1988) is approximately 12 g per kg CO_2. It would seem on the basis of the present study that Temelli et al. (1988) may have underestimated the solubility of orange oil in CO_2. Temelli et al. (1988) did not report any measurements to confirm a close approach to equilibrium in their continuous extraction experiments. Stahl and Gerard (1985) measured the solubility isotherm of limonene at 40 °C, obtaining a value in agreement with Temelli et al (1988) at a pressure of 8 MPa but a solubility value 9 times greater at 10 MPa. Reverchon (1997) has discussed the inconsistencies among the terpene solubilites in SC-CO_2 reported in the literature.

The present work was not designed to determine orange oil solubility. However, it appears that the samples collected in the early stages of extraction contained concentrations of orange oil of a similar order to the saturation value.

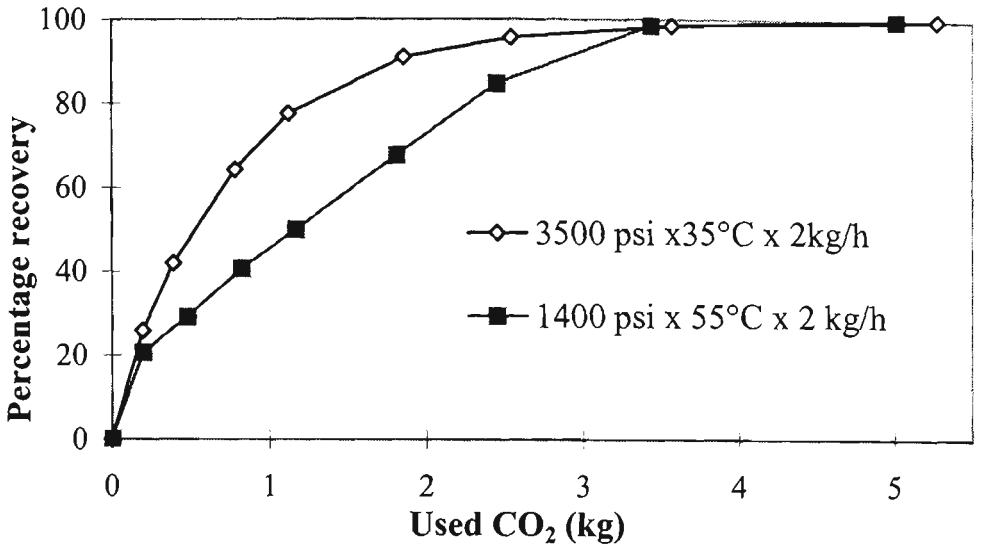


Fig 5.10. Percentage recovery yield of Valencia orange oil refined with CO_2 and Silica gel.

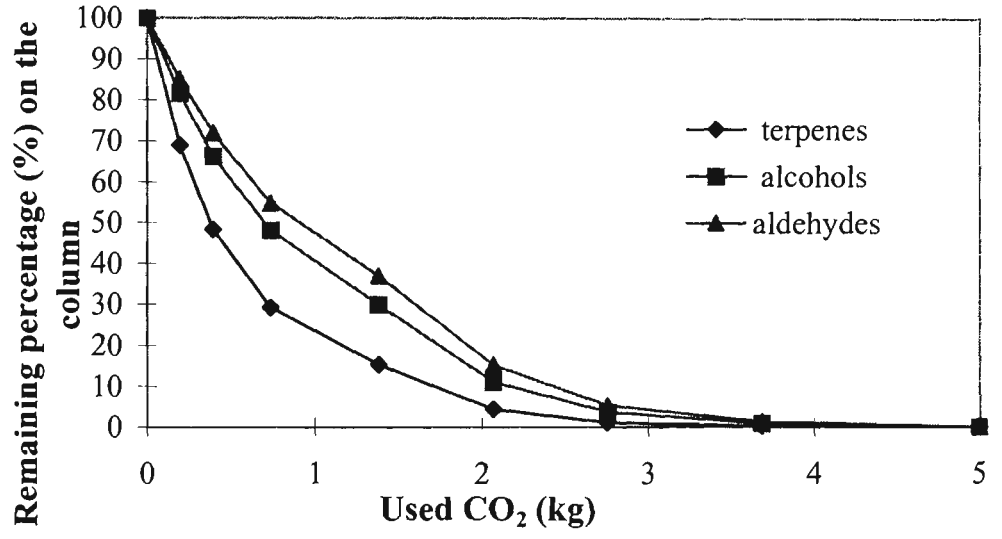


Fig 5.11. Desorption curves of major classes of orange oil constituents at 13.1MPa, 35°C and 2kg/h of CO_2.

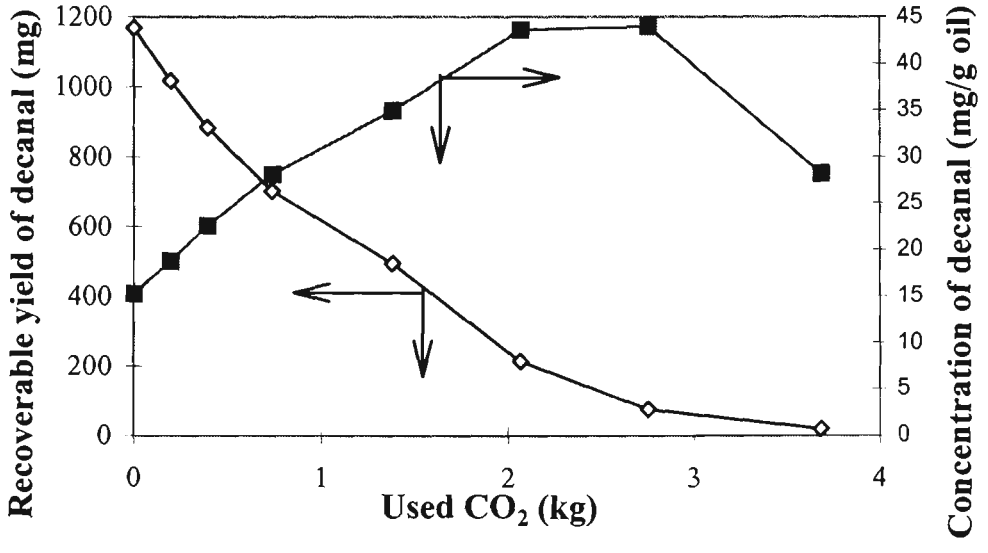


Fig. 5.12. Decanal in remaining adsorbate during extraction at 13.1MPa, 35°C and 2kg/h of CO_2.

5.4.2.2 Desorption curves of oil components

The time courses of desorption of each of the 24 identified compounds have been measured, in 22 different experiments. The result for the 528 different desorption curves are tabulated in Appendix 2. Fortunately the compounds tended to behave as groups, and for convenience they have been divided into three groups - alcohols, aldehydes and terpenes, The first two groups are the major components responsible for flavour in orange oil. All of the desorption curves were of the form shown in Fig 5.11. The data of the Y -axis represent the remaining percentage of the total recoverable amount of the component on the column. From the point of view of separation, the important features of the curves are their differences.

The oxygenated compounds were always desorbed later than the terpenes, which means that the adsorbate remaining on the column was always richer in oxygenated compounds than was the previously extracted oil under all CO_2 conditions in this study. The adsorbate remaining on the column is therefore the higher value product. Its composition is the composition of the fraction yet to be collected and is referred to as the recoverable concentrate. The ratio of aldehydes and alcohols to terpenes increased as desorption progressed and the ratio depended on the temperature and pressure of the CO_2 as well as the CO_2 flow rate.(see appendix 2). Fig 5.12 is a typical desorption curve showing that as desorption progressed the concentration of decanal in the adsorbate increased but the amount of adsorbate decreased until decanal started to be exhausted. This shows very clearly the different conditions needed for high yield or for high concentration. The yield and concentration curves depended strongly on the

temperature and pressure of the CO_2. These observations are discussed in detail in sections 5.4.3 and 5.4.4.

5.4.2.3 Recovery of aldehydes and decanal

In this study the total amount of aldehydes is defined as the total amount of n-octanal, n-nonanal, citronellal, decanal, geranial, neral and perrillaldehyde. The amounts recoverable in 5 kg CO_2 were calculated by linear interpolation or extrapolation of the extraction curves and expressed as percentages of the total feed aldehydes. Decanal, the major component of the aldehydes, was treated in a similar manner. Figures 5.13, 5.14, 5.15 and 5.16 are the isotherm results for percentage recovery of major feed aldehydes and decanal as functions of pressure, together with estimates of the response surfaces generated by regression analysis.

The calculation points of the model curves are marked on the figures to enable the curves to be easily distinguished from each other; the marked points on the model curves are not experimental data. The percentage recovery of total feed aldehydes by extraction with 5 kg CO_2 can be expected to increase with increasing CO_2 pressure since this corresponds to increasing density and solvent power of the CO_2. The percentage recovery could be expected to approach the asymptote of 100% as pressure increases. The measured data of Fig 5.13 are consistent with the expected behaviour,

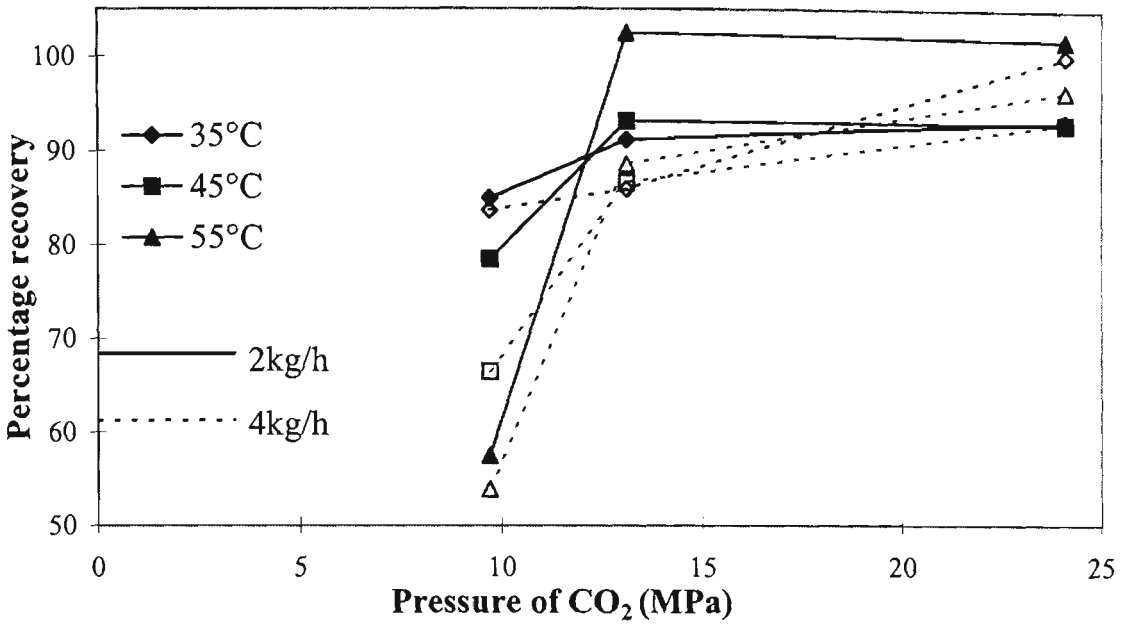


Fig 5.13. Percentage recovery of total feed aldehydes using 5 kg CO_2, isotherms as functions of CO_2 pressure (observed).

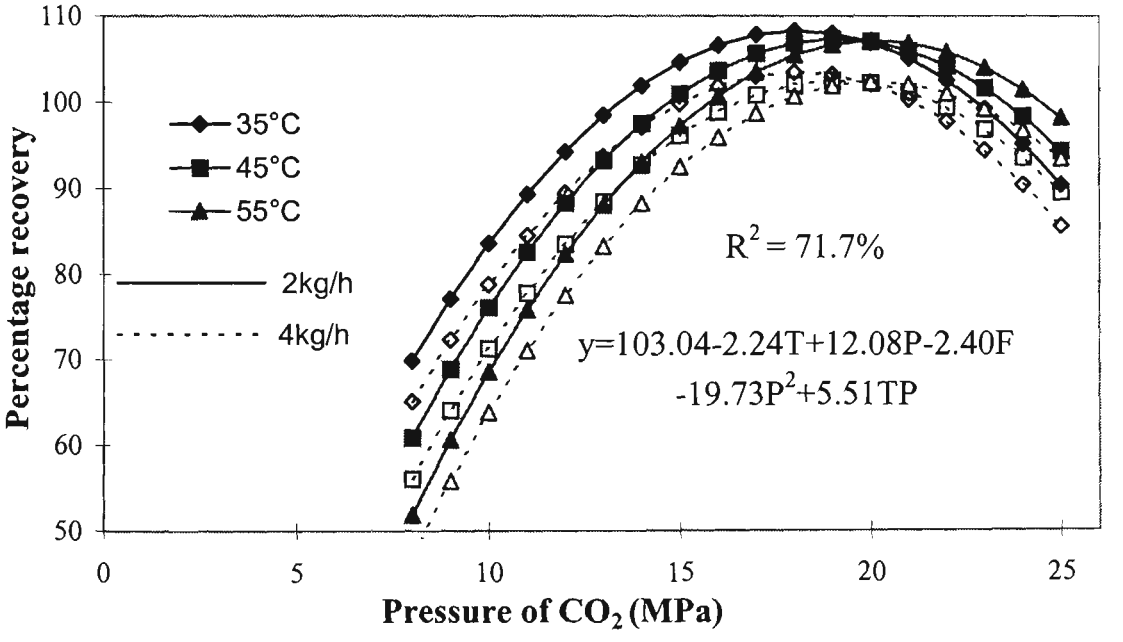


Fig 5.14. Percentage recovery of total feed aldehydes using 5 kg CO_2, isotherms as functions of CO_2 pressure (modelled). T, P and F in this model represent the coded temperature, pressure and flowrate of CO_2. R^2 is the percentage of variance accounted for by the model.

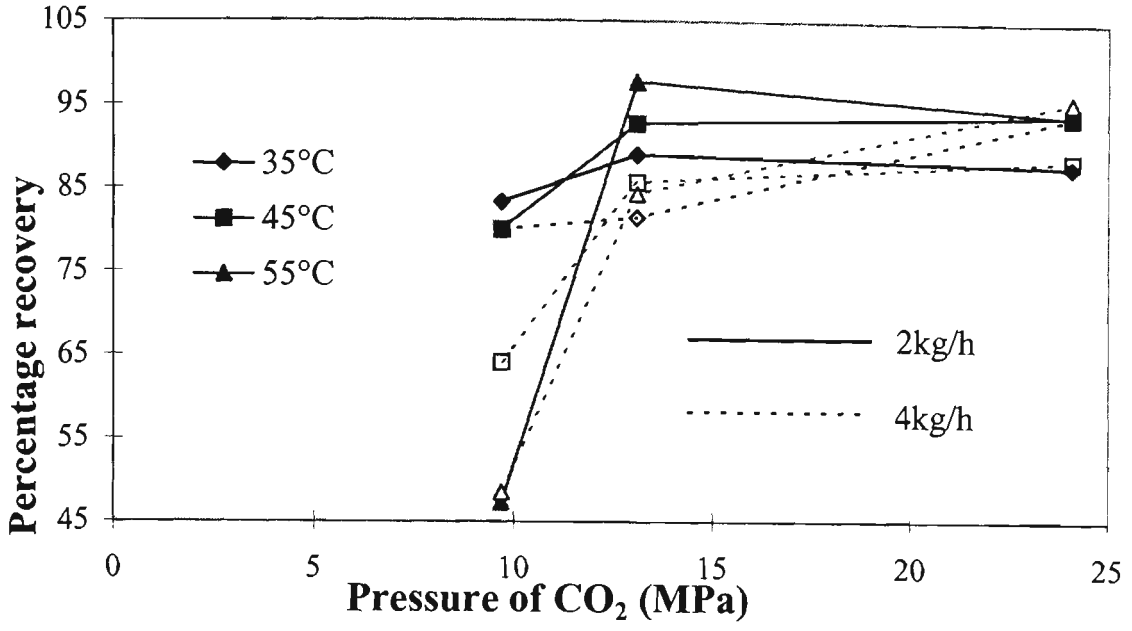


Fig. 5.15. Percentage recovery of total feed decanal using 5 kg CO_2, isotherms as functions of CO_2 pressure (observed).

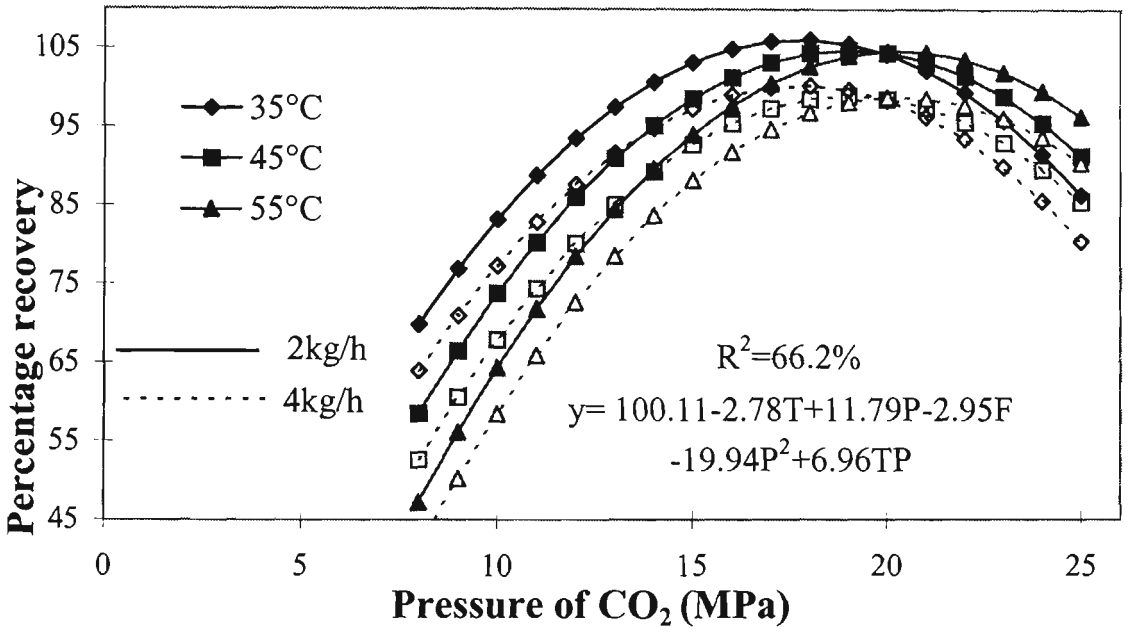


Fig 5.16. Percentage recovery of total feed decanal using 5 kg CO_2, isotherms as functions of CO_2 pressure (modelled), T, P and F in this model represent the coded temperature, pressure and flowrate of CO_2. R^2 is the percentage of variance accounted for by the model.

except where the percentage recovery slightly exceeded 100% due to experimental error. For aldehydes, the higher the flow rate, the lower the recoverable adsorbate. As discussed in section 5.4.2.1 the amount recoverable in 5 kg CO_2 was close to an asymptotic value which did not vary with time or amount of CO_2, and therefore it is most likely that the effect of CO_2 flow rate on aldehyde yield was caused by increased losses in the separation cell with increased CO_2 flow rate.

The regression model shown in Fig 5.14 predicts values close to most of the measured data (accounts for 71.7% of the variance of the data), but has a physically unrealistic form because only linear and quadratic functions of pressure are included. The model predicts a maximum value, rather than an asymptote. If more data were measured at pressures over 13 MPa it is likely that the present model would fit poorly. The model could be improved by including a function of pressure which approaches an asymptote. The data and response surface for the recovery of decanal are similar in form to the total aldehyde results. The fact that at the higher CO_2 flow rate, the decanal recovery was lower can be explained as discussed for total aldehydes.

It is noteworthy that use of CO_2 density as a process variable instead of temperature or pressure consistently improved the agreement of the results with linear regression models, as shown by the percentage of the variance accounted for by the model. This suggests that, in the case of total recovery of adsorbate, density interacted less with the other process variables than either pressure or temperature did, allowing simple models involving density to achieve a better fit. Figures 5.17 and 5.18 show CO_2 desorption yields as functions of density at three temperatures. This enables the effect of density

on CO_2 solvent properties to be seen separately from the effect of temperature. At higher temperature, higher total recovery yields of aldehyde adsorbate were obtained. This can be interpreted as a temperature effect on the adsorption equilibria of bound oxygenated compounds. The increase in desorption at higher temperature implies that the desorption process for aldehydes is endothermic, according to the Van't Hoff equation (Denbigh, 1966): $d \ln K/d T = \Delta H/RT^2$ where in this case, K is the equilibrium constant for desorption of an oxygenated compound, ΔH is the heat of desorption and T is the temperature (Kelvin).

The percentage recovery by extraction with 5 kg CO_2 can be expected to approach the asymptote of 100% as density increases, as was argued for the effect of pressure, and the measured data are consistent with that conclusion. The regression model of Figure 5.18 accounts for 84% of the variance of the data and, within the range of the data, has a physically realistic form. Within the ranges of densities used in these experiments, expression of the degree of compression of CO_2 as density rather than pressure appears to lead to response surfaces of simpler mathematical form.

Recovery of total feed aldehydes as a function of density (isobars at three different pressures) is shown in Fig 5.19. The percentage recovery by extraction with 5 kg CO_2 at a fixed pressure is not necessarily expected to approach an asymptote of 100% as density increases. This is because, as density increases at a given pressure, the temperature of the CO_2 must decrease because the condition of fixed pressure requires that the temperature be lower at higher densities. The lower temperature opposes the desorption of bound oxygenated compounds, counteracting the effect of increased

density to varying degrees. For example at the lowest pressure of 9.66 MPa and temperatures of 55 °C and 45 °C, the CO_2 densities of 0.299 and 0.4439g/ml respectively are too low to counteract the effect of decreased temperature, and the highest recovery yield at 9.66 MPa was observed at the lowest temperature (35 °C) in contrast to the results obtained at the other two pressures. The regression model, which includes the first and second powers of the density as well as of the pressure, is able to account for 87.2% of the measured variance.(see Figure 5.20).

5.4.2.4 Recovery of alcohols and linalool

In the case of alcohols, the desorption by CO_2 conformed less to simple models than was the case for aldehydes (from comparison of R^2 values). From experimental data (Figure 5.21), at the lowest pressure used, 9.66 MPa, as the temperature increased, the recovery yields decreased. At the two higher pressures, the relationship between yield and temperature was apparently more complex.

As for aldehydes, the percentage recovery could be expected to approach an asymptote of 100% as pressure increases, and the measured data were consistent with this. However, the regression model (Figure 5.22), with the first and second powers of pressure, predicts a maximum value rather than an asymptote. The model is therefore physically unrealistic. Nevertheless, the model can account for 61% of the observed variance. A model which included the flow rate as a variable showed a similar ability to account for the variance (60.9%) suggesting that flow rate had no real effect on recovery of alcohols.

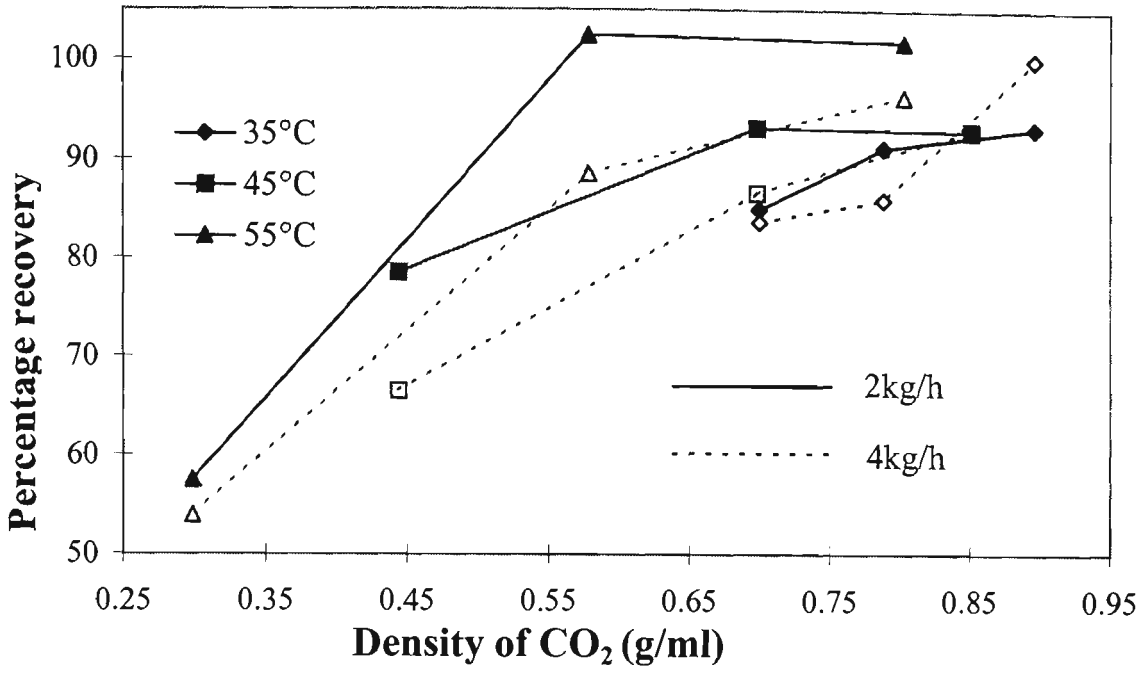


Fig. 5.17. Percentage recovery of total feed aldehydes using 5 kg CO_2, isotherms as functions of CO_2 density (observed).

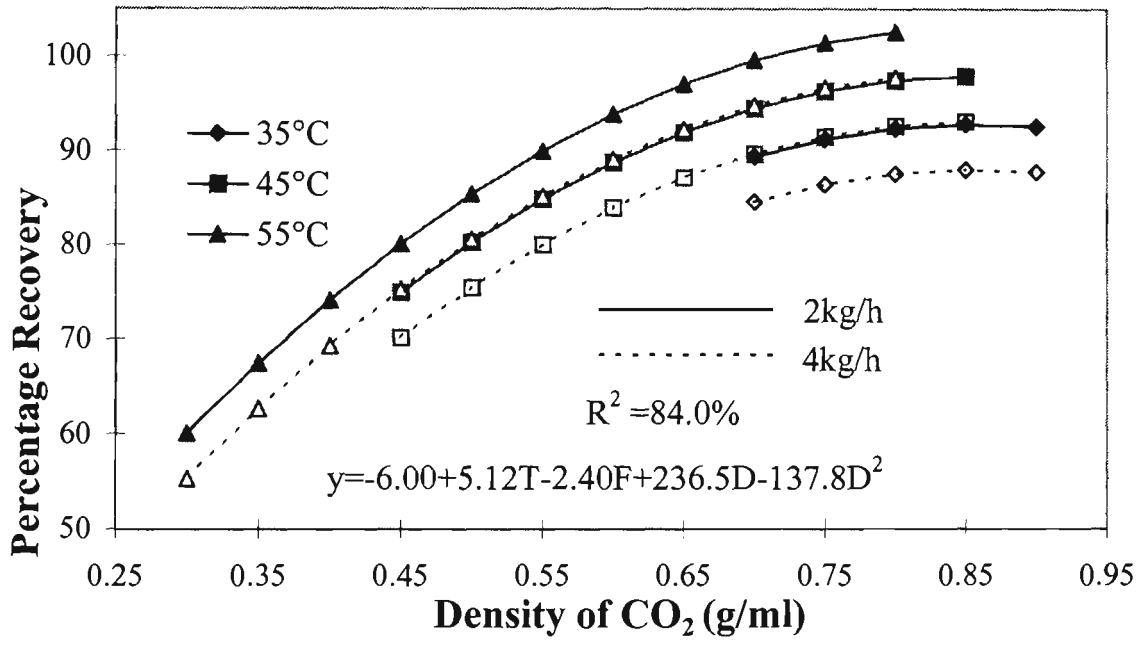


Fig 5.18. Percentage recovery of total feed aldehydes using 5 kg CO_2, isotherms as functions of CO_2 density (modelled), T, and F in this model represent the coded temperature and flowrate of CO_2 and D is the density of CO_2. R^2 is the percentage of variance accounted for by the model.

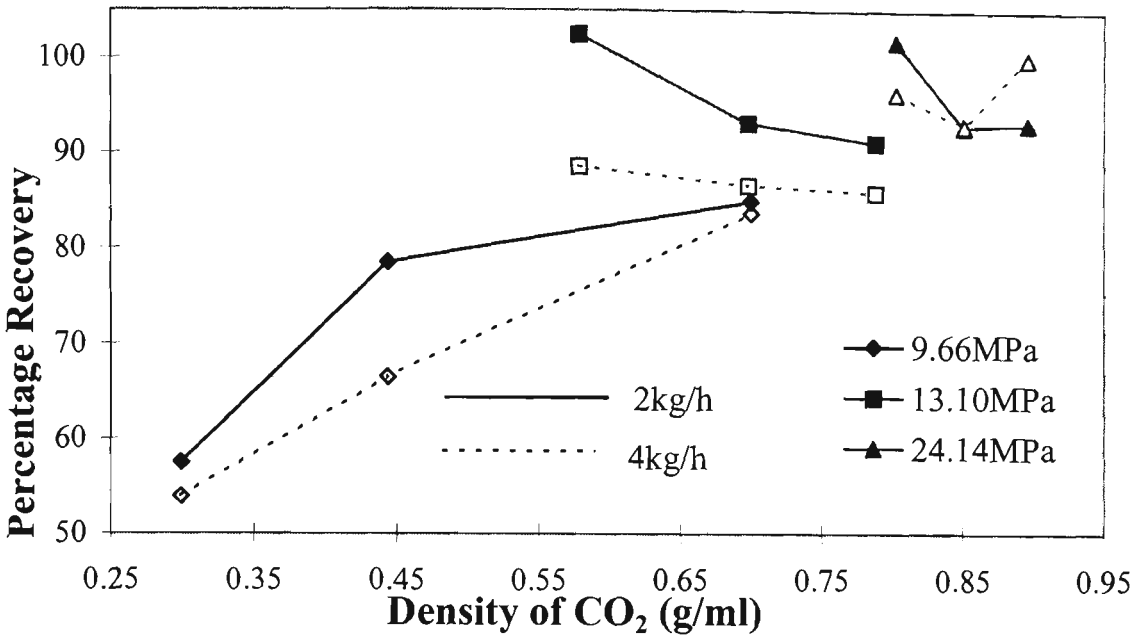


Fig 5.19. Percentage recovery of total feed aldehydes using 5 kg CO_2, isobars as functions of CO_2 density (observed).

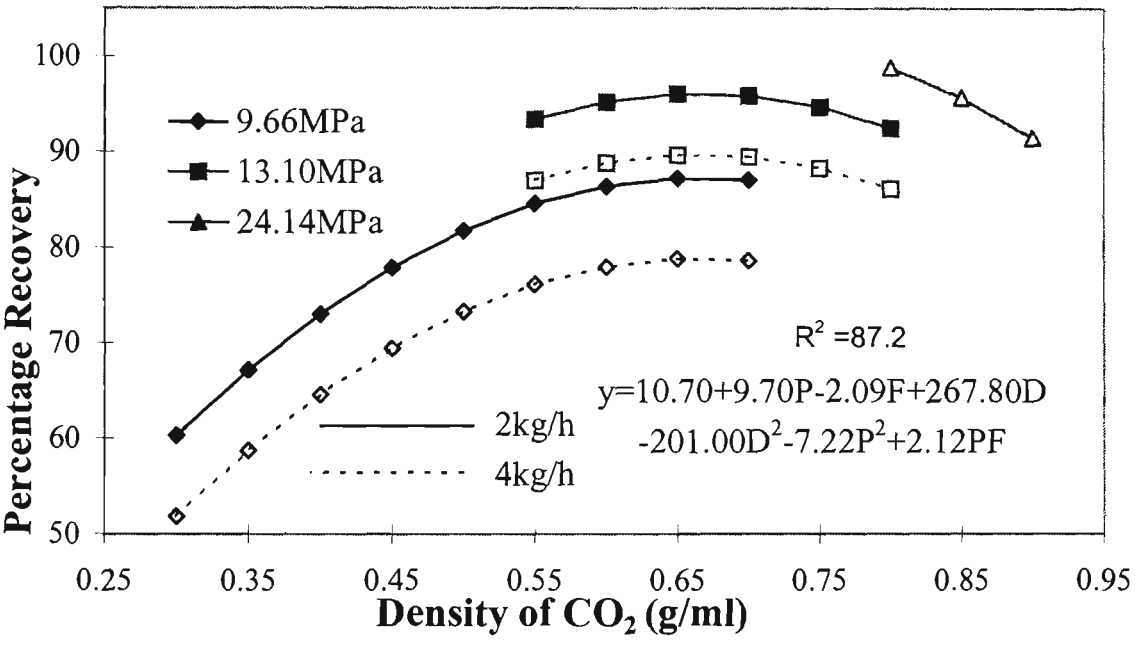


Fig 5.20. Percentage recovery of total feed aldehydes using 5 kg CO_2, isobars as functions of CO_2 density (modelled). P and F in this model represent the coded value of pressure and flowrate of CO_2 and D is the density of CO_2. R^2 is the percentage of variance accounted for by the model. At 24.14 MPa the two recovery curves overlap.

