SUPERCRITICAL FLUID EXTRACTION OF CANOLA SEED

by

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Abstract

The extraction of oil from fixed beds of Canola seed (Brassica napus and Brassica campestris) was studied using carbon dioxide at temperatures and pressures ranging from 25 to 90°C and 10-36 MPa respectively.

The highest oil solubility in the CO_2 (11 mg/g CO_2) was observed at 36 MPa and 55°C. The equilibrium oil concentration in the CO_2 phase, was found to be independent of the oil concentration in the seed phase. The extracts were found to be essentially free from phosphorus (<7ppm) and their fatty acid content did not change significantly as the extraction progressed.

The total amount of oil recovered from the seeds by CO_2 extraction depended upon the seed pre-treatment. For commercially flaked seed, this amount was comparable to that recoverable by conventional hexane extraction.

The CO_2 extraction of simple triglycerides at 36 MPa and 55°C was investigated. The solubilities of tripalmitolein, triolein, and trieicosenoin were 20 mg/g CO_2, 10 mg/g CO_2, and 4 mg/g CO_2 respectively. The composition of CO_2 extracts of an equimolar mixture of the above triglycerides was also studied. It was found that the concentration of each triglyceride in the extract was equal to the product of its mole-fraction in the mixture and its solubility in the CO_2.

Equations governing the mass transfer from the Canola seed to the CO_2 solvent were developed. A transient one-dimensional mathematical model based on these equations was used to obtain concentration profiles of oil in both the solvent and seed

phases, and to determine the overall volumetric mass transfer coefficient. The calculated concentrations and extraction rates were in good agreement with experimental results. The overall volumetric mass transfer coefficient for the initial constant rate period was found to be proportional to the 0.54 power of interstitial velocity.

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I. INTRODUCTION

1.1 Introduction

Supercritical fluid extraction(SFE), also referred to as "dense gas extraction", "dense phase extraction" and "supercritical gas extraction", is a high pressure solvent extraction process characterized by the use of supercritical fluids in place of conventional liquid solvents.

An understanding of the term "supercritical fluid" can be obtained by referring to the pressure-temperature phase diagram of a pure substance shown in Fig. 1.1. The lines A-TP, TP-C and TP-B divide the diagram into three regions representing three phases: solid, liquid and vapor. Along each of the lines two phases exist in equilibrium with each other while at point TP, the triple point, three phases co-exist. The line TP-C, which separates the liquid and vapor regions of the diagram, terminates at the critical point C. Beyond this point, the substance, which will no longer exist as a liquid or vapor is usually referred to as a supercritical fluid. While in the supercritical state, the substance resembles both a gas in terms of its viscosity and diffusivity and a liquid in terms of its density (Table 1.1). Although a variety of substances can be used in their supercritical state for extraction, common gases such as carbon dioxide, nitrous oxide and ethane have received the greatest attention.

The solvation capacity of a supercritical fluid (at constant T) is strongly dependent on its density which in turn

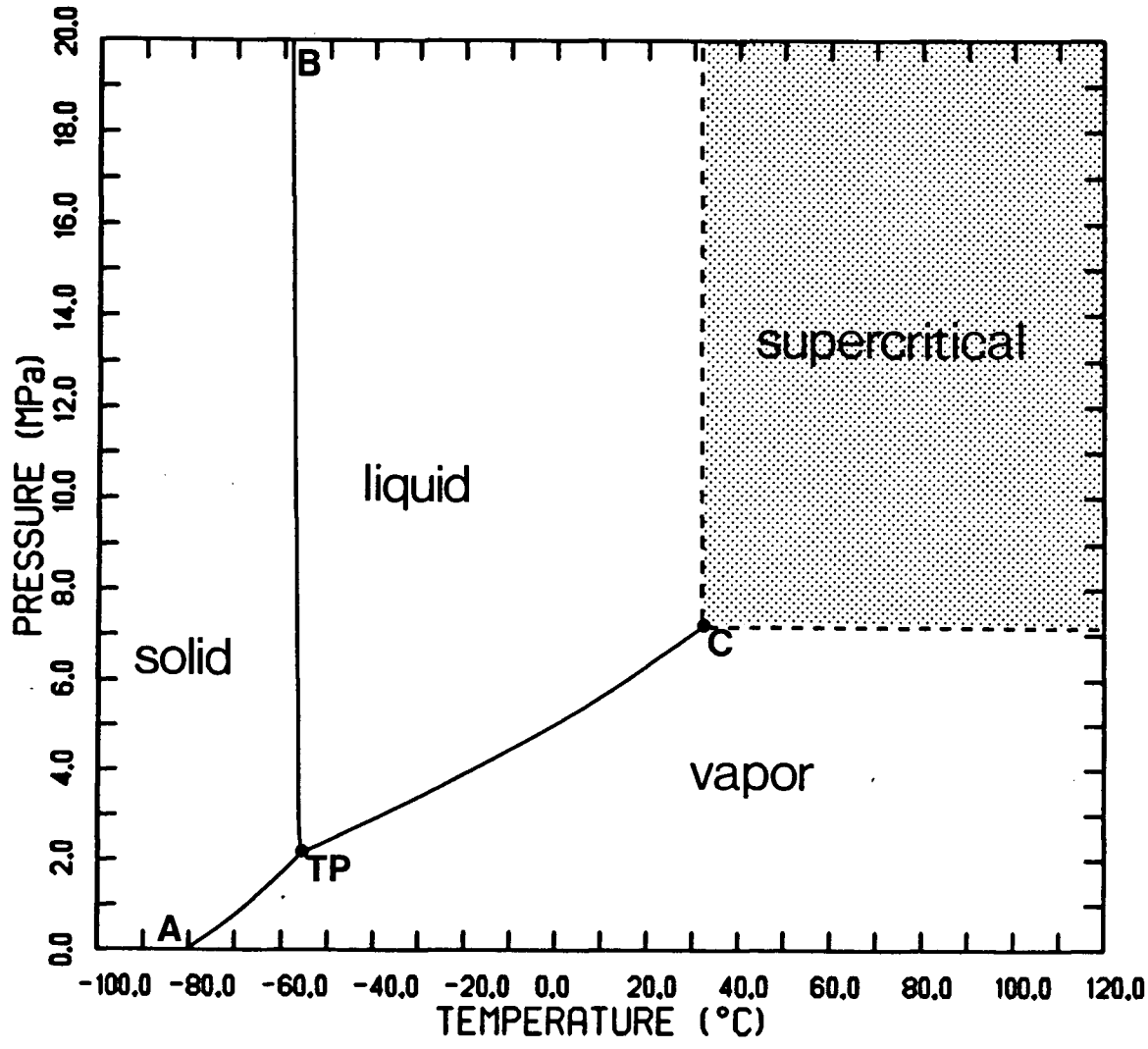

FIGURE 1.1 Phase diagram for carbon dioxide showing the relationship of the supercritical state to the solid, liquid and vapor states. The triple point is designated as TP and the critical point as C.

is proportional to the external pressure applied to the fluid (Brogle, 1982). It follows therefore that the solvent power of a supercritical fluid can be controlled by varying the pressure at which the process is carried out. This phenomenon is illustrated in Fig. 1.2. The solubility of napthalene in CO_2 at 55°C and 10.0 MPa is about 25 X 10^{-4} moles/mole CO_2. As the pressure of the carbon dioxide is increased, the napthalene mole fraction rises. At 25.0 MPa the solubility of the napthalene approaches 500 X 10^{-4} moles/mole CO_2. This represents a twenty-fold increase in solubility. Compounds extracted by a high pressure supercritical fluid can thus be recovered from the fluid by a simple pressure reduction. Additionally, effective separations can also be achieved by a variation of temperature rather than pressure.

TABLE 1.1 Typical values of viscosity, density and diffusivity for liquid, gaseous and supercritical carbon dioxide.

CO_2 state	viscosity g/cm s	density g/cm^3	diffusion coefficient cm^2/s
liquid	(a) 1.5 X 10^{-2}	(b) 0.9	(c,d) 10^{-5}
gas	(a) 1.4 X 10^{-4}	(b) 0.002	(c,d) 10^{-1}
supercritical fluid	(a) 9.1 X 10^{-4}	(b) 0.9	(c,d) 10^{-3}-10^{-4}

Source: (a) Newitt et al. 1956; , 1956; (b) Vukalovich and Altunin, 1968; (c) Randall, 1982; (d) Gere, 1983.

The ability to vary the density is an integral feature of the supercritical fluid extraction (SFE) process. Consequently, the process is most often carried out near the critical point, where the density variation is the greatest.

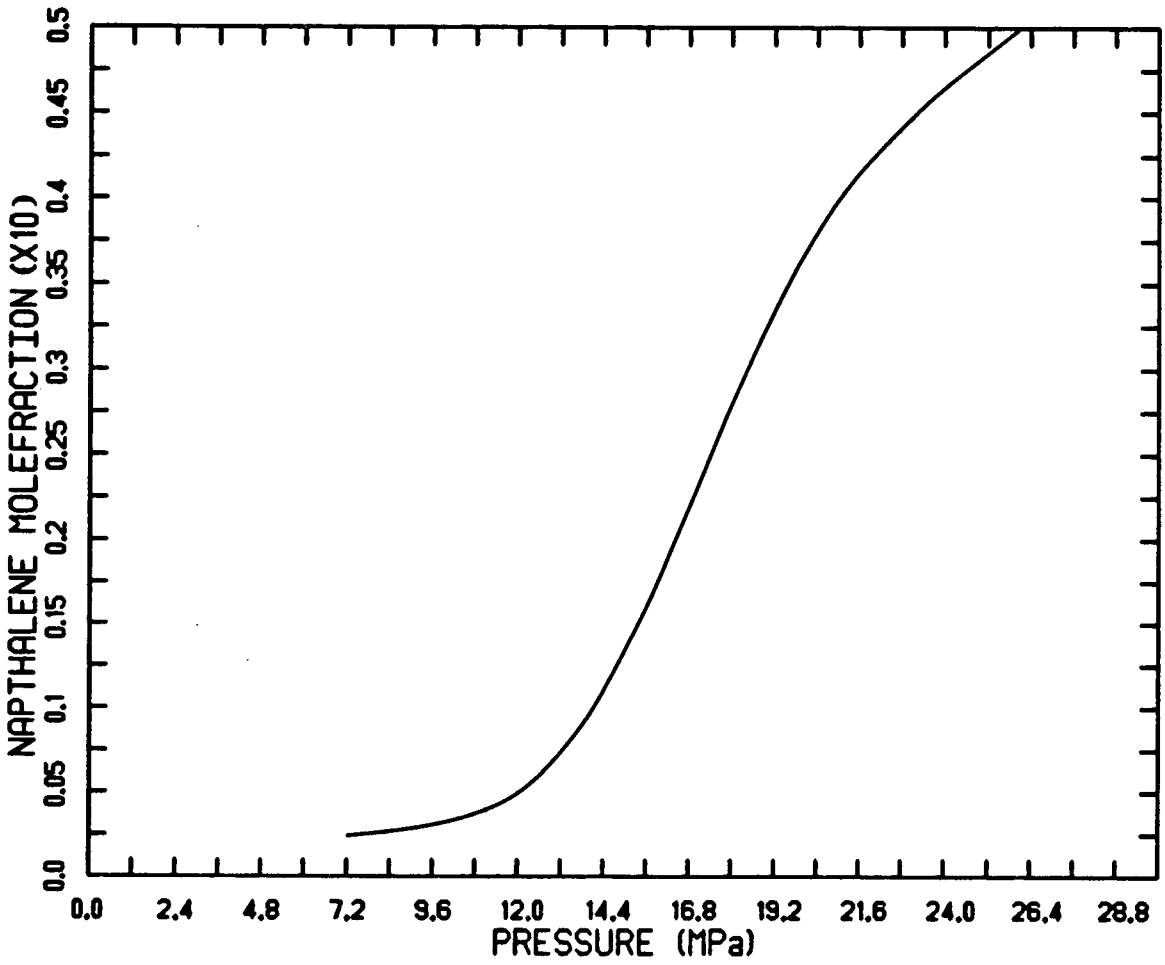

FIGURE 1.2 Molefraction solubility of napthalene in carbon dioxide at 55 °C (Paul and Wise, 1971).

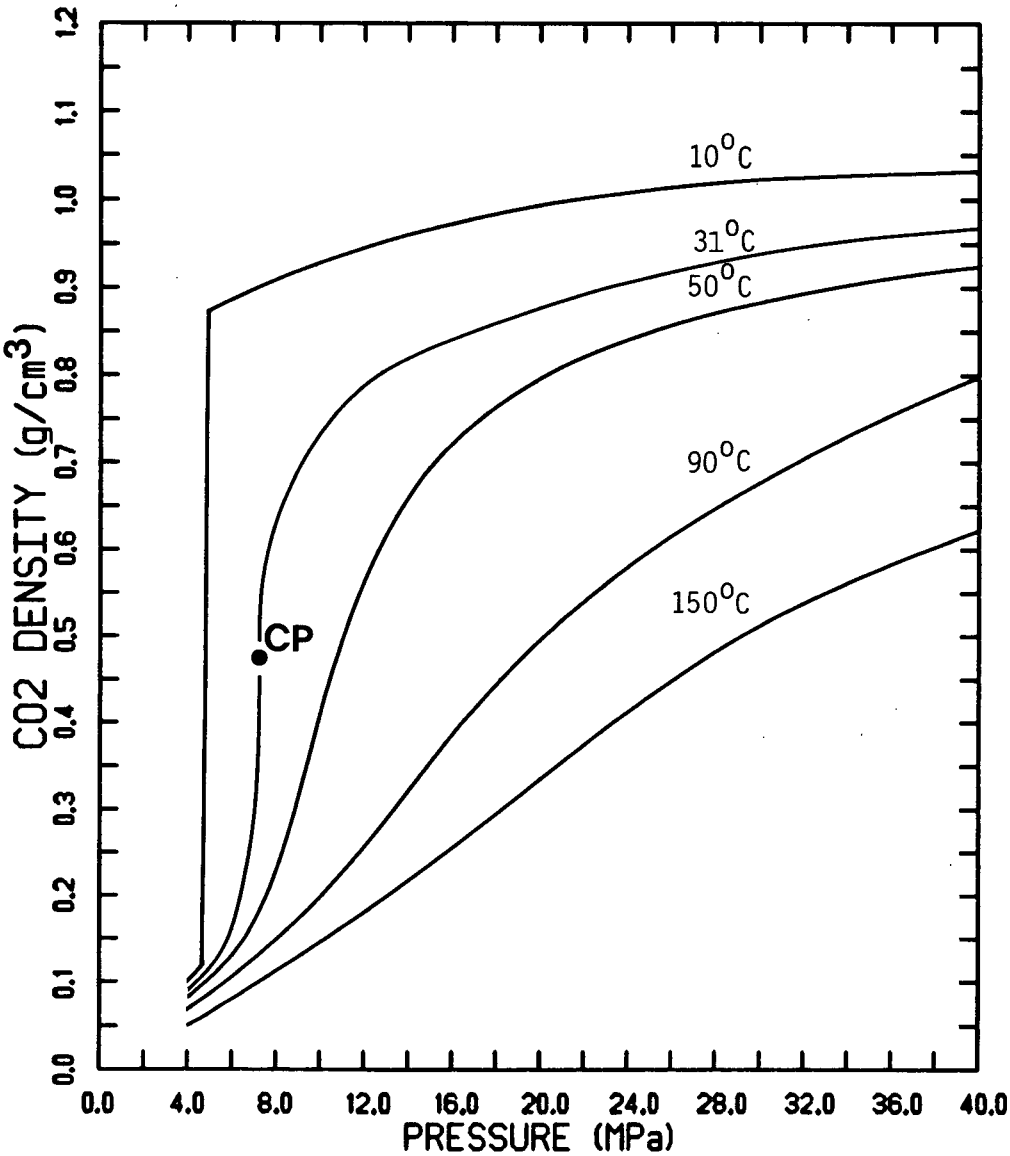

FIGURE 1.3 Density of carbon dioxide as a function of pressure at different temperatures. The critical point (CP) of the CO_2 is indicated on the diagram (Newitt et al., 1956).

Figure 1.3 shows the density of carbon dioxide as a function of temperature. At the critical point the variation of the fluid density with pressure is so great that large density variations can even be observed at different heights in a fluid column due to the gravitational field (Balzarini and Ohrn, 1972).

In practice, supercritical fluid extraction may employ a mixture of two or more fluids. Alternatively, the extraction process may use a fluid which has had small amounts of conventional solvents added to it. The added compounds act to modify the solvation behavior of the supercritical fluid and allow it to be 'tailored' for a specific extraction. These compounds are commonly referred to as "entrainers" (Brunner and Peter, 1982). For a more detailed outline of the theoretical and practical aspects of SFE the reader is referred to the review article by Williams(1981) or the January 1982 issue of Separation Science and Technology (Marcel Dekker, Inc.)

1.2 Background

1.2.1 Supercritical Fluid Extraction

Although Hannay and Hogarth reported the unusual solvent power of supercritical fluids over 100 years ago, supercritical fluids have only recently gained prominence. The long leadtime is, in part, attributable to the difficulty in understanding the physical nature of supercritical fluids. Moreover, for years the price of petroleum-based solvents which are conventially used in solvent extraction, has been low, thereby discouraging research into alternative technologies.

During the last decade, however, this situation has changed. The cost of petroleum solvents has in some cases increased twenty-fold. Furthermore, as the oil crisis of 1973 has shown, the supply of these products is not secure.

During the same period, there has been a growing public concern regarding health issues. Many food additives have become suspect and petroleum solvent residues have become far less acceptable (Hellyar and de Filippi, 1982). Supercritical fluid extraction (SFE), on the other hand, can utilize safe, low-toxicity gases such as carbon dioxide for the extraction of food products (Caragay, 1981; Hubert and Vitzthum, 1978).

In light of these recent trends, the interest in SFE is growing and some promising results have been obtained. For example, edible oils have been collected and purified by SFE. Since the gases used for extraction are very volatile, virtually no solvent residue remains in the collected extract. De-solventizing, a costly and sometimes lengthy procedure when using conventional solvents is greatly simplified (Milligan and Tandy 1974).

Although several gases can be used for extraction purposes, carbon dioxide is most commonly used. The preference for CO_2 is due in large part to its physical and chemical properties. It is non-flammable and non toxic. Its critical temperature is low (31°C) and the extraction can be carried out at pressures of 10-40 MPa which is well within the capabilities of current technology. Furthermore, numerous compounds are soluble in carbon dioxide (Calame and Steiner 1982; de Filippi, 1982).

Other advantages are the low cost and high availability of CO_2.

As the knowledge of SFE increases, its applications are becoming more diverse. To date it has been used experimentally in biotechnology for removing fatty acids from aqueous solutions (Shimshick 1983), in agriculture for producing the insecticide pyrethrum (Stahl and Shutz, 1980), in perfumery for the extraction of volatile aromatics from flowers (Calame and Steiner, 1982) and in pharmaceutics for the extraction of psycho-active drugs (Stahl and Gerrard, 1982). In the food industry SFE has been used to extract oil from soybeans (Friedrich et al., 1982), corn germ (Christianson et al., 1984) and rapeseed (Bunzenberger et al., 1984). Additionally, the process is being used commercially for de-caffeinating coffee (Zosel, 1978; Williams, 1981) and for producing hop extracts used in the making of beer (Vollbrecht, 1982; Hubert and Vitzthum, 1978, Gardner,1982). SFE has been used by the petroleum industry on a large scale for tertiary oil recovery (Holm and Josendal, 1974; Stalkup, 1978) and experimentally for improving low grade crudes (Humphrey et al., 1984; Gearhart and Garwin, 1976).

1.2.2 Oilseed Production

The subject of this thesis concerns the oilseed industry, which may benefit greatly from SFE.

In the U.S., over 30 million tons of soybeans are processed each year (Hron et al., 1982). Almost all of the 6 million tons of oil from this crop is recovered using hexane. Since hexane is a petroleum product it is subject to continuously increasing

cost and uncertain availability (Friedrich and List, 1982). In addition, hexane does not only extract triglycerides, but also unwanted gums. These gums must then be removed from the oil in a separate process. It has been suggested that supercritical fluid extraction of oilseeds could produce an oil which is essentially gum-free (Friedrich and List, 1982).

In Canada, the largest oilseed crop is Canola (composed of Brassica napus and Brassica campestris). In 1981 over 2.5 million tonnes of the seed were produced (Pigden, 1983). The majority of this seed, like soya, is extracted using hexane. The extract from this process is then refined in a series of additional steps, which are outlined below.

1.2.3 Canola Seed Processing

The initial step in the processing of Canola seed (Fig. 1.4) is a cleaning procedure in which detritus harmful to the operation of the mechanical extraction equipment is removed. The seed, which is then 99% pure is preheated to 40°C and introduced into a seed crusher. In the crusher the seed is forced between two rollers operating at different speeds. The shearing and crushing action of these rollers ruptures most of the oil-containing cells. This step is a necessary pre-requisite for the subsequent processing (Othmer and Agarwal, 1955). The seed material emerging from the crusher consists of thin (> 0.2mm) flakes with a large surface area to volume ratio. These flakes are then transferred to large boilers where they are rapidly heated to 90°C and maintained at this temperature for 20-30

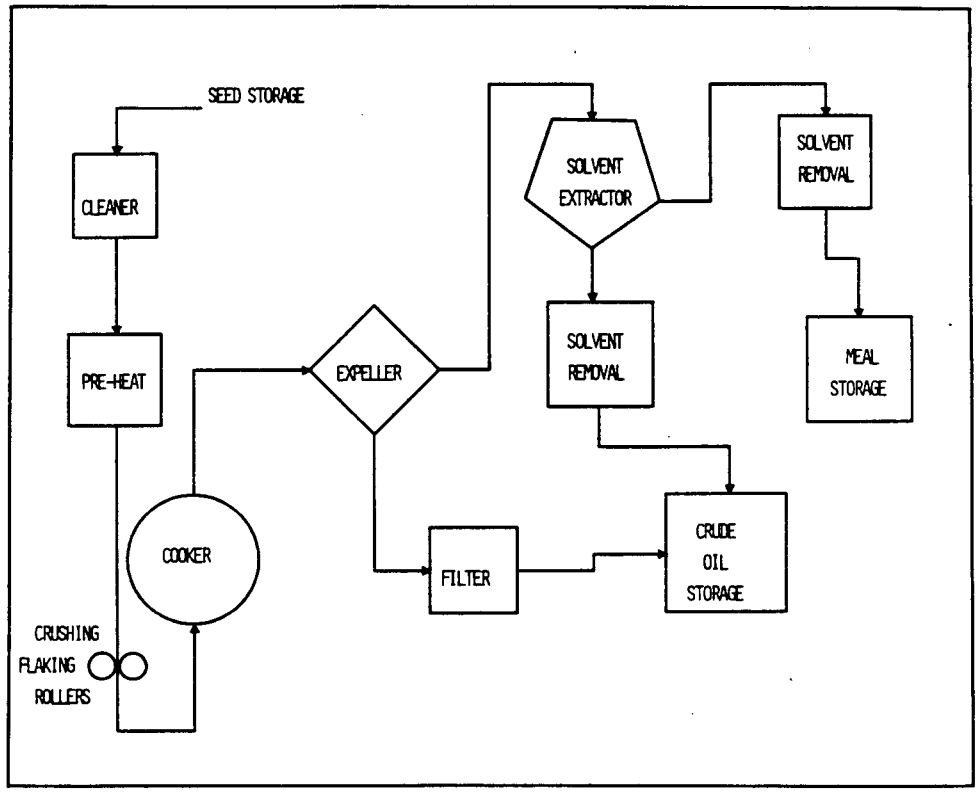

FIGURE 1.4 Steps in the processing of Canola seed (Appelqvist, 1972).

minutes. This process is known as "cooking" and serves several functions:

1) it decreases the viscosity of the oil and allows it to flow and coalesce;

2) it adjusts the moisture content of the seed;

3) it completes the rupturing of the oil cells;

4) it coagulates the proteins in the seed thereby preventing their transfer to the oil.

In addition to the above functions, the cooking of the seed also inactivates the native enzyme myrosinase which catalyzes the degradation of glucosinolates in the seed. The toxic degradation products from this reaction (isothiocyanates and oxazolidinethiones) are soluble in the oil and interfere with its subsequent hydrogenation (Rutkowski et al., 1982).

Following the cooking procedure, the seed material enters the expeller. This device, which contains a rotating screwshaft running in a cylindrical barrel, squeezes the liquid oil from the seed. The oil obtained from this operation is subsequently clarified by passing it through a series of filters or, alternatively, by centrifugation. The solid leaving the expeller which may contain from 15-18wt% oil, is then broken up and extracted with commercial hexane. After the extraction process, the hexane saturated seed "meal" is transferred to de-solventizers where the solvent is flashed from the meal by steam injection. The meal is then "finished" in a toasting process and emerges essentially free from solvent with a residual oil content of about 1 to 2wt% (Beach, 1983; Anjou, 1972). The oil obtained from the hexane extraction process is then de-

solventized and added to the expeller oil and the mixture is subsequently refined.

The following is a summary of additional Canola oil processing steps (Teasdale and Mag, 1983).

In the "refining" step (Fig. 1.5), hot (80°C) crude oil is mixed with small amounts of phosphoric acid and water to promote the precipitation of phospholipid gums. This de-gumming step is necessary since the gums tend to coagulate and precipitate during storage. After this initial de-gumming, the oil is treated with an alkali (eg. sodium hydroxide) to remove a large portion of the free fatty acids and to further reduce its phosphorus content. During alkali refining, the phosphorus level of the oil is reduced from 100-200 ppm to approximately 5 ppm (Appelqvist, 1972).

The next step, referred to as "bleaching", is carried out in preparation for subsequent hydrogenation and deodorization of the oil. During this step the oil is exposed to surface active clay which adsorbs pigments such as chlorophylls and carotenoids.

After bleaching, the oil is treated with pressurized hydrogen, in the presence of a catalyst, to saturate the fatty acids. Saturation increases the oxidative stability of the oil and increases its melting point.

The hydrogenated oil is next subjected to steam distillation for deodorizing. In the process, flavor and odor compounds, free fatty acids and various degradation materials are removed from the oil. The final product is a bland tasting,

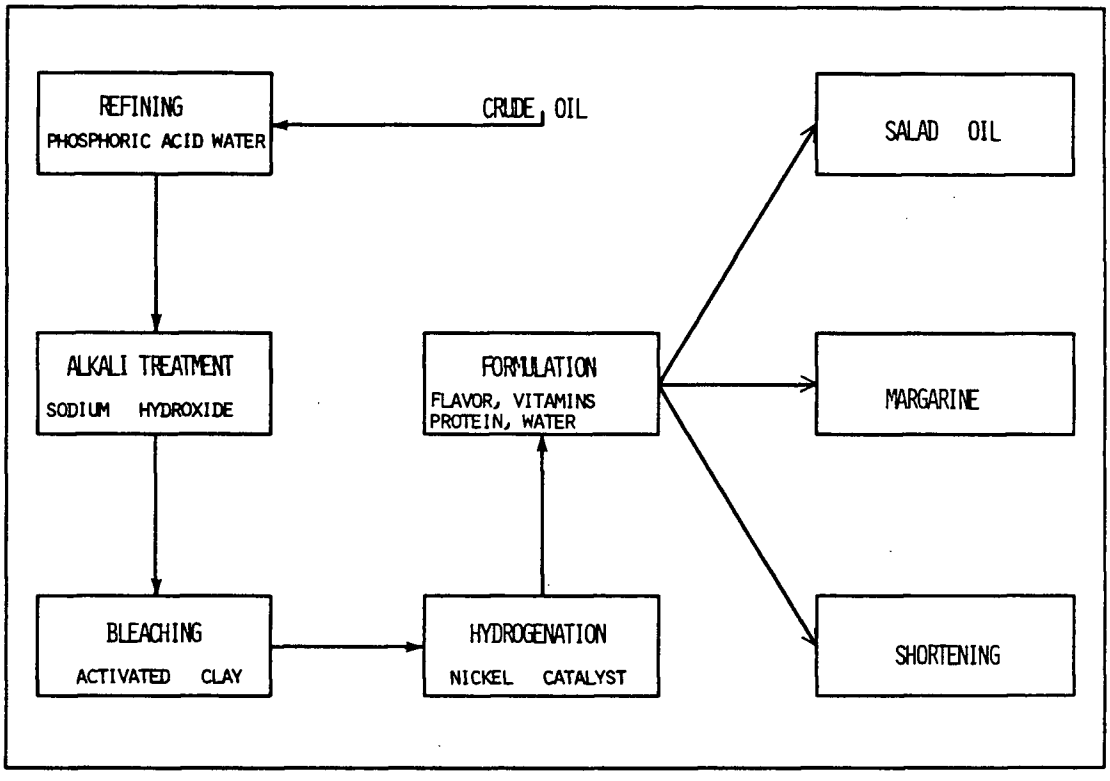

FIGURE 1.5 Steps in the processing of crude Canola oil.

light yellow oil with excellent storage stability.

The last step in Canola oil processing is called formulation. As the name implies, it is during this process that the oil is converted into its final form, i.e. salad oil, margarine or shortening. Depending on which of these products is desired, lecithin, color, vitamins, milk whey, water and salt may be added. For a detailed discussion of Canola oil processing the reader is referred to Appelqvist (1972) or Teasdale and Mag (1983)

In Table 1.2 some typical specifications for crude and refined Canola oil are listed.

TABLE 1.2 Typical specifications of commercially processed crude and refined Canola oils (Appelqvist, 1972).

	crude	refined
free fatty acids	1.0%	0.05%
water	0.3%	0.05%
phosphorus	500 ppm	< 5 ppm
sulfur	7 ppm	-
erucic acid	2%	2%

1.3 Research Objectives

In order to assess the feasibility and merits of supercritical CO_2 as a solvent in the Canola oilseed industry, a large amount of information is required.

At the beginning of this project it was decided to focus research on two main areas. First, the parameters affecting the extraction process had to be established. These parameters include temperature, pressure and flowrate of the carbon dioxide

solvent, and the physical state of the seed material. Second, the triglyceride and phospholipid content of the extracts had to be determined. Both areas are important for determining the optimum extraction conditions (which in turn can be used to compare the SFE process with the standard hexane extraction process) and for gaining insight into the extraction mechanism itself.

The specific objectives of the research were:

1) to develop laboratory equipment for investigating the extraction of Canola seed using supercritical carbon dioxide;

2) to determine how the solubility of the oil in CO_2 is affected by temperature and pressure;

3) to determine how the extraction process is affected by the mechanical condition of the Canola seeds;

4) to determine how the composition of the extract varies during the course of the extraction;

5) to determine the mass transfer coefficient for the extraction process.

The research project was carried out in a number of stages. In the initial stage, equipment was developed by which small amounts of the seed material could be extracted with a stream of carbon dioxide at different temperatures, pressures and flow rates. With the same equipment the extracts could be collected for further analysis.

In the second stage a method was developed to determine the solubility of the oil in the carbon dioxide. Experiments were then conducted to determine the relationship between oil

solubility and carbon dioxide pressure, temperature and seed oil concentration.

In the following stage, the effect of seed treatment on the extraction process was investigated. The seed treatment techniques which were used ranged from commercial flaking to the use of high pressure gas to burst the seeds.

In the succeeding section, analytical techniques were developed to gain information on the fatty acid composition and phosphorus content of the carbon dioxide extracts of Canola seed.

In the final stage, the equations governing the extraction process were developed and solved numerically. The results predicted by this mathematical model were then compared with experimental data.

II. LITERATURE REVIEW

2.1 Introduction

A brief review of the general literature pertaining to supercritical fluid extraction (SFE) is presented before discussing the literature dealing specifically with supercritical extraction of oilseeds. The final section of this chapter contains a discussion of the anatomy of the Canola seed.

2.2 Review Of SFE Literature

The fact that supercritical fluids could act as solvents was first reported by Hannay and Hogarth in 1879. They demonstrated that a salt, potassium iodide, could be dissolved in supercritical ethanol and they observed that the salt's solubility was largely dependent on the pressure of the ethanol. These results were subsequently confirmed (Tyrer,1910) and it was shown that the dense gas/salt solution was electrically conductive (Krans, 1922). During this time it was also suggested that supercritical water in the earth's crust was of importance in geological processes (Niggli, 1912). In 1945 Katz and Whaley (as cited in Randal,1982) showed that natural gas could be used to separate hydrocarbon mixtures. In the late 50's Zhuze demonstrated that supercritical fluids could be used for removing ashphaltenes and resins from petroleum (1958), extracting ozocerite wax from raw ore (1959) and recovering lanolin from wool grease (1958). In the following decade, several articles were published dealing with SFE in the petroleum industry (Ellis and Valteris, 1965; Zhuze, 1960).

Extensive surveys of the solubilities of compounds in supercritical fluids were reported (Giddings et al., 1968; McLaren et al., 1968; Giddings et al., 1969) and attempts were made to predict solubilities in supercritical fluids based on Hildebrand solubility parameters (Giddings et al., 1969; Czubryt et al., 1970). In 1971 Paul and Wise published the first comprehensive review of the theory and applications of the technique entitled "The Principles of Gas Extraction" and in 1978 the first symposium devoted entirely to the subject was held in Germany. During this symposium, papers were presented on the theoretical aspects of SFE (Schneider), empirical methods for determining the solubilities of compounds in supercritical fluids (Stahl et al.), the applications of SFE in the food and flavor industry (Zosel, Hubert and Vitzthum), and criteria for the design and construction of full-scale SFE plants (Eggers). Zosel also discussed the principles of supercritical fluid separations and their practical aspects. Zosel also describes how, by using supercritical ethane with pressure programming, he was able to separate a sample of mixed triglycerides into fifty fractions.

Since this symposium, many additional papers have appeared on both the practical and theoretical aspects of SFE. One notable group of papers on the latter subject deals with attempts to predict the equilibrium solubilities of compounds in supercritical fluids (Vetere, 1979; King et al., 1983; King and Bott, 1982). The ability to predict solubility would eliminate the need for difficult and costly experimental procedures.

Usually these predictions are based on the equations of state (King and Bott, 1982). The virial expansion developed by Rowlinson and Richardson (1959) has been used in this regard with some success, but it is limited to solids and gases. A more useful method for predicting solubilities involves a modification of the Redlich-Kwong equation (King et al., 1983). Although the equations can be used with liquids as well as solids, their solutions require complex calculations. Mackay and Paulaitus (1979) have reported that multicomponent equilibrium solubilities can, in principle, be calculated using extensions of existing theory. However, the current understanding of the liquid and supercritical states is insufficient to make this presently possible (King and Bott, 1982).

For a detailed account of the early history of SFE the reader is referred to Booth and Bidwell (1949), for an extensive survey of the various applications of SFE to Randall (1982) and Williams (1981) and for information on the theoretical aspects of the process, to Paulaitus et al., 1983) and Schneider (1978).

2.3 Supercritical Fluid Extraction Of Oilseeds

One of the first extensive research projects dealing with oilseed extraction using supercritical carbon dioxide, was undertaken by Stahl et al. in 1980. The authors investigated the effect of temperature and pressure on the equilibrium solubility of the oil in the carbon dioxide, and the effect of pre-treatments on the extraction rate and oil recovery. This equipment consisted essentially of a temperature controlled

fixed bed extractor which could be filled with seed material and flushed with a stream of high pressure CO_2. At the outlet of the extractor a two stage pressure reduction sampling system was used to collect the oil. During the extraction, a stream of liquid or supercritical carbon dioxide was passed through the extractor which was filled with seed. The concentration of oil in the outlet stream was determined by measuring the mass of oil extracted and the volume of carbon dioxide used. To determine whether the exit oil concentration had reached equilibrium, the following method was used: the extraction was begun and the extractor outlet concentrations were measured. When the concentration of oil at the extractor outlet became constant the values were noted and the extraction was stopped. The bed of seeds was then sectioned into nine segments of equal length and each segment subjected to a hexane extraction in order to determine the remaining oil content. If the section of seedbed at the extractor outlet retained its initial concentration, then the stream of carbon dioxide passing through this section must have been saturated with oil. The concentration of oil in the exit stream (previously recorded) was therefore assumed to have reached saturation.