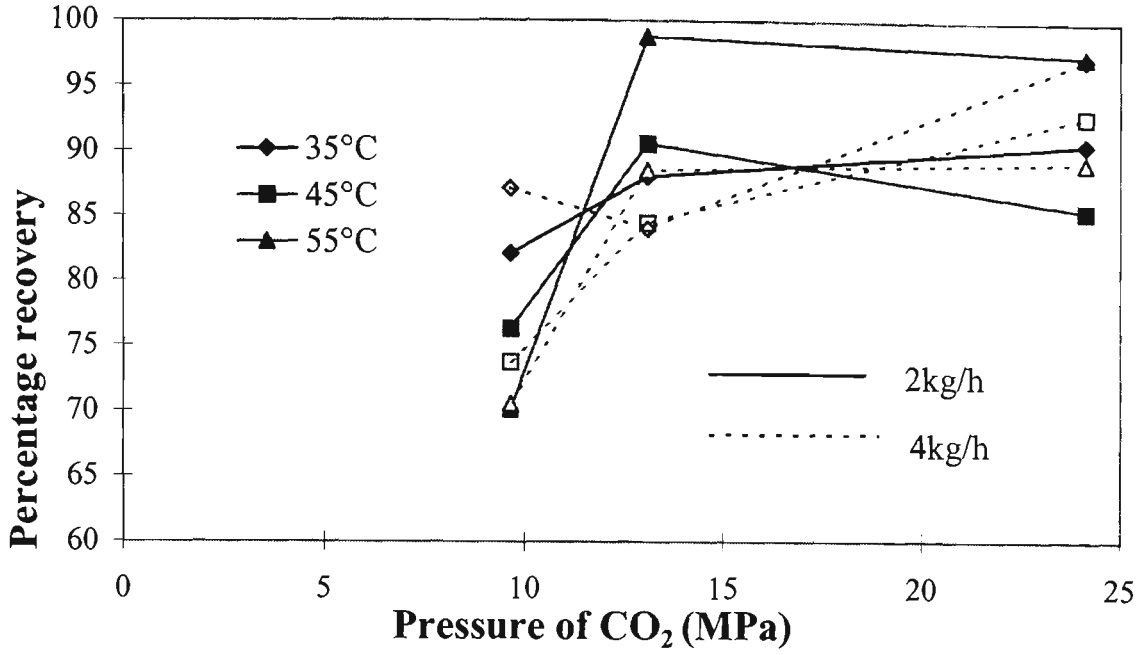


Fig 5.21. Percentage recovery of total feed alcohols using 5 kg CO_2, isotherms as functions of pressure (observed).

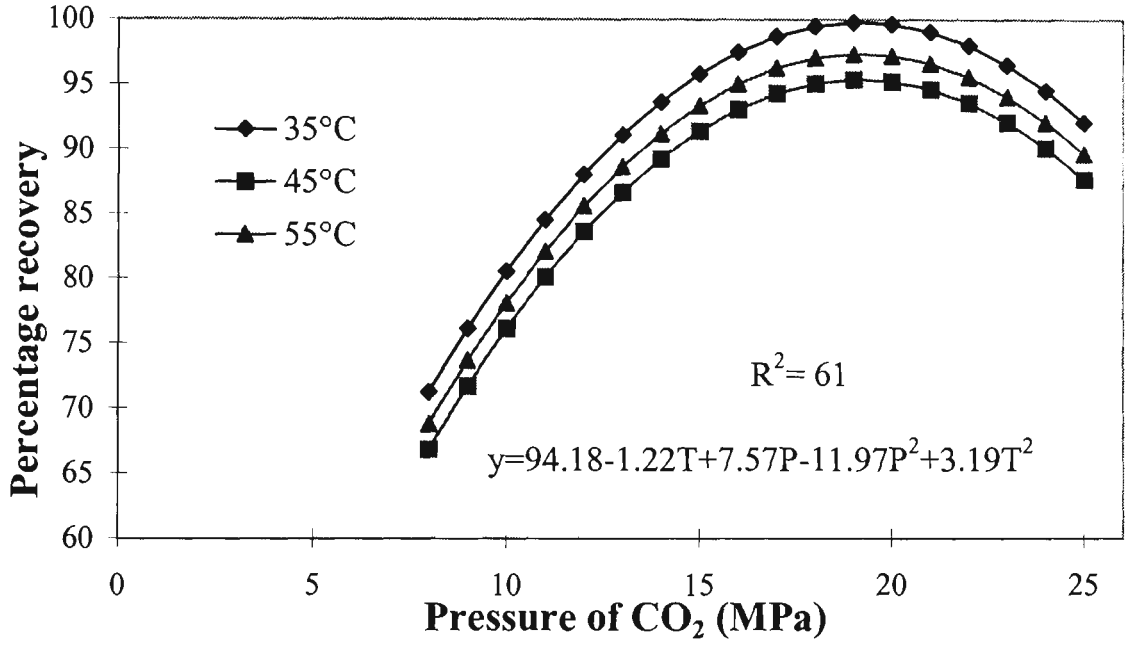


Fig 5.22. Percentage recovery of total feed alcohols using 5 kg CO_2, isotherms as functions of pressure (modelled). P and T in this model represent the coded pressure and temperature of CO_2. Flowrate is not a variable in this model. R^2 is the percentage of variance accounted for by the model.

As was seen in aldehyde recovery models, when the x axis variable was changed from pressure to density for isothermal data, the model fit improved greatly and the form of the model was more realistic in that it did not have a maximum in the range of the experiment. This can be seen by comparing Figure 5.21 to Figure 5.24. As for aldehydes, the dependence of yield on temperature suggests an endothermic desorption process for alcohols.

Recovery of total feed alcohols as a function of density (isobars at three different pressures) is shown in Fig 5.25. The percentage recovery by extraction with 5 kg CO_2 at a fixed pressure is not necessarily expected to approach an asymptote of 100% as density increases. This is because the condition of fixed pressure requires that the temperature be lower at higher densities. The measured data reflect the expected complexity of the relationship.

The regression model, which includes the first and second powers of the density as well as of the pressure, is able to account for 68.1% of the measured variance. The model predicts a maximum recovery for each isobar, but the measured data can neither confirm nor disprove the prediction. The flow rate is not included as a variable in the model plotted in Fig 5.26 because a model including flow rate is only able to account for 66.6% of the measured variance, therefore its effect was found to be not statistically significant. This suggests that flow rate had no real effect on recovery of alcohols.

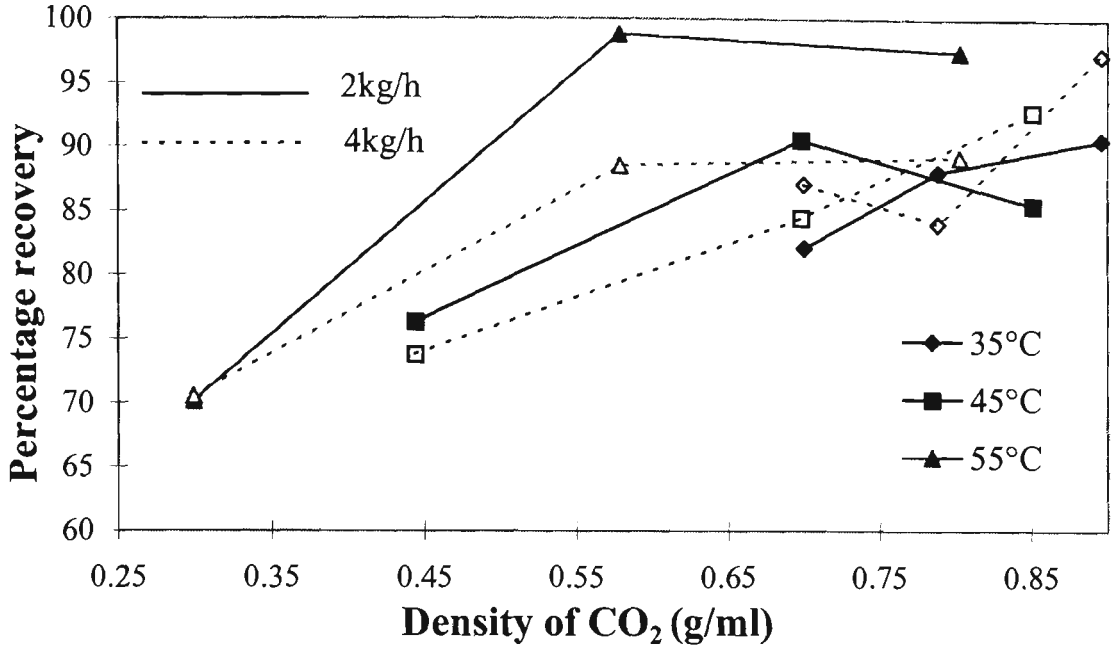


Fig 5.23. Percentage recovery of total feed alcohols using 5 kg CO_2, isotherms as functions of CO_2 density (observed).

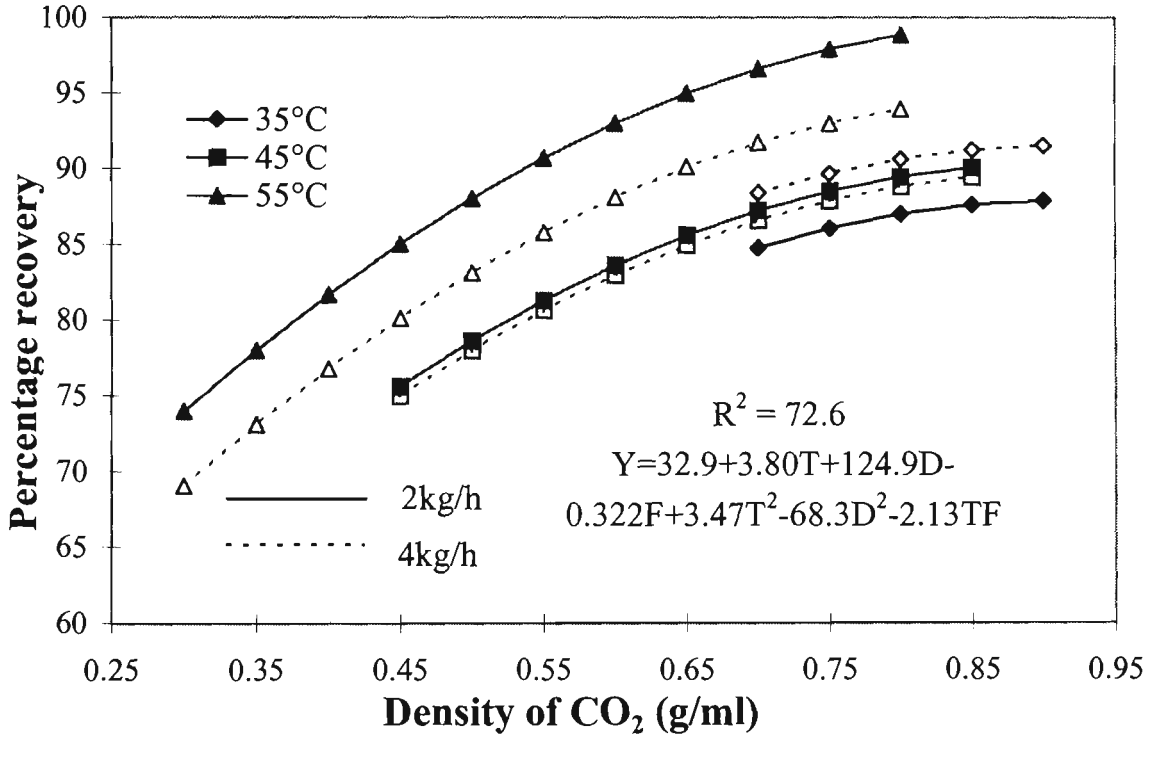


Fig 5.24. Percentage recovery of total feed alcohols using 5 kg CO_2, isotherms as functions of density (modelled). P and F in this model represent the coded pressure and flowrate of CO_2 and D is the density of CO_2. R^2 is the percentage of variance accounted for by the model.

5.4.3 Production of ten-fold concentrates by fractional CO_2 extraction

5.4.3.1 Production of ten-fold aldehyde concentrate

Figure 5.27 shows the recoverable yield of aldehydes as a ten-fold concentrate, as a function of pressure at the three process temperatures. Figure 5.28 shows the corresponding estimate of the response surface as a simple function of pressure, temperature and CO_2 flow rate. The ten-fold concentrate is defined as a concentrate with an aldehyde/terpene ratio ten times that of feed oil, and the yield is expressed as a percentage of the amount of aldehydes in the feed oil. Considering the procedure used to calculate the yield of ten-fold concentrate was more complex than the calculations of total recoverable yield, and that a value of zero was observed for one extraction run, it is perhaps not surprising that the fit with the model was not as good as for the total recovery yield. However, some trends are still seen from the modelled isothermal curves and most cases of experimental data, e.g. that the lower the temperature, the better the separation that could be achieved. This indicates that lower temperatures improved the separability of the components of orange oil and can be explained by the effect of temperature on the equilibrium content of desorption as discussed in Section 5.4.2.3. If the binding of oxygenated compounds is exothermic (or the desorption is endothermic), retention of oxygenated compounds is favoured by lower temperatures.

The effect of CO_2 flow rate was small and uncertain, as shown by the model.

The observed data for replicates (Appendix 5) show relatively poor reproducibility, indicating sensitivity to experimental variations such as column packing. Despite this

apparent low reproducibility, most of the measured isotherms show a maximum, which is not unexpected given the effects of pressure on both the aldehyde and terpene extraction curves.

The regression model, $Y=54.19-14.99T-2.56P+0.85F-19.17P^2-6.77TP+4.32PF$ which includes the first and second powers of the pressure, also predicts maxima in the isotherms, but can only account for 61.5% of the measured variance. This may be the result of the poor reproducibility for this response variable amplified by the complex calculations. In summary, the model and the measured data both indicate that the lower the desorption temperature the higher the yield.

Fig 5.29 shows the yield of ten-fold concentrate of aldehyde as a function of density at the three process temperatures. In this form the data could not be so readily modelled by regression analysis. No simple function including density as a variable could account for more than 40% of the variance. There was no model found which was adequately predicted the experimental data.

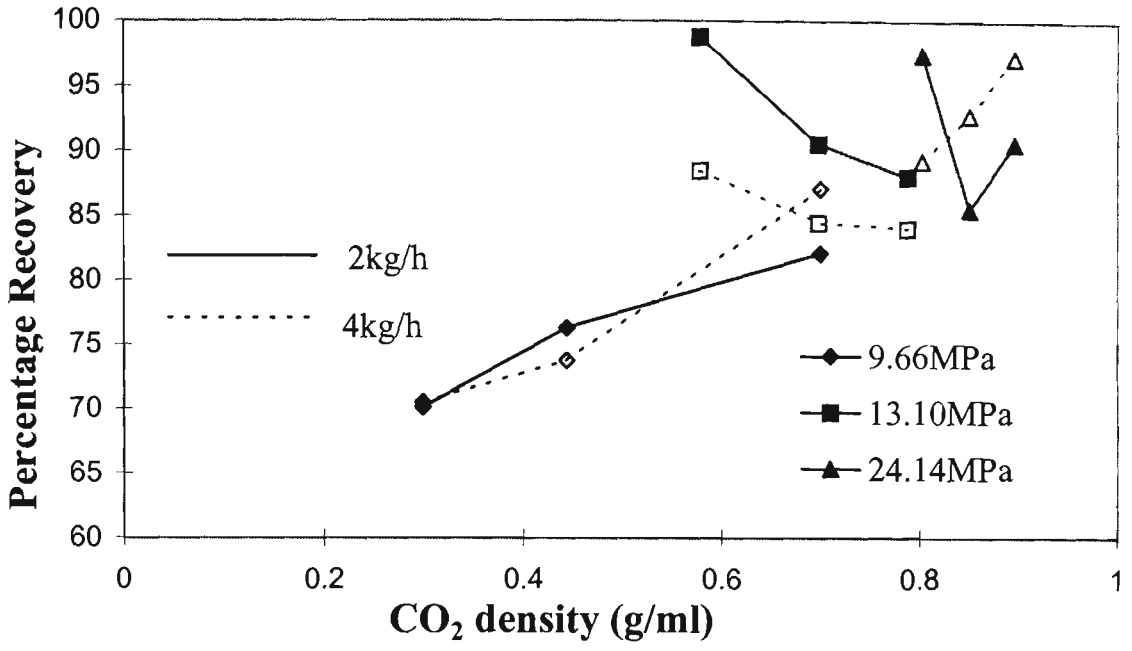


Fig 5.25. Percentage recovery of total feed alcohols using 5 kg CO_2, isobars as functions of density (observed).

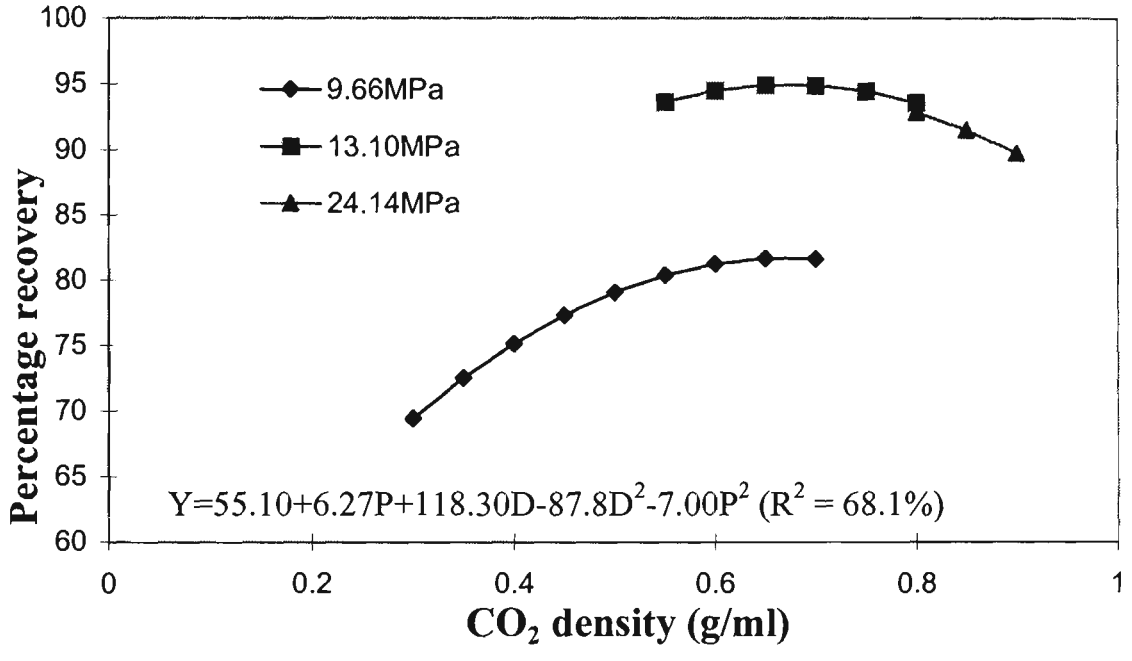


Fig. 5.26. Percentage recovery of total feed alcohols using 5 kg CO_2, isobars as functions of density (modelled). P and D in this model represent the coded value of pressure and density of CO_2. R^2 is the percentage of variance accounted for by the model. Flowrate is not a variable in this model.

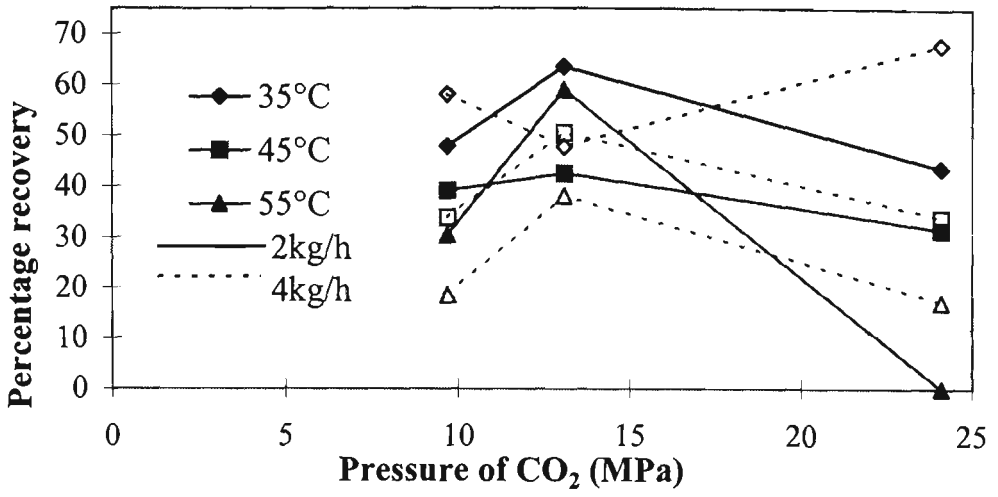


Fig 5.27. Percentage recovery of aldehydes 10 times concentrated as the feed (ald/ter) (observed), isotherms as functions of pressure.

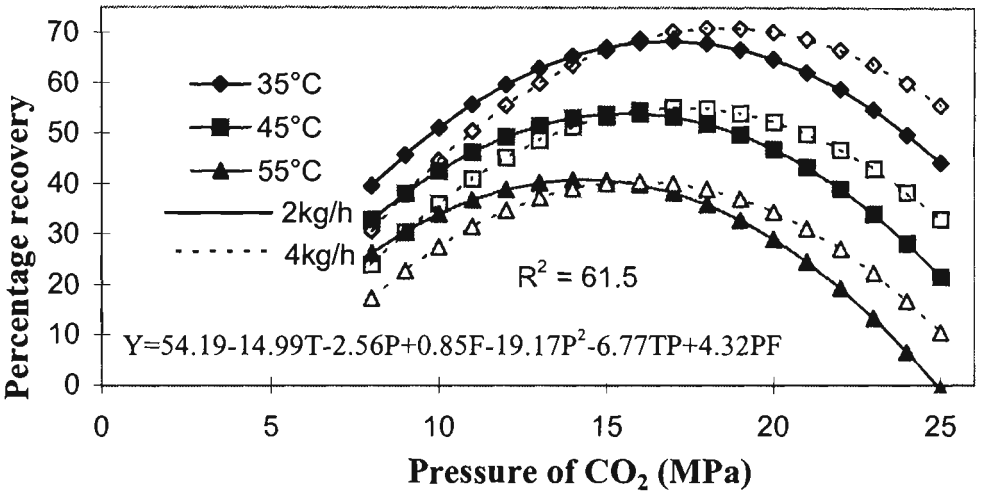


Fig 5.28. Percentage recovery of aldehydes 10 times concentrated as the feed (ald/ter) (modelled), isotherms as functions of pressure. T, P and F in this model represent the coded temperature, pressure and flowrate of CO_2. R^2 is the percentage of variance accounted for by the model.

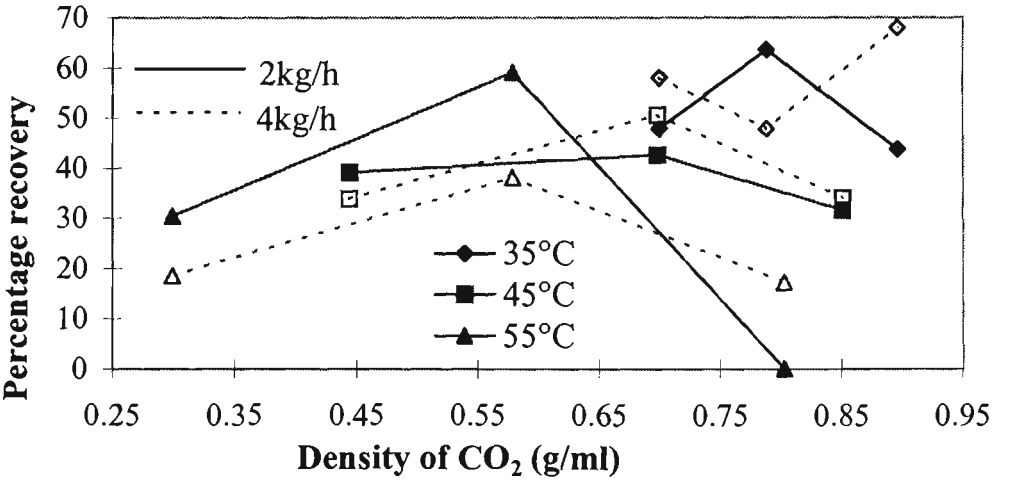


Fig. 5.29. Percentage recovery of aldehydes 10 times concentrated as the feed (ald/ter) (observed), isotherms as functions of density.

5.4.3.2 Production of ten-fold decanal concentrate

This response variable is defined as the percentage of the amount of decanal in the feed oil recoverable as a concentrate with a decanal/limonene ratio ten times that of feed oil. Figure 5.30 shows the recoverable yield of decanal in the form of a ten-fold concentrate, as a function of pressure at the three process temperatures. Figure 5.31 shows the corresponding estimate of the response surface as a simple function of pressure, temperature and CO_2 flow rate. The curves are very similar to the curves presented in 5.4.3.1 and the equation to the estimate of the response surface, $Y=60.56-15.13T-1.43P+1.55F-20.95P^2-5.84TP+4.26PF$ is also very similar to the equation in 5.4.3.1. The regression equation could account for 54.4% of the observed variance. The advantage of using this response variable for the process is that it can be calculated from measurements of the concentrations of only the major aldehyde and terpene respectively, thus allowing measurement by simpler methods than those employed in the present work. There seems to be no significant loss of information in using this variable in place of the yield of ten-fold concentrated aldehydes (5.4.3.1). In feed oil decanal was 46.08% of total aldehyde, and limonene was 97.50% of total terpene, while in the most concentrated fraction decanal was 47.14% of total aldehyde and limonene was 97.65 of total terpene. It would be expected that there would be a slight increase in error variance associated with measuring decanal by itself, as opposed to measuring total aldehydes which is a substantially larger quantity. This may be reflected in the slight reduction in percentage of variance accounted for by the decanal model when compared to the aldehyde model. The similarity of the values for

regression coefficients indicates that most of the aldehydes behaved very similarly to decanal.

5.4.3.3 Production of ten-fold alcohol concentrate

Figure 5.32 shows the percentage yield of ten-fold concentrate of alcohols as a function of pressure at the three process temperatures. The definition of ten-fold concentrate is similar to the definition for aldehydes (section 5.4.3.1) as is the definition of percentage yield. Fig 5.33 shows the best simple regression model of the response surface,

$y=20.59-7.49T-10.65P-7.24F+3.48TP$ ($R^2 = 73$).

The response surface slopes steadily downwards as pressure or temperature increases and also slopes downwards as CO_2 flow rate increases. This indicates that at a given temperature the selectivity of silica gel for adsorption of alcohols relative to terpenes decreases as the pressure increases, and therefore as the density increases. This behaviour is in line with the general observation that the selectivity of SC-CO_2 as a solvent decreases as its pressure or density increases (Chapters 1, 2 and 3). The selectivity of the desorption also decreases as temperature increases, which is to say that the desorption of alcohols is favoured by higher temperatures. Assuming that the desorption of alcohols is close to equilibrium at any point of time, the temperature effect can be explained if the desorption process is endothermic, according to the Van't Hoff equation (Section 5.4.2.3). The negative effect of flow rate on selectivity is also not surprising as higher flow rates can reduce the chromatographic effect in adsorption/desorption systems by increasing the deviation from equilibrium.

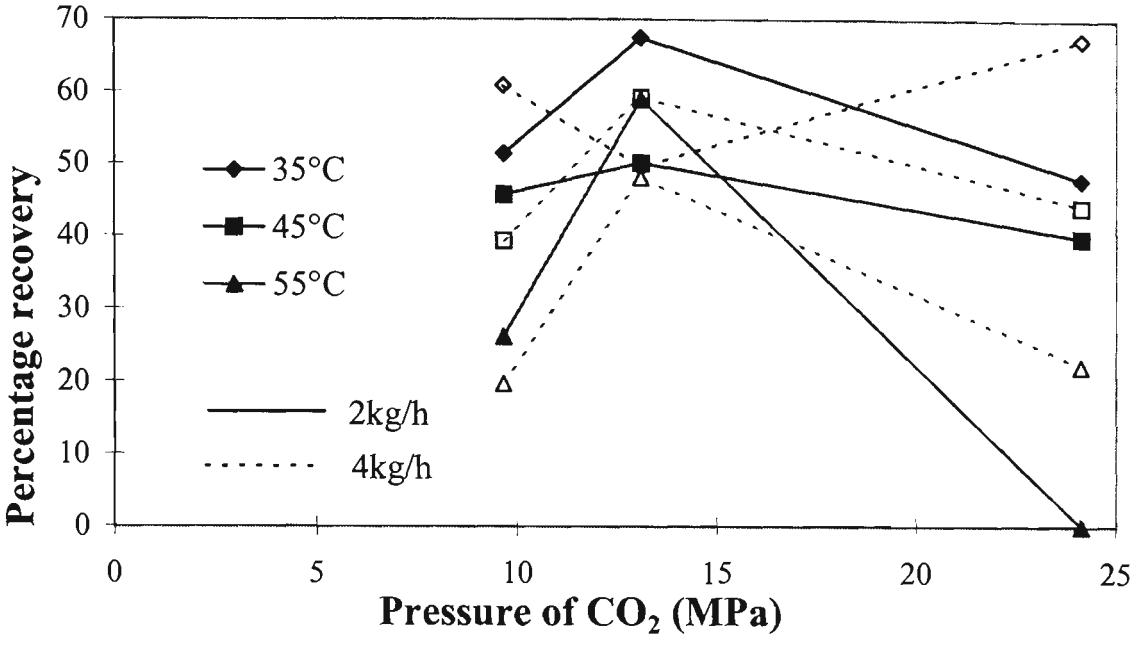


Fig 5.30. Percentage recovery of decanal 10 times concentrated as the feed (dec/lim) (observed), isotherms as functions of pressure.

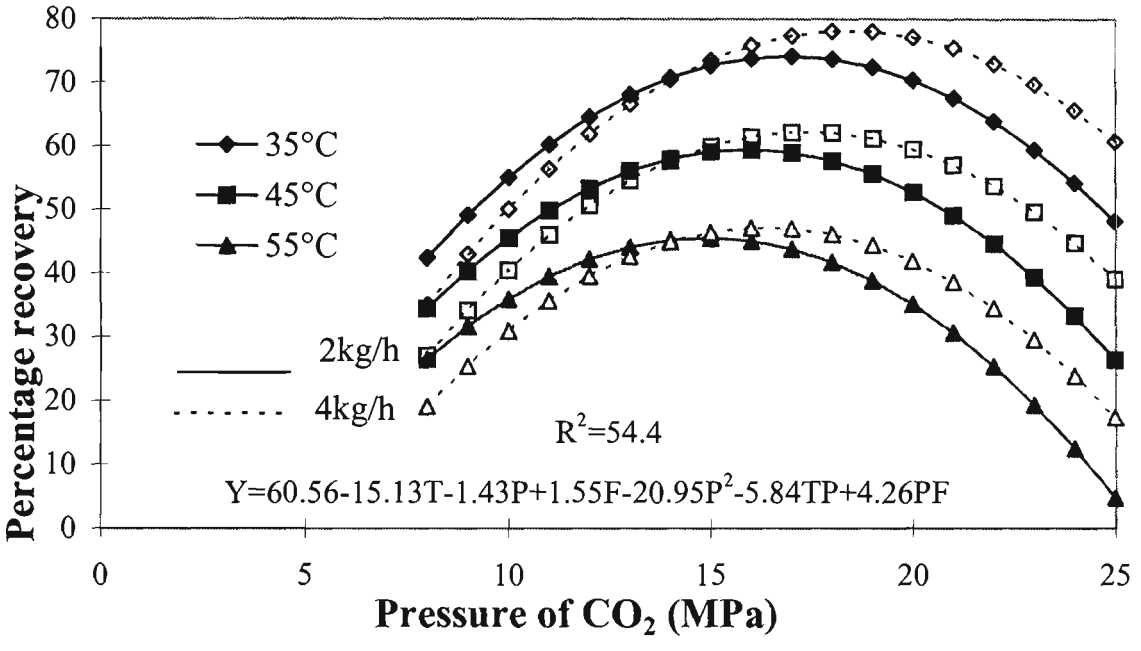


Fig 5.31. Percentage recovery of decanal 10 times concentrated as the feed (dec/lim) (modelled), isotherms as functions of pressure of CO_2. T, P and F in this model represent the coded temperature, pressure and flowrate of CO_2. R^2 is the percentage of variance accounted for by the model.

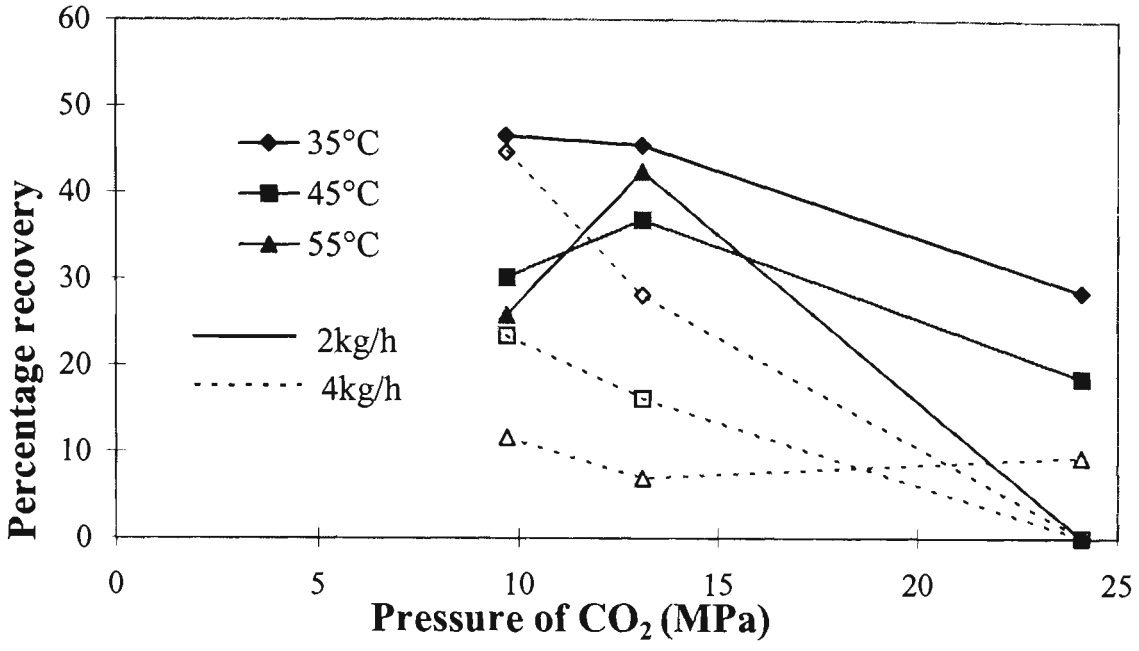


Fig 5.32. Percentage recovery of alcohols 10 times concentrated as the feed (alc/ter) (observed), isotherms as functions of pressure.