Figures 2.1 and 2.2 indicate the solubilities of seed oils as a function of temperature and pressure as determined by the authors. From Fig. 2.1 it can be seen that the solubility of sunflower seed oil in supercritical carbon dioxide depends on the system pressure in an almost linear manner, reaching about 3wt% at 70 MPa. Figure 2.2 shows that the solvation capacity of

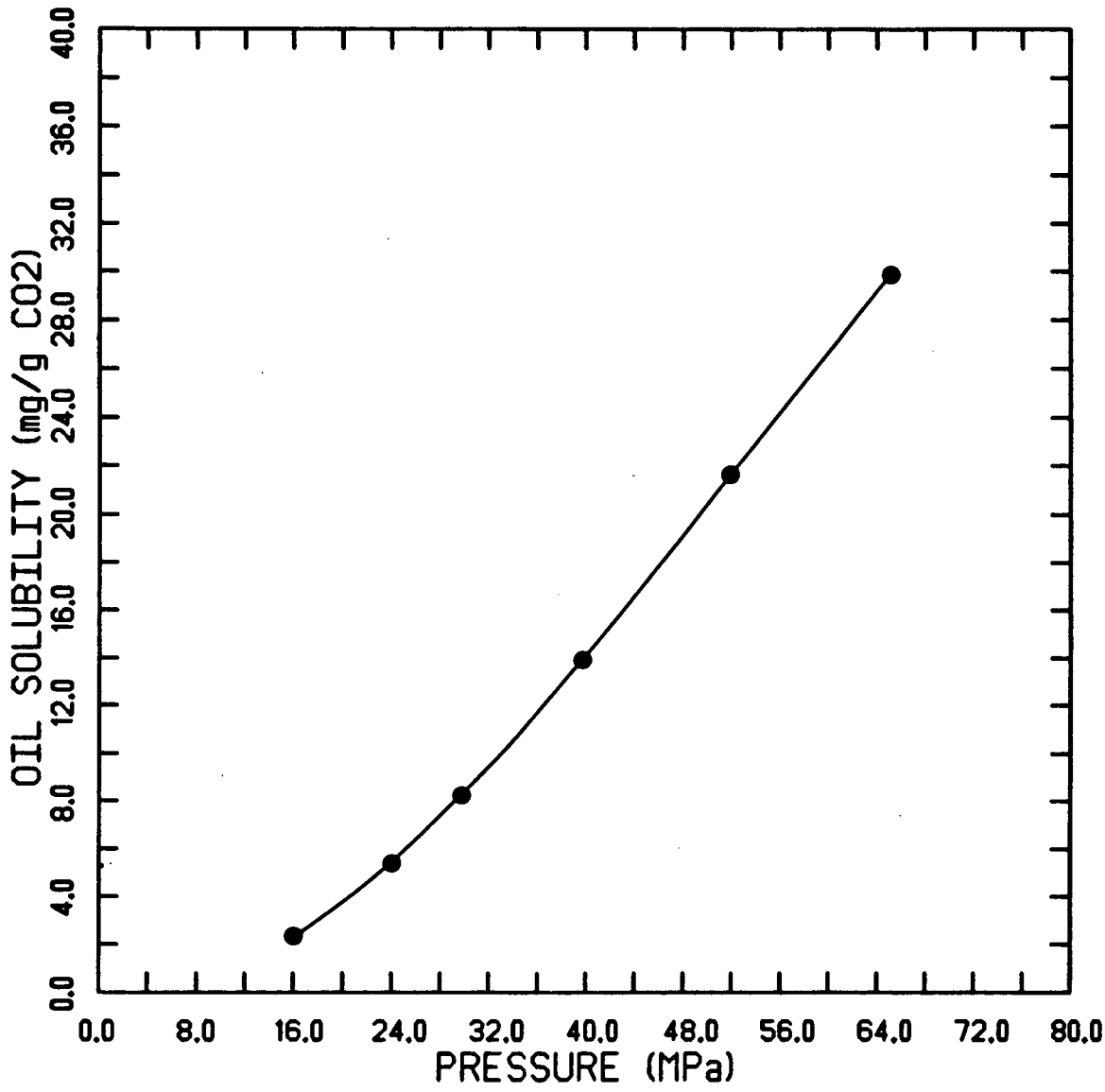

FIGURE 2.1 Solubility of Sunflower seed oil in carbon dioxide at 40 °C as a function of pressure (Stahl et al., 1980).

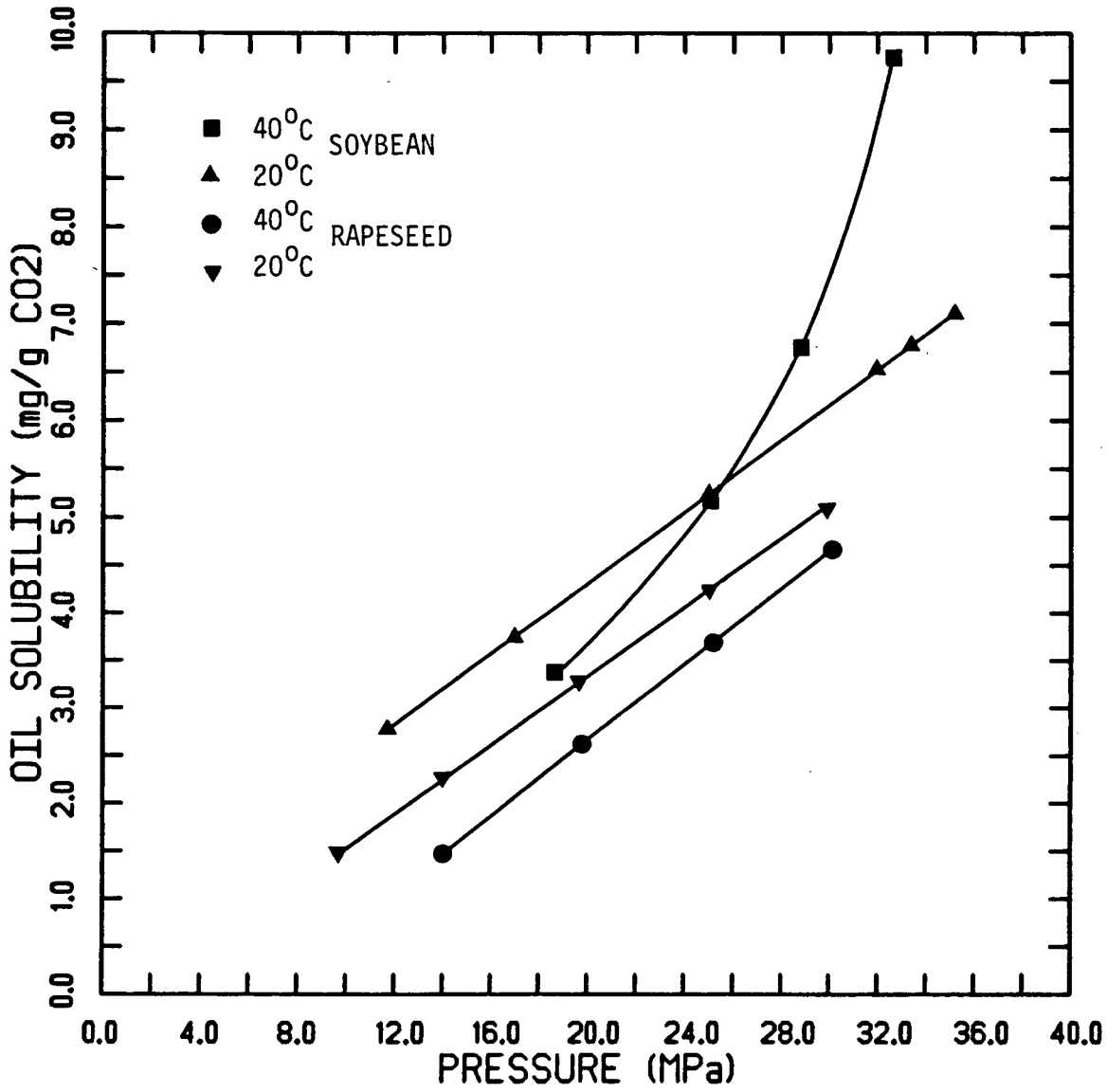

FIGURE 2.2 solubility of Soybean and Rapeseed oils in liquid carbon dioxide at 20°C and supercritical carbon dioxide at 40°C as a function of pressure (■▲Stahl et al., 1980; ●▼·Bunzenberger et al., 1984).

liquid carbon dioxide is also, although to a lesser extent, dependent on pressure. At pressures below 25 MPa, liquid carbon dioxide has a higher solvation capacity, for soybean oil, than supercritical carbon dioxide whereas at higher pressures the opposite is true. The "solubility crossover" effect has also been reported for other vegetable oils (de Filippi, 1982). The effect is not unexpected and can be explained by considering the pressure-density function of CO_2 (Fig. 2.3). At 20 MPa and 20 °C the density of liquid carbon dioxide is 0.94g/cm^3. At the same pressure but at 40 °C supercritical carbon dioxide has a density of only 0.84g/cm^3. However, at a pressure of 30 MPa, the density of the liquid and supercritical carbon dioxide are very similar (0.99g/cm^3 vs. 0.92g/cm^3). Since the solvation capacity of carbon dioxide is greater at higher densities (Brogle, 1982; de Filippi, 1982), it can dissolve more oil at 20 MPa in the liquid state than in the supercritical state. However, the solvation capacity is also dependent on temperature. At the higher pressures the densities of the liquid and supercritical carbon dioxide are more nearly equal and consequently temperature plays a dominant role in the solvation process.

Stahl et al. (1980) also studied the effect of seed preparation on the extraction process. The seed samples were treated prior to extraction using three different methods. When rapeseed was chopped to a very small

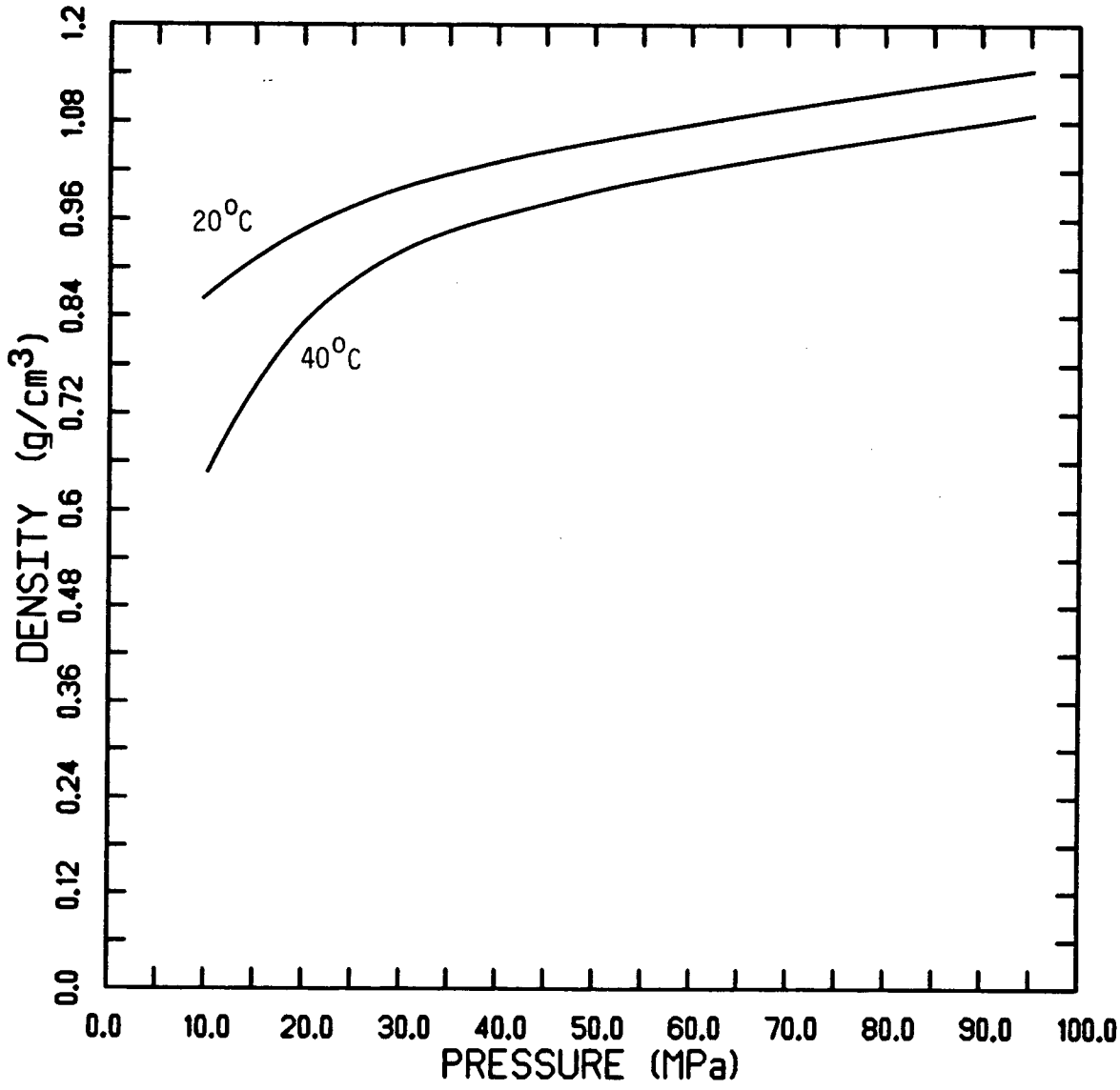

FIGURE 2.3 Density of carbon dioxide at 20 °C and 40 °C as a function of pressure (Newitt et al., 1956).

size (<0.4 mm), up to 98% of the oil could be removed while less than 1% of the oil was removed from whole seeds. They reported that not only the size of the particles in the crushed seed affect the extraction, but the shape of these particles was also significant. It is also apparent from the authors' data that the degree of crushing had a substantial effect on the solubility of soybean oil in the CO_2. Depending upon the crush, the solubility varied from 6 to 8 mg oil/g CO_2. No explanation is given as to why the solubility of the oil would be affected by seed condition, and no experimental conditions other than carbon dioxide temperature were reported for these experiments.

In a more recent paper by List et al. (1982) the extraction of soybean flakes using supercritical carbon dioxide was studied both from a physical and a chemical perspective. The extractions were performed in semi-batch mode using a procedure similar to that of Stahl et al. (1980). During the extractions, the collected oil was periodically sampled and analyzed with respect to free fatty acids, unsaponifiables and phospholipids. The authors found that the average phospholipid content of the carbon dioxide extracted oil was low in comparison to the oil produced by hexane extraction (0.17% vs 1.5%) and suggest therefore that carbon dioxide extracted oil would not require de-gumming. The concentration of phospholipids in the supercritical carbon dioxide extracts were found to increase as the extraction proceeded. Thus, the authors noted that, in a commercial process, there would be little merit in extracting the seed beyond a certain point since the oil removed in the

later stages of extraction is of poorer quality.

In addition to chemical tests, the oils, which were extracted using hexane and supercritical carbon dioxide, were also compared by means of sensory evaluation techniques. It was found that the carbon dioxide extracts were not significantly different in taste or odor from the hexane extracts.

Bunzenberger et al. (1984) reported on the supercritical fluid extraction of rapeseed. The authors present equilibrium oil solubility and extraction rate data at different pressures and temperatures using both liquid and supercritical carbon dioxide. The method used to obtain equilibrium values was similar to that used by Stahl et al. (1980). For comparison purposes the reported oil solubility data are plotted in Fig. 2.2. Although the data indicate that the oil solubility in the liquid and supercritical carbon dioxide increases with increasing pressure, the effect is not as pronounced as expected from the work of Stahl et al.(1980) and de Filippi (1982). In addition, the solubility of the rapeseed oil is significantly lower than those reported for other vegetable oils (de Filippi,1982; List et al., 1982; Stahl and Quirin, 1982). No reasons for such low solubilities are suggested by the authors. One possible explanation for the low values may lie in the oil itself. Unlike most other vegetable oils, certain cultivars of rapeseed contain high percentages of erucic acid (Sonntag, 1979). An oil containing triglycerides formed from this high molecular weight fatty acid would be expected to exhibit a lower solubility in CO_2 (Brunner and Peter, 1982). This explanation

cannot be substantiated however, since information on the species of seed or oil composition was not provided.

In a recent paper, Brunner and Peter (1982) reported the equilibrium solubilities of pure triglycerides and palm oil in various supercritical fluids. The authors indicate that over the range of temperatures and pressures studied (70,75°C, 10-40 MPa) nitrous oxide and ethane proved to be more effective solvents for triglycerides than either carbon dioxide or tri-fluoro-chloromethane. The reason for these differences is not readily apparent and can not be explained in terms of density (ρCO_2 = 0.645g/cm^3, ρC_2H_6 = 0.37g/cm^3) or polarity ($C_2H_6 < N_2O < CF_3Cl$). These results are also contrary to suggestions by Paul and Wise (1971) that compounds exhibit similar solubilities in fluids with similar physical properties.

A further aspect of Brunner and Peter's research involved the effect of ethanol entrainer on the solubilities of palm oil in supercritical carbon dioxide. The authors found that, compared with pure carbon dioxide under identical conditions (70°C, 20 MPa), 10% ethanol in the carbon dioxide increased the solubility of the oil by a factor of 20. With the same concentration of ethanol at a higher carbon dioxide pressure (50°C, 30 MPa) the authors reported oil solubilities in excess of 10 wt%. The use of an entrainer is significant in other respects as well. Due to energy considerations, the recovery of solutes from a supercritical solvent is more economically carried out by raising the temperature of the mixture than by decreasing its pressure. If a pure supercritical solvent is

used, solute separation by changing the temperature is rarely feasible because the required temperature increases are so large that they lead to the degradation of the compounds. By using a suitable entrainer, this problem may be overcome.

2.4 Seed Anatomy

Two species of Canola seed, i.e. Brassica napus and Brassica campestris are grown in Canada. The latter has a slightly shorter growing period and a lower oil content. The seeds of B. Napus (Fig. 2.4) are generally larger (2-3mm), than those of B. Campestris(1.5-2.5mm). The anatomy and location of lipid rich areas in both species are however similar.

As seen from Fig. 2.5, the seeds consist of three distinct regions: seed coat, cotyledons and radicle. The dense fibrous seed coat is composed of structural carbohydrates, mucilage and lignin(Yiu et al., 1982). This coat (Fig. 2.6) comprises from 12-20wt% of the entire seed weight (Bengtsson et al., 1972). The surface of the coat is observed to be highly reticulated (Fig. 2.7) and may be somewhat porous (Stanly et al., 1976). The endosperm tissue, which is 1 to 2 cells thick, is located on the inner surface of the seed coat (Van Caeseele et al., 1982). This endosperm tissue is rich in oil and is probably the reason why the seed coat contains appreciable amounts of oil (20wt%)(Anjou, 1972; Yiu et al., 1982). The embryo is composed of the cotyledons which are embryonic leaves and the radicle from which the root develops (Bengtsson et al., 1972).

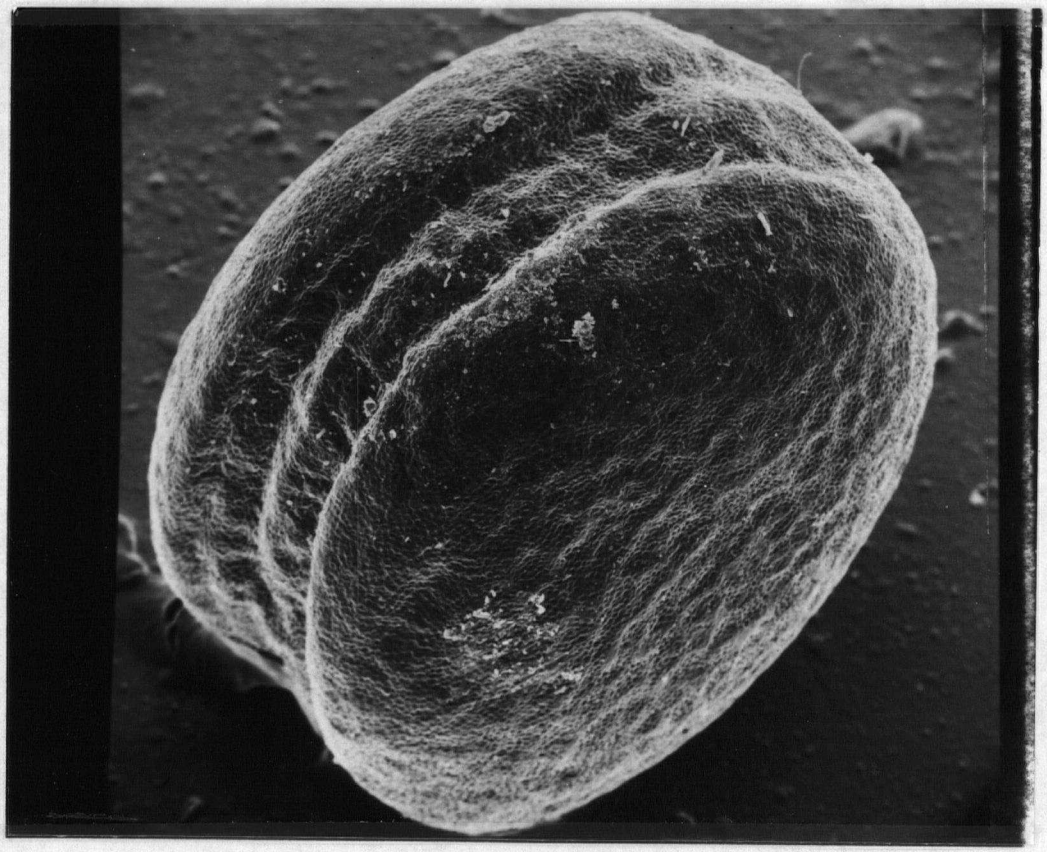

FIGURE 2.4 Photograph of a typical Brassica napus seed. (magnification 40X)

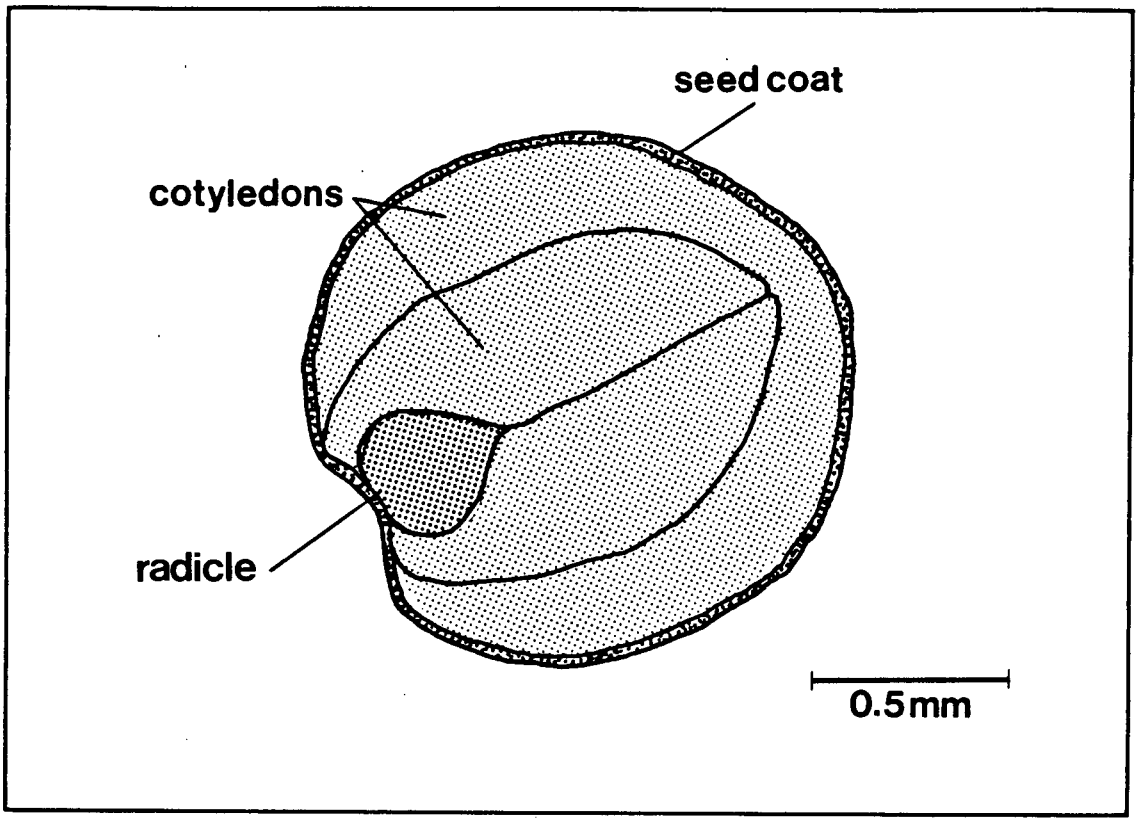

Figure 2.5 Lateral section through a Brassica napus seed showing the seed's basic anatomy. (Stanly et al., 1976).

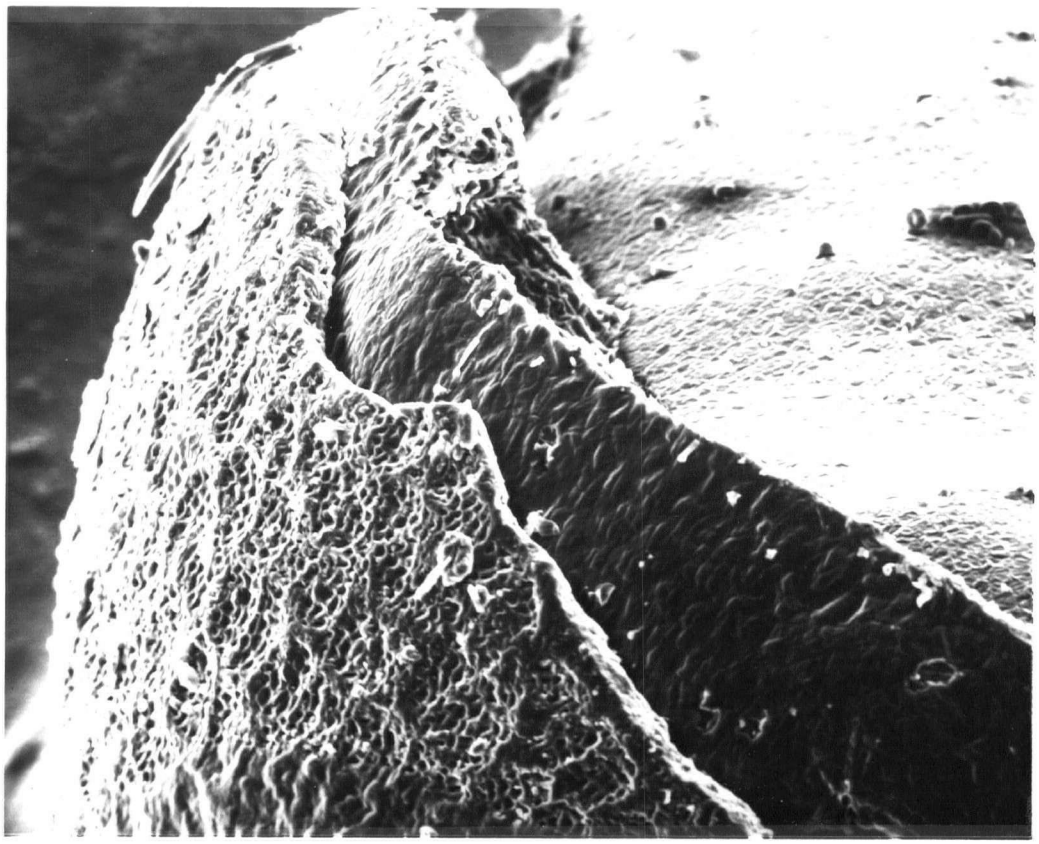

FIGURE 2.6 Scanning electron micrograph of a section of a B. napus seed fragment showing the seeds outer coat (Magnification 200X).

Canola seeds contain approximately 40wt% hexane extractable material by weight (Hofsten 1970; Khan and Hanna, 1983). Generally 95-98wt% of this oil is in the form of triacylglycerols. The remaining 2-5wt% of the oil is composed of mono- and di-acylglycerols, phospho- and galactolipids, sterol esters, waxes and free fatty acids (Appelqvist, 1972). The oil is distributed evenly throughout the cotyledons and the radical (Yiu et al., 1982) in the form of small droplets (0.5-1μm) bounded by a thin layer of protein. The oil droplets are located in the cytoplasm of the cells (Hofsten, 1970, Stanly et al., 1976).

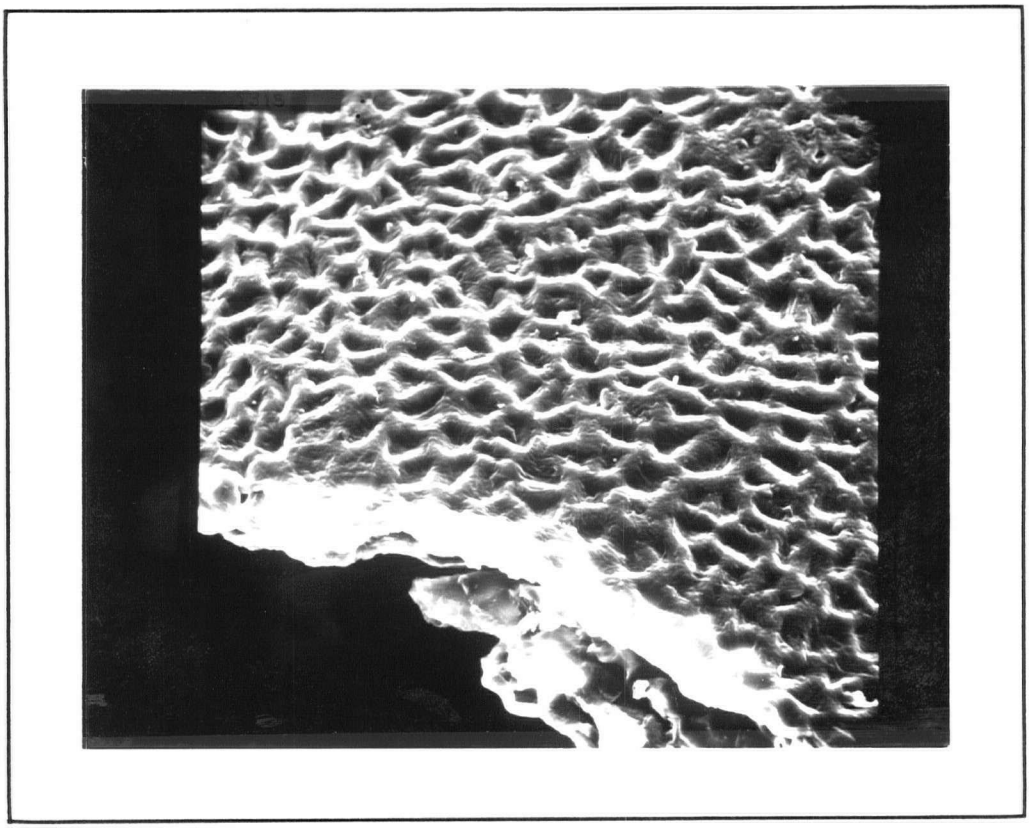

FIGURE 2.7 Scanning electron micrograph of a section of B. napus seed showing the seeds reticulated outer coat (Magnification 400X).

III. EXTRACTION MODEL

3.1 Introduction

In order to allow for the efficient design of extraction equipment, two basic types of information are necessary: (i) the equilibrium distribution of solute between the feed and the solvent phase and (ii) the mass transfer rate of solute from the feed to the solvent. Both of these can, in principle, be obtained by using a laboratory-scale fixed bed extractor.

3.2 Extraction From A Fixed Bed

Consider the fixed bed extractor depicted in Fig 3.1. The solute-free solvent enters at one end of the bed and exits at the opposite end. The bed consists of a mixture of solid inert material and solute. Initially the solute is distributed evenly throughout the bed. The bed can be considered to consist of a series of thin sections having uniform solute concentrations. Provided a driving force exists, the solute is transferred from the 'solid' phase to the solvent. With time, the concentration of solute in the solvent phase increases, while the solute concentration in the 'solid' phase decreases. As the solvent passes through succeeding sections, the concentration of solute progressively increases resulting in a solvent phase concentration profile as shown in Fig. 3.2a. A corresponding concentration profile also develops in the 'solid' phase (Fig. 3.2b). As the extraction proceeds, the fixed bed will gradually become depleted of solute causing the concentration profile in both phases to vary with time (Fig. 3.2a,b). The shape of the

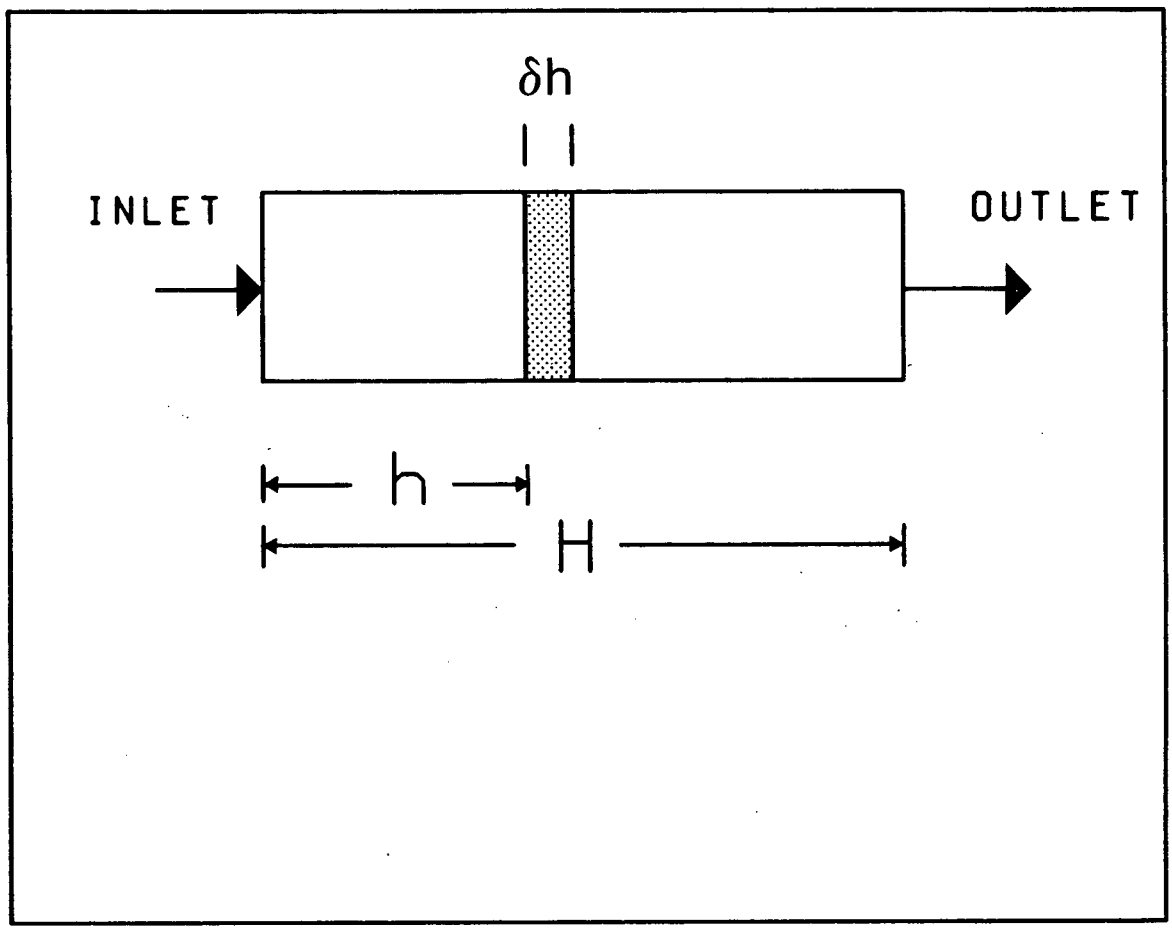

FIGURE 3.1 Schematic diagram of a fixed-bed extraction vessel.