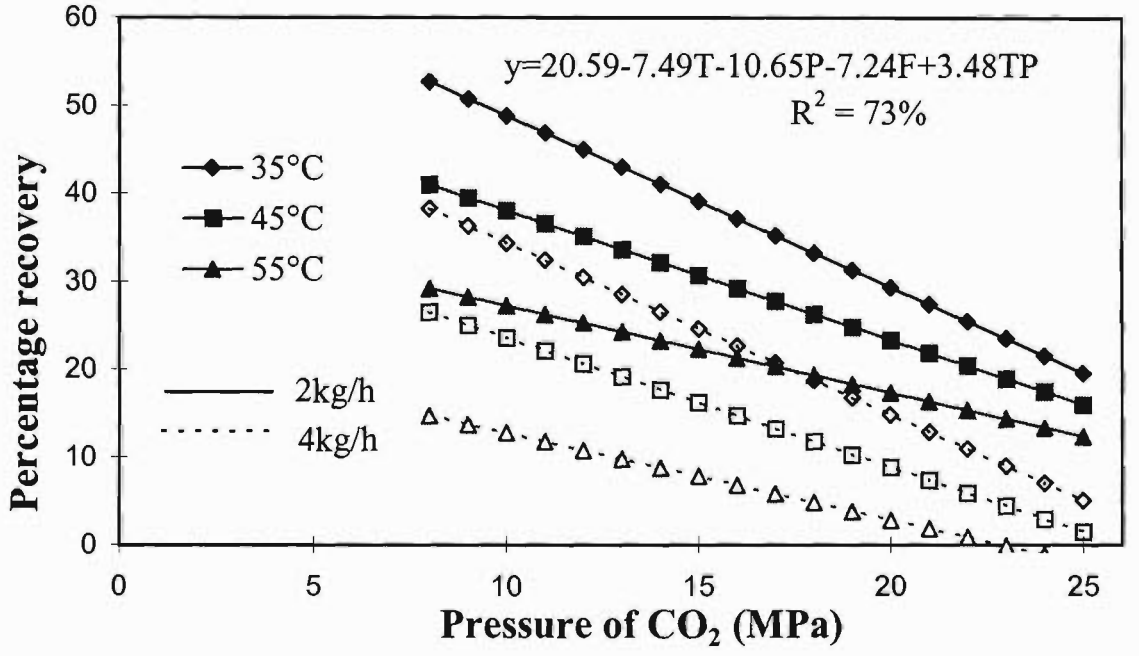


Fig 5.33. Percentage recovery of alcohols 10 times concentrated as the feed (alc/ter, modelled), isotherms as functions of pressure. T, P and F in this model represent the coded temperature, pressure and flowrate of used CO_2. R^2 is the percentage of variance accounted for by the model.

The observed data for replicates as mentioned above show relatively poor reproducibility, indicating sensitivity to experimental variations such as column packing. Despite this apparent low reproducibility, and in contrast to the data for aldehyde concentrates, most of the measured isotherms do not show a maximum, but suggest that the pressure for maximum yield of alcohol concentrate is outside the range studied. A lower density of CO_2 favours retention of alcohols on the silica gel while terpenes are desorbed. The regression model of best fit is consequently one without the second power of the pressure, and showing a linear trend to higher yields at lower pressures.

Outside the measured range, especially at lower pressures, this model is probably physically unrealistic; at lower pressures it is expected that the lower solubility of alcohols would reduce the recoverable yield of alcohol concentrate.

It can be concluded that the optimum pressure for recovering alcohol concentrates is lower than the optimum for aldehyde concentrates. This agrees with the conclusion of Temelli et al. (1990) that linalool is more difficult than decanal to separate from terpene compounds. It can also be concluded that the lower the temperature the greater the yield of alcohol concentrates.

5.4.3.4 Production of ten-fold linalool concentrate

Figure 5.34 shows the percentage yield of ten-fold concentrate of linalool as a function of pressure at the three process temperatures. The definition of ten-fold concentrate is

similar to the definition for decanal (section 5.4.3.2) as is the definition of percentage yield. Fig 5.35 shows the best simple regression model of the response surface. The family of curves is similar to the family of curves presented in 5.4.3.3 and the equation to the estimate of the response surface,

$Y=22.17-14.48T-8.89P-4.34F+7.9T^2$

is also similar to the equation in 5.4.3.3 in that the signs of all the coefficients are the same although the magnitudes differ somewhat. This contrasts with the close similarity observed between the decanal response surface and the total aldehydes response surface described in Section 5.4.3.2. The result may indicate that linalool is less representative of all the alcohols than decanal is representative of all the aldehydes. The regression equation could account for 70.4% of the observed variance. In feed oil, linalool was about 62.36% of total alcohol and limonene was 97.50% of total terpene. In the most concentrated fraction linalool was 71.05% of total alcohol and limonene was 97.65% of total terpene. It would be expected that there would be a slight increase in error variance associated with measuring linalool by itself, as opposed to measuring total alcohols which is a larger quantity. This may be reflected in the slight reduction in percentage of variance accounted for by the linalool model when compared to the alcohol model. The advantage of using the yield of ten-fold concentrate of linalool as the response variable for the process, rather than the equivalent total alcohol variable, is that it can be calculated from measurements of the concentrations of only the major alcohol and terpene respectively, thus allowing measurement by simple methods.

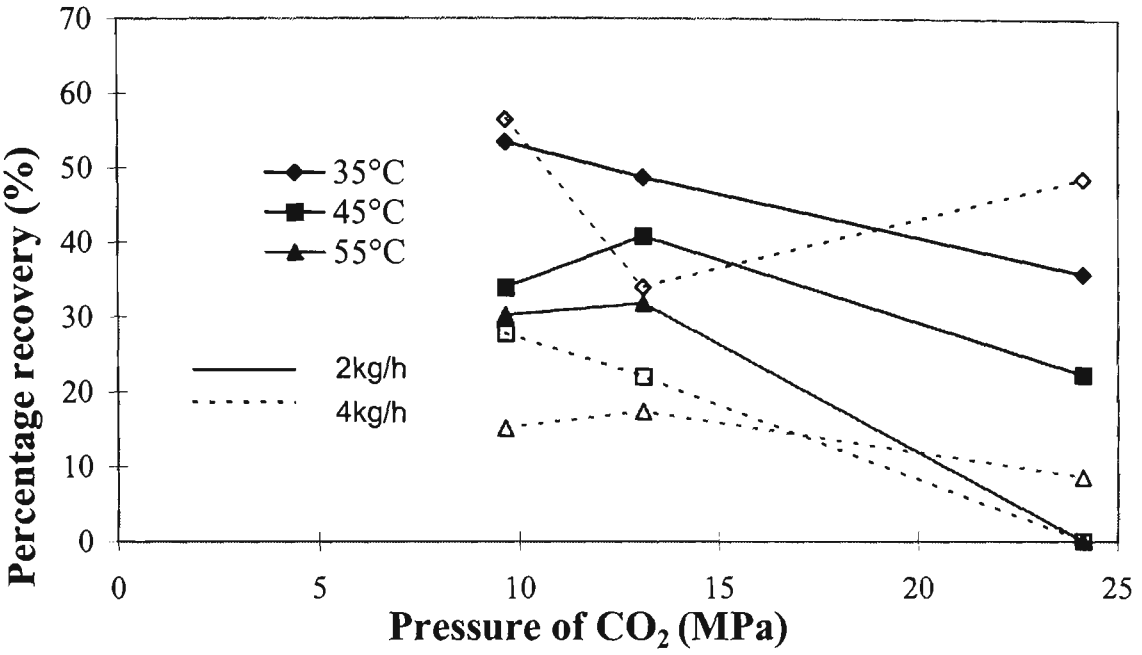


Fig. 5.34. Percentage recovery of linalool 10 times concentrated as the feed (lin/lim, observed), isotherms as functions of pressure.

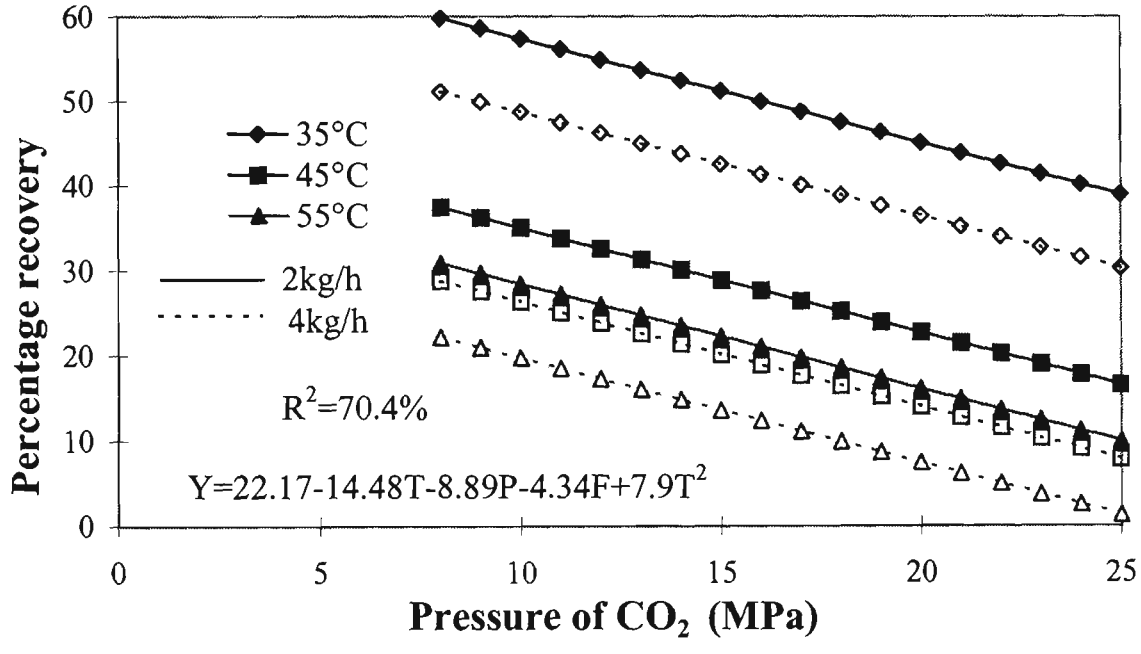


Fig. 5.35. Percentage recovery of linalool 10 times concentrated as the feed (lin/lim)(modelled), isotherms as functions of pressure of CO_2. T, P and F in this model represent the coded temperature, pressure and flowrate of CO_2. R^2 is the percentage of variance accounted for by the model.

5.4.4 Production of fifteen-fold concentrates by fractional CO_2 extraction

5.4.4.1 Production of fifteen-fold aldehyde concentrate

Fig 5.36 shows the percentage yield of aldehydes as a fifteen-fold concentrate as a function of pressure at the three process temperatures. Fig 5.37 shows a corresponding family of curves lying on the response surface described by the equation,

$Y=28.48-6.25T-8.98P+1.12F-8.45P^2-2.4TP+1.97PF$

This response surface accounts for 66.2% of the observed variance.

These curves show similar characteristics to the curves for ten-fold concentrates of aldehydes (section 5.4.3.1) with pressure and temperature having the major effects. The effect of flow rate was small and of doubtful significance. In most of the measured isotherms and in all of the model isotherms there was an optimum pressure for recovering aldehyde concentrate which was the result of the interaction of the effects of CO_2 solvent properties on the desorption curves of terpenes and aldehydes respectively. Again there was a marked reduction in yield of concentrate with increased temperature, which would be expected according to the Van't Hoff equation (Section 5.4.2.3). if the desorption of aldehydes was endothermic and if the desorption process was close to equilibrium.

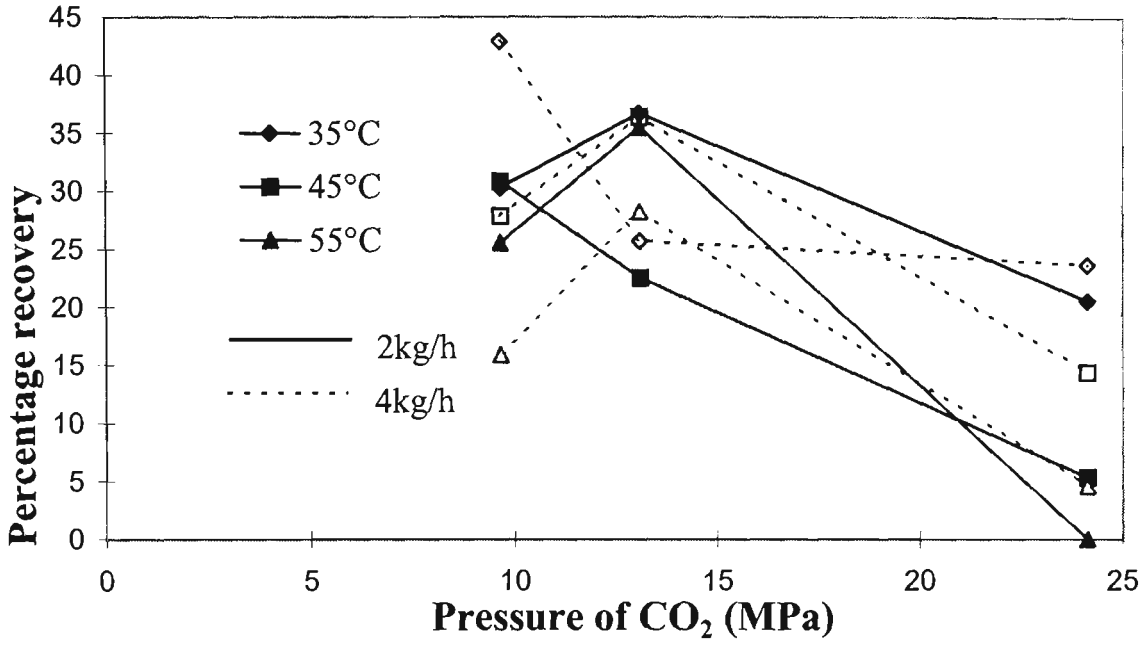


Fig.5.36. Percentage recovery of aldehydes 15 times concentrated as the feed (ald/ter) (observed), isotherms as functions of pressure.

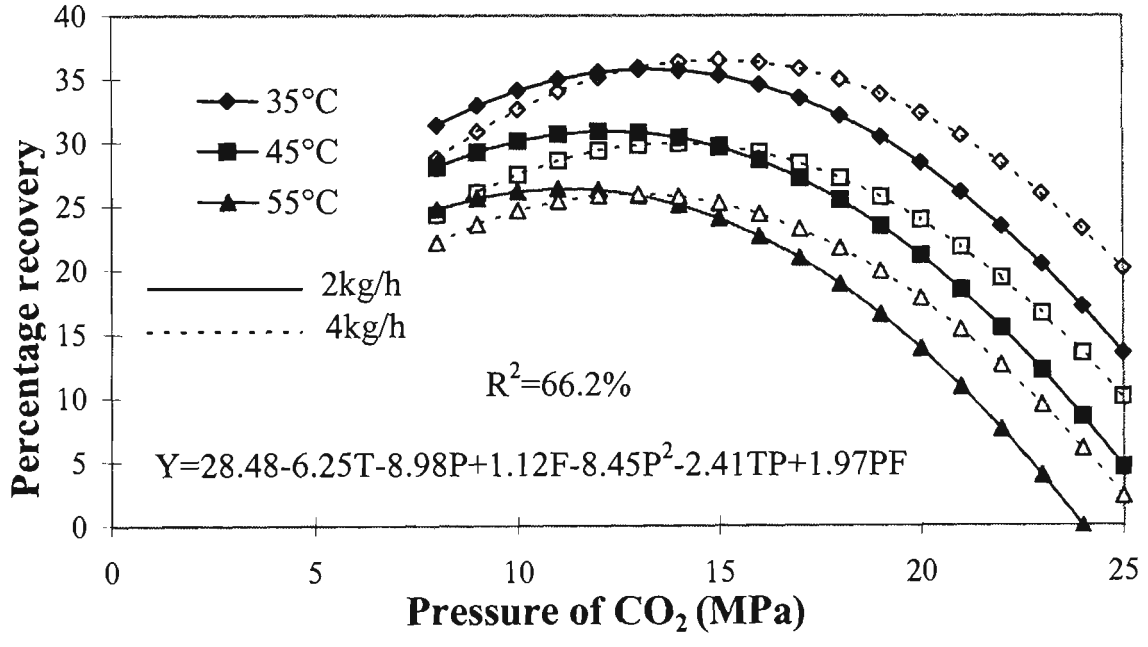


Fig. 5.37. Percentage recovery of aldehydes 15 times concentrated as the feed(ald/ter) (modelled), isotherms as functions of pressure of CO_2. T, P and F in this model represent the coded temperature, pressure and flowrate of CO_2. R^2 is the percentage of variance accounted for by the model.

5.4.4.2 Production of fifteen-fold decanal concentrate

Fig 5.38 shows the percentage yield of decanal as a fifteen-fold concentrate as a function of pressure at the three process temperatures. Fig 5.39 shows a corresponding family of curves lying on the response surface described by the equation,

$Y=36.65-9.76T-7.92P+2.90F-11.32P^2-4.16TP+3.31PF$

This response surface accounts for 58.6% of the observed variance. As was observed for ten-fold concentrates of decanal, the use of decanal and limonene concentrations in place of total aldehydes and terpenes, respectively, produced only minor changes in the data and response surface (Section 5.4.4.1). This means that this more easily measured response variable could be used to represent the production of fifteen-fold concentrate of total aldehydes.

5.4.4.3 Production of fifteen-fold alcohol concentrate

Fig 5.40 shows the percentage yield of alcohols as a fifteen-fold concentrate as a function of pressure at the three process temperatures. Fig 5.41 shows a corresponding family of curves lying on the response surface described by the equation,

$Y = 6.67-3.51T-8.52P-4.22F+5.37P^2$

This response surface accounts for 76.2% of the observed variance.

These curves show similar characteristics to the curves for ten-fold concentrates of alcohols (section 5.4.3.3). In the range studied, the higher the pressure, temperature or flow rate the lower the yield of concentrate. Again these trends can be attributed to higher pressure CO_2 being a less selective solvent, to the desorption of alcohols being

endothermic, and to the action of higher solvent flow rates in reducing chromatographic separation.

5.4.4.4 Production of fifteen-fold linalool concentrate

Fig 5.42 shows the percentage yield of linalool as a fifteen-fold concentrate as a function of pressure at the three process temperatures. No simple regression model could be found which could account for more than 50% of the observed variance, so no attempt has been made to estimate the Response Surface. This inability to fit a model is probably the result of the experimental errors becoming too large in relation to the small amounts of concentrated linalool being measured.

5.4.5 Organoleptic assessment of selected samples

Organoleptic testing was carried out by staff of Keith Harris & Co. Ltd. Samples from thirteen desorbed fractions with decanal contents over 3.0% were blended together in proportion to the masses of the fractions to produce a pooled sample containing 4.61% decanal. The fractions had been collected under a range of operating conditions which are detailed in Appendix 6. Using an evaluation panel of three trained assessors, the flavour of the pooled sample was compared with a ten fold orange oil produced by vacuum distillation. The SC-CO_2 refined oil was found to have superior flavour. The fractions were then individually assessed for aroma and five were identified as promising because of the lack of "oily/peely" aroma and the presence of aldehydic,

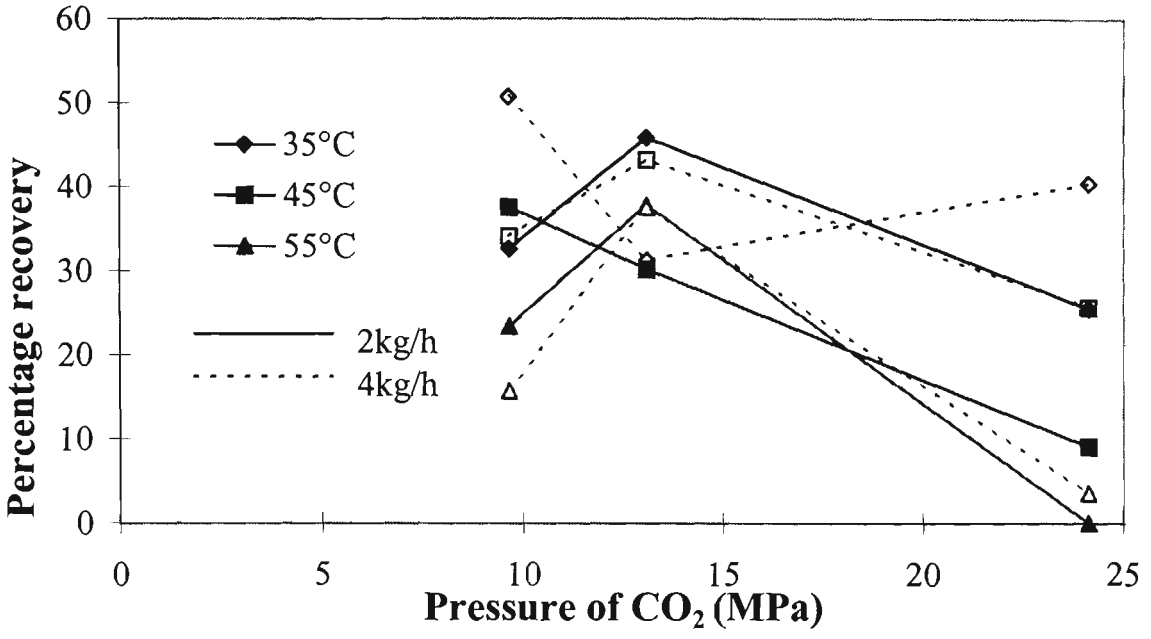


Fig 5.38. Percentage recovery of decanal 15 times concentrated as the feed (dec/lim) (observed), isotherms as functions of pressure.

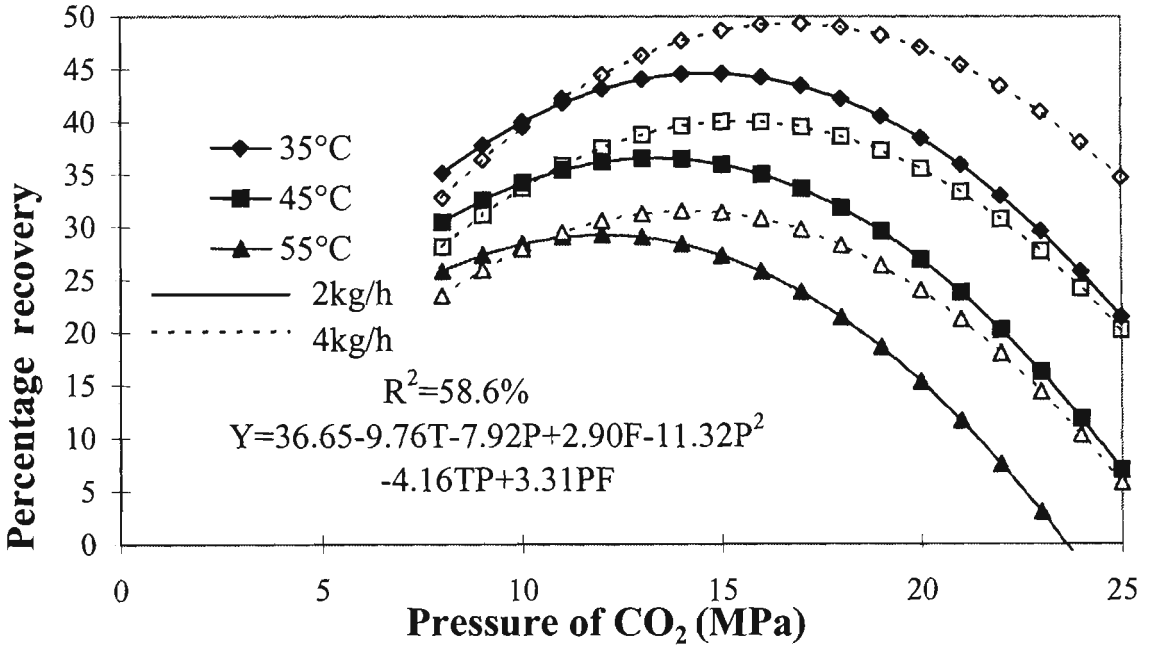


Fig 5.39. Percentage recovery of decanal 15 times concentrated as the feed (dec/lim) (modelled), isotherms as functions of pressure of CO_2. T, P and F in this model represent the coded value of temperature, pressure and flowrate of CO_2. R^2 is the percentage of variance accounted for by the model.

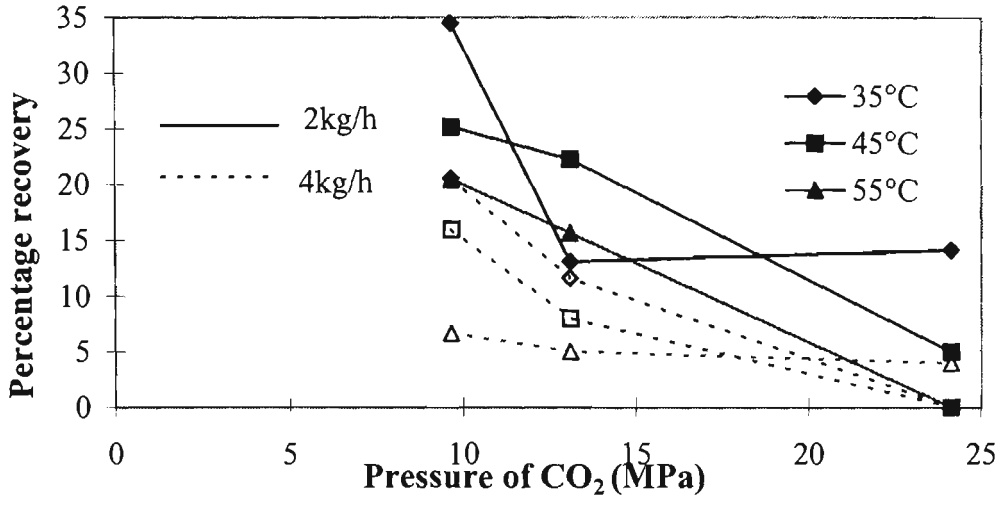


Fig 5.40. Percentage recovery of alcohols 15 times concentrated as the feed (alc/ter) (observed), isotherms as functions of pressure.

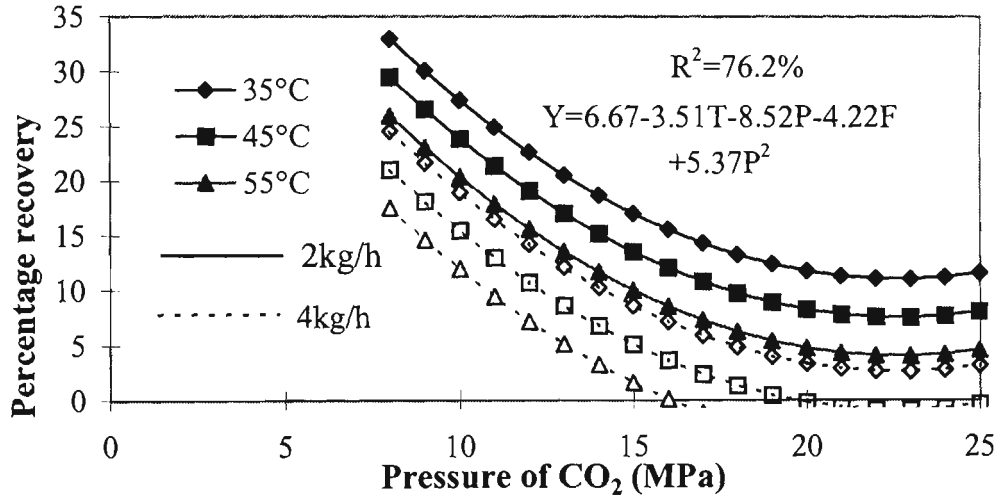


Fig 5.41. Percentage recovery of alcohols 15 times concentrated as the feed(alc/ter) (modelled), isotherms as functions of pressure of CO_2. T, P and F in this model represent the coded temperature, pressure and flowrate of CO_2. R^2 is the percentage of variance accounted for by the model.

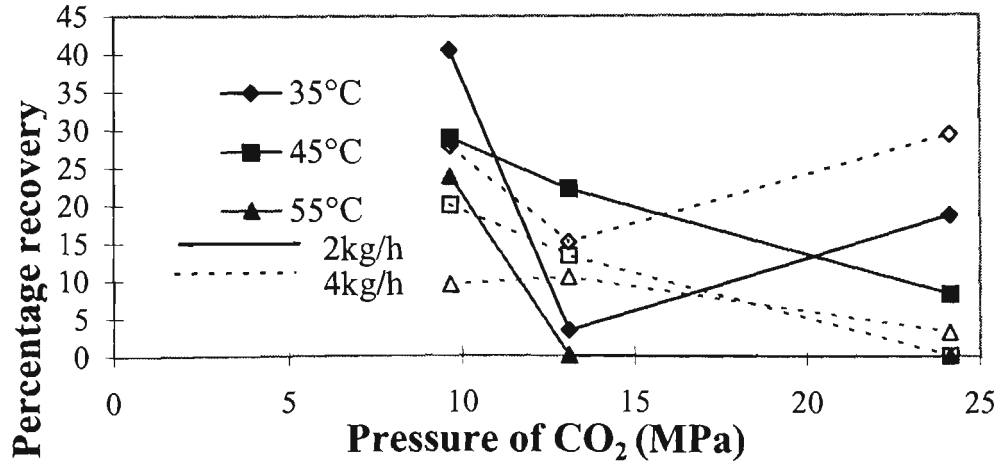


Fig 5.42. Percentage recovery of linalool 15 times concentrated as the feed (lin/lim) (observed), isotherms as functions of pressure of CO_2.

sweet, or "fresh peely" characteristics. The identity, concentration and aroma properties of these five samples are listed in Appendix 6.

5.4.6 Summary of factors affecting process performance

5.4.6.1 Effects of CO_2 density (and pressure)

Table 5.5 lists the best fit regression models of the response surfaces for the various response variables concerned with total recovery of adsorbed oil. In all cases, the regression coefficient of the pressure is positive with F probability < 0.001 and the coefficient of the square of the pressure is negative with F probability smaller than 0.05 in 87.5% of the cases, reflecting the fact that the solubility of these components increases with increasing CO_2 pressure as expected and observed, but that the trend to increasing recovery with increasing pressure must flatten out as total recovery is approached. This increase in solubility of orange oil components with increasing CO_2 pressure (or density) has been measured by Goto et al (1997).

Table 5.6 presents some linear regression models of the amount of adsorbate recoverable as 10-fold concentrates, where the adsorbate is characterised as major aldehydes, decanal, major alcohols, or linalool, concentrated relative to total mass, major terpenes or limonene as indicated. In all cases, the regression coefficient of the pressure was negative and in most cases the regression coefficient of the square of the pressure was also negative. Thus all the fitted models suggest that the lower the CO_2 pressure, the better the separation. This conclusion is in agreement with the finding of

Barth et al. (1994) and Chouchi et al. (1996). Furthermore this finding is in line with the adsorption equilibrium constants for limonene and linalool on silica gel reported by Goto et al. (1997). Inspection of their results, plotted on a logarithmic scale, reveals that the distance between the log plots increased as density decreased. According to the discussion of selectivity detailed in section 3.4.3, this means that selectivity can expected to increase at lower CO_2 densities. The use of density and square of density terms in the models of the present work consistently worsened the agreement of the linear regression models with the results. This indicates that, in the case of recovery of concentrates, the relationship between yield and CO_2 density was not simple.

5.4.6.2 Effects of extraction temperature

As shown in Tables 5.5 and 5.6, the regression coefficient of the temperature was almost always negative. The negative effect of temperature on yield was much greater for recovery of concentrate than for recovery of total adsorbate. This indicates that lower temperatures improved the separability of the components of orange oil (Table 5.6) and also the total adsorbate recoverable in 5 kg of CO_2 (Table 5.5). The former result can be explained if, as expected, the binding of oxygenated compounds is exothermic, so that retention of oxygenated compounds is favoured by lower temperatures. The latter result appears to arise from the increased solvent power of CO_2 associated with its lower temperature, and hence higher density. When density and temperature were simultaneously defined process variables, the regression coefficient of temperature was not negative. This can be attributed to the density effect being removed from the temperature term in the model. Goto et al. (1997) reported

adsorption equilibrium constants for linalool and limonene as a function of CO_2 density at 40°C, 50°C and 60°C. The results were presented on a logarithmic scale which tended to obscure the differences between the isotherms. However, close inspection of the data reveals that lower temperatures favoured adsorption, supporting the view that the adsorption process was exothermic.

5.4.6.3 Effects of CO_2 flow rate

Table 5.5 shows that the regression coefficient of the flow rate is relatively significant and negative in all models describing aldehydes or decanal recoverable in 5 kg CO_2. The regression coefficient of the flow rate was not significant for recoverable alcohols or linalool, and has been excluded from the tabulated models. For aldehydes, the higher the flow rate, the lower the recoverable adsorbate. As discussed in section 5.4.2.3 this phenomenon cannot be attributed to a rate process in the column as the desorption curves had already become flat by the time 5 kg of CO_2 had been used. If the effect is truly significant it must be attributed to loss of aldehyde product in the separator cell, which may be increased when the CO_2 flow rate is increased.

Table 5.6 shows that with the yield of alcohol or linalool concentrates as the response variable, the regression coefficient of the flow rate was negative, which can be explained as the reduction of the chromatographic separation effect as solvent flow rate increased.

However, with the yield of aldehyde or decanal concentrates as the response variable, the regression coefficient of the flow rate was small and positive. This is an interesting result. Higher flow rate seemed to favour the retention of aldehydes on the silica gel, relative to terpenes and alcohols, resulting in a higher yield of aldehyde concentrate. The physical properties of the aldehyde molecules are not thought to be substantially different from the terpenes and alcohols as they have a similar range of molecular weights. Therefore it is unlikely that the mass transfer properties of the aldehydes differed greatly from those of terpenes and alcohols. It is more likely that the delayed desorption of aldehydes relative to terpenes and alcohols resulted from a slow chemi-sorption/desorption process which did not occur with the other classes of compounds. Another indication that adsorption of aldehydes on silica may be slow was the lack of affinity of Celite (a form of silica) for aldehydes in the rapid experiment reported in Section 4.4.2. Dugo (1995) reported that Celite did adsorb aldehydes from lemon oil. This could explain why aldehydes were the first oxygenated compounds to break through the silica gel column during loading and were also retarded during extraction, relative to alcohols, when the CO_2 flow rate was increased. The possibility of using different adsorption/desorption rates to achieve fractionation of essential oils does not appear to have been investigated, and may need further research.