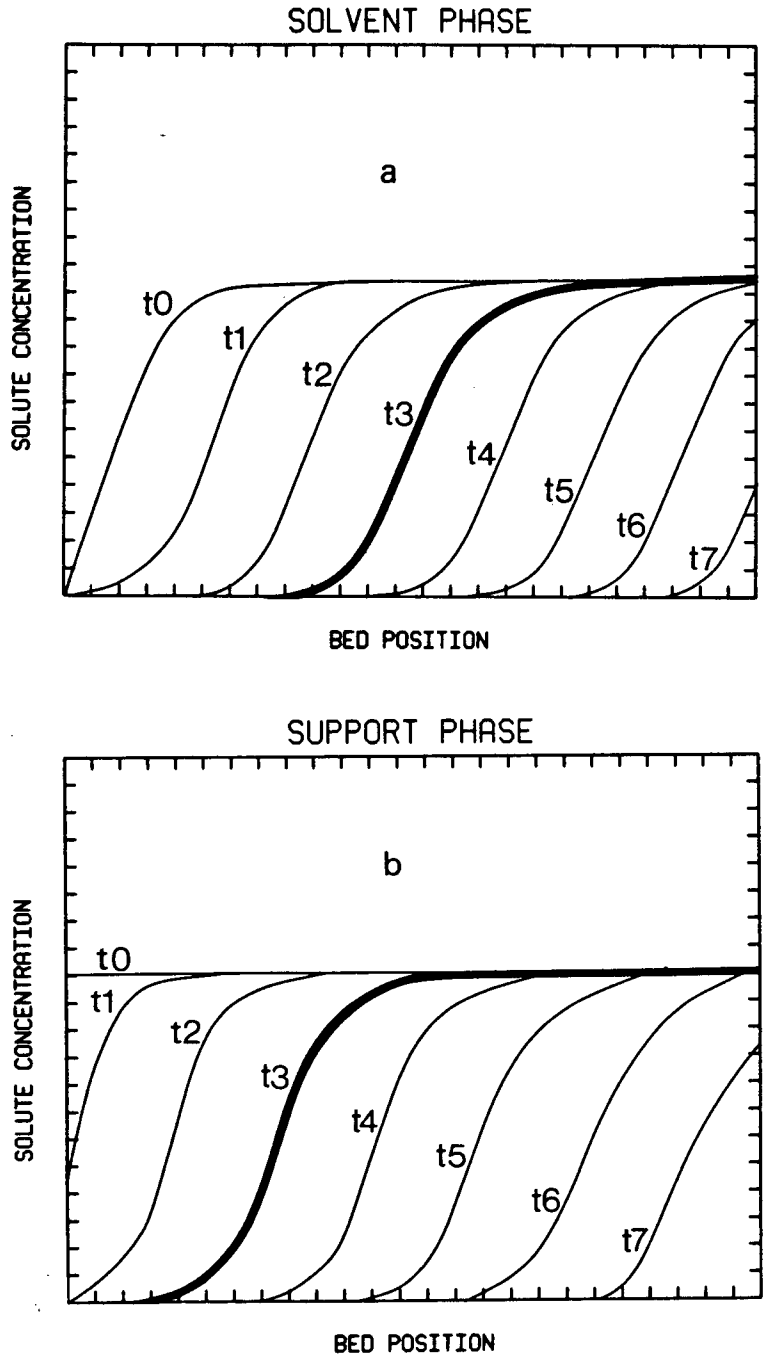

FIGURE 3.2 Solute concentration profiles in the solvent phase (a) and the 'solid' phase (b) in a fixed bed extractor at different times; where t0 denotes the beginning of the extraction.

concentration profile in each phase is dependent on the mass transfer area, mass transfer coefficient, solvent flowrate and the solubility of the solute in the solvent.

From Fig.3.2a it can be seen that the solute concentration in the solvent phase at the bed outlet also varies with time, giving a curve of the type shown in Fig.3.3a. In the present work, the integrated form of this curve is obtained experimentally (Fig.3.3b) and is called the 'extraction curve' for the process. It is understood that the slope at any point on the extraction curve corresponds to the concentration of solute in the solvent phase at the extractor outlet.

3.3 Mass Balance Equations

In the schematic diagram shown in Fig. 3.1 carbon dioxide enters through one end of the bed and exits via the opposite end. The mass of oil in the carbon dioxide phase in any element (δh) of the extractor is,

$$\text{Oil(mass)} = (A \delta h \epsilon \rho y) \quad . \quad . \quad . \quad . \quad . \quad .[3.1]$$

where:

h - distance along the bed [m]

δh - length of element [m]

ϵ - voidage of the bed of seeds [dimensionless]

ρ - density of the solvent phase [kg/m^3]

A - cross sectional area of the extractor [m^2]

y - concentration of oil in the solvent phase [kg oil/kg CO_2]

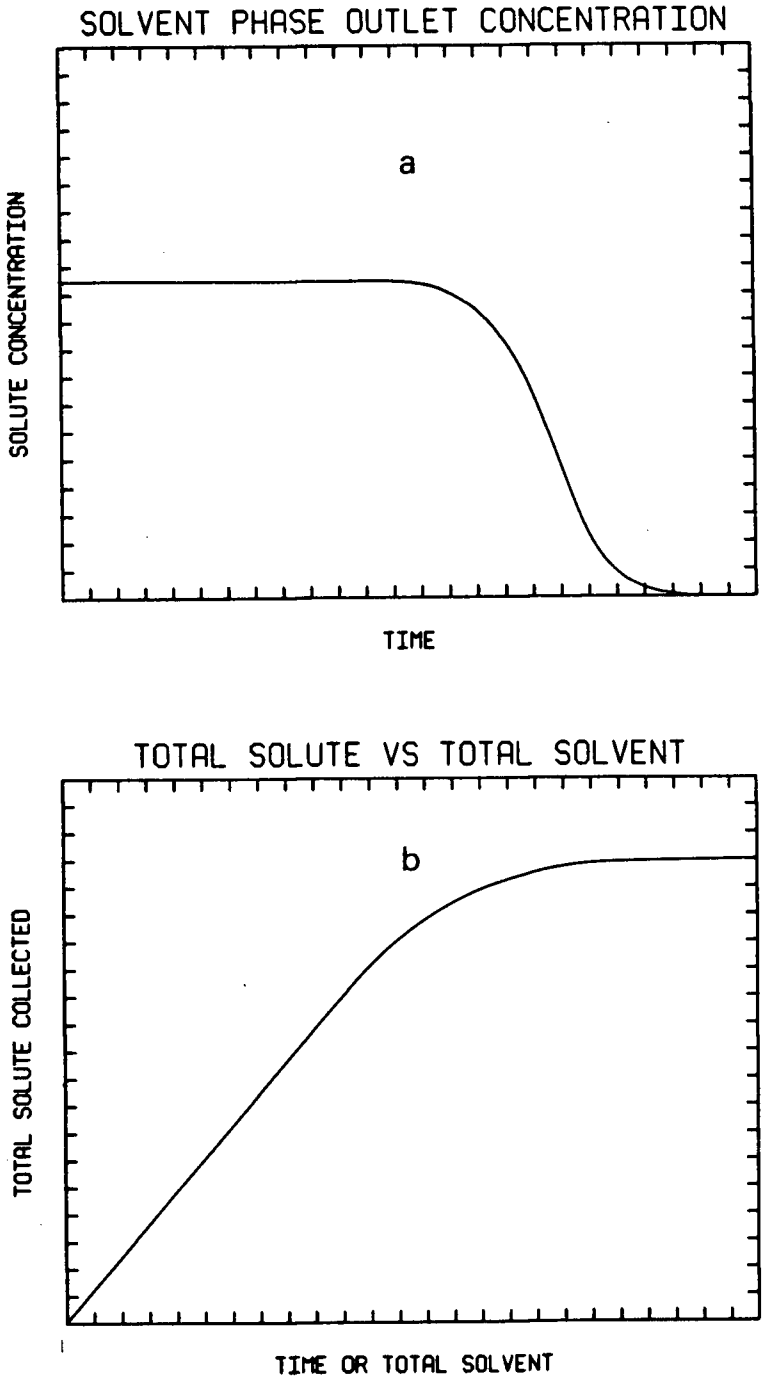

FIGURE 3.3 Concentration of solute in the solvent phase (a) at extractor outlet as a function of time. Cumulative mass of solute (b) extracted as a function of time or cummulative solvent passed through the bed.

An oil balance on the solvent phase can be written as:

$$\frac{\partial(A\delta h\epsilon\rho y)}{\partial t} = -\rho UA\frac{\partial y}{\partial h}\delta h + ApA\delta hK(y^*-y) \quad . \quad .[3.2]$$

where:

U - superficial solvent velocity [m/s]

Ap - surface area available for mass transfer per unit volume of bed [m^2/m^3]

K - overall mass transfer coefficient [$kgCO_2/m^2s$]

y* - concentration of oil in the solvent which is in equilibrium with seeds having oil concentration x [kg oil/kg CO_2]

x - concentration of oil in the seeds [kg oil/kg oil-free seed]

t - time [s]

Four assumptions are implicit in Eq. 3.2: (i) a uniform oil concentration in the CO_2 exists across the bed, (ii) the amount of axial mixing in the solvent phase is negligible compared with the convective flow, (iii)the oil concentration in the solvent phase is small, (iv) an overall mass transfer coefficient can be used to represent the extraction process. If the solvent density and flowrate are constant, Eq. 3.2 reduces to

$$\epsilon\rho\ \frac{\partial y}{\partial t} = -\rho U\ \frac{\partial y}{\partial h} + ApK(y^*-y) \quad . \quad . \quad . \quad . \quad . \quad [3.3]$$

A similar material balance can be written for oil in the seed phase of the extractor leading to:

$$\frac{\partial(x(1-\epsilon)\rho_s)}{\partial t} = -ApK(y^*-y). \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad [3.4]$$

where:

ρ_s -density of oil-free seeds [kg/m^3]

The boundary conditions for Eqs. 3.3 and 3.4 are:

t=0	0<h<H	$x=x_o$
t>0	h=0	y=0

where H and x_o denote the total bed height of seeds and the initial oil content of the seeds, respectively. In order for Eqs. 3.3 and 3.4 to be solved, the equilibrium relationship between the solvent phase oil concentration and the seed oil concentration, i.e. $y^*=f\{x\}$, must be known. This relationship may be determined experimentally by pre-extracting seed samples to obtain a range of x concentrations and then contacting each sample with solvent and measuring the oil concentration in both phases after equilibrium conditions have been established.

3.4 Microscopic Extraction Model

When Canola seed is crushed the oil contained within the seed is liberated. The crushed material is thus composed of fractured pieces of seed and exposed liquid oil. In the unextracted state it is assumed that the oil associated with the seed is found in three locations (Fig. 3.5).

1) on the surface of the seed particles and exposed to the flowing stream of solvent;

2) in the interstices between the seed particles;

3) within the tissue of the seed particles.

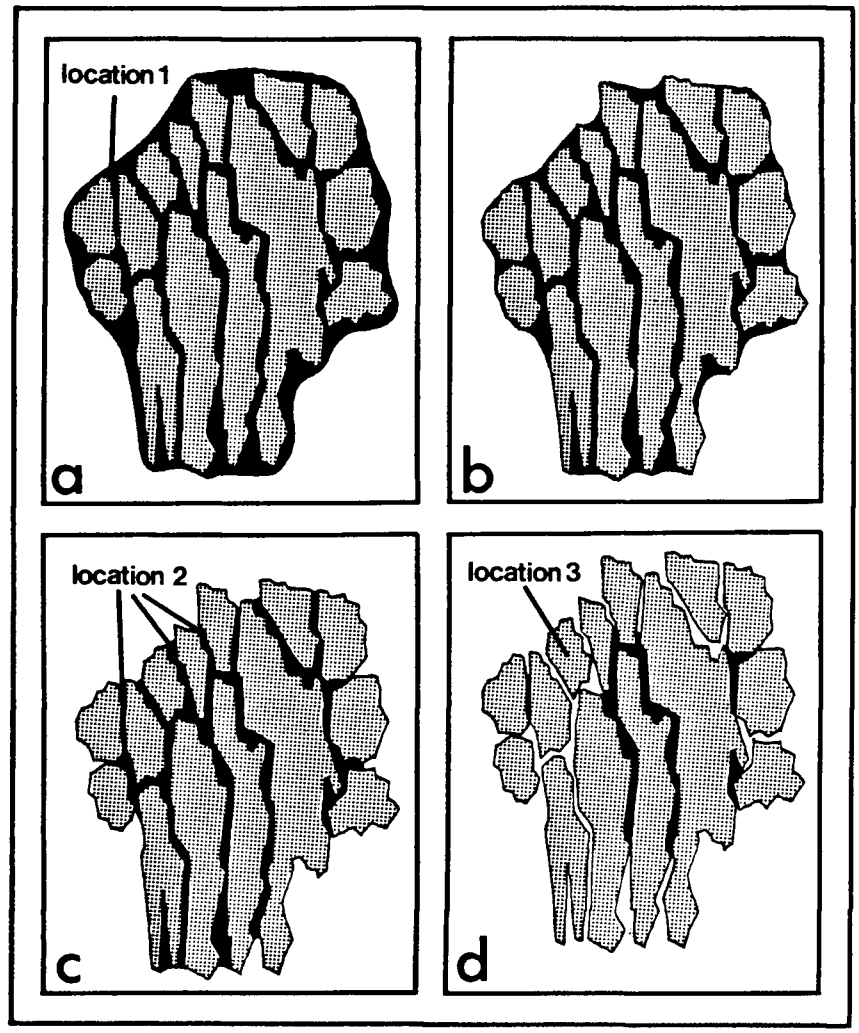

FIGURE 3.4 Cross-sectional diagram of an imaginary cluster of seed particles, at four stages during the extraction. The black areas of the diagram represent pools of liquid oil, while the shaded areas represent oil within intact seed tissue. a) The particles are covered with a layer of oil. b) Bare oil-free areas of the seed begin to appear. c) The majority of surface of seed particle is oil-free, diffusion of oil from the narrow interstices becomes significant. d) The entire outside surface of the cluster is oil-free. At this stage all oil extraction is from the the narrow interstices between the seed particles.

3) within the tissue (cells) of the seed particles.

The surface oil is removed first. The oil in the narrow interstices is primarily removed after the surface oil has been depleted and the channels to the interstitial oil become exposed to the CO_2 solvent. The oil within the undamaged seed tissue (cells) is not extracted by CO_2.

Consider a small cluster of seed fragments in the bed. Let the concentration of oil in the seed cluster at the beginning of the extraction be represented by x_0. At this time the surface of the seed fragments are covered with a layer of oil (see Fig. 3.4a). As the extraction proceeds, the surface area of the oil layer remains constant while its thickness decreases (Fig. 3.4b) until bare or oil-free regions appear on the outside of the cluster. Let the corresponding mean concentration of oil in the seed cluster at this point be denoted by x_1 . At a later time, the oil concentration reaches a value of x_2 (where $x_2 < x_1$) and most of the outside surface of the cluster is oil-free (Fig 3.4c). The remaining oil is contained within the seed tissue and the small spaces between the seed fragments. The concentration value x_3 represents the amount of oil within the intact seed tissue which can not be extracted by CO_2. Upon further extraction, the outside surface of the cluster becomes completely bare (Fig. 3.4d). The oil remaining in the cluster at this stage is removed from the spaces between the seed particles by molecular diffusion.

From Eq. 3.3 it can be seen that the rate of mass transfer

is proportional to the mass transfer coefficient (K), the driving force (y*-y) and the surface area available for mass transfer (Ap). In the model being suggested here, two different mass transfer coefficients were used. The first one, K_1 , governs the transfer of oil from the surface layer (location 1) to the solvent. The second coefficient, K_2, governs oil transfer to the solvent by diffusion from the spaces between the particles (location 2). The value of the first coefficient, K_1 , was expected to be greater than the value of the second coefficient, K_2.

Until the seed-oil concentration reaches a value of x_1 , the oils' surface area does not change significantly and, in the model, it is assumed to be constant. When the concentration of oil falls below x_1 , the area of bare seed surface increases and the area of surface oil available for mass transfer, where K_1 is applicable, correspondingly diminishes. In the model, the decrease of surface area between $x=x_1$ and $x=0$ is assumed to be a linear function of the oil concentration (Fig. 3.5a).

For $x_2 < x < x_1$, the surface of the seed is partly covered with oil. Because of this, both mass transfer coefficients must be considered. However, since K_2 is expected to be very much smaller than K_1 , K_2 was set to zero for the concentration range $x_2 < x < x_1$. When the concentration of oil in the seed particles fell below x_2, only K_2 was considered (Fig. 3.5b).

In Fig. 3.5c the relationship between ApK and seed oil concentration (x) is shown.

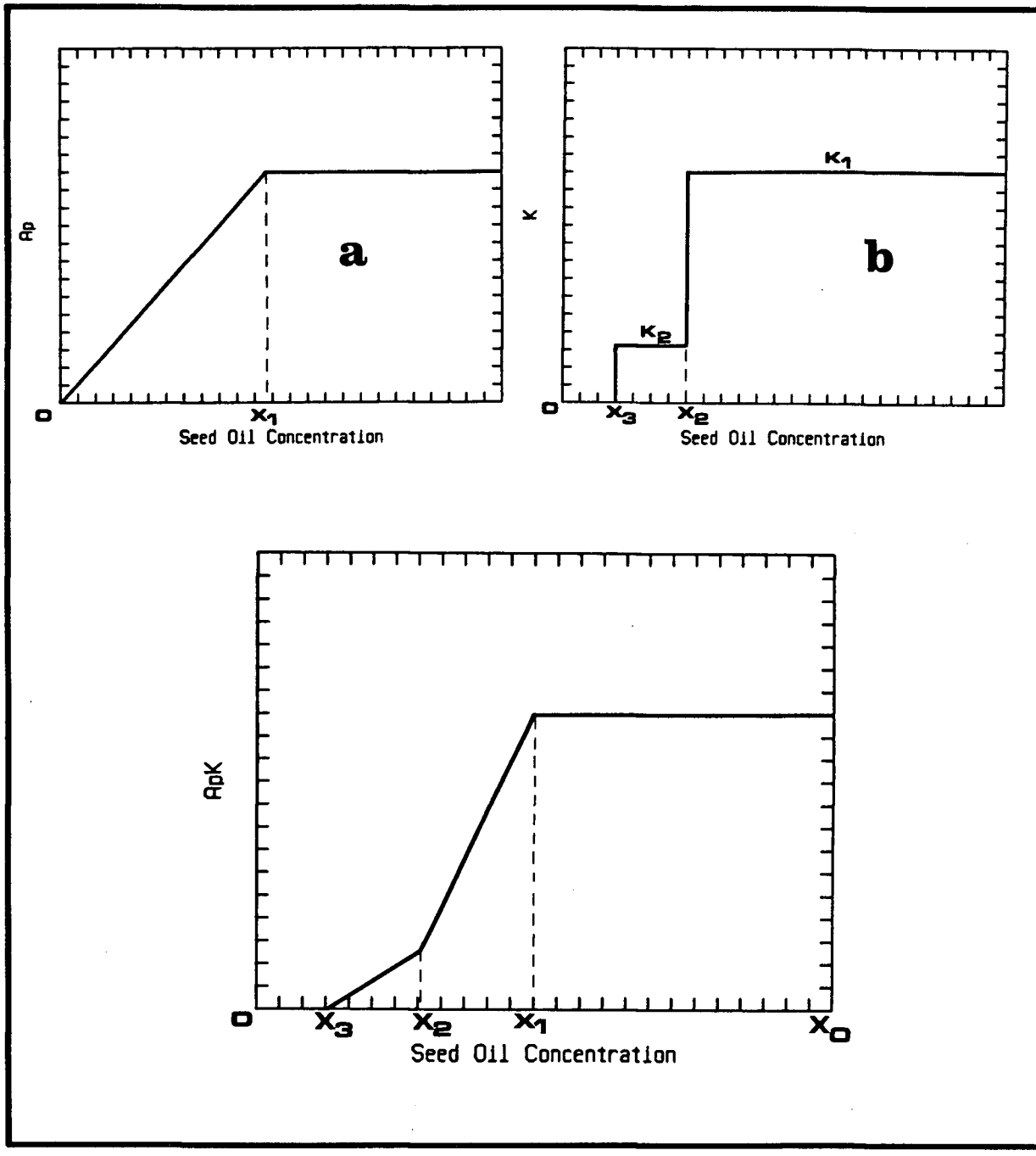

FIGURE 3.5 a) Oil surface area (Ap) availalble for mass transfer on seed cluster as a function of seed oil concentration; b) Mass transfer coefficient (K) as a function of seed oil concentration; c) the product of the mass transfer coefficient and the oil surface area as a function of seed oil concentration.