Table 5.5. Estimates of Response Surface of total yields using 5 kg CO_2 *

		R^2(%)	constant	T	P	F	D	D^2	P^2	T^2	TP	PF	TF
Aldehydes	coefficient	71.7	1.0304	-0.0224	0.1208	-0.024			-0.1973		0.0551		
(Figure 5.14)	F probability			0.15	<.001	0.145			0.001		0.041		
Aldehydes	coefficient	87.2	0.107		0.097	-0.0209	2.678	-2.01	-0.0722			0.0212	
(Figure 5.20)	F probability				<.001	0.038	<.001	0.001	0.006			0.095	
Aldehydes	coefficient	84	-0.06	0.0512		-0.024	2.365	-1.378					
(Figure 5.18)	F probability			0.061		0.058	<.001	0.003					
Alcohols	coefficient	61	0.9418	-0.0122	0.0757				-0.1197	0.0319			
(Figure 5.22)	F probability			0.451	<.001				0.006	0.087			
Alcohols	coefficient	68.1	0.551		0.0627		1.183	-0.878	-0.07				
(Figure 5.26)	F probability				<.001		0.003	0.097	0.029				
Alcohols	coefficient	72.6	0.329	0.038		-0.00322	1.249	-0.683		0.0347			-0.0213
(Figure 5.24)	F probability			0.372		0.747	<0.001	0.06		0.15			0.13
Decanal	coefficient	66.2	1.0011	-0.0278	0.1179	-0.0295			-0.1994		0.0696		
(Figure 5.16)	F probability			0.123	<.001	0.123			0.003		0.028		
Decanal **	coefficient	85.8	-0.155		0.0969	-0.0265	3.431	-2.614	-0.0458			0.021	
	F probability				<.001	0.024	<.001	<.001	0.021			0.135	
linalool **	coefficient	62.4	0.944	-0.02	0.0713				-0.1101	0.0318	0.0241		
	F probability			0.128	<.001				0.005	0.179	0.191		
linalool **	coefficient	71.5	0.484		0.0401		1.242	-0.848	-0.0451				
	F probability				<.001		<.001	0.081	0.078				

* Full recovery in these models is represented by a yield of 1.0

** No figures for these models

Table 5.6. Estimates of the Response Surface describing recoveries of components 10 times as concentrated as feed *

		R^2(%)	constant	T	P	F	D	D^2	P^2	T^2	TP	PF
Aldehyde - [ald]/[ter] (Figure 5.28)	coefficient	61.5	0.5419	-0.1499	-0.0256	0.0085			-0.1917		-0.0677	0.0432
	F probability			<.001	0.093	0.915			0.015		0.069	0.1
Aldehyde - [ald]/[ter]**	coefficient	37.2	0.377		-0.2267	0.0085	-1.19	1.78	-0.098			0.0432
	F probability				0.181	0.933	0.003	0.196	0.607			0.19
Alcohol -[alc]/[ter] (Figure 5.33)	coefficient	73	0.2059	-0.0749	-0.1065	-0.0724					0.0348	
	F probability			0.003	<.001	<.001					0.221	
Alcohol - [alc]/[ter]**	coefficient	68.3	-0.196	-0.1227		-0.0724	2.104	-2.048				
	F probability			0.005		0.001	0.002	0.005				
Alcohol - [alc]/[ter]**	coefficient	77.6	-0.2101		-0.2101	-0.0724	0.6					
	F probability				<.001	<.001	<.001					
decanal - [decanal]/oil **	coefficient	57	0.4709	-0.1303	-0.0754				-0.1762		-0.066	
	F probability			0.002	0.004				0.038		0.108	
decanal - [decanal]/oil **	coefficient	40.6	-0.178		-0.2182		0.729					
	F probability				0.011		0.01					
linalool -[linalool]/oil**	coefficient	56.7	0.0143	-0.0558	-0.114	-0.0332			0.1515			
	F probability			0.056	<.001	0.117			0.031			
decanal - [dec]/[lim] (Figure 5.31)	coefficient	54.4	0.6056	-0.1513	-0.0143	0.0155			-0.2095		-0.0584	0.0426
	F probability			<.001	0.236	0.709			0.018		0.157	0.148
linalool - [lin]/[lim] (Figure 5.35)	coefficient	70.4	0.2217	-0.1448	-0.0889	-0.0434				0.079		
	F probability			<.001	<.001	0.043				0.062		

* Full recovery in these models is represented by a yield of 1.0

** No figures for these models

Chapter 6

Conclusions and Recommendations

General Conclusions

At the commencement of this study it was not known what basic factors would limit the extraction and/or fractionation of these two oils by dense carbon dioxide under the experimental conditions used.

These basic questions can now be answered. Under the conditions studied in this work it was found that there was no significant mass transfer limitation on extraction of rice bran oil. There may have been some mass transfer effects near the point of exhaustion of oil in rice bran, but throughout most of each extraction the process appeared to be limited by equilibrium solubility phenomena which are discussed more fully below. There was also no evidence of channelling through the rice bran bed or of disruption of the bed structure as a result of pressure drop across the bed. It appears that the cellular structure of the rice bran matrix was well suited to SC-CO_2 extraction

In the case of desorption of orange oil from a silica gel matrix, a similar set of basic questions can be answered as a result of this study. Firstly, the absence of a flow rate effect on most of the response variables indicates that mass transfer limitation of the desorption was not significant. The observed effect of flow rate on yield of aldehyde concentrate was explained as a chemi-sorption effect in Chapter 5. It appears that the

high diffusivity of dense carbon dioxide enabled the desorption to occur without significant mass transfer limitation.

In contrast to the work with rice bran, there appear to have been some problems with the structure of the bed of silica gel. The low reproducibility of the duplicate runs has been attributed to channelling in the silica gel bed which consisted of particles of approximately 10^{-4} m diameter, similar to the particle size of rice bran. One possibly important difference was the depth of the bed packed into the column. In the case of rice bran it was approximately 74 cm while the silica gel bed was only 22 cm deep. The general absence of flow rate effects in all experiments indicates that higher flow rates would produce similar results but in a shorter time.

Rice Bran Extraction with Dense Carbon Dioxide

With an hourly flow of carbon dioxide equal to eight to ten times the mass of rice bran, the extraction of the oil of the rice bran was virtually complete in six hours under the conditions studied in the present work. The extraction appeared to be limited by the equilibrium solubility of the rice bran oil and the rates of extraction of the various oil components were largely determined by their partition coefficients between the oil phase and the dense CO_2 phase. The early extraction of FFAs would allow partial refinement of the rice bran oil by discarding the early fractions, but the water of rice bran was present in all oil fractions, diminishing the value of the oil. By addition of a separation stage after the extraction stage it was possible to produce a raffinate oil virtually free of water and with FFA concentrations reduced by half.

Under these conditions the concentration of oryzanol in the raffinate was increased by approximately one tenth relative to the whole rice bran oil, while α-tocopherol was maintained at the same level present in the whole oil. However, the sterol concentrations were reduced by about one fifth. These phenomena also appeared to be determined by the equilibrium partition coefficients of the various oil components.

Orange Oil Refining with Supercritical Carbon Dioxide and Silica Gel

Of fourteen materials investigated, silica gel showed the greatest adsorptive capacity for the oxygenated compounds of orange oil. Loading orange oil onto a bed of silica gel up to the point of break-through of oxygenated compounds produced an adsorbate approximately four-fold concentrated relative to the feed oil. In the present work, the desorption of the adsorbate using supercritical CO_2 was monitored and conclusions were reached concerning the effects of the process variables on the amount and concentration of extract.

In all experiments the extraction of oxygenated compounds was retarded relative to terpenes, producing extracts with progressively higher concentrations of oxygenated compounds. High CO_2 density increased the solubility of orange oil components and thus hastened the process, but reduced the selectivity of the extraction. In the range studied, the lowest temperature maximised the separation of oxygenated compounds

from terpenes. The CO_2 flow rate, in the range studied, had only minor effects on the extracts, except in the case of the recovery of aldehyde concentrates.

It can be concluded that, with the exception of aldehydes, the desorption of the components of orange oil was limited by the respective equilibrium desorption isotherms. For the aldehydes, however, the higher CO_2 flow rate favoured retention of the adsorbed aldehydes relative to the other oil components. It was concluded that desorption of aldehydes was limited partly by a slow chemical desorption step under the conditions studied.

The use of Response Surface Methodology in this study of the refining of orange oil successfully allowed the effects of three process variables to be estimated from a relatively small number of experimental runs. However, there was not enough reproducibility between replicate runs to allow accurate statistical comparison of various models of the response surfaces

Recommendations

Both the rice bran and silica gel beds were physically suitable for extraction and separation with dense carbon dioxide. Future work should investigate higher carbon dioxide flow rates to determine conditions at which mass transfer limitation becomes significant, as the commercially optimum conditions of both processes will lie in this region.

Refining rice bran oil with an adsorbent to further reduce FFA and concentrate the high value components is recommended for future study.

Desorption of orange oil from silica gel showed low reproducibility which should be investigated further with a deeper bed of silica gel and controlled pressure packing before larger scale work is undertaken. Adsorption of orange oil onto silica gel concentrated the flavour compounds four-fold, which may have some commercial application and should be investigated further.

Desorption of orange oil flavour compounds from silica gel into dense carbon dioxide produced useful quantities of ten-fold concentrates, with the optimum conditions being low temperature and low density of carbon dioxide. As the optimum conditions may well be at temperature and pressure values lower than investigated in this study, future work should explore a lower range of temperatures and pressures.

Desorption of the aldehyde components of orange oil from silica gel was slower than desorption of other components. Future work should investigate whether the aldehydes can be separated from the other flavour compounds using high rate desorption from silica gel.

References

Adams, R. P. Compounds sorted in order of elution times on DB - 5. In *Identification of essential oils by ion trap mass spectroscopy*; Academic Press, Inc.: San Diego, **1989**.

Adasoglu, N.; Dincer, S.; Bolat, E. Supercritical -fluid extraction of essential oil from Turkish lavender flowers. The Journal of Supercritical Fluids, **1994**, *7*, 93-99.

Adhikari, S.; Adhikari, J. Indian ricebran lecithin. *A.O.C.S.* **1986**, 1367-1369.

Al-Wandawi, H.; Abdul-Rahman, M.; Al-Shaikhly, K. Tomato processing wastes as essential raw materials source. *J.Agric.Food Chem.* **1985**, *33*, 804-807.

AOAC Method 926.12: Moisture and volatile matter in oils and fats. In *Official methods of Analysis of the Association of Official Analytical Chemists*, 15th. ed., Herlich, K., Ed.; AOAC, Arlington, VA **1990**.

AOAC. Method 940.28: Fatty Acids (Free) in Crude and Refined Oils. In: *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., Helrich, K. (Ed.); AOAC, Arlington, **1990**.

Arul, J.; Tardif, R.; Boudreau, A.; McGinnis, D.S.; Lencki, R.W. Solubility of milk fat triglycerides in supercritical carbon dioxide. *Food Res. Int.* **1994**, *27*, 459-467.

Baaliouamer, A.; Meklati, B. Y.; Fraisse, D.; Scharff, C. Analysis of leaf oils from four varieties of sweet orange by combined gas chromatography-mass spectrometry. *Flavour and Frangrance Journal* **1988,** *3*, 47-52.

Bamberger, T.; Erickson, J.C.; Cooney, C.L. Measurement and model prediction of solubilities of pure fatty acids, pure triglycerides, and mixtures of triglycerides in supercritical carbon dioxide. *J. Chem. Eng. Data* **1988**, *33*, 327-333.

Barth, D.; Chouchi, D; Della Porta, G; Reverchon, E; Perrut, M. Desorption of lemon peel oil by supercritical carbon dioxide: Deterpenation and psoralens elimination. *J. Supercritical Fluids* **1994**, *7*, 177-183.

Bondioli, P.; Mariani, C.; Lanzani, A.; Fedeli, E.; Mossa, A.; Muller, A. Lampante olive oil refining with supercritical carbon dioxide. *J. Amer. Oil Chem. Soc.*, **1992**, *69*, 477-480.

Braddock, R. J.; Cadwallader, K. R. Citrus by-products manufacture for food use. *Food Tech.* **1992**, *46*, 105-110.

Braddock, R. J.; Kesterson, J. W. Concentration of oxygenated flavor compounds in citrus oils. *Proceedings of the Florida State Horticultural Society* **1976,** *89*, 196-198.

Braverman, J. B. S.; Solomiansky, L. Separation of terpeneless essential oils by the chromatographic method. *Perfumery & Essential Oil Res.* **June 1957**, 284-287.

Brunetti, L.; Daghetta, A.; Fedeli, E.; Kikic, I; Zanderighi, L. Deacidification of olive oils by supercritical carbon dioxide. *J. Amer. Oil Chem. Soc.* **1989**, *66,* 209-217.

Brunner,G. Industrial process development: countercurrent multistage gas extraction perches. *Proceedings fourth international Symposium on Supercritical Fluids,* Sendai **1997**, ISBN4- 925085-02-6, Volume C, p745-756.

Brunner, G.; Peter S. On the solubility of glycerides and fatty acids in compressed gases in the presence of an entrainer. *Separation science and technology* **1982**, *17*, 199-214.

Bruno, T. J.; Ely, J.F. Supercritical fluid technology: reviews in modern theory and applications. CRC Press: Boston, **1991**.

Buckholz, L. L.; Daun, H. Instrumental and sensory characteristics of orange oil oxidation. *J. Food Sci.* **1978**, *43*, 535 -543

Bulley, N.R.; Fattori, M.; Meisen, A.; Moyls, L. Supercritical fluid extraction of vegetable oil seeds. *J. Amer. Oil Chem. Soc.* **1984**, *61*, 1362-1365.

Chamblee, T. S.; Clark, B. C.; Radford, T.; Iacobucci, G. A. General method for the high-performance liquid chromatographic prefraction essential oils and flavor mixtures for gas chromatographic - mass spectrometric analysis: Identification of new constituents on cold pressed lime oil. *Journal of Chromatography.* **1985,** *330*, 141-151.

Chamblee, T. S.; Clark, B. C.; Brewster, G.B.; Radford, T.; Iacobucci, G. A. Quantitative analysis of the volatile constituents of lemon peel oil. Effects of silica gel chromatography on the composition of its hydrocarbon and oxygenated fractions. *J. Agric. Food Chem.* **1991,** *39*, 162-169.

Chouchi, D.; Barth, D. Rapid identification of some coumarin derivatives in deterpenated citrus peel oil by gas chromatography. *Journal of Chromatography A* **1994**, *672,* 177-183.

Chouchi, D.; Barth, D.; Nicoud, R. M. Fractionation of citrus cold-pressed oils by supercritical CO_2 desorption. *Presented at the Third International Symposium on Supercritical Fluids*, Strasbourg, France, **1994,** pp183-188.

Chouchi, D.; Barth, D.; Reverchon, E.; Della Porta, G. Supercritical CO_2 desorption of bergamot peel oil. *Ind. Eng. Chem. Res.* **1995**, *34*, 4508-4513.

Chouchi, D.; Barth, D.; Reverchon, E.; Della Porta, G. Bigarade peel oil fractionation by Supercritical carbon dioxide desorption. *J. Agric. Food Chem.* **1996**, *44*, 1100-1104.

Chrastil, J. Solubility of solids and liquids in supercritical gases. *J. Phys. Chem.* **1982**, *86*, 3016-3021.

Clark, B.J.; Mailer, R. How Carlton and United made hop extract history. *Brewers' Guardian*, **1981**, October, 13-17.

Cleenewerck, B.; Dijkstra, A. J. The total degumming process - theory and industrial application in refining and hydrogenation. Fett - Wissenschaft - Technologie, **1992**, *94*, 317-322.

Crandall, P. G.; Kesterson, J. W.; Dennis, S. Storage stability of carotenoids in orange oil. *J. Food Sci.* **1983**, *48*, 924-927.

del Valle, J.M.; Aguilera, J.M. An improved equation for predicting the solubility of vegetable oils in supercritical CO_2. *Ind. Eng.Chem.Res.* **1988**, *27*, 1551-1553.

Denbigh, K. Equilibria of reactions involving gases. In *Chemical equilibrium*, Denbigh, K.(Eds.), Cambridge University Press, London, **1966.**

Dobbs, J. M.; Johnston, K.P. Selectivities in pure and mixed supercritical fluid solvents. *Ind. Eng. Chem. Res.* **1987**, *26*, 1476-1482.

Dobbs, J. M.; Wong, J. M.; Lahiere, R. J.; Johnston, K.P. Modification of supercritical fluid phase behavior using polar co-solvents. *Ind. Eng. Chem. Res.* **1987**, *26*, 56-65.

Draper, N. R.; Smith, H. *Applied Regression Analysis*, 2nd ed.; John Wiley and Sons: New York, **1981**.

Dugo, G. The composition of the volatile fraction of the Italian citrus essential oils. *Perfumer and Flavorist*, **1994**, *19*, 29-51.

Dugo, P.; Mondello, L.; Bartle, K. D.; Clifford, A. A.; Breen, D. G. P. A.; Dugo, G. Deterpenation of sweet orange and lemon essential oils with supercritical carbon dioxide using silica gel as an adsorbent. *Flavour & Fragrance Journal* **1995**, *10*, 51-58.

Eggers, R.; Sievers, U.; Stein, W. High pressure extraction of oil seed. *J. Amer. Oil Chem. Soc.* **1985,** *62, 1222-1230.*

Evelein, K.A.; Moore, R.G.; Heidemann, R.A. Correlation of the phase behavior in the systems hydrogen sulfide-water and carbon dioxide-water. *Ind. Eng. Chem. Proc. Des. Dev.* **1976**, *15*, 423-428.

Fattori, M.; Bulley, N.R.; Meisen, A. CO_2 extraction of canola seed: oil solubility and effect of seed treatment. *J. Amer. Oil Chem. Soc.* **1988**, *65*, 968-974.

Feigl, F. Spot tests in organic analysis. Elsevier Publ. Co. New York, NY.

Ferrer, O. J.; Matthews, R. F. Terpene reduction in cold-pressed orange oil by frontal analysis-displacement adsorption chromatography. *J Food Sci.* **1987**, *52*, 801-805.

Fleisher, A. Citrus hydrocarbon-free essential oils. *Perfumer & Flavorist* **1994,** *19*, 11-15.

Friedrich, J.P.; List, G.R. Characterisation of soybean oil extracted by supercritical carbon dioxide and hexane. *J. Agric. Food Chem.* **1982**, *30*, 192-193.

Grimmett, C. The use of liquid carbon dioxide for extracting natural products. *Chemistry and Industry*. **1981**, 16 May.

Galdi, M.; Carbone, N.; Valencia, M. E. Comparison of ferric glycinate to ferrous sulfate in model infant formulas: kinetics of vitamin losses. *J Food Sci.* **1989**, *54*, 1530-1533.

Hargrove, K.L. Processing and utilisation of rice bran in the United States. In: *Rice Science and Technology*; Marshall, W.E.; Wadsworth, J.I. (Eds.); Marcel Dekker, New York, **1994**.

Hartman, L.; Lago, R. C.A. The composition of lipids from rice hulls and from the surface of rice caryopsis. *J. Sci. Fd Agric.* **1976**, *27*, 939-942.

Hawthorne, S. B.; Krieger, M.S.; Miller, D. J. Analysis of flavor and fragrance compounds using supercritical fluid extraction coupled with gas chromatography. *Anal. Chem.* **1988**, *60,* 472-477.

Hiaki T.; Miyagi, H.; Tsuji, T.; Hongo, M. Vapor-liquid equilibria for supercritical carbon dioxide + butanol systems at 313.2 K, presented at The 4th International Symposium on Supercritical Fluids, Sendai, **1997**.

Hierro, M.T.G.; Santa-maria, G. Supercritical fluid extraction of vegetable and animal fats with CO_2 - A mini review. *Food Chemistry*, **1992**, *45*, 189-192.

Ikushima, Y.; Hatakeda, K.; Ito,S.; Saito, N.; Asano,T.; Goto,T. A supercritical carbon dioxide extraction from mixtures of triglycerides and higher fatty acid methyl esters using a gas-diffusion-type system. *Ind. Eng. Chem. Res.* **1988**, *27*, 818.

Kimball, D.A. Citrus Processing: Quality control and technology, 1991, Van Nostrand Reinhold, New York.

King M.B. and Bott T.R., **1993,** Introduction *In*: M.B.King and T.R.Bott (Eds.), Extraction of natural products using near-critical solvent,pp 1-31, Blackie, Glasgow.

Kiran, E,; Levelt Sengers,J. M. H. (Eds.) Supercritical Fluids: Fundamentals for applications, Kluwer, Dordrecht, **1994**.

Kirchner, J. G.; Miller, J. M. Preparation of terpeneless essential oils - a chromatographic process. *Ind. Eng. Chem.* **1952,** *44*, 318-321.

Lack E.; Seidlitz H. Commercial scale decaffeination of coffee and tea using supercritical carbon dioxide, *In*: *Extraction of Natural Products Using Near-Critical Solvent*; King, M. B.; Bott, T. R., Eds.; Blackie: Glasgow, **1993**.

Lee, A. K. K.; Bulley, N. R.; Fattori, M.; Meisen, A. Modelling of supercritical carbon dioxide extraction of canola oilseed in fixed beds. *J. Amer. Oil Chem. Soc.* **1986,** *63*, 921-925.

List, G. R.; Friedrich, J. P. Oxidative stability of seed oils extracted with supercritical carbon dioxide. *J. Amer. Oil Chem. Soc.* **1989**, *66,* 98-101.

List, G. R.; Friedrich, J. P. Processing characteristics and oxidative stability of soybean oil extracted with supercritical carbon dioxide at 50 °C and 8000 psi. *J. Amer. Oil Chem. Soc.* **1985,** *62*, 82-84.

Lucien, F.P.; Liong, K. K.; Cotton, N. J.; Macnaughton, S. J.; Foster, N. R. Separation of biomolecules using supercritical fluid extraction. *Australasian Biotechnology,* **1993**, *3,* 143-146.

Lund, E. D.; Coleman, R. L. Concentrated orange flavouring powder based on methanol-treated starch. *IFFA,* **Sept/Oct 1977**, 193-195.

Maheshwari, P.; Nikolov, Z.L.; White, T.W; Hartel, R. Solubility of fatty acids in supercritical carbon dioxide. *J. Amer. Oil. Chem. Soc.* **1992**, *69*, 1069-1076.

Matthews, R. F.; Braddock, R. J. Recovery and applications of essential oils from oranges. *Food Tech* **1987**, 41, 57-61.

Matthews, R. F.; Tseng, D. J.; Gregory, J. F.; Wet, C. I.; Littell, R. C. Concentration of aqueous orange essence by sorption on styrene-divinylbenzene resin. *Proc. Fla. State Hort. Soc.* **1991**, *104*, 81-84.

Meireles, M. A. A.; Nikolov, Z. L. Extraction and fractionation of essential oils with liquid carbon dioxide. In: *Spices, herbs and edible fungi*; Charalambous G., Ed.; Elsevier: Amsterdam, 1994.

McHugh, M.A.; Krukonis, V.J. *Supercritical Fluid Extraction: Principles and Practice*, 1st ed, Butterworths , Boston, **1986**.

Moharram,Y.G.; Ahmed, S. F. Messailam Utilization of tomato seed as a source of oil and protein, *Alex. Jour. Agric. Res.*, **1980**, *28,* 147-154.

Moyler, D. A. Extraction of flavours and fragrances with compressed CO_2. In *Extraction of Natural Products Using Near-Critical Solvents;* King, M. B.; Bott, T. R., Blackie Academic & Professional: Glasgow, **1993**.

Myers, R. H.; Montgomery, D. C. Response Surface Methodology: Process and product optimization using designed experiments, John wiley and Sons, Inc, New York, **1995**.

Nicolosi, R.J.; Rogers, E.J.; Ausman, L.M.; Orthoefer, F.T. Rice bran oil and its health benefits. In: *Rice Science and Technology*; Marshall, W.E.; Wadsworth, J. L., Eds., Marcel Dekker: New York, **1994**.

Nilsson, W.B.; Gauglitz, E.J.; Hudson, J.K. Solubilities of methyl oleate, oleic acid, oleyl glycerols, and oleyl glycerol mixtures in supercritical carbon dioxide. *J. Amer. Oil Chem. Soc.* **1991**, *68*, 87-91.

Nilsson, W.B.; Seaborn, G.T.; Hudson, J.K. Partition coefficients for fatty acid esters in supercritical fluid CO_2 with and without ethanol. *J. Amer. Oil Chem. Soc.* **1992**, *69*, 305-308.

Orthoefer, F. T. Rice bran oil: Production and utilisation. *Food Tech Europe,* **Dec. 1994/Jan. 1995,** 140-144.

Palmer, M.V.; Ting, S.S.T. Applications for supercritical fluid technology in food processing. *Food Chem.* **1995**, *52*, 345-352.

Paulatis, M.E.; Krukonis, V.J.; Kurnik, R. T.; Reid, R. C. Supercritical Fluid Extraction. *Rev. Chem. Eng.* **1982**, *1*, 179.

Dense carbon dioxide extraction and fractionation of by-product food oils dispersed in a porous or cellulosic solid matrix. Presented at the 4th International Symposium on Supercritical Fluids, Sendai, Japan, 633, **1997.**

Perre, C.; Delestre, G.; Schrive, L.; Carles, M. Deterpenation process for citrus oils by supercritical CO_2 extraction in a packed column. Presented at the 3rd International Symposium on Supercritical Fluids, Strasbourg, France, 465-469, **1994.**

Perry, R H ; Chilton, C H., Chemical Engineers' Handbook, 5th edition, McGraw-Hill, Sydney, **1973**, 16 - 23.

Pino, J.; Sanchez, M.; Sanchez, R.; Roncal, E. Chemical composition of orange oil concentrates. *Die Nahrung*, **1992**, *36*, 539-542.

Pocklington, W.D.; Dieffenbacher, A. Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. *Pure and Applied Chem.* **1988**, *60*, 877-892.

Proctor, B. E.; Kenyon, E. M. Objective evaluation of odour deterioration in orange oil. *Food Technology*, **1949**, *3*, 387-392.

Ramsay, M.E.; Hsu, J.T.; Novak, R.A,; Reightler, W.J. Processing rice bran by supercritical fluid extraction. *Food Technol.* **1991**, *30*, 98-104.

Reverchon, E. Supercritical fluid extraction and fractionation of essential oils and related products. *The Journal of Supercritical Fluids,* **1997**, *10*, 1-37.

Reverchon, E.; Donsi, G.; Osseo, L. S. Modeling of supercritical fluid extraction from herbaceous matrices. *Ind. Eng. Chem. Res.* **1993**, *32*, 2721-2726.

Reverchon, E.; Senatore, F. Supercritical carbin dioxide extraction of chamomile essential oil and its analysis by gas chromatography-mass spectormetry. *J. Agric. Food Chem.* **1994**, *42*, 154-158.

Rizvi, S.S.H.;. Benado, A.L.; Zollweg, J.A; Daniels, J.A. Supercritical fluid extraction: fundamental principles and modeling methods. *Food technology.* **1986,** 40, (6), 55-65.

Rizvi, S.S.H.; Daniels, J.A.; Benado, A.L.; Zollweg, J.A. Supercritical fluid extraction: operating principles and food applications. *Food technology.* **1986,** *40,* **(7),** 57-64.

Rogers, E.J.; Rice S. M.; Nicolosi, R. J.; Carpenter, D. R.; McClelland, C. A.; Romanczyk, L.J. Identification and quantitation of γ - Oryzanol components and simultaneous assessment of tocols in rice bran oil. *J. Amer. Oil Chem. Soc.* **1993**, *70*, 301-307.

Saito, N.; Ikushima, Y.; Hatakeda, K.; Ito, S.; Goto, T. Fractional extraction of rice bran oil and their ester with supercritical carbon dioxide. *J. Agric. Chem. Soc. Japan*, **1991**, *65*, 153-161.

Sato, M.; Goto, M.; Hirose, T. Adsorption Process for the Fractionation of Citrus Oil by Supercritical Carbon Dioxide. In: *Fundamentals of Adsorption*, LeVan, M. D. (Ed); Kluwer Academic Publishers, Boston, **1996a.**

Sato, M.; Goto, M.; Hirose, T. Fractional extraction with supercritical carbon dioxide for the removal of terpenes from citrus oil. *I & EC Res.* **1995**, *34*, 3941-3946.

Sato, M.; Goto, M.; Hirose, T. Fractionation of citrus oil by supercritical fluid extraction tower. Presented at the Third International Symposium on Supercritical Fluids, Strasbourg, France, 83-88, **1994.**

Sato, M.; Goto, M.; Hirose, T. Supercritical Fluid Extraction on Semibatch Mode for the Removal of Terpene in Citrus Oil. *Ind. Eng. Chem. Res.* **1996b**, *35,* 1906-1911.

Sato, M.; Goto, M.; Kodama, A.; Hirose, T. Fractionation of Citrus Peel Oil by Supercritical Pressure Swing Adsorption. Presented at the Fourth Italian Conference on Supercritical Fluids and their Applications, Capri, Italy, 39-46, **1997**.

Sato, M.; Goto, M.; Kodama, A.; Tanoue, N.; Hirose, T. Fractionation of Citrus Oil by Cyclic Adsorption Process in Supercritical CO_2. In: *High Pressure Chemical Engineering*; Von Rohr, P. R. and Trepp, C. (eds); Elsevier Science, **1996c.**

Seetharamaiah, G.S.; Prabhakar, J.V. Oryzanol content of ricebran oil. *J. Food Sci. Technol.* **1986**, *23,* 270-273.

Schmitt, W.J.; Reid, R.C. The use of entrainers in modifying the solubility of phenanthrene and benzoic acid in supercritical carbon dioxide and ethane. *Fluid Phase Equilib.* **1986**, *32*, 77.

Schneider, G. M. High - pressure investigations of fluid mixtures - basis of supercritical fluid technology. presented at the 4th International Symposium on Supercritical Fluids, Sendai, 795, **1997**.

Schultz, W.G.; Randall, J.M. Liquid carbon dioxide for selective aroma extraction. *Food Technology*, **1970**, 24, 1282-1286.

Shaw, P. E. Review of quantitative analyses of citrus essential oils. *J. Agric. Food Chem.* **1979**, *27*, 246-257.

Shaw, P. E.; Moshonas, M. G. Mass spectormetry for the identification of citrus flavor components, *Mass spectrometry reviews*, **1985**, 4, 397-420.

Shen, Z.; Palmer, M. V.; Ting, S.S.T.; Fairclough, R.J. Pilot scale extraction of rice bran oil with dense carbon dioxide. *J. Agric. Food Chem.* **1996**, *44*, 3033-3039.

Shin, D. H; Shimoda, M; Kawano, T; Osajima, Y Supercritical carbon dioxide extraction of terpene hydrocarbons from constructed citrus peel oil. *J. Jap. Soc. for Food Sci. & Technol.* **1992,** 39, 377-382.

Sivala, K.; Rao, V.V.; Mukherjee, R.K. Mathematical modelling of rice bran oil expression. *J. Food Proc. Eng.*, **1991,** *14*, 51-68.

Sivala, K.; Bhole, N. G.; Mukherjee, R.K., Effect of moisture on rice bran oil expression, *J.Agric.Engng Res.***1991**, *50*, 81-91.

Speek, A.J.; Schriver, J.; Schreurz, W.H.P. Vitamin E composition of some seed oils as determined by high performance liquid chromatography. *J. Food Sci.* **1985,** *50*, 121-124.

Spiro, M ; Kandiah, M, Extraction of ginger rhizome: partition constants and other eqilibrium properties in organic solvents and in supercritical carbon dioxide, *International Journal of Food Science and Technology*, **1990**, *25*, 566-575.

Stahl, E.; Schutz, E.; Mangold, K. Extraction of seed oils with liquid and supercritical carbon dioxide. *J. Agric. Food Chem.* **1980**, *28*, 1153-1157.

Stahl, E; Quirin, K.W. Dense gas extraction on a laboratory scale: A survey of some recent results. *J. Fluid Phase Equilib.* 1983, 10, 269.

Stahl, E.; Quirin, K. W.; Glatz, A.; Gerard, D; Rau, G. New developments in the field of high-pressure extraction of natural products with dense gases. *Ber. Bunsenges, Phys. Chem.*, **1984**,*88*, 900-907.

Standard G1: Edible fats and oils. In *Food Standards Code*, ANZFA Australian Govt. Publishing Service, Canberra, **1997**.

Sygiyama Kenkichi, and Saito Muneo, Simple microscale supercritical fluid extraction system and its application to gas chromatography-mass spectrometry of lemon peel oil, *Journal of Chromatography*, **1988**, 442, 121-131.

Taniguchi, M.; Tsuji, T.; Morimoto, H.; Shibata, M.; Kobayashi, T. Treatment of rice bran with supercritical carbon dioxide. *Nippon Shokuhin Kogyo Gakkaishi* **1987**, *34,* 102-108.

Taniguchi, M.; Tsuji, T., Shibata, M.; Kobayashi, T. Extraction of oils from wheat germ with supercritical carbon dioxide. *Agric. Biol. Chem.* 1985, **49**, 2367-2372.

Tateo, F. Use of Synthetic polymeric adsorbents for processing and recovering essential citrus-fruit oils. Flavour '81, Walter de Gruyter & Co, Berlin, **1981** 671-682.

Tateo, F Production of Concentrated Orange Oils Using Thin Film Evaporator. *J. Ess. Oil Res.* **1990**, 2, 7-13.

Taylor, S.L.; King, J.W.; List, G.R. Determination of oil content in oilseeds by analytical supercritical fluid extraction. *J. Amer. Oil Chem. Soc.* **1993**, *70*, 437-439.

Temelli, F. Extraction of triglycerides and phospholipids from canola with supercritical carbon dioxide and ethanol. *J. Food Sci.* **1992**, *57*, 440-457.

Temelli, F.; Braddock, R. J.; Chen, C. S.; Nagy, S.; Supercritical carbon dioxide extraction of terpenes from orange essential oil, In *Supercritical Fluid Extraction and Chromatography*; Charpentier, B.A; Sevenants M.R., Eds.; ACS Symp., **1988**, Series No.366.

Temelli, F; Chen, C S; Braddock, R J (1988b) Supercritical Fluid Extraction in Citrus Oil Processing. *Food Technol.*, June **1988**, 145-150.

Ting, S. S.T.; Macnaughton, S. J.; Tomasko, D. L.; Foster, N. R. Solubility of naproxen in supercritical carbon dioxide with and without cosolvent. Ind. Eng. Chem. Res., **1993**, *32*, 1471-81.

Ting, S. S.T.; Tomasko, D. L.; Macnaughton, S. J.; Foster, N. R. Chemical - physical interpretation of cosolvent effects in supercritical fluids. *Ind. Eng. Chem. Res.*, **1993**, *32*, 1482-1487.