3.5 Mass Transfer Coefficients

Equations 3.3 and 3.4 can be solved numerically provided the equilibrium relationship between the solvent and the seeds is known and the parameters U, ρs, ρ, ϵ and Ap are given. The only unknown variable is the mass transfer coefficient and it must be assumed for the purpose of the numerical solution. The correct value of K is then found by matching the predicted and measured oil concentrations in the solvent at the extractor outlet as a function of time.

It is known that the mass transfer coefficient depends on the solvent flowrate, the state of the seeds and the temperature and pressure at which the extraction is carried out (Treybal, 1968). If the seed material is crushed or otherwise pre-treated, the surface area available for mass transfer (Ap) is difficult to measure. In this case it is customary to determine the product ApK which is usually called the "volumetric mass transfer coefficient".

3.6 Computer Simulation

The extraction of Canola seed using CO_2 can be simulated on a computer. The solution of Eqs. 3.3 and 3.4 gives the oil concentration profiles along the bed in both the solvent and seed phase as a function of time.

In order to validate the computer model, the predictions made by the model must be compared with experimental data. This can be done using two procedures. In the first, the calculated bed-oil concentration profiles are compared with the

experimental profiles from the real extractors. This procedure however, is quite lengthy and involved since it involves sectioning the experimental seed-bed and performing an oil-content analysis on each section.

In the second procedure the experimental extraction curves are compared with the corresponding extraction curves generated by the model. The extraction curves can be calculated either from the oil concentration in the seed phase or from the oil concentration in the solvent phase. Both of these methods are outlined below.

The total mass of oil extracted at any time can be predicted by using the bed oil concentration profiles, which are calculated from the model. The mass of oil extracted (M_e) equals the total mass of oil in the bed before the extraction begins (M_o) less the oil which remains in the bed (M_r):

$$M_e = M_e - M_r \qquad . \; . \; . \; . \; [3.5]$$

Initially the total mass of oil is distributed evenly throughout the bed and is given by:

$$M_o = (1-\varepsilon)A\rho_s H x_o \qquad . \; . \; . \; . \; [3.6]$$

After the extraction begins however, an oil concentration profile develops in the bed. The total mass of oil remaining in a small section (δh) of the bed with concentration x_i is m_{ri}

$$m_{ri} = (1-\varepsilon)A\rho_s x_i \delta h \qquad . \; . \; . \; . \; [3.7]$$

and the total oil content of the bed is

$$M_r = \sum_{i=1}^{i=n} (1-\epsilon) A \rho_s x_i \, \delta h \quad . \quad . \quad . \quad . \quad [3.8]$$

As δh approaches zero, the total amount of oil is given by

$$M_r = \int_0^H (1-\epsilon) A \rho_s \, x \, dh \quad . \quad . \quad . \quad . \quad [3.9]$$

By rearranging Eq. [3.6]

$$\frac{M_o}{Hx_0} = (1-\epsilon)\rho_s A$$

and substituting into Eq. [3.9] the following result is obtained:

$$M_r = \frac{M_o}{Hx_0} \int_0^H x \, dh \quad . \quad . \quad . \quad . \quad [3.10]$$

Thus from eq. [3.5]

$$M_e = M_o - \frac{M_o}{Hx_0} \int_0^H x \, dh \quad . \quad . \quad . \quad . \quad [3.11]$$

and upon rearranging

$$M_e = M_o \left(1 - \frac{1}{Hx_o} \int_0^H x \, dh \right) \quad . \quad . \quad . \quad . \quad [3.12]$$

The oil concentration along the bed (x=f{h}) at any time can be predicted using the computer model. This oil concentration profile can then be used in conjunction with Eq. [3.12] to calculate the 'extraction curve' (described in section 3.2) for the computer simulation.

Alternatively the extraction curve can be generated from the solvent phase oil concentration. Consider the solvent concentration at the extractor outlet. The amount of oil carried

out of the extractor with the solvent phase, (δm), during any time period δt is:

$$\delta m = \dot{m}\, y_o(t)\ \delta t \quad . \quad . \quad . \quad . \quad [3.13]$$

where:

$\dot{m}$ mass flowrate of the solvent [kg/s]

$y_o(t)$ concentration of oil in the solvent phase at the extractor outlet at a given time [kg oil/kg CO_2]

The total amount of oil removed from the bed after time T is given by

$$M_T = \dot{m} \int_0^T y_o(t)dt \quad . \quad . \quad . \quad . \quad [3.14]$$

The oil concentration at the extractor outlet can be used in conjunction with Eq. 3.14 to generate the extraction curve.

By comparing the predicted extraction curve with the experimentally obtained curve, the validity of the computer model can be assessed. Once the validity of the model has been established, the parameters x_1 , x_2 and ApK can be adjusted to obtain the best agreement between the computer generated and experimentally obtained extraction curves.

IV. EXPERIMENTAL EQUIPMENT AND PROCEDURES

4.1 Introduction

In order to study the phenomenon of Canola seed extraction using supercritical CO_2, special equipment and procedures were developed. The following is a description of the extraction equipment and the various materials used during the extractions. In addition, the specific treatments which were applied to the seed prior to extraction and the techniques used to determine various qualitative features of both the seed material and oils are discussed. Also presented in this chapter are the analytical chemical techniques which were used to determine the fatty acid composition and phosphorus content of the oils.

4.2 Experimental Equipment

Since supercritical fluid extraction is a relatively new procedure, equipment suitable for this work was not available and consequently had to be developed. The basic requirements of this equipment were to pump a stream of carbon dioxide at different pressures, temperatures and flow rates through a sample of seed material. It became apparent that the equipment could be developed by adapting an existing high pressure liquid chromatograph(HPLC). Figure 4.1 is a photograph of the complete experimental extraction system.

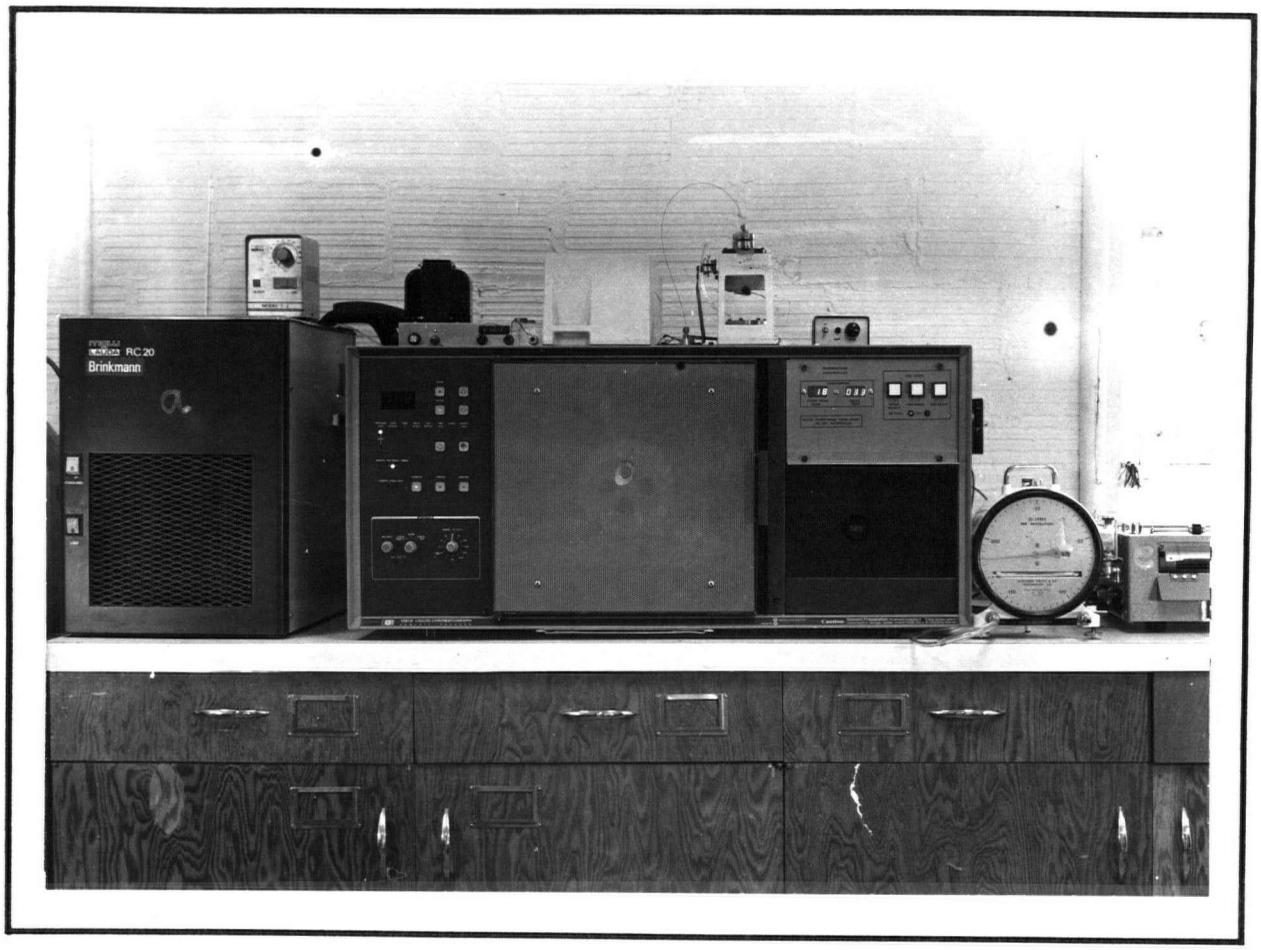

FIGURE 4.1 Photograph of the complete experimental supercritical fluid extraction system showing: Lauda-Brinkman circulating cooler (a), modified HP-1081B liquid chromatograph (b), extract collection system (c), volumetric flow-meter (d), dual pen chart recorder (e).

4.2.1 High Pressure Liquid Chromatograph

The Hewlett-Packard 1081B is a self-contained, microprocessor controlled instrument incorporating a single reciprocating diaphragm pump and a solvent flow system. Flow rates can be selected to fall between 0.1 mL/min and 9.9 mL/min against a maximum pressure of 40 MPa. The chromatograph also incorporates a small oven with a temperature range from ambient to 99 °C.

4.2.2 Solvent Flowpath

The general operation of the extraction system is shown in Fig.4.2. Liquid carbon dioxide from a storage cylinder (a) passes through the shutoff valve (b), a Nupro 7 μm sintered metal filter (c) and then into the cooled diaphragm pump head (d). The cooled liquid CO_2 then flows through a pressure-flow monitoring device (e) and into the HPLC oven. In the oven the CO_2 temperature can be raised or lowered to the desired value by passing it through 7 m of stainless steel tubing (f). The carbon dioxide then enters the extraction vessel (g). From the vessel, the carbon dioxide passes through a 2 μm frit (h) and into a flow restricter (i) where its pressure is reduced to about 0.1 MPa. The carbon dioxide then flows through 0.6 m of narrow bore (0.25 mm ID), fused silica tubing into the sampling section (j) where the liquid solute is collected. The CO_2 is subsequently directed through a wet gas-meter (k) and finally vented to the atmosphere.

All of the tubing within the system was 0.1 mm ID stainless steel and the connections were made using 1/16" stainless steel

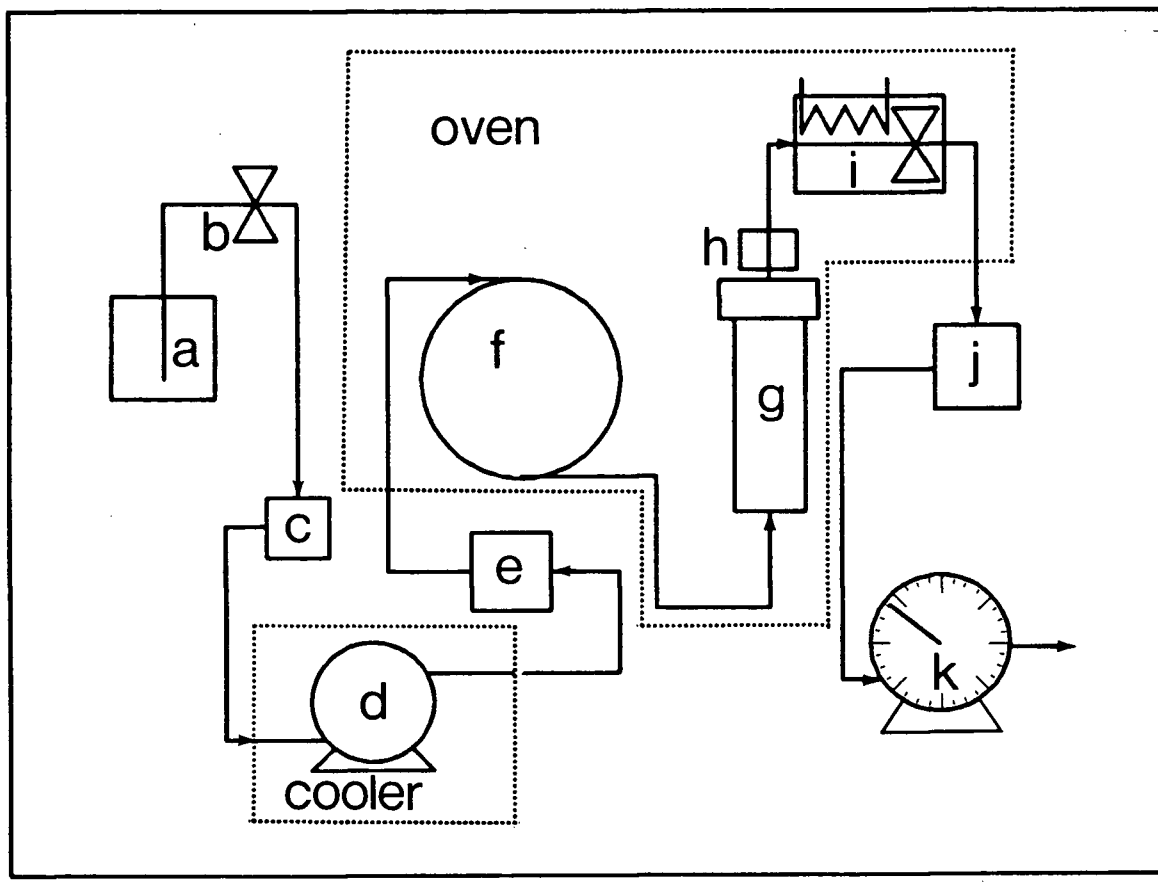

FIGURE 4.2 Schematic diagram of the experimental supercritical fluid extraction system. a) solvent (CO_2) reservoir, b) shut-off valve, c) sintered steel filter, d) diaphragm pump, e) flow and pressure transducer, f) temperature equilibration coil, g) extraction vessel, h) sintered steel filter, i) temperature controlled restricter valve, j) sample collection vessel, k) volumetric flow meter.

Swagelok fittings.

4.2.3 Pressure And Flow Control

The design of the 1081B is such that its microprocessor acts as an interface between the user and the solvent flow system. Thus, no direct manual control of the pumping system is possible. Instead, 'requests' are made to the processor via the keyboard. Provided the system is not in the 'error' or 'not ready' condition, the pump will then be activated.

The flow system in the 1081B consists of a small, piston-driven diaphragm pump (Fig. 4.3) and a feedback flow control loop. The flow rate through the pump is a function of stroke length which, in turn, is controlled by a small stepper motor. The pressure within the system is monitored continuously by a pressure transducer in the hexane-filled pulse damper (Fig. 4.2, e). The flow is also measured in this damper by monitoring the pressure-decay rate of each pump pulse.

The outputs from the pressure transducer, stepper motor and piston stroke length are used by the microprocessor to calculate the flow rate. This value is then compared with the flowrate setpoint and instructions are sent to the stepper motor to take appropriate action. This feedback flow control is executed every 0.6 sec.

The flow system can be operated in one of two modes. In the first mode, the pumping system tracks the flow setpoint while the system pressure is allowed to 'float'; the system responds only if the pressure goes above the maximum setpoint value. In the second mode, the system tracks the pressure setpoint

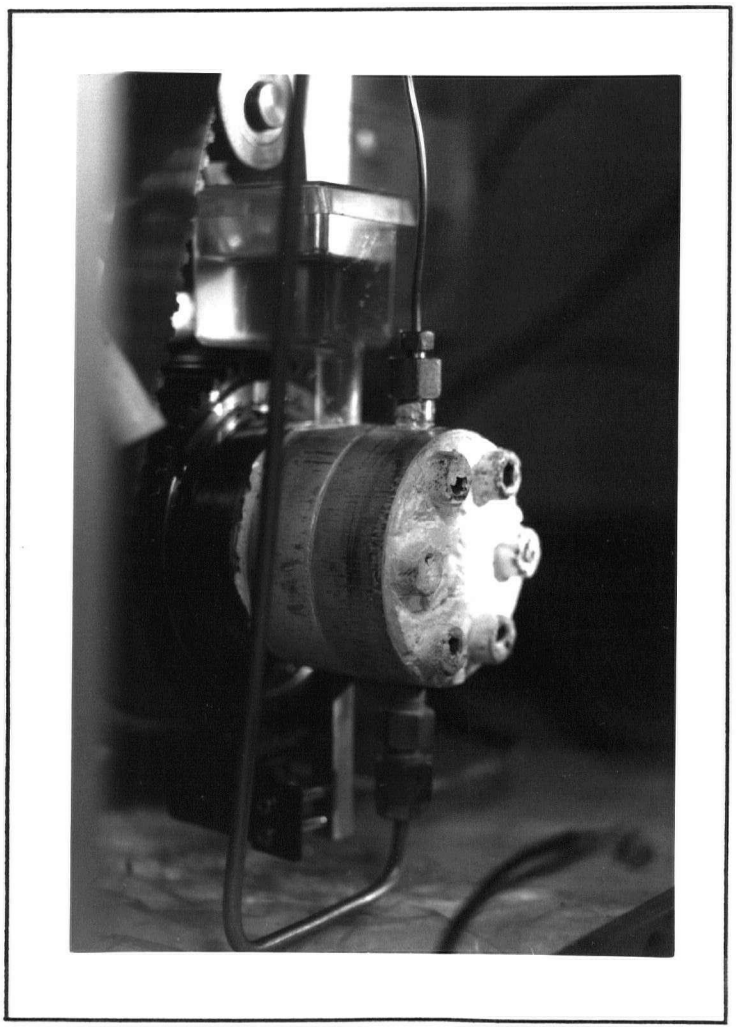

FIGURE 4.3 Photograph of the HPLC pump with the cooling collar removed.

allowing the flow rate to 'float'. If the requested flowrate is significantly higher than the maximum flow possible for a given pressure, the system will operate in the second mode.