Tseng, D. J.; Matthews, R. F.; Gregory, J. F.; Wet, C. I.; Littell, R. C. Concentration of aqueous orange essence by sorption on styrene-divinylbenzene resin. *Proc. Fla.State. Hort. Soc.* **1991**, *104*, 81-84.

Tzamtzis, N. E.; Liodakis, S E; Parissakis, G K. The Deterpenation of orange and lemon oils using preparative adsorption chromatography. *Flavour & Fragrance Journal*, **1990,** *5*, 57-67.

Van Dijck, W. J. D.; Ruys, A. H. Terpeneless oils - a new method of production. *Perfumery and Essential Oil Record*, March **1937**, 91-94.

Vora, J. D.; Matthews, R. F.; Grandall, P. G.; Cook, R. Preparation and chemical composition of orange oil concentrates. *J. Food Sci.* **1983**, 48, 1197-1199.

Wankat, P. C. Large - scale chromatography. *In Handbook of separation process technology*; Rousseau, R. W., (Ed.); John Wiley & Sons, New York, **1987**.

Wilson, C.W.; Shaw, P. E. Glass capillary gas chromatography for quantitative determination of volatile constituents in cold-pressed grapefruit oil. *J. Agric. Food Chem.* **1980**, *28*, 919-922.

Wolford, R.W.; Kesterson, J. W.; Attaway, J. A. Physicochemical properties of citrus essential oil from Florida. *J.Agr. Food Chem.* **1971**, *19* (6), 1097-1105.

Wong, J. M.; Johnston, K. P. Solubilization of Biomolecules in carbon dioxide based supercritical fluids. Biotechnology Progress **1986**, *2*(1), 29-39.

Yamauchi, Y.; Saito, M. Fractionation of lemon-peel oil by semi-preparative supercritical fluid chromatography. *J Chromatography*, **1990,** 505, 237-246.

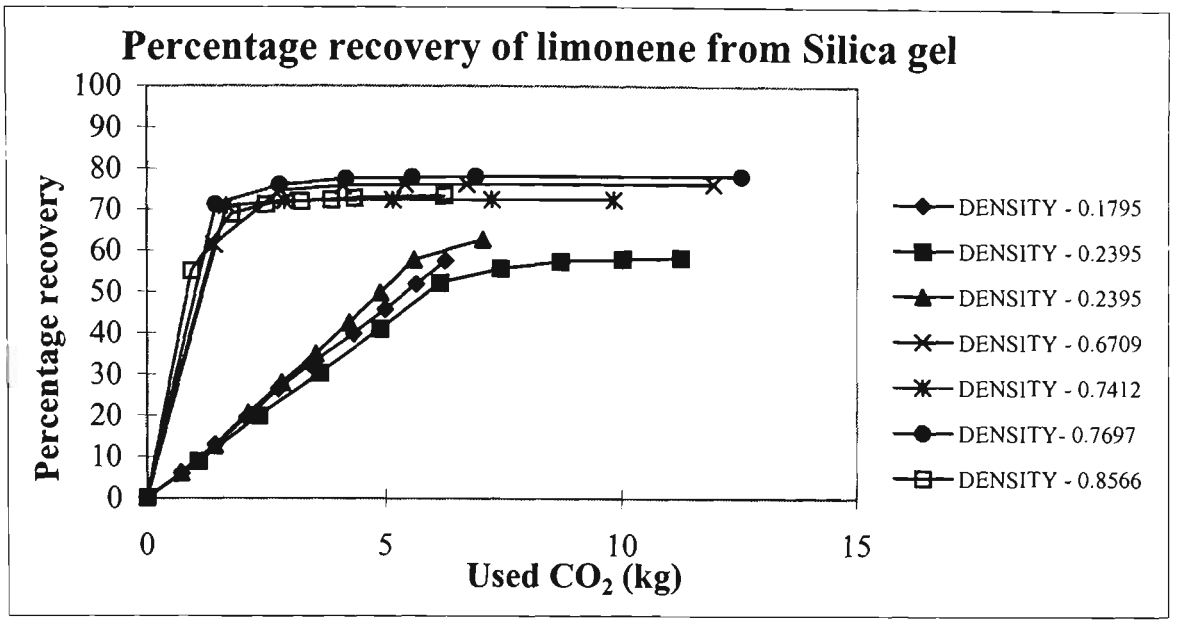
Owusu-Yaw, J.; Matthews, West, P F. Alcohol deterpenation of orange oil. *J Food Sci.*, **1986**, *51*, 1180-1182.

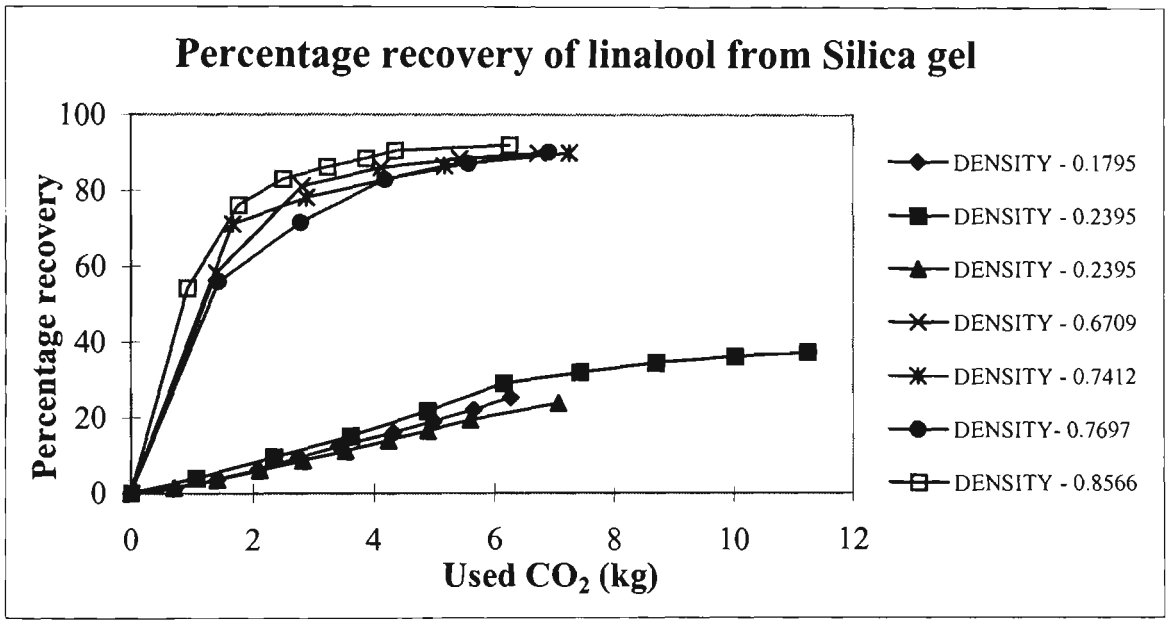
Yun, J.S.L.; Liong; K.K.; Gurdial, G.S.; Foster N.R. Solubility of cholesterol in supercritical carbon dioxide. *Ind. Eng. Chem. Res.* **1991**, *30,* 2476-2482.

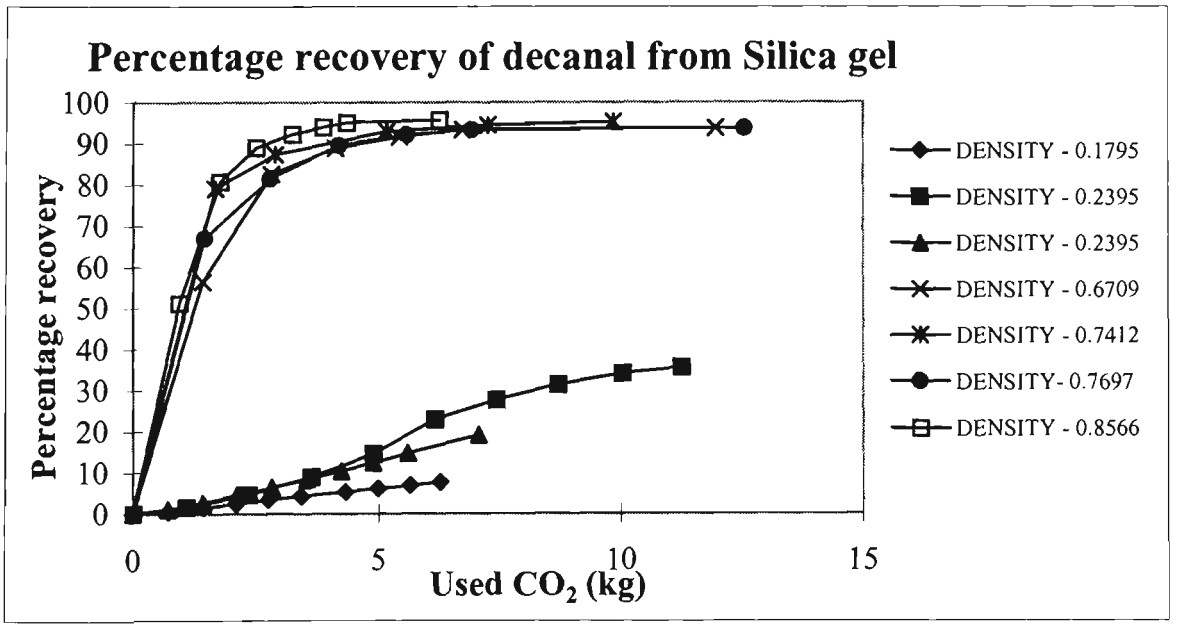
Zhao, W.; Shishikura, A.; Fujimoto, K.; Arai, K.; Saito, S. Fractional extraction of rice bran oil with supercritical carbon dioxide. *Agric. Biol. Chem.* **1987,** *51*, 1773-77.

Ziegler, G.R.; Liaw Y.J.; Deodorisation and deacidification of edible oils with dense carbon dioxide. *J. Amer. Oil Chem. Soc.* **1993**, *70,* 947-953.

Appendix 1


Percentage recovery of limonene from Silica gel
Percentage recovery
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
Used CO_2 (kg)
DENSITY - 0.1795
DENSITY - 0.2395
DENSITY - 0.2395
DENSITY - 0.6709
DENSITY - 0.7412
DENSITY- 0.7697
DENSITY - 0.8566


Percentage recovery of linalool from Silica gel
Percentage recovery
100
80
60
40
20
0
0
2
4
6
8
10
12
Used CO_2 (kg)
DENSITY - 0.1795
DENSITY - 0.2395
DENSITY - 0.2395
DENSITY - 0.6709
DENSITY - 0.7412
DENSITY- 0.7697
DENSITY - 0.8566


Percentage recovery of decanal from Silica gel
Percentage recovery
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
Used CO_2 (kg)
DENSITY - 0.1795
DENSITY - 0.2395
DENSITY - 0.2395
DENSITY - 0.6709
DENSITY - 0.7412
DENSITY- 0.7697
DENSITY - 0.8566

Appendix 2

CO_2 conditions: 24.14 MPa x 45 °Cx 2 kg/h

	Feed oil	terpene frac.	CO_2 1*	CO_2 2*	CO_2 3	CO_2 4	CO_2 5	CO_2 6	CO_2 7	CO_2 8	CO_2 9
weight of frac.(g)	484.18	355.10	29.06	20.88	11.62	7.76	6.02	4.17	9.47	3.46	1.82
used CO_2 (kg)			0.32	0.22	0.12	0.17	0.18	0.28	0.71	0.81	2.81
					concentration(mg/g fraction)						
α-pinene	3.80	3.97	3.61	3.44	3.28	3.22	3.09	3.05	2.86	2.53	2.20
sabinene	1.89	2.01	1.68	1.55	1.47	1.46	1.35	1.24	1.10	0.94	0.69
β-pinene	0.14	0.15	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.10	0.09
β-myrcene	15.68	16.47	15.08	14.66	14.25	13.95	13.57	13.29	12.61	11.27	9.52
n-octanal	0.99	0.00	2.91	4.15	5.15	5.53	5.81	6.11	6.77	6.55	5.12
α-phellandrene	0.18	0.20	0.18	0.18	0.18	0.17	0.18	0.17	0.16	0.15	0.13
carene	0.82	0.86	0.79	0.77	0.77	0.76	0.74	0.73	0.69	0.62	0.52
limonene	921.06	956.81	876.52	867.29	842.64	824.10	819.60	798.61	761.63	700.38	601.17
n-octanol	1.40	0.00	4.39	4.94	5.39	5.71	6.23	6.69	8.05	11.38	18.10
linalool	3.74	0.00	11.50	14.53	16.84	18.00	19.42	20.71	24.15	29.72	33.97
n-nonanal	0.30	0.00	0.75	1.08	1.36	1.47	1.59	1.68	1.94	1.90	0.00
limonene oxide (c	0.83	0.00	1.90	2.68	3.39	3.76	4.08	4.45	5.21	6.49	7.88
limonene oxide(tr	0.41	0.00	1.19	1.83	2.40	2.69	2.94	3.15	3.75	4.47	4.12
citronellal	0.35	0.00	0.91	1.35	1.72	1.86	1.98	2.07	2.37	2.36	1.87
α-terpineol	0.86	0.00	2.52	2.84	3.10	3.28	3.58	3.82	4.61	6.56	11.29
decanal	2.71	0.00	6.94	10.24	13.22	14.56	15.99	17.05	21.58	24.67	21.14
carveol(trans)	0.41	0.00	0.92	1.24	1.53	1.72	1.98	2.22	2.86	4.26	7.67
carveol(cis)	0.29	0.00	0.82	0.95	1.09	1.17	1.30	1.39	1.72	2.53	4.48
neral	0.29	0.00	0.81	1.10	1.36	1.46	1.61	1.70	2.04	2.55	2.87
carvone	0.59	0.00	1.08	1.45	1.78	2.04	2.33	2.59	3.21	4.44	6.25
geranial	0.57	0.00	1.55	2.10	2.57	2.81	3.24	3.44	4.19	5.46	6.87
perrillaldehyde	0.68	0.00	2.69	3.24	2.39	2.58	2.69	3.11	4.89	8.30	12.24
valencene	0.73	0.76	0.65	0.65	0.67	0.66	0.69	0.69	0.68	0.64	0.53
δ-cadinene	0.15	0.16	0.14	0.15	0.15	0.15	0.16	0.17	0.18	0.17	0.18

Frac.: fraction

*(spilt some)

CO_2 conditions: 13.10 MPa x 45 °Cx 4 kg/h										
	Feed oil	terpene frac.	CO2 1	CO2 2*	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8
weight of frac.(g)	499.52	380.21	54.12	6.83	22.46	2.65	1.53	0.90	0.35	0.86
used CO_2 (kg)			0.415	0.415	0.73	0.73	1.61	1.51	2.14	3.35
concentration(mg/g fraction)										
α-pinene	3.75	3.11	2.67	2.81	2.23	2.09	1.43	1.06	0.74	0.81
sabinene	1.90	1.72	1.28	1.34	1.09	1.04	0.72	0.55	0.44	0.43
β-pinene	0.14	0.12	0.11	0.12	0.10	0.09	0.07	0.06	0.06	0.07
β-myrcene	15.65	14.67	12.40	13.39	11.19	10.26	6.74	5.07	4.17	4.33
n-octanal	0.99	0.00	2.51	4.32	6.45	13.86	19.92	17.23	14.76	10.69
α-phellandrene	0.20	0.18	0.16	0.17	0.15	0.16	0.15	0.15	0.15	0.19
carene	0.82	0.76	0.65	0.70	0.62	0.62	0.47	0.36	0.30	0.28
limonene	913.32	956.32	853.16	845.82	825.44	696.22	443.81	333.20	277.82	283.34
n-octanol	1.39	0.00	6.34	10.24	7.66	5.71	5.15	5.98	6.93	7.54
linalool	3.72	0.00	14.04	21.61	18.76	26.65	55.75	67.06	73.10	72.64
n-nonanal	0.29	0.00	0.66	1.03	1.84	3.72	0.00	0.00	0.00	0.00
limonene oxide (cis)	0.71	0.00	1.66	2.44	4.30	10.07	19.20	20.79	17.59	14.89
limonene oxide(trans)	0.38	0.00	1.08	1.60	3.31	8.59	16.37	17.37	16.78	14.06
citronellal	0.35	0.00	0.85	1.41	2.55	5.24	7.82	7.02	6.28	4.48
α-terpineol	0.86	0.00	3.69	5.50	5.21	4.56	5.61	6.92	8.11	9.46
decanal	2.69	0.08	6.56	10.24	19.78	44.68	75.77	72.85	67.08	47.02
carveol(trans)	0.35	0.00	0.95	1.46	1.98	3.93	9.02	12.46	15.43	18.59
carveol(cis)	0.28	0.00	1.03	1.58	1.60	1.64	2.58	3.65	4.98	6.29
neral	0.29	0.00	1.00	1.49	1.59	2.54	5.43	7.25	8.34	9.56
carvone	0.49	0.00	0.87	1.30	1.74	3.82	9.03	11.66	14.02	15.72
geranial	0.57	0.00	1.91	3.09	3.39	4.89	15.56	20.54	25.14	28.92
perrillaldehyde	0.66	0.00	3.36	4.97	5.55	3.74	0.00	0.00	0.00	0.00
valencene	0.72	0.79	0.67	0.64	0.77	0.77	0.68	0.57	0.67	0.54
δ-cadinene	0.15	0.17	0.16	0.15	0.18	0.20	0.23	0.22	0.33	0.28

Frac.: fraction

*: spilt some

CO_2 conditions: 24.14 MPa x 35°Cx 4 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2*	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8
weight of frac.(g)	500.20	339.02	47.58	53.93	17.26	8.05	3.08	1.66	1.17	0.77	0.72
used CO_2 (kg)				0.415	0.365	0.63	0.63	1.36	1.31	1.99	3.35
				concentration(mg/g fraction)							
α-pinene	3.75	3.00	2.72	2.61	2.14	1.97	1.91	1.53	1.54	1.47	1.45
sabinene	1.90	1.65	1.55	1.25	1.09	0.98	0.96	0.82	0.84	0.81	0.83
β-pinene	0.14	0.12	0.11	0.11	0.09	0.09	0.09	0.06	0.07	0.07	0.07
β-myrcene	15.65	14.06	13.24	12.15	10.45	10.09	10.08	8.04	7.71	7.65	8.00
n-octanal	0.99	0.00	0.08	3.35	6.58	6.27	7.50	11.90	12.44	9.76	6.81
α-phellandrene	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.14	0.14	0.14	0.15
carene	0.82	0.74	0.68	0.65	0.59	0.55	0.55	0.49	0.47	0.45	0.45
limonene	905.26	947.63	928.12	823.53	752.74	745.50	711.22	572.72	505.67	509.12	544.15
n-octanol	1.39	0.00	0.00	7.14	10.77	6.64	4.38	3.69	3.69	4.09	4.39
linalool	3.72	0.00	0.00	17.68	34.38	22.74	15.31	12.26	11.79	11.68	10.81
n-nonanal	0.29	0.00	0.04	0.84	0.00	2.10	2.71	4.68	4.71	3.81	2.72
limonene oxide (cis)	0.71	0.00	0.00	2.23	5.76	4.89	4.90	7.41	9.78	10.63	9.44
limonene oxide(trans)	0.38	0.00	0.00	1.42	4.09	3.33	3.19	5.09	6.89	7.03	5.59
citronellal	0.35	0.00	0.05	1.19	2.53	2.78	3.42	5.67	5.74	4.43	2.96
α-terpineol	0.86	0.00	0.00	4.39	7.66	5.82	4.26	4.11	4.12	4.48	4.80
decanal	2.69	0.00	0.68	8.58	18.83	25.37	33.64	56.68	56.41	44.25	29.53
carveol(trans)	0.35	0.00	0.00	1.23	2.63	2.78	2.95	4.66	6.08	7.84	9.03
carveol(cis)	0.28	0.00	0.00	1.27	2.34	1.93	1.40	1.52	1.72	2.20	2.55
neral	0.29	0.00	0.00	1.50	3.19	2.13	1.28	1.07	1.10	1.12	1.14
carvone	0.49	0.00	0.00	1.23	3.25	3.24	2.68	3.40	4.47	5.71	6.02
geranial	0.57	0.00	0.00	2.94	6.71	4.62	2.89	2.45	2.30	2.40	2.35
perrillaldehyde	0.66	0.00	0.00	4.29	8.87	8.71	6.63	4.02	3.79	4.22	8.40
valencene	0.72	0.82	0.84	0.74	0.74	0.76	0.71	0.62	0.51	0.47	0.54
δ-cadinene	0.15	0.18	0.18	0.17	0.18	0.16	0.15	0.13	0.11	0.11	0.16

Frac.: fraction

*: spilt some

dep.aft.ter.: The fraction released by slow pressurization and depressurization with CO_2

CO_2 conditions: 13.10 MPa x 35 °Cx 2 kg/h											
	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3*	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8
weight of frac.(g)	487.88	331.59	53.751	22.22	14.96	14.15	10.83	9.25	3.18	1.08	0.75
used CO_2 (kg)				0.197	0.197	0.343	0.643	0.687	0.687	0.930	1.567
				concentration(mg/g fraction)							
α-pinene	3.75	2.73	2.68	2.52	2.44	2.20	2.20	2.03	1.79	1.44	0.32
sabinene	1.90	1.54	1.53	1.26	1.30	1.15	1.14	1.03	0.95	0.80	0.24
β-pinene	0.14	0.11	0.11	0.10	0.10	0.09	0.10	0.09	0.09	0.08	0.02
β-myrcene	15.65	13.13	13.01	11.97	11.72	10.77	10.73	9.93	9.04	7.44	2.28
n-octanal	0.99	0.00	0.03	2.34	3.28	4.40	6.53	8.76	10.44	10.44	4.23
α-phellandrene	0.20	0.16	0.16	0.16	0.15	0.15	0.15	0.14	0.15	0.14	0.06
carene	0.82	0.68	0.66	0.63	0.63	0.60	0.60	0.56	0.51	0.41	0.12
limonene	907.71	954.75	939.30	866.25	853.60	831.69	793.35	733.26	634.65	494.04	162.17
n-octanol	1.39	0.00	0.00	5.29	6.23	7.20	8.72	10.33	12.07	13.71	8.19
linalool	3.72	0.04	0.00	12.58	16.14	20.61	28.39	34.04	38.55	39.66	17.72
n-nonanal	0.29	0.00	0.03	0.66	0.88	1.15	0.00	0.00	0.00	0.00	1.93
limonene oxide (cis)	0.71	0.00	0.00	1.65	2.31	3.35	4.69	6.33	7.89	9.29	5.06
limonene oxide(trans)	0.38	0.00	0.00	1.01	1.52	2.32	3.34	4.57	5.63	6.12	2.97
citronellal	0.35	0.00	0.00	0.91	1.28	1.84	2.64	3.60	4.25	4.20	1.97
α-terpineol	0.86	0.00	0.00	3.40	3.89	4.77	5.69	7.45	9.16	11.60	8.35
decanal	2.69	0.00	0.55	6.79	8.92	12.85	19.10	30.39	43.37	53.15	28.22
carveol(trans)	0.35	0.00	0.00	0.98	1.17	1.57	2.08	2.97	4.16	6.39	4.98
carveol(cis)	0.28	0.00	0.00	1.08	1.25	1.54	1.86	2.52	3.18	4.24	3.06
neral	0.29	0.00	0.00	1.07	1.39	1.86	2.33	2.78	3.07	3.34	1.92
carvone	0.49	0.00	0.00	0.96	1.24	1.76	2.38	3.37	4.67	6.61	4.75
geranial	0.57	0.00	0.00	2.18	2.80	3.95	5.03	6.06	7.03	7.75	4.54
perrillaldehyde	0.66	0.00	0.00	3.37	3.95	5.11	6.41	8.92	11.49	15.36	10.90
valencene	0.72	0.90	0.91	0.79	0.76	0.84	0.77	0.72	0.62	0.57	0.32
δ-cadinene	0.15	0.19	0.19	0.18	0.17	0.19	0.17	0.17	0.15	0.13	0.08

Frac.: fraction

*: spilt some

dep.aft.ter.: The fraction released by slow pressurization and depressurization with CO_2

CO_2 conditions: 24.14 MPa x 45 °Cx 2 kg/h											
	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8
weight of frac.(g)	493.16	332.1	39.276	25.79	15.56	20.62	12.68	12.48	4.50	1.09	1.54
used CO_2 (kg)				0.232	0.232	0.413	0.363	0.877	0.827	1.240	2.117
				concentration(mg/g fraction)							
α-pinene	3.75	2.88	2.84	2.94	2.78	2.35	2.23	2.03	1.75	1.48	2.47
sabinene	1.90	1.59	1.63	1.41	1.35	1.24	1.15	1.06	0.93	0.82	1.10
β-pinene	0.14	0.11	0.12	0.11	0.11	0.10	0.10	0.09	0.09	0.09	0.10
β-myrcene	15.65	13.50	13.78	13.34	13.05	11.60	11.08	10.33	9.06	8.19	9.93
n-octanal	0.99	0.00	0.00	2.21	3.51	4.48	5.50	7.02	7.68	7.20	5.22
α-phellandrene	0.20	0.17	0.17	0.17	0.17	0.16	0.15	0.16	0.15	0.15	0.18
carene	0.82	0.71	0.70	0.70	0.70	0.62	0.60	0.58	0.52	0.46	0.55
limonene	907.71	955.70	939.70	866.64	850.40	835.78	821.09	775.56	668.07	540.95	622.47
n-octanol	1.39	0.00	0.00	4.99	6.37	6.25	6.10	6.40	8.52	14.17	13.42
linalool	3.72	0.03	0.03	10.71	14.97	16.17	17.75	24.10	40.17	54.04	39.98
n-nonanal	0.29	0.00	0.00	0.63	0.96	1.31	1.57	1.99	0.00	0.00	0.00
limonene oxide (cis)	0.71	0.00	0.00	1.47	2.30	3.08	3.98	5.54	7.62	8.93	7.17
limonene oxide(trans)	0.38	0.00	0.00	0.85	1.49	2.18	3.03	4.49	6.13	5.87	3.98
citronellal	0.35	0.00	0.00	0.77	1.28	1.78	2.21	2.93	3.27	2.89	1.77
α-terpineol	0.86	0.00	0.00	3.07	3.79	4.07	4.08	4.37	5.53	8.66	8.66
decanal	2.69	0.00	0.39	6.33	9.90	13.85	17.23	24.13	32.38	30.16	16.80
carveol(trans)	0.35	0.00	0.00	0.94	1.26	1.54	1.88	2.67	4.45	7.42	6.78
carveol(cis)	0.28	0.00	0.00	0.96	1.22	1.34	1.36	1.52	2.19	3.86	3.62
neral	0.29	0.00	0.00	0.77	1.12	1.35	1.57	2.37	3.78	5.29	3.62
carvone	0.49	0.00	0.00	0.83	1.14	1.45	1.90	3.09	5.39	7.65	5.97
geranial	0.57	0.00	0.00	1.59	2.24	2.79	3.06	4.56	7.90	11.58	8.49
perrillaldehyde	0.66	0.00	0.00	3.03	3.90	4.37	4.78	3.48	4.58	12.12	10.57
valencene	0.72	0.91	0.82	0.69	0.68	0.74	0.78	0.76	0.65	0.62	0.44
δ-cadinene	0.15	0.19	0.17	0.16	0.16	0.17	0.18	0.18	0.16	0.16	0.14

Frac.: fraction

*: spilt some

dep.aft.ter.: The fraction released by slow pressurization and depressurization with CO_2

CO_2 conditions: 9.66 MPa x 45 °Cx 2 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	484.44		348.84	26.51	21.10	25.43	12.55	6.09	1.43	1.04	0.52	2.91	13.09
used CO_2 (kg)				0.185	0.185	0.320	0.320	0.640	0.590	0.910	1.500		
concentration(mg/g fraction)													
α-pinene	3.75		3.27	3.21	3.13	3.02	2.75	2.38	1.60	1.05	0.71	0.03	2.73
sabinene	1.90		1.77	1.58	1.55	1.49	1.38	1.19	0.79	0.55	0.39	0.00	1.62
β-pinene	0.14		0.13	0.12	0.13	0.12	0.12	0.10	0.09	0.06	0.04	0.00	0.11
β-myrcene	15.65		14.91	14.26	14.28	13.87	13.03	11.46	7.94	5.05	3.67	0.12	14.12
n-octanal	0.99		0.00	1.91	3.45	3.99	5.10	10.24	17.00	16.31	10.61	0.32	0.02
α-phellandrene	0.20		0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.21	0.25	0.00	0.16
carene	0.82		0.77	0.74	0.75	0.75	0.72	0.66	0.48	0.31	0.22	0.00	0.71
limonene	910.28		956.10	896.84	891.93	894.78	880.60	806.36	574.60	339.85	248.00	20.28	933.41
n-octanol	1.39		0.00	2.82	3.26	3.33	3.73	7.09	18.19	35.21	45.43	1.63	0.00
linalool	3.72		0.00	7.36	10.43	11.48	13.58	31.88	91.29	141.21	138.64	4.73	0.02
n-nonanal	0.29		0.02	0.51	0.85	0.97	1.21	0.00	0.00	0.00	0.00	0.27	0.02
limonene oxide (cis)	0.71		0.00	1.25	2.25	2.57	3.09	5.81	10.15	9.78	9.19	0.52	0.00
limonene oxide(trans)	0.38		0.00	0.81	1.68	2.00	2.59	5.75	10.71	13.02	13.23	0.46	0.00
citronellal	0.35		0.00	0.63	1.13	1.29	1.58	3.25	6.68	8.35	6.79	0.22	0.00
α-terpineol	0.86		0.00	1.67	1.84	1.85	2.04	3.76	9.62	17.60	23.06	0.98	0.00
decanal	2.69		0.00	4.63	7.62	8.59	10.46	22.25	53.91	96.33	121.82	4.71	0.51
carveol(trans)	0.35		0.00	0.57	0.81	0.88	1.05	2.17	5.43	10.05	12.63	0.57	0.00
carveol(cis)	0.28		0.00	0.48	0.54	0.55	0.62	1.15	2.86	5.47	7.19	0.29	0.00
neral	0.29		0.00	0.52	0.81	0.90	1.06	2.24	6.33	10.73	10.47	0.37	0.00
carvone	0.49		0.00	0.62	0.96	1.08	1.32	2.93	7.73	11.34	12.38	0.65	0.00
geranial	0.57		0.00	1.02	1.48	1.58	1.89	3.93	15.28	28.32	41.34	0.78	0.00
perrillaldehyde	0.66		0.00	1.68	1.17	1.17	1.29	2.13	5.31	7.23	0.00	0.57	0.00
valencene	0.72		0.74	0.62	0.56	0.56	0.67	1.28	1.69	1.25	0.79	0.03	0.85
δ-cadinene	0.15		0.16	0.13	0.12	0.13	0.16	0.33	0.55	0.55	0.49	0.02	0.18

Frac.: fraction dep.aft.ter.: The fraction released by slow pressurization and depressurization with CO2

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 24.14 MPa x 45 °Cx 4 kg/h												
	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2*	CO2 3	CO2 4	CO2 5	CO2 6*	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	484.18	347.89		39.50	21.27	26.92	8.69	2.19	1.67	0.33	0.62	1.59	16.41
used CO_2 (kg)				0.331	0.285	0.620	0.620	1.240	1.240	1.810	3.050		
concentration(mg/g fraction)													
α-pinene	3.75	3.40		2.96	2.62	2.78	2.79	2.47	1.99	1.12	1.25	0.00	3.10
sabinene	1.90	1.82		1.50	1.32	1.31	1.29	1.15	0.92	0.59	0.56	0.00	1.75
β-pinene	0.14	0.13		0.12	0.11	0.11	0.11	0.10	0.08	0.06	0.06	0.00	0.12
β-myrcene	15.65	15.17		13.51	12.37	12.76	12.55	11.27	9.09	6.62	6.97	0.06	14.87
n-octanal	0.99	0.00		2.93	3.74	4.75	6.72	9.43	7.99	6.96	4.40	0.05	0.08
α-phellandrene	0.20	0.17		0.16	0.16	0.16	0.15	0.17	0.14	0.12	0.13	0.00	0.17
carene	0.82	0.79		0.70	0.66	0.68	0.68	0.63	0.51	0.37	0.36	0.00	0.75
limonene	910.28	955.02		848.98	831.62	816.83	646.64	473.98	569.36	453.22	457.41	9.24	924.38
n-octanol	1.39	0.00		6.26	6.59	6.04	5.45	5.89	5.58	6.55	6.70	0.08	0.00
linalool	3.72	0.00		15.10	18.42	17.43	15.97	17.69	16.27	16.05	13.21	0.14	0.00
n-nonanal	0.29	0.00		0.73	0.96	1.34	2.02	3.24	3.02	2.83	2.01	0.03	0.06
limonene oxide (cis)	0.71	0.00		2.01	2.94	3.66	5.24	9.19	9.95	11.13	5.82	0.15	0.00
limonene oxide(trans)	0.38	0.00		1.28	1.99	2.51	3.73	6.57	6.64	6.28	4.56	0.04	0.00
citronellal	0.35	0.00		0.96	1.32	1.74	2.50	3.80	3.30	2.89	1.87	0.02	0.05
α-terpineol	0.86	0.00		3.52	4.15	3.90	3.60	4.50	4.64	5.70	6.58	0.10	0.00
decanal	2.69	0.00		6.82	9.88	13.87	21.85	37.96	35.00	33.19	21.57	0.28	1.08
carveol(trans)	0.35	0.00		1.02	1.41	1.66	2.37	5.46	7.45	11.20	15.04	0.25	0.04
carveol(cis)	0.28	0.00		1.08	1.30	1.23	1.22	1.86	2.28	3.12	3.99	0.05	0.00
neral	0.29	0.00		1.09	1.51	1.46	1.35	1.71	1.72	1.80	1.77	0.02	0.00
carvone	0.49	0.00		0.99	1.54	1.82	2.33	4.63	5.88	7.96	9.16	0.17	0.00
geranial	0.57	0.00		2.11	3.04	2.96	2.76	3.54	3.69	4.02	3.95	0.05	0.00
perrillaldehyde	0.66	0.00		3.25	4.37	4.54	2.84	4.16	5.02	11.21	12.20	0.16	0.00
valencene	0.72	0.76		0.63	0.67	0.68	0.65	0.66	0.58	0.60	0.67	0.00	0.78
δ-cadinene	0.15	0.16		0.14	0.15	0.15	0.15	0.16	0.16	0.19	0.26	0.00	0.17