Liquid carbon dioxide at room temperature is difficult to pump because of its high compressibility (Vukalovich and Altunin, 1968). For this reason the CO_2 utilized in the present experiments was cooled to approximately -5 °C by circulating cold ethylene glycol solution (at about 0.25 L/min) through an aluminum collar fitted to the pump-head (Fig. 4.4). The temperature of the glycol solution was lowered to -20°C in a Lauda-Brinkman circulating bath. The pump (with the cooling collar) and the coolant flow lines were insulated with 1 cm thick refrigeration foam-rubber tubing (Fleck Bros. Ltd., Appendix III).

4.2.4 Extraction Vessels

Four extraction vessels (autoclaves) were used during the course of this work (Fig. 4.5). All of the vessels were fabricated 'in-house' from 316 stainless steel round stock, with the exception of vessel #4, which was a standard unpacked HPLC column (Supelco Corp., Appendix III) each of the vessels can be envisaged as being basically a thick-walled tube with a flanged top. Figure 4.6 is a machine drawing of the medium size vessel.

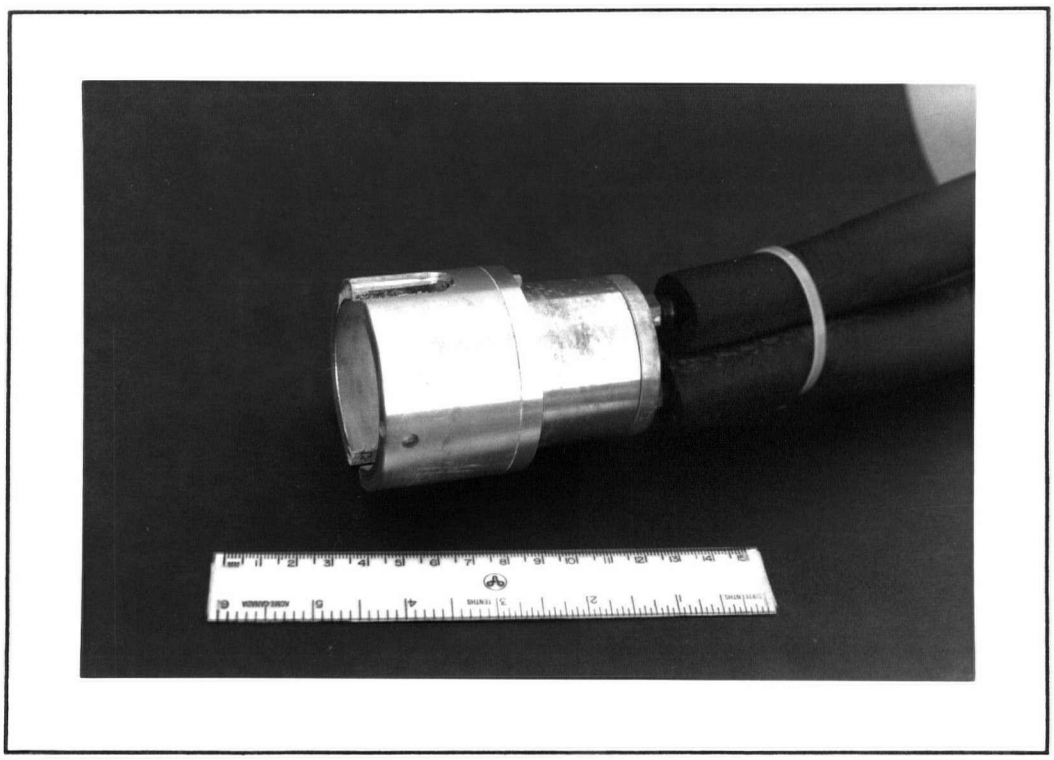

FIGURE 4.4 Photograph of the aluminum collar which was used to cool the pumphead on the HPLC. The coolant delivery and return lines, with insulation, can be seen at the right.

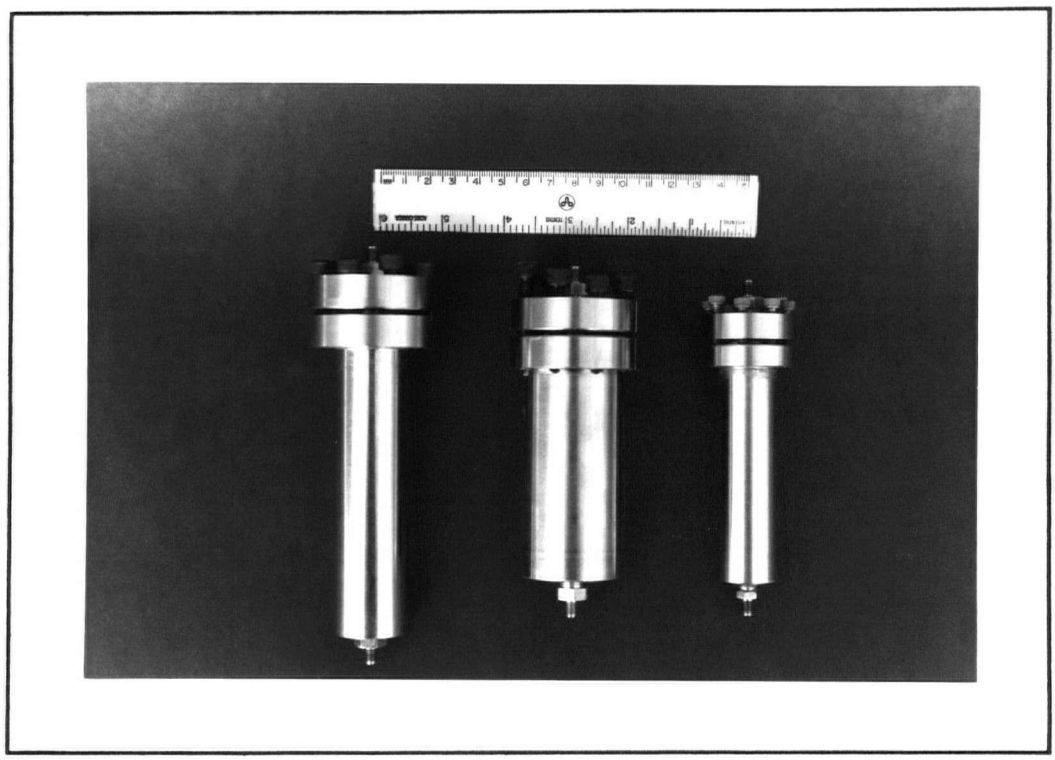

FIGURE 4.5 Photograph of the three extraction vessels used in the experiments. In the photograph the tops of the vessels are installed.

TABLE 4.1 Dimensions of the extraction vessels used in the experiments.

vessel number	inside diameter (cm)	inside length (cm)	vessel volume (cm^3)	vessel wall thickness (cm)
1	1.27	8.2	10.4	0.6
2	1.27	11.4	14.4	0.6
3	2.54	8.2	41.6	0.8
4	0.48	30.4	5.4	0.1

The tops of the vessels were fabricated with a 'sealing ridge' around their periphery. A seal between the top and the body of the autoclave was made by securing the top, with its 'sealing ridge' to the sealing surface of the vessel, using eight stainless steel cap screws (Figs. 4.7 and 4.8). The bolt size for the small vessel was 10/24 (Unified National Coarse (UNC)) and for the large vessels 1/4" UNC. Since stainless steel tends to 'weld' to itself, the bolts were lightly lubricated with a copper-containing anti-seize compound (Jet-Lube SS-30, Fleck Bros. Ltd., Appendix III) prior to each use. Each of the 10/24 bolts was tightened to 50 in-lbs. The 1/4" bolts were tightened to 100 in-lbs.

The bottom and top of the extraction vessels were fitted with 1/8" National Pipe Thread (NPT) X 1/16" Swagelok tube fittings (Columbia Valve and Fitting, Appendix III). Solvent flow-line connections to and from the vessels were made via these fittings. In Table 4.1 the size specifications for each of

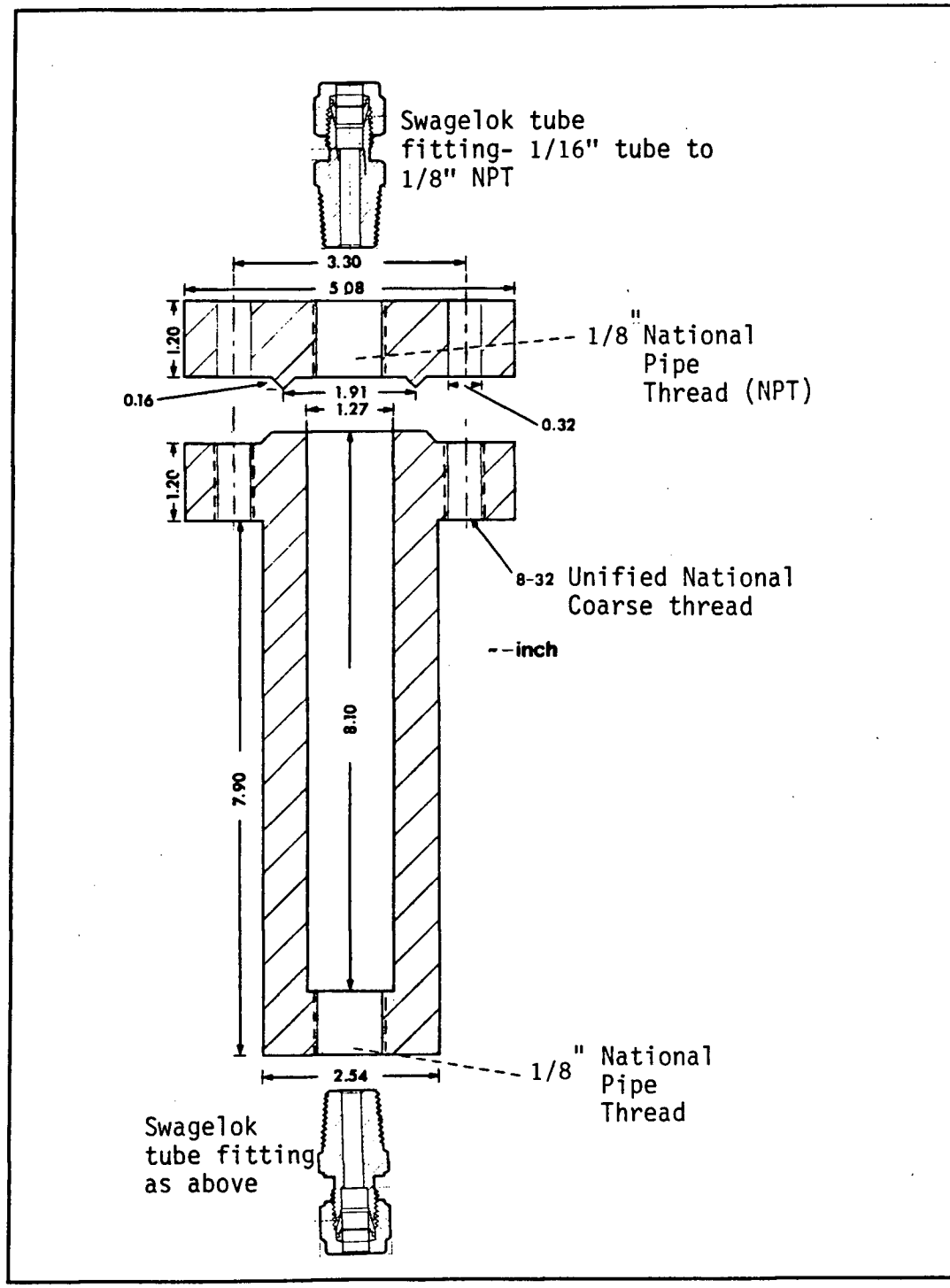

FIGURE 4.6 Cross-sectional view of extractor vessel #1 (Table 4.1). All measurements shown are in cm, except were indicated.

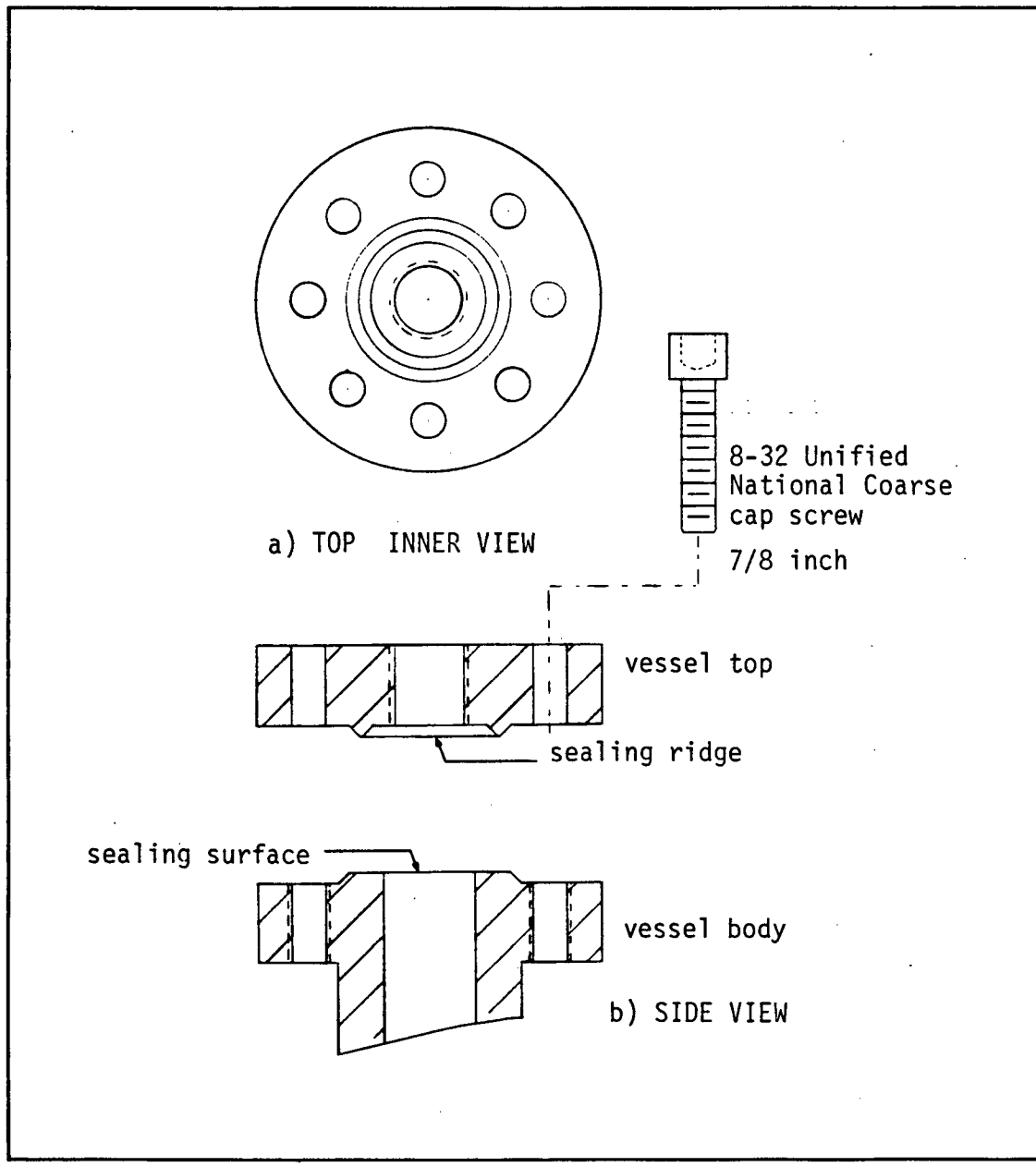

FIGURE 4.7 Details of the extraction vessel seal. a) view from the inner surface of the extractor top showing the circular sealing ridge. b) cross-sectional view of a portion of the extractor and top, showing the sealing surface and sealing ridge.

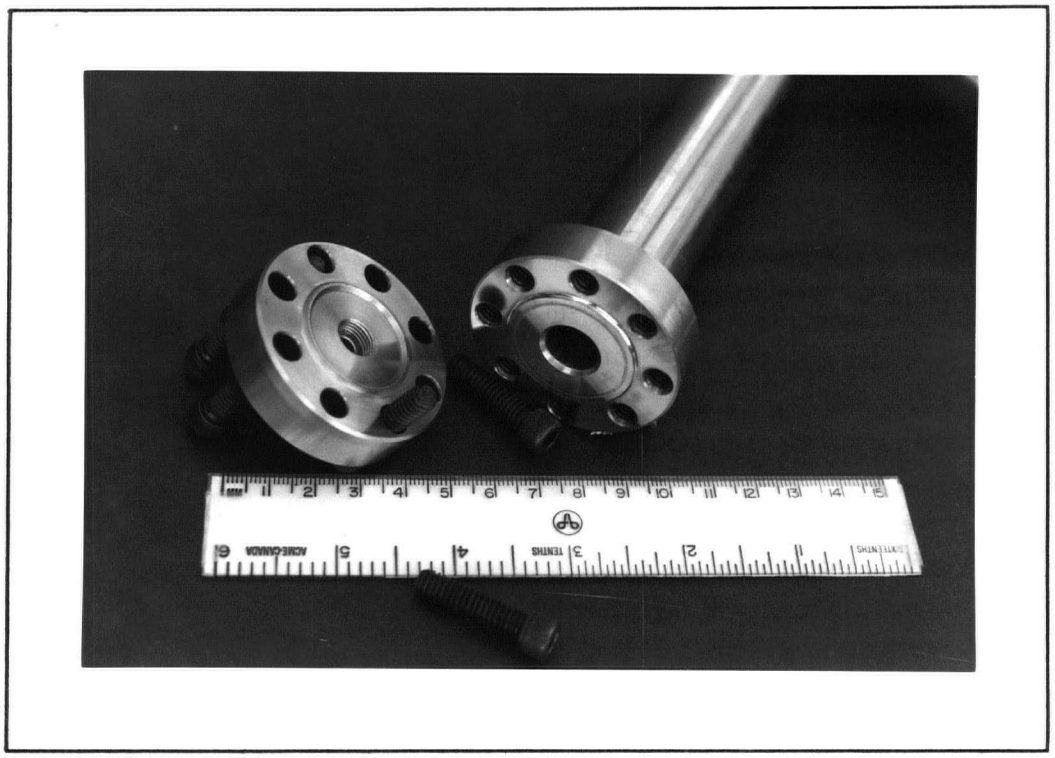

FIGURE 4.8 Photograph of details of the sealing arrangement on vessel #2.

the extraction vessels are listed.