Frac.: fraction dep.aft.ter.: The fraction released by slow pressurization and depressurization with CO2

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 9.66 MPa x 45 °Cx 4 kg/h												
	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	494.59		380.31	49.07	19.00	12.78	1.13	0.77	0.70	0.82	0.92	2.06	7.93
used CO_2 (kg)				0.317	0.317	0.633	0.683	1.367	1.317	2.200	3.417		
				concentration(mg/g fraction)									
α-pinene	3.75	3.78		3.57	2.88	2.67	1.70	0.79	0.55	0.37	0.28	0.00	2.97
sabinene	1.90	1.94		1.47	1.24	1.20	0.90	0.57	0.41	0.30	0.22	0.00	1.69
β-pinene	0.14	0.14		0.14	0.12	0.12	0.09	0.07	0.04	0.04	0.04	0.00	0.12
β-myrcene	15.65	16.01		14.97	13.33	12.76	9.92	5.84	4.19	3.02	2.15	0.05	14.66
n-octanal	0.99	0.00		2.37	3.89	5.80	13.90	30.64	35.43	33.83	26.78	0.44	0.30
α-phellandrene	0.20	0.18		0.17	0.16	0.16	0.16	0.19	0.18	0.19	0.22	0.00	0.17
carene	0.82	0.83		0.77	0.71	0.71	0.63	0.52	0.43	0.34	0.23	0.00	0.74
limonene	899.15	947.18		876.40	866.29	854.72	748.82	500.90	357.50	254.19	174.26	8.18	929.91
n-octanol	1.39	0.00		4.87	8.45	9.74	14.36	15.52	14.61	14.43	14.89	0.31	0.00
linalool	3.72	0.00		10.39	20.23	23.21	32.14	58.38	70.35	78.83	84.37	1.66	0.05
n-nonanal	0.29	0.00		0.57	0.84	1.30	3.06	0.00	0.00	0.00	0.00	0.20	0.16
limonene oxide (cis)	0.71	0.00		1.42	2.43	3.20	8.18	21.16	27.40	27.01	25.22	0.58	0.04
limonene oxide(trans)	0.38	0.00		0.96	1.89	2.44	7.01	20.12	26.89	33.07	34.42	0.55	0.03
citronellal	0.35	0.00		0.68	1.09	1.77	4.38	10.43	12.88	13.45	12.14	0.21	0.21
α-terpineol	0.86	0.00		2.64	4.09	4.79	8.15	11.33	12.14	13.66	15.11	0.32	0.00
decanal	2.69	0.14		5.18	7.10	10.78	33.16	90.49	121.32	141.42	146.27	2.72	2.53
carveol(trans)	0.35	0.00		0.80	1.19	1.48	3.16	7.59	10.82	14.95	18.96	0.49	0.06
carveol(cis)	0.28	0.00		0.77	1.21	1.43	2.51	3.64	4.21	4.87	6.16	0.12	0.00
neral	0.29	0.00		0.67	1.27	1.49	2.36	4.52	5.87	7.18	9.30	0.17	0.00
carvone	0.49	0.00		0.75	1.14	1.40	2.70	7.05	10.30	12.89	15.35	0.46	0.04
geranial	0.57	0.00		1.32	2.40	2.82	5.07	9.74	11.64	15.50	28.68	0.33	0.00
perrillaldehyde	0.66	0.00		2.43	3.31	3.73	6.78	7.39	7.91	8.25	10.58	0.22	0.00
valencene	0.72	0.75		0.61	0.51	0.74	1.50	2.45	2.39	1.99	1.52	0.02	0.87
δ-cadinene	0.15	0.16		0.15	0.13	0.18	0.40	0.69	0.75	0.68	0.66	0.00	0.18

Frac.: fraction dep.aft.ter.: The fraction released by slow pressurization and depressurization with CO2

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	493.28	381.82		43.65	19.29	15.10	5.34	2.30	1.52	1.24	1.10	2.65	7.37
used CO_2 (kg)				0.297	0.347	0.693	0.643	1.337	1.487	2.030	3.217		
							concentration(mg/g fraction)						
α-pinene	3.75	3.53		3.37	2.95	2.81	2.27	2.07	1.85	1.56	1.11	0.02	3.24
sabinene	1.90	1.83		1.52	1.36	1.22	1.08	0.95	0.88	0.68	0.60	0.00	1.77
β-pinene	0.14	0.13		0.13	0.12	0.11	0.10	0.09	0.08	0.06	0.06	0.00	0.13
β-myrcene	15.65	15.13		14.18	12.94	12.41	10.71	9.68	8.53	7.44	6.30	0.11	15.12
n-octanal	0.99	0.00		3.55	5.34	6.15	7.37	10.49	10.69	9.23	7.20	0.17	0.34
α-phellandrene	0.20	0.17		0.18	0.17	0.16	0.14	0.14	0.14	0.13	0.14	0.00	0.17
carene	0.82	0.78		0.75	0.70	0.68	0.60	0.58	0.53	0.46	0.37	0.00	0.76
limonene	349.10	957.46		837.39	800.83	776.74	738.27	625.54	551.40	489.16	422.93	11.38	915.25
n-octanol	1.39	0.00		7.10	8.99	7.76	6.15	5.55	5.47	5.77	6.83	0.15	0.00
linalool	3.72	0.00		16.39	24.46	22.83	19.51	17.98	17.19	16.41	15.21	0.31	0.00
n-nonanal	0.29	0.00		0.82	1.20	1.70	2.27	3.49	3.75	3.46	2.94	0.08	0.20
limonene oxide (cis)	0.71	0.00		2.22	4.26	5.30	6.75	9.80	11.56	13.03	8.69	0.21	0.08
limonene oxide(trans)	0.38	0.00		1.51	3.04	3.74	4.77	6.87	7.97	8.19	7.00	0.11	0.05
citronellal	0.35	0.00		1.03	1.67	2.09	2.88	4.27	4.57	3.93	3.23	0.07	0.20
α-terpineol	0.86	0.00		3.88	5.26	4.93	4.40	4.44	4.78	5.45	7.18	0.17	0.00
decanal	2.69	0.08		7.52	12.68	17.27	25.91	40.86	44.26	39.66	32.23	0.80	2.60
carveol(trans)	0.35	0.00		1.17	1.93	2.44	3.37	5.60	7.58	10.25	15.72	0.44	0.11
carveol(cis)	0.28	0.00		1.23	1.75	1.74	1.66	1.93	2.33	2.81	4.36	0.09	0.00
neral	0.29	0.00		1.12	1.93	1.96	1.86	1.86	1.97	2.00	2.15	0.04	0.00
carvone	0.49	0.00		1.12	2.08	2.67	3.32	4.78	5.86	7.40	9.75	0.29	0.10
geranial	0.57	0.00		2.23	3.91	4.09	3.84	4.08	4.00	4.28	4.70	0.10	0.00
perrillaldehyde	0.66	0.00		3.63	5.43	6.08	3.91	4.05	5.12	5.96	12.60	0.29	0.00
valencene	0.72	0.76		0.63	0.65	0.67	0.69	0.67	0.59	0.59	0.67	0.00	0.77
δ-cadinene	0.15	0.16		0.15	0.15	0.15	0.16	0.17	0.14	0.17	0.20	0.00	0.16

CO_2 conditions: 24.14 MPa x 45 °Cx 4kg/h

Frac.: fraction

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 13.10 MPa x 45 °Cx 2 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	495.00	372.43		27.98	17.60	22.82	11.81	9.31	2.51	0.97	0.91	2.96	10.96
used CO_2 (kg)				0.195	0.195	0.390	0.440	0.680	0.630	0.970	1.650		
				concentration(mg/g fraction)									
α-pinene	3.75	3.92		3.59	3.46	3.35	3.14	2.83	2.34	1.83	1.24	0.25	3.42
sabinene	1.90	1.99		1.56	1.50	1.45	1.37	1.23	0.99	0.89	0.65	0.12	1.84
β-pinene	0.14	0.14		0.13	0.13	0.13	0.12	0.11	0.10	0.10	0.10	0.01	0.13
β-myrcene	15.65	16.30		15.02	14.75	14.37	13.60	12.32	10.35	8.23	6.45	1.12	15.65
n-octanal	0.99	0.01		3.02	4.19	5.26	6.59	7.93	7.94	6.94	5.70	0.96	0.17
α-phellandrene	0.20	0.20		0.19	0.19	0.19	0.18	0.18	0.16	0.18	0.16	0.03	0.20
carene	0.82	0.85		0.79	0.79	0.78	0.75	0.69	0.58	0.47	0.36	0.06	0.80
limonene	896.97	951.53		867.43	862.81	848.37	815.64	745.48	633.40	500.24	413.39	51.01	914.12
n-octanol	1.39	0.00		4.75	5.15	5.35	6.18	9.30	16.62	28.24	34.25	5.93	0.01
linalool	3.72	0.03		12.07	14.44	16.25	21.30	33.03	46.47	51.66	47.35	6.77	0.03
n-nonanal	0.29	0.01		0.74	1.02	1.27	1.68	2.07	0.00	0.00	0.00	0.46	0.09
limonene oxide (cis)	0.71	0.00		1.81	2.57	3.20	4.14	5.22	7.29	9.82	6.72	1.92	0.02
limonene oxide(trans)	0.38	0.00		1.25	1.90	2.47	3.31	4.08	5.39	6.44	6.15	0.88	0.01
citronellal	0.35	0.00		0.91	1.30	1.68	2.20	2.90	3.22	3.01	2.51	0.40	0.10
α-terpineol	0.86	0.00		2.68	2.85	2.99	3.44	4.98	8.75	16.25	22.66	3.97	0.01
decanal	2.69	0.05		6.76	9.26	11.99	16.75	26.46	37.48	41.25	35.90	5.91	1.54
carveol(trans)	0.35	0.00		0.90	1.09	1.28	1.71	2.77	5.02	8.59	12.47	2.42	0.05
carveol(cis)	0.28	0.00		0.85	0.92	0.98	1.17	1.81	3.31	6.08	8.68	1.52	0.00
neral	0.29	0.00		0.88	1.11	1.29	1.76	2.75	3.60	4.15	4.16	0.60	0.00
carvone	0.49	0.00		0.95	1.23	1.49	2.15	3.55	5.07	6.58	8.34	1.51	0.03
geranial	0.57	0.00		1.70	2.06	2.47	3.28	5.65	8.44	11.10	12.20	1.64	0.00
perrillaldehyde	0.66	0.00		2.67	3.03	2.21	2.66	3.55	10.12	16.42	22.16	4.61	0.01
valencene	0.72	0.74		0.62	0.63	0.66	0.70	0.69	0.66	0.62	0.68	0.12	0.79
δ-cadinene	0.15	0.16		0.14	0.15	0.16	0.17	0.18	0.18	0.24	0.27	0.05	0.17

Frac.: fraction dep.aft.ter.: The fraction released by slow pressurization and depressurization with CO2

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 13.10 MPa x 45 °Cx 2 kg/h												
	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	492.36	378		44.74	22.84	18.15	3.16	1.29	0.88	0.72	0.82	1.97	5.39
used CO_2 (kg)				0.337	0.287	0.623	0.623	1.247	1.297	1.970	3.267		
concentration(mg/g fraction)													
α-pinene	3.75	3.94		3.67	3.33	3.09	2.25	1.50	1.18	0.81	0.60	0.00	3.29
sabinene	1.90	2.00		1.57	1.46	1.34	1.02	0.70	0.56	0.32	0.27	0.00	1.81
β-pinene	0.14	0.14		0.14	0.13	0.13	0.10	0.08	0.07	0.07	0.08	0.00	0.13
β-myrcene	15.65	16.47		15.25	14.58	13.93	10.92	7.28	5.71	4.33	3.55	0.05	15.69
n-octanal	0.99	0.04		3.23	4.90	5.66	12.46	18.29	16.75	12.82	9.43	0.13	0.35
α-phellandrene	0.20	0.22		0.21	0.21	0.21	0.21	0.23	0.25	0.20	0.26	0.00	0.22
carene	0.82	0.86		0.80	0.79	0.77	0.68	0.54	0.43	0.33	0.28	0.00	0.80
limonene	910.84	956.74		859.61	858.89	852.64	733.02	497.72	387.22	306.40	252.86	6.42	919.52
n-octanol	1.39	0.00		6.62	7.63	5.85	5.80	9.51	12.68	16.14	19.90	0.34	0.00
linalool	3.72	0.00		14.28	20.39	18.17	23.14	46.76	56.78	62.28	64.64	0.94	0.04
n-nonanal	0.29	0.00		0.73	1.09	1.49	3.69	0.00	0.00	0.00	0.00	0.06	0.15
limonene oxide (cis)	0.71	0.00		1.55	2.56	3.40	8.17	16.49	15.67	15.37	13.17	0.36	0.05
limonene oxide(trans)	0.38	0.00		1.13	2.16	3.02	7.95	16.33	22.96	25.19	25.81	0.23	0.03
citronellal	0.35	0.00		0.85	1.36	1.82	4.49	7.74	7.70	6.01	4.73	0.06	0.21
α-terpineol	0.86	0.00		3.53	4.12	3.37	3.90	7.13	11.89	14.75	22.58	0.30	0.00
decanal	2.69	0.23		6.45	10.01	15.19	41.84	79.29	83.31	71.22	56.86	0.80	2.11
carveol(trans)	0.35	0.00		0.89	1.28	1.58	3.81	9.63	14.00	18.45	25.03	0.50	0.07
carveol(cis)	0.28	0.00		1.05	1.30	1.14	1.51	3.20	4.77	6.88	10.35	0.14	0.00
neral	0.29	0.00		0.88	1.41	1.41	2.29	4.76	6.43	7.94	10.07	0.14	0.00
carvone	0.49	0.00		0.84	1.28	1.56	3.56	8.33	11.51	14.30	16.91	0.37	0.05
geranial	0.57	0.00		1.75	2.60	2.62	4.55	10.49	15.21	18.09	24.09	0.28	0.00
perrillaldehyde	0.66	0.00		3.13	3.89	3.69	2.90	5.56	7.37	10.96	13.64	0.28	0.00
valencene	0.72	0.77		0.60	0.65	0.77	1.17	1.21	1.30	0.86	0.75	0.00	0.81
δ-cadinene	0.15	0.17		0.15	0.16	0.20	0.33	0.45	0.76	0.53	0.54	0.00	0.18

Frac.: fraction dep.aft.ter.: The fraction released by slow pressurization and depressurization with CO2

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 24.14 MPa x 55 °Cx 2 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	491.03	383.76		15.17	15.67	22.48	13.79	12.80	5.22	1.74	1.06	2.77	4.56
used CO_2 (kg)				0.205	0.155	0.360	0.310	0.670	0.670	0.980	1.700		
concentration(mg/g fraction)													
α-pinene	3.75	3.95		3.54	3.26	3.14	3.11	2.95	2.70	2.27	2.12	0.08	3.30
sabinene	1.90	2.01		1.43	1.31	1.25	1.30	1.13	1.09	1.18	0.95	0.04	1.80
β-pinene	0.14	0.15		0.13	0.13	0.12	0.13	0.12	0.11	0.10	0.09	0.00	0.13
β-myrcene	15.65	16.53		14.74	14.07	13.69	13.52	12.92	12.19	11.05	10.30	0.32	15.49
n-octanal	0.99	0.00		4.01	4.98	5.32	5.70	5.92	5.75	5.35	4.67	0.15	0.26
α-phellandrene	0.20	0.22		0.21	0.20	0.20	0.21	0.21	0.21	0.23	0.22	0.00	0.21
carene	0.82	0.86		0.78	0.76	0.74	0.74	0.71	0.68	0.61	0.56	0.02	0.78
limonene	907.18	965.09		856.42	839.22	827.93	812.29	784.02	747.42	677.41	636.84	30.06	927.38
n-octanol	1.39	0.00		7.73	8.07	7.84	7.85	7.69	7.40	7.09	6.22	0.20	0.00
linalool	3.72	0.00		17.78	20.43	20.66	20.90	20.50	19.62	18.26	15.58	0.45	0.00
n-nonanal	0.29	0.00		0.90	1.21	1.37	1.53	1.70	1.69	1.67	1.50	0.05	0.16
limonene oxide (cis)	0.71	0.00		2.02	2.88	3.36	3.92	4.65	5.57	3.89	3.73	0.32	0.00
limonene oxide(trans)	0.38	0.00		1.58	2.35	2.79	3.33	3.79	4.21	4.41	4.04	0.10	0.00
citronellal	0.35	0.00		1.07	1.46	1.65	1.87	2.04	2.09	2.02	1.69	0.05	0.15
α-terpineol	0.86	0.00		4.25	4.70	4.72	4.91	5.05	4.99	4.81	4.03	0.14	0.00
decanal	2.69	0.17		8.18	11.39	13.51	16.49	19.32	20.27	19.89	16.76	0.50	2.16
carveol(trans)	0.35	0.00		1.20	1.54	1.77	2.15	2.87	4.34	7.69	12.33	0.52	0.06
carveol(cis)	0.28	0.00		1.35	1.53	1.58	1.72	1.86	2.03	2.44	2.70	0.10	0.00
neral	0.29	0.00		1.19	1.53	1.65	1.78	1.87	1.92	1.91	1.83	0.06	0.00
carvone	0.49	0.00		1.11	1.50	1.78	2.14	2.71	3.47	4.46	5.39	0.22	0.00
geranial	0.57	0.00		2.25	2.96	3.35	3.56	3.81	4.18	4.13	3.76	0.12	0.00
perrillaldehyde	0.66	0.00		3.86	5.22	4.97	5.67	6.47	6.83	4.48	4.05	0.12	0.00
valencene	0.72	0.77		0.59	0.68	0.70	0.71	0.69	0.68	0.67	0.65	0.02	0.79
δ-cadinene	0.15	0.16		0.16	0.18	0.19	0.18	0.18	0.19	0.18	0.21	0.00	0.17

Frac.: fraction dep.aft.ter.: The fraction released by slow pressurization and depressurization with CO2

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 9.66 MPa x 45 °Cx 2 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	489.49	375.59		21.92	17.20	24.05	8.18	11.08	1.65	0.57	0.81	2.61	10.38
used CO_2 (kg)				0.214	0.164	0.378	0.378	0.757	0.707	1.035	1.742		
concentration(mg/g fraction)													
α-pinene	3.75	3.88		3.45	3.55	3.85	3.26	3.01	2.03	1.29	0.61	0.02	3.23
sabinene	1.90	1.97		1.53	1.62	1.92	1.61	1.37	0.97	0.64	0.38	0.00	1.82
β-pinene	0.14	0.14		0.13	0.14	0.15	0.13	0.12	0.11	0.09	0.03	0.00	0.13
β-myrcene	15.65	16.27		14.39	15.12	16.40	14.20	13.20	8.76	5.58	3.58	0.09	15.57
n-octanal	0.99	0.00		2.80	3.45	4.59	6.23	11.07	20.19	17.15	10.12	0.21	0.43
α-phellandrene	0.20	0.21		0.19	0.21	0.21	0.20	0.21	0.22	0.23	0.28	0.00	0.20
carene	0.82	0.85		0.75	0.80	0.86	0.79	0.77	0.56	0.36	0.22	0.00	0.78
limonene	911.32	953.83		878.92	886.16	885.05	873.83	822.06	549.42	351.09	230.96	12.03	930.17
n-octanol	1.39	0.00		4.99	5.82	6.88	7.14	9.63	20.78	25.48	27.63	0.55	0.00
linalool	3.72	0.00		11.90	13.79	14.97	14.00	17.29	61.12	117.19	150.79	3.25	0.00
n-nonanal	0.29	0.00		0.66	0.79	1.05	1.38	2.58	0.00	0.00	0.00	0.14	0.26
limonene oxide (cis)	0.71	0.00		1.42	1.71	2.09	2.52	4.88	15.19	16.30	12.84	0.29	0.00
limonene oxide(trans)	0.38	0.00		0.96	1.21	1.54	2.03	4.52	17.21	19.69	16.28	0.30	0.00
citronellal	0.35	0.00		0.82	1.02	1.37	1.80	3.34	8.58	9.24	6.85	0.12	0.28
α-terpineol	0.86	0.00		2.63	3.02	3.49	3.65	5.15	13.43	19.86	23.29	0.51	0.00
decanal	2.69	0.10		5.84	7.01	8.96	11.62	22.77	75.96	106.22	113.03	2.19	3.88
carveol(trans)	0.35	0.00		0.77	0.88	1.02	1.11	1.78	6.07	9.36	11.52	0.31	0.00
carveol(cis)	0.28	0.00		0.82	0.96	1.10	1.15	1.60	4.16	6.43	8.37	0.16	0.00
neral	0.29	0.00		0.81	0.96	1.05	1.00	1.28	3.95	8.11	12.32	0.23	0.00
carvone	0.49	0.00		0.79	0.90	1.02	1.04	1.53	5.89	10.88	13.94	0.42	0.00
geranial	0.57	0.00		1.59	1.84	0.20	1.94	2.49	7.12	13.37	22.43	0.37	0.00
perrillaldehyde	0.66	0.00		2.44	2.73	3.15	3.29	4.78	13.75	22.96	28.41	0.41	0.00
valencene	0.72	0.77		0.54	0.58	0.63	0.72	0.96	1.18	0.96	0.70	0.00	0.84
δ-cadinene	0.15	0.16		0.12	0.13	0.14	0.17	0.25	0.42	0.46	0.50	0.00	0.18

Frac.: fraction

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 9.66 MPa x 55 °Cx 4 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	489.95		376.18	33.55	10.97	14.78	14.13	11.72	1.27	0.63	1.09	0.92	6.49
used CO_2 (kg)				0.351	0.351	0.652	0.652	1.303	1.303	1.955	3.258		
concentration(mg/g fraction)													
α-pinene	3.75		3.96	4.01	4.88	4.60	2.66	0.85	1.10	0.66	0.15	0.00	3.24
sabinene	1.90		2.01	1.76	1.88	1.88	1.35	0.56	0.59	0.35	0.14	0.00	1.82
β-pinene	0.14		0.15	0.14	0.16	0.16	0.13	0.07	0.07	0.04	0.00	0.00	0.13
β-myrcene	15.65		16.56	16.05	17.94	18.36	14.54	7.31	5.69	3.45	1.43	0.04	15.51
n-octanal	0.99		0.00	2.26	3.50	5.15	5.68	8.40	22.73	30.92	17.48	0.40	0.28
α-phellandrene	0.20		0.22	0.22	0.24	0.25	0.21	0.20	0.41	0.57	0.65	0.02	0.20
carene	0.82		0.86	0.83	0.89	0.90	0.80	0.58	0.51	0.39	0.20	0.00	0.79
limonene	911.32		952.76	887.60	904.31	901.41	907.08	871.40	610.02	311.70	118.97	16.20	924.82
n-octanol	1.39		0.00	4.12	6.05	6.92	6.50	7.12	10.47	17.37	27.66	0.62	0.00
linalool	3.72		0.00	9.22	13.14	15.32	15.47	23.26	58.98	102.92	138.55	3.32	0.00
n-nonanal	0.29		0.00	0.55	0.64	0.73	0.83	1.91	0.00	0.00	0.00	0.33	0.15
limonene oxide (cis)	0.71		0.00	1.11	1.36	1.49	1.55	3.45	12.09	15.91	18.64	0.58	0.00
limonene oxide(trans)	0.38		0.00	0.75	1.05	1.32	1.59	4.18	16.83	33.94	41.15	0.85	0.00
citronellal	0.35		0.00	0.62	0.70	0.79	0.89	2.08	7.34	12.59	12.77	0.30	0.18
α-terpineol	0.86		0.00	2.32	2.60	2.61	2.49	3.62	7.75	14.48	33.88	0.54	0.00
decanal	2.69		0.09	5.00	4.73	4.72	4.88	14.44	73.10	148.04	206.03	4.91	2.38
carveol(trans)	0.35		0.00	0.64	0.64	0.65	0.64	1.41	5.73	12.58	19.89	0.50	0.00
carveol(cis)	0.28		0.00	0.68	0.74	0.72	0.67	1.06	2.25	4.27	7.08	0.15	0.00
neral	0.29		0.00	0.57	0.66	0.72	0.71	1.37	4.01	7.78	13.54	0.28	0.00
carvone	0.49		0.00	0.67	0.65	0.67	0.65	1.47	5.54	10.41	14.55	0.47	0.00
geranial	0.57		0.00	1.08	1.20	1.25	1.18	2.36	6.96	20.58	32.55	0.48	0.00
perrillaldehyde	0.66		0.00	2.13	2.04	1.85	1.68	2.90	3.80	0.00	10.25	0.26	0.00
valencene	0.72		0.77	0.60	0.31	0.20	0.15	0.92	5.54	8.51	5.03	0.11	0.94
δ-cadinene	0.15		0.16	0.14	0.08	0.05	0.04	0.27	1.68	2.80	1.96	0.04	0.20

Frac.: fraction

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 13.10 MPa x 55 °Cx 2 kg/h												
	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	489.01	378.87		18.04	18.58	25.14	12.28	8.54	2.31	0.75	0.84	1.55	6.47
used CO_2 (kg)				0.218	0.118	0.387	0.337	0.673	0.723	1.060	1.733		
concentration(mg/g fraction)													
α-pinene	3.75	3.92		3.59	3.45	3.28	3.11	2.74	1.93	1.55	1.45	0.11	3.36
sabinene	1.90	1.99		1.66	1.45	1.56	1.50	1.13	0.99	0.82	0.74	0.04	1.83
β-pinene	0.14	0.14		0.13	0.13	0.13	0.12	0.11	0.09	0.09	0.11	0.00	0.13
β-myrcene	15.65	16.35		15.12	14.84	14.28	13.68	12.27	9.42	7.14	6.13	0.33	15.69
n-octanal	0.99	0.00		4.04	4.67	5.64	6.86	7.76	7.25	5.99	5.01	0.24	0.14
α-phellandrene	0.20	0.22		0.22	0.20	0.20	0.21	0.22	0.25	0.33	0.42	0.02	0.20
carene	0.82	0.85		0.80	0.80	0.76	0.76	0.69	0.53	0.42	0.37	0.02	0.79
limonene	911.32	952.59		864.35	864.73	854.78	813.43	740.70	587.30	448.68	382.90	18.83	919.85
n-octanol	1.39	0.00		5.61	5.91	6.96	9.79	13.70	17.27	16.40	13.07	0.43	0.00
linalool	3.72	0.00		14.12	16.03	20.72	26.35	31.34	35.39	25.91	17.64	0.65	0.03
n-nonanal	0.29	0.00		1.00	1.08	1.30	1.71	2.13	0.00	2.52	2.16	0.09	0.12
limonene oxide (cis)	0.71	0.00		2.06	2.39	3.09	4.29	6.22	9.74	7.47	5.57	0.66	0.00
limonene oxide(trans)	0.38	0.00		1.82	2.06	2.80	3.96	5.72	8.59	9.18	6.57	0.22	0.00
citronellal	0.35	0.00		1.08	1.27	1.60	2.16	2.95	3.63	2.98	2.05	0.07	0.09
α-terpineol	0.86	0.00		3.07	3.26	3.84	5.34	8.88	15.88	18.20	21.55	0.46	0.00
decanal	2.69	0.08		8.65	9.59	12.07	18.40	30.48	45.95	44.30	31.15	1.08	2.47
carveol(trans)	0.35	0.00		1.05	1.19	1.47	2.18	3.72	7.41	11.73	19.22	0.85	0.00
carveol(cis)	0.28	0.00		0.98	1.06	1.24	1.73	3.04	5.90	7.88	9.27	0.25	0.00
neral	0.29	0.00		0.94	1.13	1.49	2.09	2.98	4.33	4.58	3.97	0.10	0.00
carvone	0.49	0.00		1.03	1.17	1.50	2.17	3.96	7.79	11.63	14.10	0.54	0.00
geranial	0.57	0.00		1.73	2.10	2.83	4.03	6.46	10.56	11.64	12.06	0.29	0.00
perrillaldehyde	0.66	0.00		2.95	3.20	3.70	5.15	9.57	20.24	28.37	27.44	0.94	0.00
valencene	0.72	0.76		0.58	0.61	0.68	0.75	0.76	0.79	0.89	0.83	0.03	0.84
δ-cadinene	0.15	0.16		0.15	0.16	0.18	0.20	0.23	0.28	0.37	0.37	0.00	0.18

Frac.: fraction

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 13.10 MPa x 55 °Cx 2 kg/h

	Feed oil	terpene	dep.aft.te	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.
weight of frac.(g)	486.86	329.02	34.54	18.34	7.51	10.26	8.30	15.78	15.23	12.29	1.27	
used CO_2 (kg)				0.198	0.283	0.345	0.345	0.640	0.640	0.985	1.575	
						concentration(mg/g fraction)						
α-pinene	3.75	3.89	3.46	4.17	4.18	4.45	4.50	3.78	2.27	0.98	0.48	2.76
sabinene	1.90	1.97	1.82	1.92	1.71	1.70	1.64	1.41	0.90	0.48	0.26	1.56
β-pinene	0.14	0.14	0.13	0.15	0.14	0.15	0.16	0.14	0.11	0.07	0.02	0.12
β-myrcene	15.65	16.20	14.98	16.29	15.73	16.32	17.07	16.21	12.55	8.07	3.16	13.90
n-octanal	0.99	0.00	0.00	1.88	2.04	2.47	3.32	4.24	4.29	9.79	30.44	0.11
α-phellandrene	0.20	0.21	0.19	0.22	0.21	0.21	0.23	0.23	0.22	0.25	0.61	0.18
carene	0.82	0.84	0.77	0.84	0.79	0.81	0.84	0.81	0.71	0.61	0.40	0.72
limonene	911.32	938.69	924.37	799.72	904.67	904.95	899.33	891.85	888.83	862.44	268.87	903.87
n-octanol	1.39	0.00	0.00	3.46	3.65	4.22	5.19	6.18	6.07	8.82	27.94	0.00
linalool	3.72	0.00	0.00	7.31	7.60	8.74	10.92	13.30	13.73	28.44	120.82	0.00
n-nonanal	0.29	0.00	0.00	0.45	0.43	0.45	0.53	0.62	0.69	2.18	0.00	0.10
limonene oxide (cis)	0.71	0.00	0.00	0.94	0.91	0.98	1.12	1.22	1.20	3.04	14.04	0.00
limonene oxide(trans)	0.38	0.00	0.00	0.61	0.62	0.71	0.90	1.14	1.30	4.73	38.56	0.00
citronellal	0.35	0.00	0.00	0.50	0.46	0.48	0.54	0.62	0.67	11.41	11.49	0.08
α-terpineol	0.86	0.00	0.00	1.88	1.71	1.74	1.95	2.16	2.24	4.41	24.09	0.00
decanal	2.69	0.00	0.24	3.96	3.34	3.26	3.55	3.81	3.94	14.28	129.19	2.13
carveol(trans)	0.35	0.00	0.00	0.54	0.47	0.46	0.52	0.57	0.60	1.37	12.29	0.00
carveol(cis)	0.28	0.00	0.00	0.56	0.48	0.49	0.54	0.59	0.61	1.09	6.78	0.00
neral	0.29	0.00	0.00	0.43	0.40	0.41	0.49	0.57	0.59	1.46	9.74	0.00
carvone	0.49	0.00	0.00	0.55	0.47	0.46	0.53	0.58	0.59	1.43	10.34	0.00
geranial	0.57	0.00	0.00	0.83	0.73	0.74	0.83	0.92	0.95	2.33	19.30	0.00
perrillaldehyde	0.66	0.00	0.00	1.65	1.41	1.34	1.46	1.54	1.51	2.75	14.50	0.00
valencene	0.72	0.77	0.69	0.51	0.31	0.25	0.24	0.21	0.19	0.93	10.15	1.02
δ-cadinene	0.15	0.16	0.15	0.11	0.08	0.06	0.06	0.05	0.05	0.28	3.56	0.22

Frac.: fraction

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 24.14 MPa x 55 °Cx 4 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	512.03		369.81	46.20	22.53	27.24	10.20	3.90	1.38	0.91	0.80	2.57	8.25
used CO_2 (kg)				0.296	0.296	0.692	0.642	1.333	1.283	1.975	3.258		
concentration(mg/g fraction)													
α-pinene	3.75		3.95	3.56	3.45	3.25	2.95	2.31	1.73	1.34	1.30	0.05	3.21
sabinene	1.90		2.00	1.62	1.58	1.58	1.45	0.94	0.94	0.79	0.77	0.02	1.78
β-pinene	0.14		0.14	0.14	0.13	0.13	0.12	0.10	0.08	0.09	0.10	0.00	0.13
β-myrcene	15.65		16.41	14.85	14.64	14.05	13.16	10.99	9.05	7.61	7.22	0.18	15.20
n-octanal	0.99		0.00	2.95	4.56	5.29	5.94	6.50	6.00	4.55	3.59	0.13	0.03
α-phellandrene	0.20		0.20	0.19	0.20	0.19	0.19	0.17	0.20	0.21	0.23	0.00	0.18
carene	0.82		0.85	0.78	0.79	0.76	0.72	0.60	0.50	0.41	0.40	0.00	0.78
limonene	911.32		962.81	875.37	861.36	838.43	801.07	701.00	569.80	490.07	466.68	13.88	913.89
n-octanol	1.39		0.00	4.82	5.80	5.71	6.11	9.48	14.84	19.89	22.24	0.72	0.00
linalool	3.72		0.00	12.38	16.17	16.59	17.90	24.90	34.77	37.59	32.33	0.75	0.04
n-nonanal	0.29		0.00	0.74	1.15	1.45	1.76	2.14	0.00	0.00	0.00	0.06	0.03
limonene oxide (cis)	0.71		0.00	1.89	3.14	3.86	4.98	7.92	6.39	5.51	0.00	0.43	0.00
limonene oxide(trans)	0.38		0.00	1.40	2.44	3.11	4.06	5.88	6.78	6.18	4.64	0.10	0.00
citronellal	0.35		0.00	0.88	1.42	1.75	2.10	2.47	2.42	1.89	1.41	0.04	0.00
α-terpineol	0.86		0.00	2.79	3.32	3.30	3.62	5.95	9.76	13.98	16.90	0.59	0.00
decanal	2.69		0.00	6.98	11.34	14.74	19.28	25.48	26.14	20.67	14.96	0.55	0.60
carveol(trans)	0.35		0.00	1.02	1.52	1.89	2.80	5.94	10.03	14.31	17.05	0.67	0.03
carveol(cis)	0.28		0.00	0.90	1.10	1.17	1.38	2.53	4.22	6.46	7.65	0.23	0.00
neral	0.29		0.00	0.89	1.23	1.34	1.55	2.36	3.38	3.99	3.81	0.07	0.00
carvone	0.49		0.00	1.04	1.55	1.85	2.52	4.65	6.79	8.77	8.92	0.34	0.00
geranial	0.57		0.00	1.71	2.40	2.56	3.00	5.02	7.37	9.47	9.11	0.20	0.00
perrillaldehyde	0.66		0.00	2.81	3.62	3.96	2.94	8.25	12.98	16.75	18.67	0.71	0.03
valencene	0.72		0.75	0.67	0.64	0.68	0.70	0.73	0.70	0.73	0.76	0.02	0.91
δ-cadinene	0.15		0.16	0.16	0.15	0.16	0.17	0.20	0.21	0.26	0.27	0.00	0.19

Frac.: fraction

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 24.14 MPa x 35 °Cx 2 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by co
weight of frac.(g)	493.00		371.92	24.48	15.33	21.24	12.64	12.82	4.47	2.64	1.48	2.72	10.08
used CO_2 (kg)				0.196	0.196	0.392	0.342	0.733	0.683	1.025	1.708		
concentration(mg/g fraction)													
α-pinene	3.75		3.88	3.58	3.46	3.28	3.16	2.93	2.55	2.08	1.51	1.69	3.38
sabinene	1.90		1.98	1.70	1.61	1.54	1.51	1.40	1.12	0.81	0.65	0.88	1.86
β-pinene	0.14		0.14	0.13	0.13	0.13	0.12	0.12	0.11	0.09	0.08	0.07	0.13
β-myrcene	15.65		16.26	14.98	14.55	13.98	13.61	12.82	11.27	9.33	7.70	7.45	15.63
n-octanal	0.99		0.00	2.15	3.12	4.30	5.64	7.65	10.05	10.07	7.69	6.69	0.79
α-phellandrene	0.20		0.20	0.19	0.18	0.17	0.17	0.18	0.14	0.10	0.12	0.15	0.19
carene	0.82		0.84	0.79	0.77	0.75	0.74	0.72	0.64	0.53	0.44	0.42	0.78
limonene	919.12		960.76	879.73	861.38	849.23	830.50	790.19	707.96	597.04	506.77	461.95	931.39
n-octanol	1.39		0.00	4.69	5.71	6.29	6.72	7.40	9.20	12.11	16.43	16.08	0.03
linalool	3.72		0.00	10.14	13.77	16.88	19.67	23.98	33.25	47.10	48.97	41.43	0.06
n-nonanal	0.29		0.00	0.56	0.78	1.07	1.42	1.93	2.47	0.00	0.00		0.32
limonene oxide (cis)	0.71		0.00	1.40	2.02	2.76	3.60	4.73	7.10	9.93	10.70	7.97	
limonene oxide(trans)	0.38		0.00	0.77	1.23	1.81	2.49	3.40	5.13	6.95	6.64	5.03	
citronellal	0.35		0.00	0.69	1.03	1.47	1.95	2.71	3.54	3.53	2.72	2.25	0.13
α-terpineol	0.86		0.00	2.58	3.15	3.54	3.86	4.37	5.60	7.65	10.73	10.21	0.10
decanal	2.69		0.16	5.14	7.40	10.35	13.91	20.13	30.62	36.35	30.73	27.13	0.45
carveol(trans)	0.35		0.00	0.75	1.03	1.30	1.57	2.05	3.16	5.04	7.76	6.80	0.03
carveol(cis)	0.28		0.00	0.82	1.05	1.23	1.36	1.58	2.08	3.11	4.58	4.23	4.22
neral	0.29		0.00	0.71	1.04	1.33	1.61	2.06	2.97	3.72	3.74	2.98	0.12
carvone	0.49		0.00	0.81	1.11	1.43	1.81	2.50	3.98	5.98	8.16	6.38	0.00
geranial	0.57		0.00	1.41	2.03	2.60	3.07	4.08	6.08	8.62	9.16	7.22	0.00
perrillaldehyde	0.66		0.00	2.60	3.29	3.85	4.54	3.57	4.54	6.56	14.53	14.25	0.10
valencene	0.72		0.77	0.67	0.67	0.66	0.67	0.68	0.64	0.63	0.56	0.50	0.00
δ-cadinene	0.15		0.16	0.15	0.14	0.15	0.15	0.16	0.15	0.17	0.15	0.13	0.00

Frac.: fraction

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 9.66 MPa x 35 °Cx 4 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	502.13	387.99		49.06	16.25	10.77	3.09	1.89	1.23	0.82	1.19	3.44	12.28
used CO_2 (kg)				0.396	0.346	0.692	0.642	1.333	1.333	2.075	3.308		
concentration(mg/g fraction)													
α-pinene	3.75	3.96		3.60	3.29	2.93	2.29	1.63	1.40	1.23	1.38	1.79	3.47
sabinene	1.90	2.01		1.68	1.54	1.37	1.08	0.76	0.69	0.63	0.66	0.98	1.91
β-pinene	0.14	0.14		0.14	0.13	0.12	0.10	0.07	0.07	0.07	0.07	0.08	0.14
β-myrcene	15.65	16.49		15.04	13.98	12.66	10.12	7.40	6.64	6.46	7.31	8.25	16.02
n-octanal	0.99	0.00		2.63	5.13	9.58	17.70	21.64	18.05	12.96	8.16	7.17	0.50
α-phellandrene	0.20	0.20		0.19	0.18	0.15	0.13	0.11	0.12	0.12	0.14	0.17	0.19
carene	0.82	0.86		0.79	0.77	0.73	0.65	0.49	0.43	0.39	0.42	0.44	0.80
limonene	908.05	955.69		871.45	834.66	783.54	645.97	476.27	432.91	427.78	471.86	491.36	921.98
n-octanol	1.39	0.00		5.59	9.92	9.74	9.70	11.52	13.16	14.80	15.36	14.49	0.00
linalool	3.72	0.00		12.79	25.68	29.48	42.14	55.66	58.04	55.57	46.96	42.26	0.04
n-nonanal	0.29	0.00		0.64	1.06	2.12	0.00	0.00	0.00	0.00	0.00		0.26
limonene oxide (cis)	0.71	0.00		1.64	3.36	6.14	11.76	16.09	15.33	13.34	10.71	8.11	0.00
limonene oxide(trans)	0.38	0.00		0.98	2.23	4.40	8.98	12.16	11.13	8.87	6.18	5.05	0.00
citronellal	0.35	0.00		0.82	1.66	3.31	6.37	8.02	6.91	5.09	3.20	2.80	0.29
α-terpineol	0.86	0.00		2.90	4.88	5.17	5.93	8.04	10.54	12.23	13.32	12.44	0.00
decanal	2.69	0.14		5.69	10.34	22.56	49.82	74.00	71.63	58.73	39.92	35.00	4.12
carveol(trans)	0.35	0.00		0.87	1.48	2.20	3.93	6.33	7.89	8.95	9.54	7.91	0.00
carveol(cis)	0.28	0.00		0.96	1.58	1.74	2.21	3.22	4.11	4.88	5.33	4.82	0.00
neral	0.29	0.00		0.95	1.90	2.29	3.24	4.31	4.81	4.77	4.15	3.77	0.00
carvone	0.49	0.00		0.95	1.69	2.67	4.96	8.17	9.76	10.44	10.05	8.05	0.03
geranial	0.57	0.00		1.82	3.70	4.50	6.63	9.56	10.96	11.30	10.22	9.29	0.00
perrillaldehyde	0.66	0.00		2.79	4.53	3.83	4.95	7.70	10.54	18.32	18.49	17.44	0.00
valencene	0.72	0.77		0.65	0.67	0.73	0.74	0.59	0.62	0.64	0.70	0.62	0.78
δ-cadinene	0.15	0.16		0.14	0.15	0.16	0.18	0.15	0.20	0.22	0.27	0.18	0.16

Frac.: fraction

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 13.10 MPa x 35 °Cx 4 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	498.04	374.3		48.73	18.03	14.37	3.85	1.83	1.52	1.16	2.45	2.88	13.45
used CO_2 (kg)				0.296	0.346	0.692	0.692	1.383	1.483	2.025	3.408		
concentration(mg/g fraction)													
α-pinene	3.75	3.95		3.58	3.32	3.01	2.54	2.09	2.12	2.19	2.31	2.24	3.52
sabinene	1.90	2.00		1.67	1.59	1.41	1.15	0.97	0.96	1.06	1.23	1.21	1.92
β-pinene	0.14	0.15		0.14	0.13	0.12	0.10	0.09	0.09	0.09	0.09	0.09	0.13
β-myrcene	15.65	16.42		14.97	14.07	12.95	11.15	9.39	9.55	10.74	10.86	10.31	16.02
n-octanal	0.99	0.00		2.79	5.15	7.99	12.33	13.29	9.97	5.57	3.16	2.83	0.70
α-phellandrene	0.20	0.20		0.19	0.18	0.16	0.15	0.13	0.14	0.16	0.17	0.16	0.19
carene	0.82	0.85		0.78	0.77	0.73	0.65	0.54	0.52	0.55	0.55	0.52	0.80
limonene	915.47	956.39		871.70	839.19	790.82	692.29	580.05	575.19	641.81	635.85	662.84	930.38
n-octanol	1.39	0.00		4.72	7.06	8.57	10.15	13.30	14.81	13.24	11.97	11.44	0.00
linalool	3.72	0.00		12.44	20.21	25.22	33.39	46.29	44.49	30.78	21.57	19.63	0.04
n-nonanal	0.29	0.00		0.72	1.25	1.89	3.25	0.00	0.00	1.97	1.17	1.02	0.32
limonene oxide (cis)	0.71	0.00		1.78	3.34	5.26	8.22	9.93	8.75	5.80	3.89	3.54	0.05
limonene oxide(trans)	0.38	0.00		1.11	2.29	3.70	5.92	7.09	5.79	3.33	1.77	1.55	0.03
citronellal	0.35	0.00		0.91	1.73	2.73	4.31	4.79	3.66	2.15	1.27	1.13	0.40
α-terpineol	0.86	0.00		2.61	3.83	4.77	5.80	7.66	8.69	8.14	7.99	7.55	0.05
decanal	2.69	0.05		6.45	11.74	19.73	35.32	45.85	38.70	24.36	14.63	13.07	4.80
carveol(trans)	0.35	0.00		0.88	1.45	2.05	3.30	4.90	5.43	4.89	4.45	4.21	0.00
carveol(cis)	0.28	0.00		0.85	1.33	1.64	2.12	2.97	3.45	3.20	3.06	2.92	0.00
neral	0.29	0.00		0.94	1.60	1.99	2.75	3.38	3.25	2.47	1.83	1.64	0.00
carvone	0.49	0.00		1.06	1.72	2.49	4.14	5.98	6.23	5.10	3.82	3.56	0.03
geranial	0.57	0.00		1.85	3.16	4.00	5.64	7.51	7.51	5.53	4.22	3.89	0.00
perrillaldehyde	0.66	0.00		2.64	4.01	5.73	4.79	11.32	12.16	11.05	9.74	9.23	0.00
valencene	0.72	0.75		0.65	0.66	0.68	0.66	0.58	0.56	0.64	0.61	0.56	0.72
δ-cadinene	0.15	0.16		0.14	0.14	0.15	0.15	0.13	0.14	0.17	0.15	0.14	0.15

Frac.: fraction

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 9.66 MPa x 45 °Cx 4 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	497.10	383.34		32.17	23.91	18.33	3.62	2.16	0.95	0.69	0.82	3.64	11.23
used CO_2 (kg)				0.346	0.346	0.642	0.692	1.333	1.333	2.025	3.358		
concentration(mg/g fraction)													
α-pinene	3.75	3.96		3.85	3.61	3.06	2.53	1.42	0.83	0.55	0.47	0.04	3.30
sabinene	1.90	2.02		1.65	1.61	1.45	1.24	0.78	0.49	0.35	0.29	0.02	1.85
β-pinene	0.14	0.14		0.14	0.14	0.12	0.11	0.08	0.06	0.03	0.03	0.00	0.13
β-myrcene	15.65	16.49		15.62	15.24	13.78	11.83	6.99	4.27	3.14	2.60	0.21	15.61
n-octanal	0.99	0.00		2.11	3.87	6.33	13.99	29.39	25.88	18.89	11.95	0.06	0.11
α-phellandrene	0.20	0.20		0.19	0.19	0.18	0.18	0.17	0.19	0.22	0.30	0.00	0.19
carene	0.82	0.86		0.80	0.81	0.77	0.74	0.54	0.35	0.25	0.19	0.00	0.78
limonene	901.41	948.60		882.78	872.61	858.99	775.93	473.51	288.73	212.40	172.66	622.74	903.44
n-octanol	1.39	0.00		4.53	7.36	5.79	7.09	13.89	19.95	26.14	34.03	0.26	0.00
linalool	3.72	0.00		9.15	16.61	17.61	27.59	77.95	113.81	128.62	124.34	0.47	0.00
n-nonanal	0.29	0.00		0.52	0.80	1.41	3.21	0.00	0.00	0.00	0.00	0.03	0.09
limonene oxide (cis)	0.71	0.00		1.23	2.10	3.50	7.52	19.31	21.15	18.43	13.02	0.13	0.00
limonene oxide(trans)	0.38	0.00		0.70	1.42	2.79	6.60	19.62	24.12	21.74	13.55	0.03	0.00
citronellal	0.35	0.00		0.59	1.00	1.89	4.34	11.19	12.28	10.44	7.07	0.02	0.07
α-terpineol	0.86	0.00		2.37	3.45	2.95	3.93	9.21	14.09	17.00	21.58	0.20	0.00
decanal	2.69	0.00		4.54	6.53	12.55	30.31	91.02	126.43	130.18	105.96	0.29	1.98
carveol(trans)	0.35	0.00		0.67	0.97	1.22	2.37	7.27	11.08	13.26	15.50	0.14	0.00
carveol(cis)	0.28	0.00		0.71	1.05	0.96	1.33	3.05	4.83	6.15	8.45	0.07	0.00
neral	0.29	0.00		0.56	1.03	1.25	2.12	5.84	9.53	11.85	13.15	0.04	0.00
carvone	0.49	0.00		0.70	0.97	1.40	2.83	8.19	12.36	14.39	17.21	0.17	0.00
geranial	0.57	0.00		1.13	1.89	2.24	3.83	16.31	25.60	32.55	41.04	0.08	0.00
perrillaldehyde	0.66	0.00		2.18	2.96	1.94	2.43	0.00	0.00	0.00	0.00	0.21	0.00
valencene	0.72	0.75		0.59	0.48	0.69	1.31	1.41	1.00	0.79	0.70	0.00	0.78
δ-cadinene	0.15	0.16		0.14	0.12	0.17	0.33	0.41	0.37	0.39	0.44	0.00	0.16

Frac.: fraction

*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

CO_2 conditions: 9.66 MPa x 35 °Cx 2 kg/h

	Feed oil	terpene frac.	dep.aft.ter.	CO2 1	CO2 2	CO2 3	CO2 4	CO2 5	CO2 6	CO2 7	CO2 8	Sep. res.	Res.by col
weight of frac.(g)	498.40	385.73		10.97	15.42	24.38	13.29	10.58	3.49	2.44	2.18	3.72	10.21
used CO_2 (kg)				0.196	0.196	0.342	0.392	0.733	0.733	1.075	1.808		
concentration(mg/g fraction)													
α-pinene	3.80	3.95		3.82	3.61	3.48	3.32	3.03	2.48	1.86	1.98	2.36	3.45
sabinene	1.92	2.01		1.78	1.73	1.69	1.61	1.46	1.23	0.99	1.00	1.13	1.91
β-pinene	0.14	0.15		0.14	0.14	0.13	0.13	0.12	0.10	0.09	0.10	0.11	0.13
β-myrcene	15.70	16.44		15.49	15.11	14.73	14.12	12.97	11.01	9.34	9.74	10.41	16.09
n-octanal	0.99	0.00		1.98	3.27	4.50	6.23	9.66	12.01	8.66	5.24	4.89	1.49
α-phellandrene	0.20	0.20		0.19	0.19	0.18	0.18	0.18	0.17	0.14	0.15	0.17	0.19
carene	0.82	0.85		0.81	0.80	0.79	0.78	0.73	0.62	0.49	0.50	0.53	0.80
limonene	911.32	961.68		889.64	881.66	871.10	849.88	781.47	662.22	568.86	197.52		
n-octanol	1.39	0.00		3.44	4.66	5.28	6.08	8.62	14.31	19.96	21.83	20.12	0.03
linalool	3.71	0.00		8.39	12.35	13.93	16.92	25.90	51.14	76.23	69.41	63.36	0.05
n-nonanal	0.29	0.00		0.54	0.82	1.11	1.50	2.29	0.00	0.00	0.00		0.57
limonene oxide (cis)	0.71	0.00		1.31	2.13	2.85	3.89	5.95	8.82	7.22	4.98	4.38	0.09
limonene oxide(trans)	0.38	0.00		0.77	1.41	2.00	2.85	4.60	6.92	5.04	2.39	2.08	0.06
citronellal	0.35	0.00		0.66	1.12	1.57	2.14	3.19	3.86	2.88	1.80	1.64	0.79
α-terpineol	0.85	0.00		1.93	2.49	2.81	3.25	4.55	7.48	11.10	12.65	11.29	0.00
decanal	2.67	0.15		4.91	7.48	10.26	14.42	23.48	36.19	35.41	23.43	20.93	7.33
carveol(trans)	0.35	0.00		0.69	0.93	1.10	1.38	2.17	3.71	5.02	5.64	5.21	0.12
carveol(cis)	0.28	0.00		0.64	0.85	0.94	1.10	1.59	2.73	4.03	4.62	4.35	0.00
neral	0.29	0.00		0.59	0.92	1.04	1.29	2.07	4.11	6.50	5.64	5.10	0.00
carvone	0.49	0.00		0.80	1.03	1.22	1.58	2.64	5.19	8.14	8.12	7.34	0.03
geranial	0.57	0.00		1.15	1.77	1.99	2.47	3.92	7.71	12.80	13.34	11.85	0.00
perrillaldehyde	0.64	0.00		2.00	2.49	3.05	2.39	2.04	5.73	8.87	14.31	13.66	0.00
valencene	0.70	0.75		0.64	0.63	0.66	0.70	0.69	0.63	0.59	0.61	0.59	0.71
δ-cadinene	0.15	0.16		0.14	0.14	0.14	0.16	0.16	0.16	0.16	0.18	0.17	0.15

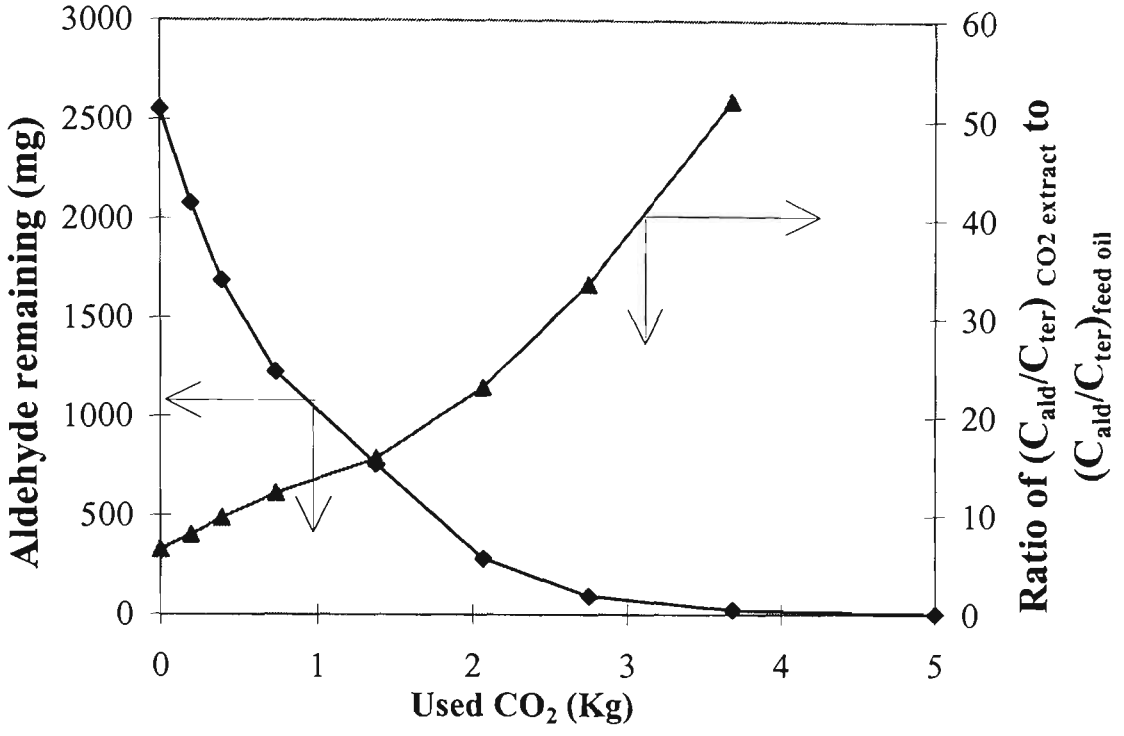
Frac.: fraction

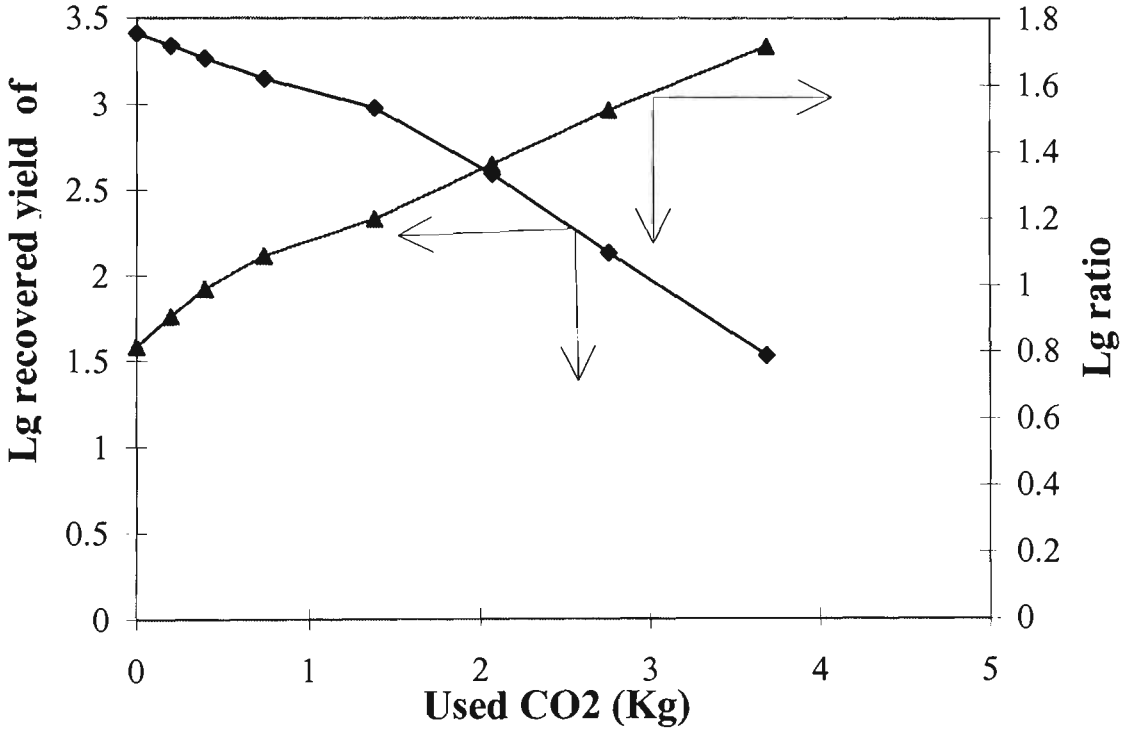
*: spilt some

Sep. res.: separator residue

Res.by col.: residue released by column depressurisation

Appendix 3

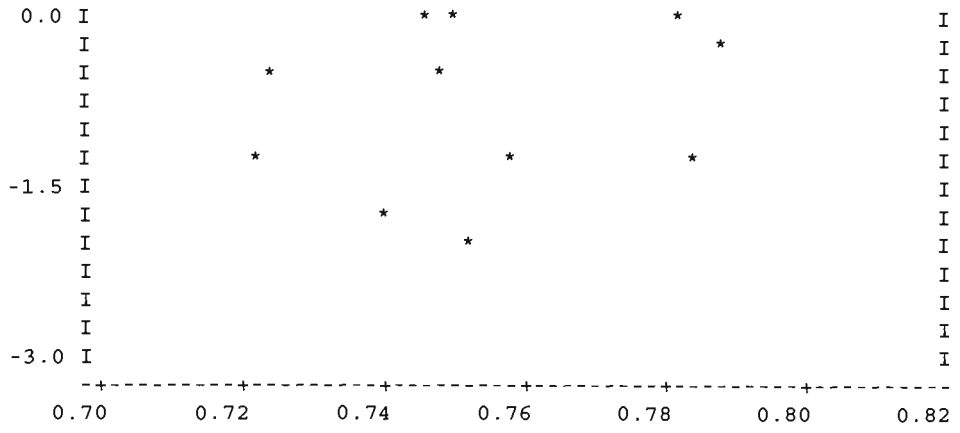

Aldehyde remaining (mg)
Ratio of (C_{ald}/C_{ter}) CO2 extract to $(C_{ald}/C_{ter})_{feed\ oil}$
Used CO_2 (Kg)
3000
2500
2000
1500
1000
500
0
60
50
40
30
20
10
0
0
1
2
3
4
5


Lg recovered yield of
Lg ratio
Used CO2 (Kg)
3.5
3
2.5
2
1.5
1
0.5
0
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5

Appendix 4a (Oil - a3.out)

```
Genstat 5  Release 3.2 (PC/Windows 95)              19 October 1997 12:40:46
Copyright 1995, Lawes Agricultural Trust (Rothamsted Experimental Station)

  1  "
 -2  .....  Aldehyde Recovery by CO2 desorption (Analysis A)
 -3  "
  4  output [pr=dots] 1
  5  units [22]
  6  open 'oil_a1.txt'; ch=2; file=in; width=90
  7  skip [ch=2] 3
  8  read [ch=2; format=!((0.1,1,0.0,-10,0.1,8,*)22)] run,p,t,d,f,\
  9   feeda,reca,a10,a15

  Identifier   Minimum      Mean   Maximum    Values   Missing
         run      1.00     11.50     22.00        22         0
           p      1400      2300      3500        22         0
           t     35.00     45.00     55.00        22         0
           d    0.2990    0.6684    0.8962        22         0
           f     2.000     3.000     4.000        22         0
       feeda      2772      2853      2988        22         0
        reca    0.6991    0.7539    0.8195        22         0
         a10         0      1117      1956        22         0
         a15       0.0     654.8    1233.0        22         0

 10  close ch=2; file=in
 11
 12  factor [lev=!(1400,1900,3500)] Pres
 13  factor [lev=!(35,45,55)] Temp
 14  factor [lev=!(2,4)] Flow
 15
 16  calc Pres = p
 17  calc Temp = t
 18  calc Flow = f
 19
 20  "
-21  .....  Proportion of Aldehyde Recovered by 5kg CO2
-22  "
 23  calc recp = reca
 24
 25  tabulate [pr=means; class=Pres,Temp,Flow] recp

                            Mean
                  Flow      2.00       4.00
     Pres         Temp
  1400.00        35.00    0.7293     0.7218
                 45.00    0.7239     0.7228
                 55.00    0.7215     0.7609
  1900.00        35.00    0.7440     0.7144
                 45.00    0.7656     0.7395
                 55.00    0.7841     0.7981
  3500.00        35.00    0.7835     0.7504
                 45.00    0.7775     0.7599
                 55.00    0.8195     0.7851

 26
 27  calc t = (t-45)/10
 28  calc p = (p-2450)/1050
 29  calc f = f-3
 30
 31  calc t2 = t**2
 32  calc p2 = p**2
 33
 34  calc tp = t*p
 35  calc tf = t*f
 36  calc pf = p*f
```

```
 37
 38  "
-39  .....  Full Model
-40  "
 41  model recp
 42  terms t + p + f + t2 + p2 + tp + tf + pf
 43  fit [pr=mod,summ,est,acc; tprob=y; fprob=y] t + p + f + t2 + p2 + pf

43.....................................................................................

***** Regression Analysis *****

 Response variate: recp
     Fitted terms: Constant + t + p + f + t2 + p2 + pf


*** Summary of analysis ***

             d.f.          s.s.          m.s.       v.r.  F pr.
Regression      6      0.015951     0.0026584       7.28  <.001
Residual       15      0.005476     0.0003651
Total          21      0.021427     0.0010203

Change         -6     -0.015951     0.0026584       7.28  <.001

Percentage variance accounted for 64.2
Standard error of observations is estimated to be 0.0191


*** Estimates of regression coefficients ***

                            estimate         s.e.      t(15)  t pr.
Constant                      0.7685       0.0129      59.44  <.001
t                             0.01882      0.00552      3.41  0.004
p                             0.02415      0.00478      5.06  <.001
f                            -0.00627      0.00413     -1.52  0.150
t2                            0.01165      0.00827      1.41  0.179
p2                           -0.0218       0.0132      -1.65  0.119
pf                           -0.00737      0.00461     -1.60  0.131


*** Accumulated analysis of variance ***

Change                          d.f.          s.s.          m.s.       v.r.  F pr.
+ t                                1     0.0042488     0.0042488      11.64  0.004
+ p                                1     0.0081643     0.0081643      22.36  <.001
+ f                                1     0.0005980     0.0005980       1.64  0.220
+ t2                               1     0.0010097     0.0010097       2.77  0.117
+ p2                               1     0.0009957     0.0009957       2.73  0.119
+ pf                               1     0.0009341     0.0009341       2.56  0.131
Residual                          15     0.0054764     0.0003651

Total                             21     0.0214269     0.0010203

 44  rkeep res=res; fitted=yhat; estim=bhat; dev=RSS; df=rdf
 45  graph [nrow=20; ncol=60] res;yhat

            -+---------+---------+---------+---------+---------+---------+
            I           *                 *                               I
        1.5 I                                                             I
            I                                         *                   I
            I       *                                                     I
            I                           *                                 I
            I             *              *           *                    I
            I        *                                  *            *    I
```



res v. yhat using symbol *

```
 46
 47  variate [22] ybar
 48  scalar [0] pedf
 49  factor [lev=18] trt; values=!(1,2,3,4, 1, 5,6,7, 6, \
 50                                8,9,10, 5, 11,12,13,14,15,16,17,\
 51                                7, 18)
 52  for i=1...18
 53   restrict recp,ybar;  trt.eq.i;  saveset=iw
 54   calc ym = mean(recp)
 55   restrict recp,ybar
 56   calc ybar$[iw] = ym
 57   calc df = nvalues(iw) - 1
 58   calc pedf = pedf + df
 59   delete [redef=y] iw,ym,df
 60  endfor
 61
 62  print run,Pres,Temp,Flow,trt,recp,yhat,ybar;\
 63    fieldw=4,7(10); deci=5(0),3(4)
```

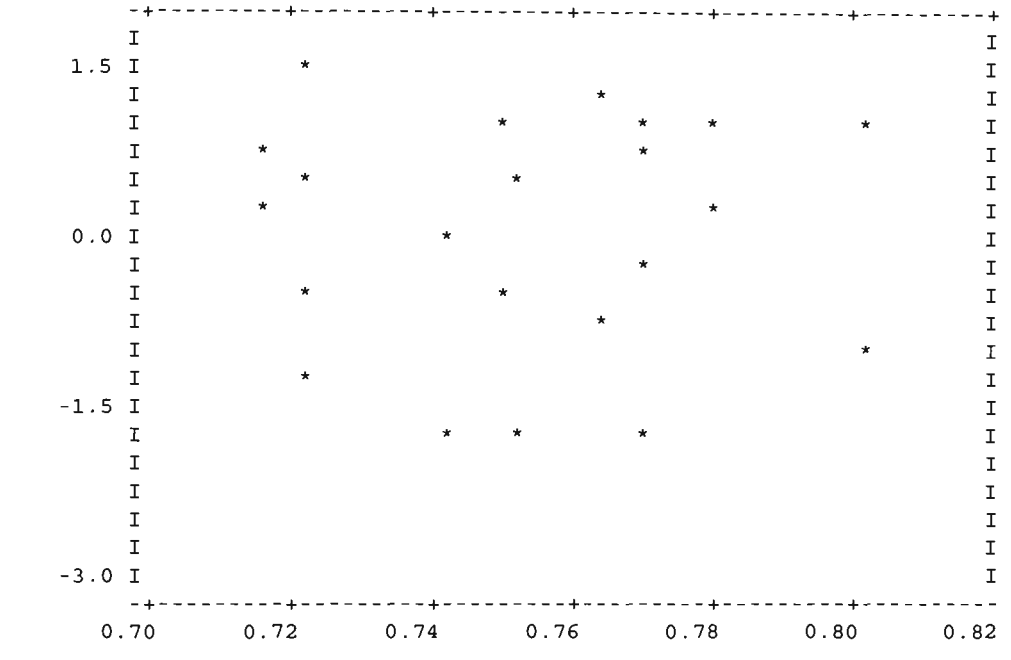
run	Pres	Temp	Flow	trt	recp	yhat	ybar
1	3500	45	2	1	0.7887	0.7845	0.7775
2	1900	45	4	2	0.7395	0.7474	0.7395
3	3500	35	4	3	0.7504	0.7500	0.7504
4	1900	35	2	4	0.7440	0.7451	0.7440
5	3500	45	2	1	0.7662	0.7845	0.7775
6	1400	45	2	5	0.6991	0.7214	0.7239
7	3500	45	4	6	0.7364	0.7572	0.7599
8	1400	45	4	7	0.7314	0.7236	0.7228
9	3500	45	4	6	0.7834	0.7572	0.7599
10	1900	45	2	8	0.7656	0.7523	0.7656
11	1900	55	4	9	0.7981	0.7779	0.7981
12	3500	55	2	10	0.8195	0.8149	0.8195
13	1400	45	2	5	0.7487	0.7214	0.7239
14	1400	55	4	11	0.7609	0.7541	0.7609
15	1900	55	2	12	0.7841	0.7827	0.7841
16	1400	55	2	13	0.7215	0.7519	0.7215
17	3500	55	4	14	0.7851	0.7877	0.7851
18	3500	35	2	15	0.7835	0.7773	0.7835
19	1400	35	4	16	0.7218	0.7165	0.7218
20	1900	35	4	17	0.7144	0.7403	0.7144
21	1400	45	4	7	0.7141	0.7236	0.7228
22	1400	35	2	18	0.7293	0.7143	0.7293

```
 64
 65  "..... Pure Error Sum of Squares
-66  "
 67  calc PESS = sum((recp - ybar)**2)
 68
 69  print rdf,   RSS
```

```
        rdf         RSS
      15.00    0.005476

 70  print pedf, PESS

       pedf        PESS
      4.000    0.002737

 71
 72  "..... Lack of Fit Sum of Squares
-73  "
 74  calc LFSS = RSS - PESS
 75  calc lfdf = rdf - pedf
 76  calc F = (LFSS/lfdf)/(PESS/pedf)
 77  calc fprob = 1-fprobability(F;lfdf;pedf)
 78
 79  "..... Lack of Fit Test
-80  "
 81  print lfdf,pedf,F,fprob;  fieldw=4(10); deci=1,1,3,4

     lfdf      pedf         F     fprob
     11.0       4.0     0.364    0.9170

 82
 83  "
-84  ..... Reduced Model
-85  "
 86  model recp
 87  terms t + p + f + t2 + p2 + tp + tf + pf
 88  fit [pr=mod,summ,est,acc; tprob=y; fprob=y] t + p + t2 + p2

88.....................................................................


***** Regression Analysis *****

 Response variate: recp
    Fitted terms: Constant + t + p + t2 + p2


*** Summary of analysis ***

             d.f.         s.s.         m.s.      v.r.  F pr.
Regression      4     0.014418    0.0036046      8.74  <.001
Residual       17     0.007008    0.0004123
Total          21     0.021427    0.0010203

Change         -4    -0.014418    0.0036046      8.74  <.001

Percentage variance accounted for 59.6
Standard error of observations is estimated to be 0.0203


*** Estimates of regression coefficients ***

                           estimate         s.e.     t(17)  t pr.
Constant                     0.7685       0.0137     55.93  <.001
t                           0.01882      0.00586      3.21  0.005
p                           0.02415      0.00508      4.76  <.001
t2                          0.01165      0.00879      1.33  0.203
p2                           -0.0218       0.0140     -1.55  0.139


*** Accumulated analysis of variance ***

Change                       d.f.         s.s.         m.s.      v.r.  F pr.
```

```
+ t                              1    0.0042488    0.0042488     10.31  0.005
+ p                              1    0.0081643    0.0081643     19.80  <.001
+ t2                             1    0.0010097    0.0010097      2.45  0.136
+ p2                             1    0.0009957    0.0009957      2.42  0.139
Residual                        17    0.0070085    0.0004123

Total                           21    0.0214269    0.0010203
```

```
  89  rkeep res=res; fitted=yhat; estim=bhat; dev=RSS; df=rdf
  90  graph [nrow=20; ncol=60] res;yhat
```



res v. yhat using symbol *

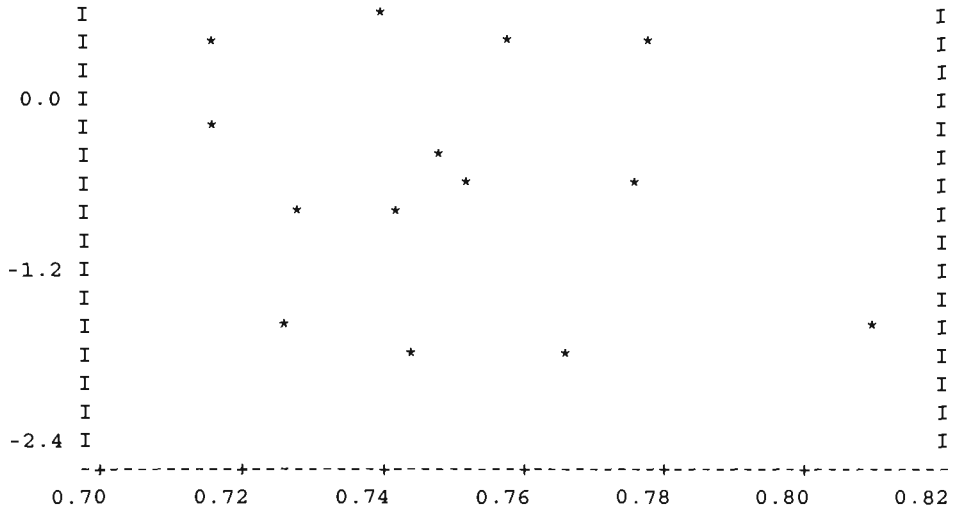
```
  91
  92  ".....  Pure Error Sum of Squares
 -93  "
  94  calc PESS = sum((recp - ybar)**2)
  95
  96  print rdf,   RSS

        rdf         RSS
      17.00    0.007008

  97  print pedf, PESS

       pedf        PESS
      4.000    0.002737

  98
  99  ".....  Lack of Fit Sum of Squares
-100  "
 101  calc LFSS = RSS - PESS
 102  calc lfdf = rdf - pedf
 103  calc F = (LFSS/lfdf)/(PESS/pedf)
 104  calc fprob = 1-fprobability(F;lfdf;pedf)
 105
 106  ".....  Lack of Fit Test
-107  "
 108  print lfdf,pedf,F,fprob;  fieldw=4(10); deci=1,1,3,4

      lfdf       pedf          F      fprob
      13.0        4.0      0.480     0.8585
```

```
109
110  tabulate [pr=means; class=Pres,Temp,Flow] yhat

                                Mean
                     Flow       2.00        4.00
        Pres         Temp
     1400.00         35.00     0.7154      0.7154
                     45.00     0.7225      0.7225
                     55.00     0.7530      0.7530
     1900.00         35.00     0.7427      0.7427
                     45.00     0.7499      0.7499
                     55.00     0.7803      0.7803
     3500.00         35.00     0.7637      0.7637
                     45.00     0.7708      0.7708
                     55.00     0.8013      0.8013

111
112  delete [redef=y] ybar,pedf
113
114  stop

******** End of job.  Maximum of 4863 data units used at line 52 (84381 left)
```

Appendix 4b (Oil-ad2.out)

```
Genstat 5  Release 3.2 (PC/Windows 95)              19 October 1997 13:01:47
Copyright 1995, Lawes Agricultural Trust (Rothamsted Experimental Station)

  1  "
 -2  .....  Aldehyde Recovery by CO2 desorption (Analysis A)
 -3  "
  4  output [pr=dots] 1
  5  units [22]
  6  open 'oil_a1.txt'; ch=2; file=in; width=90
  7  skip [ch=2] 3
  8  read [ch=2; format=!((0.1,1,0.0,-10,0.1,8,*)22)] run,p,t,d,f,\
  9   feeda,reca,a10,a15

   Identifier   Minimum      Mean   Maximum    Values   Missing
          run      1.00     11.50     22.00        22         0
            p      1400      2300      3500        22         0
            t     35.00     45.00     55.00        22         0
            d    0.2990    0.6684    0.8962        22         0
            f     2.000     3.000     4.000        22         0
        feeda      2772      2853      2988        22         0
         reca    0.6991    0.7539    0.8195        22         0
          a10         0      1117      1956        22         0
          a15       0.0     654.8    1233.0        22         0

 10  close ch=2; file=in
 11
 12  factor [lev=!(1400,1900,3500)] Pres
 13  factor [lev=!(35,45,55)] Temp
 14  factor [lev=!(2,4)] Flow
 15
 16  calc Pres = p
 17  calc Temp = t
 18  calc Flow = f
 19
 20  "
-21  .....  Proportion of Aldehyde Recovered by 5kg CO2
-22  "
 23  calc recp = reca
 24
 25  tabulate [pr=means; class=Pres,Temp,Flow] recp

                              Mean
                      Flow    2.00        4.00
         Pres         Temp
      1400.00        35.00  0.7293      0.7218
                     45.00  0.7239      0.7228
                     55.00  0.7215      0.7609
      1900.00        35.00  0.7440      0.7144
                     45.00  0.7656      0.7395
                     55.00  0.7841      0.7981
      3500.00        35.00  0.7835      0.7504
                     45.00  0.7775      0.7599
                     55.00  0.8195      0.7851

 26
 27  calc t = (t-45)/10
 28  calc p = (p-2450)/1050
 29  calc f = f-3
 30
 31  calc t2 = t**2
 32  calc p2 = p**2
 33  calc d2 =d**2
 34
 35  calc tp = t*p
 36  calc tf = t*f
```

```
 37  calc pf = p*f
 38
 39  calc dof =d/f
 40
 41  "
-42  .....  Model with density
-43  "
 44  model recp
 45  terms t + f + d + t2 + d2 + tf
 46  fit [pr=mod,summ,est,acc; tprob=y; fprob=y] t + d+ f + t2 +d2 +tf

46.....................................................................................


***** Regression Analysis *****

 Response variate: recp
     Fitted terms: Constant + t + d + f + t2 + d2 + tf


*** Summary of analysis ***

             d.f.          s.s.          m.s.       v.r.  F pr.
Regression      6      0.015804     0.0026340       7.03  0.001
Residual       15      0.005623     0.0003749
Total          21      0.021427     0.0010203

Change         -6     -0.015804     0.0026340       7.03  0.001

Percentage variance accounted for 63.3
Standard error of observations is estimated to be 0.0194


*** Estimates of regression coefficients ***

                           estimate          s.e.      t(15)  t pr.
Constant                     0.6646        0.0511      13.02  <.001
t                           0.03325       0.00629       5.28  <.001
d                             0.130         0.175       0.74  0.469
f                          -0.00521       0.00413      -1.26  0.226
t2                          0.00967       0.00836       1.16  0.266
d2                           -0.006         0.141      -0.04  0.969
tf                          0.00743       0.00559       1.33  0.203


*** Accumulated analysis of variance ***

Change                       d.f.          s.s.          m.s.       v.r.  F pr.
+ t                             1     0.0042488     0.0042488      11.33  0.004
+ d                             1     0.0097898     0.0097898      26.12  <.001
+ f                             1     0.0005980     0.0005980       1.60  0.226
+ t2                            1     0.0005039     0.0005039       1.34  0.264
+ d2                            1     0.0000006     0.0000006       0.00  0.969
+ tf                            1     0.0006631     0.0006631       1.77  0.203
Residual                       15     0.0056228     0.0003749

Total                          21     0.0214269     0.0010203

 47  rkeep res=res; fitted=yhat; estim=bhat; dev=RSS; df=rdf
 48  graph [nrow=20; ncol=60] res;yhat


           -+---------+---------+---------+---------+---------+---------+
            I             *                                             I
        1.2 I                                   *                       I
            I        *               *        *       *           *      I
            I                                        *                  I
```



res v. yhat using symbol *

```
49
50  variate [22] ybar
51  scalar [0] pedf
52  factor [lev=18] trt; values=!(1,2,3,4, 1, 5,6,7, 6, \
53                                8,9,10, 5, 11,12,13,14,15,16,17,\
54                                7, 18)
55  for i=1...18
56   restrict recp,ybar;  trt.eq.i;  saveset=iw
57   calc ym = mean(recp)
58   restrict recp,ybar
59   calc ybar$[iw] = ym
60   calc df = nvalues(iw) - 1
61   calc pedf = pedf + df
62   delete [redef=y] iw,ym,df
63  endfor
64
65  print run,Pres,Temp,Flow,trt,recp,yhat,ybar;\
66    fieldw=4,7(10); deci=5(0),3(4)
```

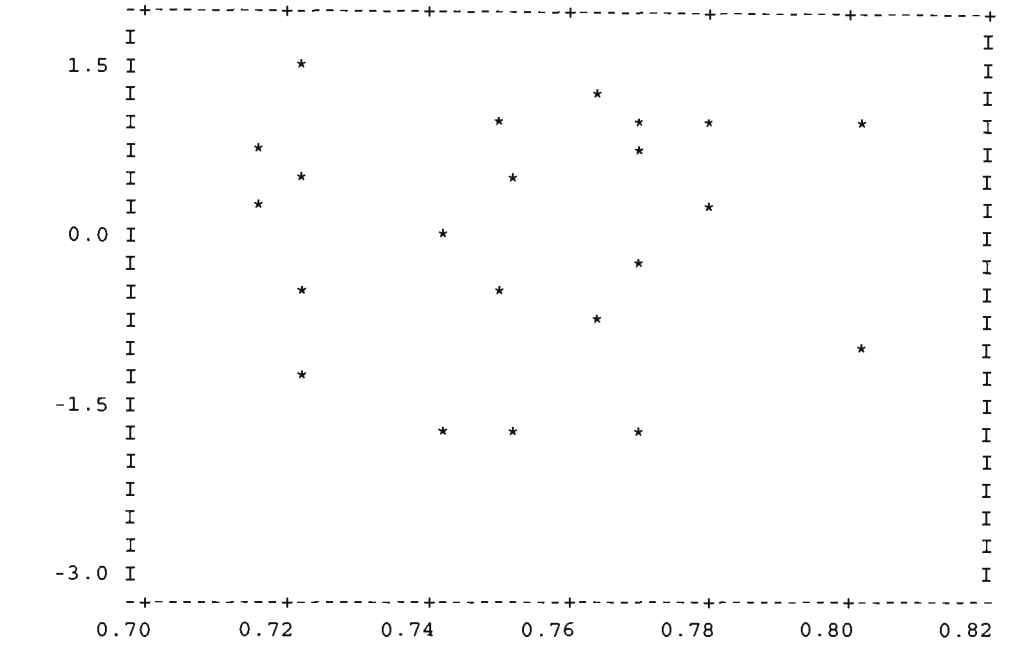
run	Pres	Temp	Flow	trt	recp	yhat	ybar
1	3500	45	2	1	0.7887	0.7762	0.7775
2	1900	45	4	2	0.7395	0.7472	0.7395
3	3500	35	4	3	0.7504	0.7402	0.7504
4	1900	35	2	4	0.7440	0.7525	0.7440
5	3500	45	2	1	0.7662	0.7762	0.7775
6	1400	45	2	5	0.6991	0.7263	0.7239
7	3500	45	4	6	0.7364	0.7657	0.7599
8	1400	45	4	7	0.7314	0.7159	0.7228
9	3500	45	4	6	0.7834	0.7657	0.7599
10	1900	45	2	8	0.7656	0.7576	0.7656
11	1900	55	4	9	0.7981	0.7829	0.7981
12	3500	55	2	10	0.8195	0.8059	0.8195
13	1400	45	2	5	0.7487	0.7263	0.7239
14	1400	55	4	11	0.7609	0.7480	0.7609
15	1900	55	2	12	0.7841	0.7785	0.7841
16	1400	55	2	13	0.7215	0.7436	0.7215
17	3500	55	4	14	0.7851	0.8103	0.7851
18	3500	35	2	15	0.7835	0.7655	0.7835
19	1400	35	4	16	0.7218	0.7164	0.7218
20	1900	35	4	17	0.7144	0.7272	0.7144
21	1400	45	4	7	0.7141	0.7159	0.7228
22	1400	35	2	18	0.7293	0.7417	0.7293

```
67
68  "....  Pure Error Sum of Squares
-69 "
```

```
 70  calc PESS = sum((recp - ybar)**2)
 71
 72  print rdf,   RSS

        rdf         RSS
      15.00    0.005623

 73  print pedf, PESS

       pedf        PESS
      4.000    0.002737

 74
 75  "..... Lack of Fit Sum of Squares
-76  "
 77  calc LFSS = RSS - PESS
 78  calc lfdf = rdf - pedf
 79  calc F = (LFSS/lfdf)/(PESS/pedf)
 80  calc fprob = 1-fprobability(F;lfdf;pedf)
 81
 82  "..... Lack of Fit Test
-83  "
 84  print lfdf,pedf,F,fprob;  fieldw=4(10); deci=1,1,3,4

     lfdf      pedf         F     fprob
     11.0       4.0     0.383    0.9062

 85
 86  "
-87  ..... Reduced Model
-88  "
 89  model recp
 90  terms t + p + f + t2 + p2 + tp + tf + pf
 91  fit [pr=mod,summ,est,acc; tprob=y; fprob=y] t + p + t2 + p2

91.....................................................................

***** Regression Analysis *****

 Response variate: recp
     Fitted terms: Constant + t + p + t2 + p2


*** Summary of analysis ***

             d.f.         s.s.         m.s.      v.r.  F pr.
Regression      4     0.014418    0.0036046      8.74  <.001
Residual       17     0.007008    0.0004123
Total          21     0.021427    0.0010203

Change         -4    -0.014418    0.0036046      8.74  <.001

Percentage variance accounted for 59.6
Standard error of observations is estimated to be 0.0203


*** Estimates of regression coefficients ***

                           estimate         s.e.     t(17)  t pr.
Constant                     0.7685       0.0137     55.93  <.001
t                           0.01882      0.00586      3.21  0.005
p                           0.02415      0.00508      4.76  <.001
t2                          0.01165      0.00879      1.33  0.203
p2                           -0.0218       0.0140     -1.55  0.139
```

```
*** Accumulated analysis of variance ***

Change                          d.f.         s.s.         m.s.      v.r.  F pr.
+ t                                1    0.0042488    0.0042488     10.31  0.005
+ p                                1    0.0081643    0.0081643     19.80  <.001
+ t2                               1    0.0010097    0.0010097      2.45  0.136
+ p2                               1    0.0009957    0.0009957      2.42  0.139
Residual                          17    0.0070085    0.0004123

Total                             21    0.0214269    0.0010203
```

```
  92  rkeep res=res; fitted=yhat; estim=bhat; dev=RSS; df=rdf
  93  graph [nrow=20; ncol=60] res;yhat
```



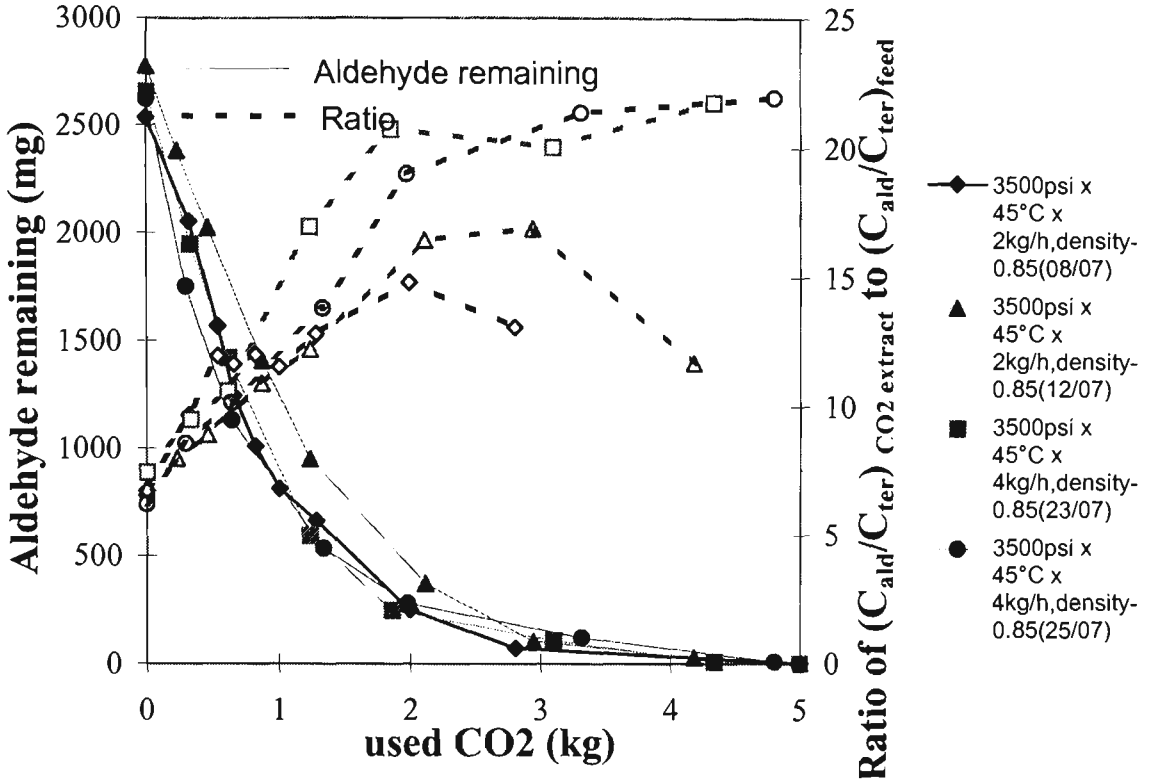
res v. yhat using symbol *

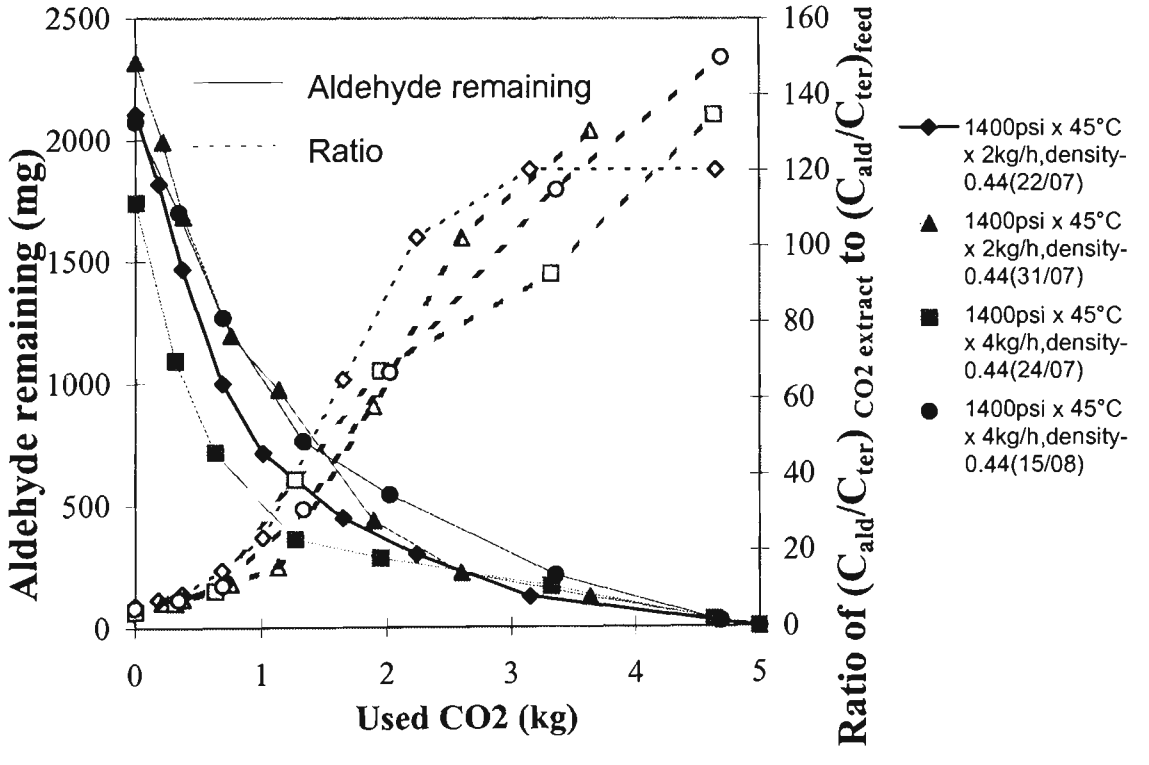
```
  94
  95  "..... Pure Error Sum of Squares
 -96  "
  97  calc PESS = sum((recp - ybar)**2)
  98
  99  print rdf,   RSS

        rdf         RSS
      17.00    0.007008

 100  print pedf, PESS

       pedf        PESS
      4.000    0.002737

 101
 102  "..... Lack of Fit Sum of Squares
-103  "
 104  calc LFSS = RSS - PESS
 105  calc lfdf = rdf - pedf
 106  calc F = (LFSS/lfdf)/(PESS/pedf)
 107  calc fprob = 1-fprobability(F;lfdf;pedf)
 108
 109  "..... Lack of Fit Test
-110  "
 111  print lfdf,pedf,F,fprob;  fieldw=4(10); deci=1,1,3,4
```

```
     lfdf       pedf          F      fprob
     13.0        4.0      0.480     0.8585

112
113  tabulate [pr=means; class=Pres,Temp,Flow] yhat

                                  Mean
                       Flow       2.00        4.00
        Pres           Temp
     1400.00          35.00      0.7154      0.7154
                      45.00      0.7225      0.7225
                      55.00      0.7530      0.7530
     1900.00          35.00      0.7427      0.7427
                      45.00      0.7499      0.7499
                      55.00      0.7803      0.7803
     3500.00          35.00      0.7637      0.7637
                      45.00      0.7708      0.7708
                      55.00      0.8013      0.8013

114
115  delete [redef=y] ybar,pedf
116
117  stop

******** End of job.  Maximum of 4884 data units used at line 55 (84360 left)
```

Appendix 5

Replications





Appendix 6: Samples to send to Keith Harris for sensory evaluation : 24 samples flushed with N_2 freshly

Concentrations of some major components of valencia orange oil (mg/g oil)

	Feed oil Sample No.	Terpene fraction Sample No.	24.14 MPa x 45°C Sample No.				9.66 MPa x55°C Sample No.	13.10 MPa x 55°C Sample No.			24.14 MPa x55°C Sample No.
comp. name	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
a-pinene	3.80	3.97	2.03	1.75	1.48	2.47	0.48	3.59	3.11	1.93	3.14
sabinene	1.92	2.01	1.06	0.93	0.82	1.10	0.26	1.66	1.50	0.99	1.25
b-myrcene	15.70	16.47	10.33	9.06	8.19	9.93	3.16	15.12	13.68	9.42	13.69
n-octanal	0.99	0.00	7.02	7.68	7.20	5.22	30.44	4.04	6.86	7.25	5.32
carene	0.82	0.86	0.58	0.52	0.46	0.55	0.40	0.80	0.76	0.53	0.74
limonene	**911.32**	**965.63**	**775.56**	**668.07**	**540.95**	**622.47**	**268.87**	**864.35**	**813.43**	**587.30**	**827.93**
n-octanol	1.39	0.00	6.40	8.52	14.17	13.42	27.94	5.61	9.79	17.27	7.84
linalool	**3.71**	**0.00**	**24.10**	**40.17**	**54.04**	**39.98**	**120.82**	**14.12**	**26.35**	**35.39**	**20.66**
limonene oxide (cis)	0.71	0.00	5.54	7.62	8.93	7.17	14.04	2.06	4.29	9.74	3.36
limonene oxide(trans)	0.38	0.00	4.49	6.13	5.87	3.98	38.56	1.82	3.96	8.59	2.79
citronellal	0.35	0.00	2.93	3.27	2.89	1.77	11.49	1.08	2.16	3.63	1.65
a-terpineol	0.85	0.00	4.37	5.53	8.66	8.66	24.09	3.07	5.34	15.88	4.72
decanal	**2.67**	**0.00**	**24.13**	**32.38**	**30.16**	**16.80**	**129.19**	**8.65**	**18.40**	**45.95**	**13.51**
carveol(trans)	0.35	0.00	2.67	4.45	7.42	6.78	12.29	1.05	2.18	7.41	1.77
carveol(cis)	0.28	0.00	1.52	2.19	3.86	3.62	6.78	0.98	1.73	5.90	1.58
neral	0.29	0.00	2.37	3.78	5.29	3.62	9.74	0.94	2.09	4.33	1.65
carvone	0.49	0.00	3.09	5.39	7.65	5.97	10.34	1.03	2.17	7.79	1.78
geranial	0.57	0.00	4.56	7.90	11.58	8.49	19.30	1.73	4.03	10.56	3.35
perrillaldehyde	0.64	0.00	3.48	4.58	12.12	10.57	14.50	2.95	5.15	20.24	4.97
valencene	0.70	0.76	0.76	0.65	0.62	0.44	10.15	0.58	0.75	0.79	0.70
Fraction weight % of total CO_2 extract		ca. 75 % of total feed oil	13.24 CO_2 5	4.77 CO_2 6	1.16 CO_2 7	1.63 CO_2 8	1.43 CO_2 8	20.86 CO_2 1	14.2 CO_2 4	2.67 CO_2 6	25.56 CO_2 3

Concentrations of some major components of valencia orange oil (mg/g oil)

	9.66 MPa x35°C Sample No.		13.10 MPa x35°C Sample No.	24.14 MPa x35°C Sample No.		9.66 MPax45°C Sample No.			13.10 MPax45°C Sample No.				
comp. name	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
a-pinene	3.03	1.86	1.79	2.08	1.51	2.38	1.60	1.01	3.14	2.83	2.34	1.83	1.24
sabinene	1.46	0.99	0.95	0.81	0.65	1.19	0.79	0.54	1.37	1.23	0.99	0.89	0.65
b-myrcene	12.97	9.34	9.04	9.33	7.70	11.46	7.94	4.90	13.60	12.32	10.35	8.23	6.45
n-octanal	9.66	8.66	10.44	10.07	7.69	10.24	17.00	15.67	6.59	7.93	7.94	6.94	5.70
carene	0.73	0.49	0.51	0.53	0.44	0.66	0.48	0.30	0.75	0.69	0.58	0.47	0.36
limonene	**781.47**	**568.86**	**634.65**	**597.04**	**506.77**	**806.36**	**574.60**	**329.51**	**815.64**	**745.48**	**633.40**	**500.24**	**413.39**
n-octanol	8.62	19.96	12.07	12.11	16.43	7.09	18.19	36.36	6.18	9.30	16.62	28.24	34.25
linalool	**25.90**	**76.23**	**38.55**	**47.10**	**48.97**	**31.88**	**91.29**	**140.92**	**21.30**	**33.03**	**46.47**	**51.66**	**47.35**
limonene oxide (cis)	5.95	7.22	7.89	9.93	10.70	5.81	10.15	9.71	4.14	5.22	7.29	9.82	6.72
limonene oxide(trans)	4.60	5.04	5.63	6.95	6.64	5.75	10.71	13.04	3.31	4.08	5.39	6.44	6.15
citronellal	3.19	2.88	4.25	3.53	2.72	3.25	6.68	8.18	2.20	2.90	3.22	3.01	2.51
a-terpineol	4.55	11.10	9.16	7.65	10.73	3.76	9.62	18.21	3.44	4.98	8.75	16.25	22.66
decanal	**23.48**	**35.41**	**43.37**	**36.35**	**30.73**	**22.25**	**53.91**	**99.20**	**16.75**	**26.46**	**37.48**	**41.25**	**35.90**
carveol(trans)	2.17	5.02	4.16	5.04	7.76	2.17	5.43	10.34	1.71	2.77	5.02	8.59	12.47
carveol(cis)	1.59	4.03	3.18	3.11	4.58	1.15	2.86	5.67	1.17	1.81	3.31	6.08	8.68
neral	2.07	6.50	3.07	3.72	3.74	2.24	6.33	10.70	1.76	2.75	3.60	4.15	4.16
carvone	2.64	8.14	4.67	5.98	8.16	2.93	7.73	11.46	2.15	3.55	5.07	6.58	8.34
geranial	3.92	12.80	7.03	8.62	9.16	3.93	15.28	29.78	3.28	5.65	8.44	11.10	12.20
perrillaldehyde	2.04	8.87	11.49	6.56	14.53	2.13	5.31	7.23	2.66	3.55	10.12	16.42	22.16
valencene	0.69	0.59	0.62	0.63	0.56	1.28	1.69	1.20	0.70	0.69	0.66	0.62	0.68
Fraction weight % of total CO_2 extract	12.78 CO_2 5	2.95 CO_2 7	4.16 CO_2 6	2.78 CO_2 7	1.56 CO_2 8	6.09 CO_2 5	1.51 CO_2 6	1.62 CO_2 7+8	12.57 CO_2 4	9.92 CO_2 5	2.67 CO_2 6	1.03 CO_2 7	0.97 CO_2 8

appendix 6 includes the two pages of sensory evaluation from Keith Harris

KEITH HARRIS & CO. LTD.
A.C.N. 000 026 282
7 SEFTON ROAD, THORNLEIGH, N.S.W. 2120
AUSTRALIA

CABLES KEHAR SYDNEY
TELEPHONE (02) 9484 1341
FAX: (02) 9481 8145

POSTAL ADDRESS
P.O. BOX 147, PENNANT HILLS
N.S.W. 2120
AUSTRALIA

14th October, 1996

Dr. Brian Innison
A.F.I.S.C.
WERRIBEE VIC

Fax No.: -03 9742 0201

Dear Brian,

Herewith our comments on samples of orange oil you submitted for evaluation..

From the analysis table we selected all fractions with a decanal content above 30% as under:

Fraction	% of total	% decanal
4	4.77	32.38
5	1.16	30.16
7	1.43	129.19
10	2.67	45.95
13	2.95	35.41
14	4.16	43.37
15	2.78	36.35
16	1.56	30.73
18	1.51	53.91
19	1.62	99.20
22	2.67	37.48
23	1.03	41.25
24	0.97	35.90
	29.28	46.13

The fractions were blended in proportion and the flavour compared with 10:1 orange oil producted by vacuum distillation.

The evaluation panel (3) all selected the fractions as being superior in quality.

Yours faithfully,

Victor Fuchs

vf1410.wri

KEITH HARRIS & CO. LTD.
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POSTAL ADDRESS
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AUSTRALIA

EVALUATION REPORT

DATE: October 15, 1996

Orange oil fractions prepared by CO_2 separation

All samples were evaluated by aroma for characteristics which are present in terpeneless orange oils. These characteristics being aldehydic,sweet, fresh peely. A group of these fractions was isolated due to the dominance of these characteristics and the lack of oil/peel characters.

These fractions were then also evaluated by taste in a sweetened acidified solution (8% sugar;0.08%aca) at a strength of approx. 15 mg/Lt and observations recorded.

SAMPLE NO	APPROX FOLD	CHARACTERISTICS/
7	40	Fresh Peel, aldehydic,sweet
12	10	Fresh oily, juicy topnote
18	20	Ripe, Juicy, Aldehydic
19	35	Aldehydic, grassy
23	15	Fresh peel, Aldehydic

All other samples exhibited differing degrees oily peely type aroma and as such did not provoke interest.