4.2.5 Flow Restricter

The purpose of the flow restricter is to maintain the pressure in the extractor at the desired value. The operation of the extraction system is affected by the characteristics of the flow restricter. In principle the device is quite simple, but a valve suitable for use at the desired high pressures and low flow rates was not available and had to be developed.

A variety of flow restricters were assessed early in the work with limited success. The simplest, a piece of 1/16" OD crimped stainless steel tubing tended to freeze up periodically and plug. Additionally, its flow rate could not be varied easily.

Two metering valves, one made by Whitey and the other by Nupro (Columbia Valve and Fitting, Appendix III) were subsequently used, but both had problems with leaking seals and could not be precisely regulated. Eventually it was found that a Parker MV-200 metering valve (Surrey Fluid and Power, Appendix III) could be used to adequately control the CO_2 flow through the system.

The Parker valve was designed as a high pressure, liquid metering valve. Its operation in the present extraction system was unconventional because the CO_2 flow was not adjusted by turning the valve stem, but by fixing it in one position and varying the temperature of the valve thereby causing the orifice to expand or contract. This modification allowed the flow resistance of the valve to be varied precisely and, as a result,

the system pressure and flow rate could be maintained constant and independent of fluid viscosity.

The valve was located in the HPLC oven and mounted in a heated aluminum block (5cm X 3cm X 2cm) (Fig. 4.9). The valve was fitted with two 1/8" NPT X 1/16" Swagelok adapters and installed so that the stream of supercritical CO_2 entered via the top of the valve and the mixture of gaseous CO_2 and liquid oil exited via the bottom. The aluminum block was insulated with about 1 cm of glass fibre wool and heated with a 100w Chromalux cartridge heater (Chromalux Can., Appendix III) installed in the block. A heating circuit based on the Radio Corporation of America (RCA) 3059 integrated circuit (refer to RCA Application Note ICAN 6182, 1978) was constructed and used to control the temperature of the heating block to within ± 0.5°C. In Fig. 4.10 the restricter valve is shown installed in the HPLC oven.

4.2.6 Extract Collection Systems

Upon passing through the restricter, the carbon dioxide within the system changes from the supercritical to the gaseous state. Commensurate with this change of state is a large decrease in the solvation capacity of the carbon dioxide. Compounds such as oils, soluble in the CO_2 on the upstream side of the restricter, precipitate downstream of the restricter. The sampling systems allowed the separation and collection of the resulting oil droplets.

Two extract sampling systems were used during the course of the experiments. The first system consisted of a 25 cm stainless steel tube (0.1mm ID) attached to the restricter by a 1/8" NPT X

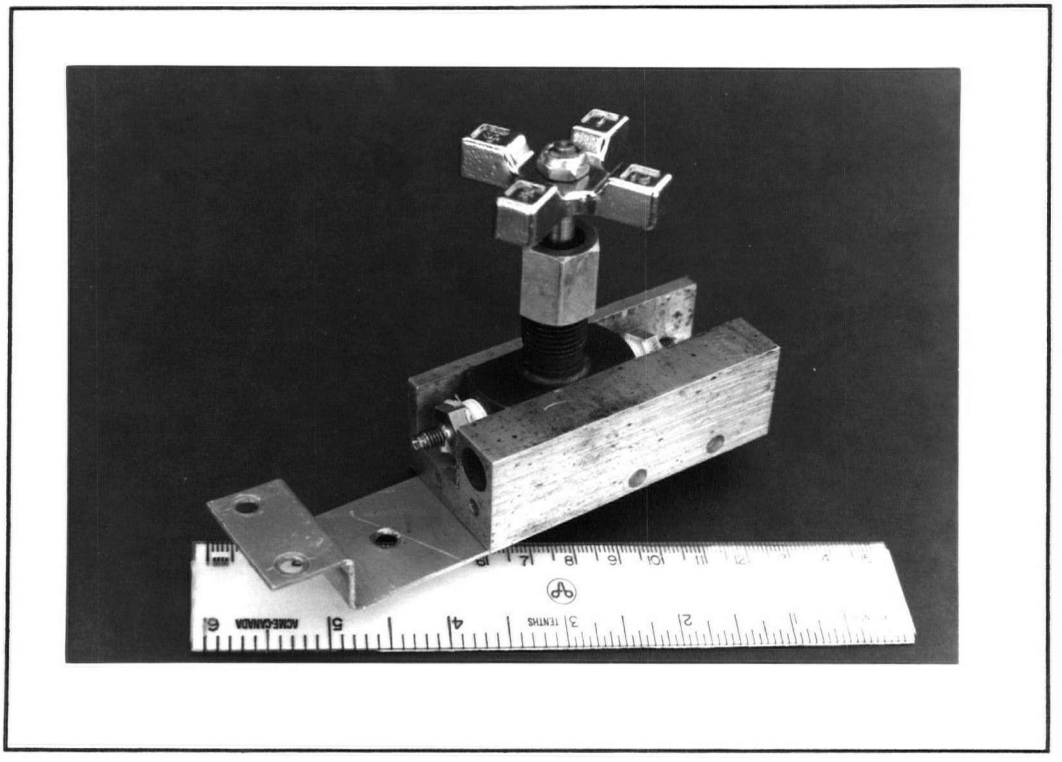

FIGURE 4.9 Photograph of the MV-200s restricter valve shown in the aluminum block which was used to regulate its temperature.

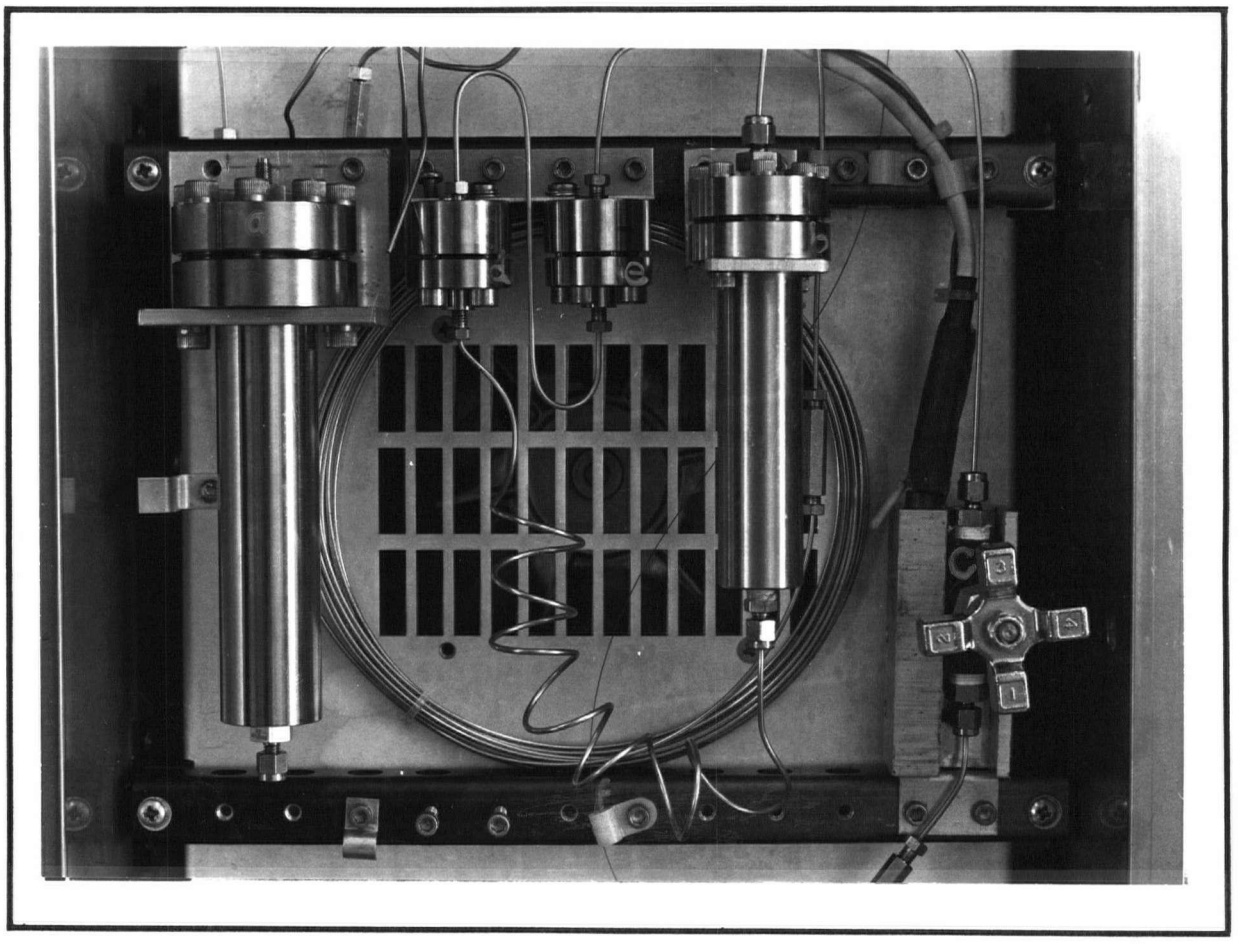

FIGURE 4.10 Photograph of the oven in the extraction system. In the picture the extraction vessels (a,b), the restricter valve with heating block (c), the stainless steel filters (d,e) and the silica sampling tube (f) are visible.

1/16" Swagelok tube union. The tube was directed to the exterior of the instrument via the oven wall. A piece of folded 7cm filter paper was secured to the end of the tube using a paper clip such that the flow of CO_2 passed into the envelope of the folded paper. This sampling system, although effective and used during the initial experiments, tended to be somewhat cumbersome, and if used improperly, led to low values for the oil collected.

The second sampling system was more complex, but allowed sequential oil samples to be collected and the carbon dioxide to be measured by a wet test meter. The system, depicted in Fig. 4.11, was as follows: a 5 cm long section of 0.1 mm ID stainless steel tubing was connected to the restricter valve using a Swagelok adapter. The opposite end of the section was connected to one end of a Valco zero dead-volume 1/16" HPLC union (Chromatographic Specialties, Appendix III). A 60 cm, 0.2 mm ID fused silica tube (Supelco Corp. Appendix III) was inserted and sealed using a 'Micro Graphite Ferrule' (Supelco Corp.). The other end of the tubing was then channelled through the oven wall to the exterior of the instrument and directed into the top of the sampling head, where it passed concentrically down and through a 5 cm, 0.8 mm OD glass capillary tube into the collection vial (Fig. 4.12). The two phase gas and entrained oil mixture, flowed through the silica tube into the sample vial where the oil was deposited. The oil-free CO_2 then flowed from the vial up a glass capillary and entered the wet test meter.

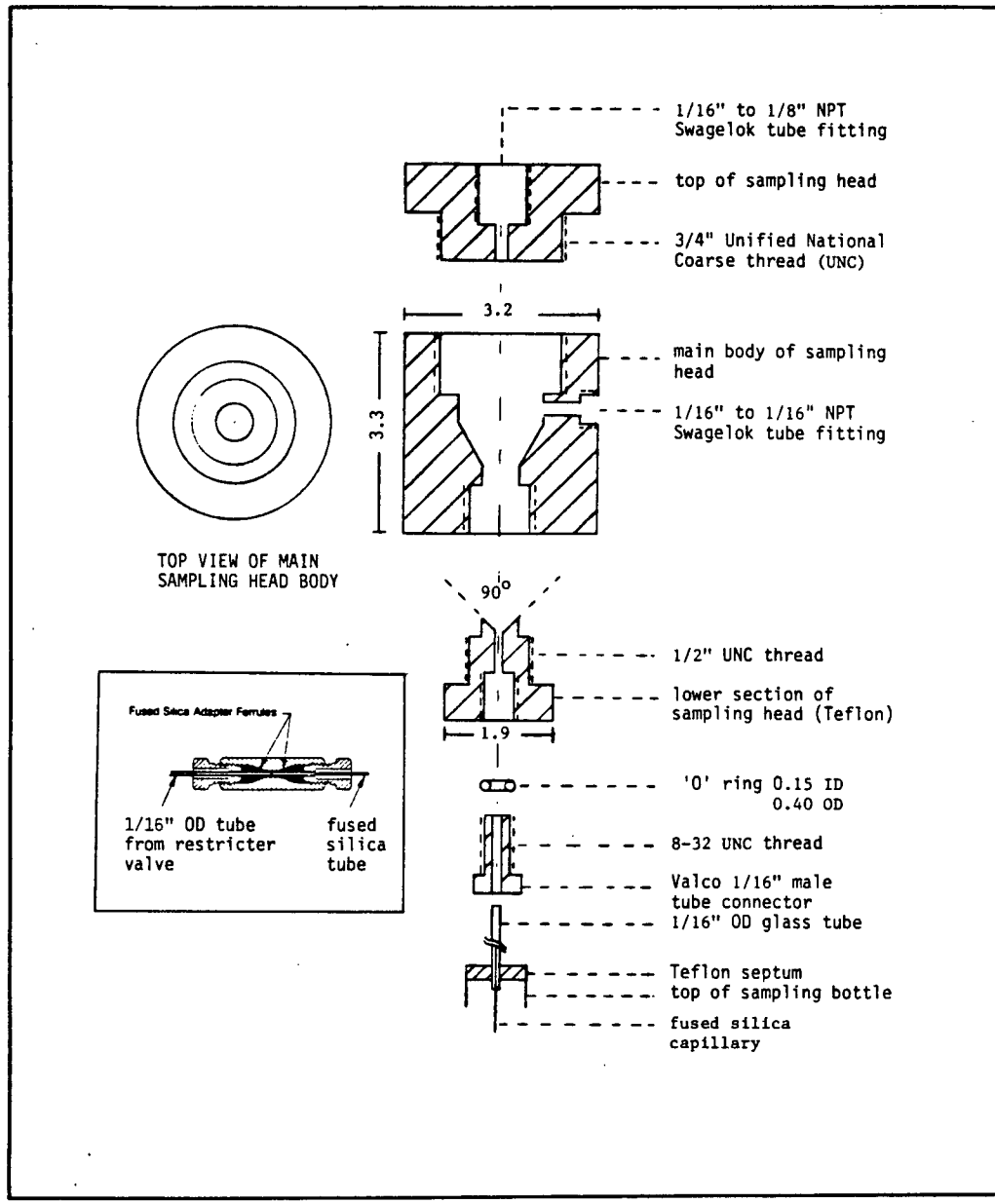

FIGURE 4.11 Cross-sectional diagram of the sampling head with a top view of the main body of the sampler. All measurements shown are in cm unless otherwise specified. All of the components of the head which are shown were made from 316 stainless steel, except where otherwise indicated. The inset is a diagram of a 1/16" Valco tube union. The union was used to couple the exit tube from the restricter to the fused silica inlet tube of the sampling system.

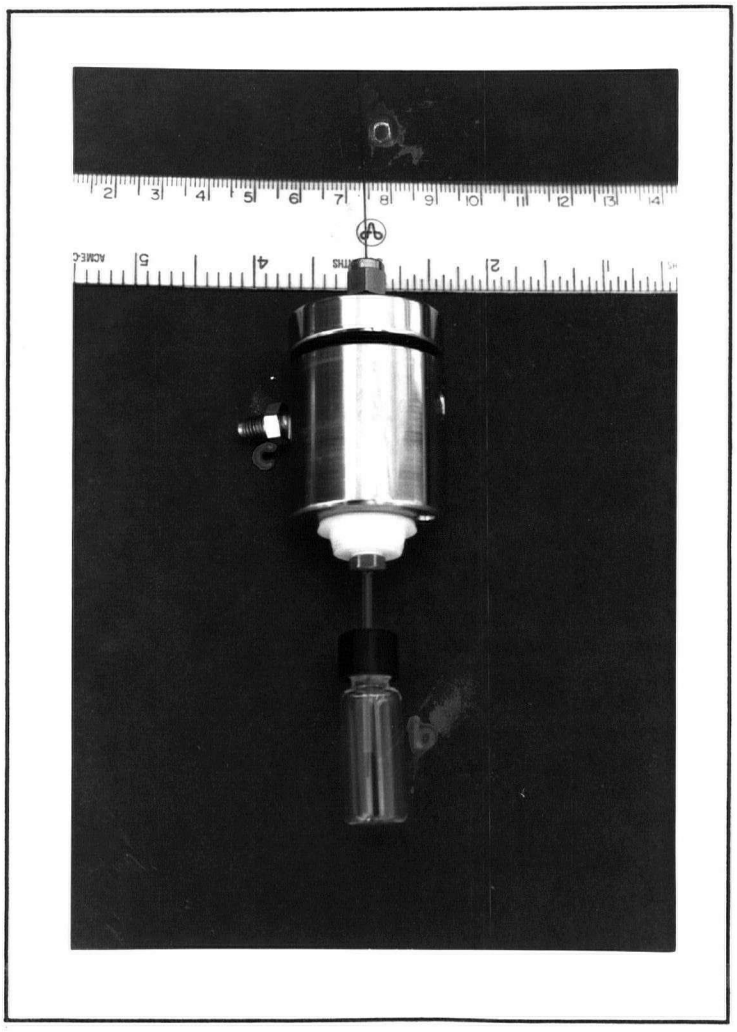

FIGURE 4.12 Photograph of the extract collection system which was used to separate and collect the oil from the CO_2. In the picture the silica tube (a), the collection vial (b) and the CO_2 exhaust fitting (c) are visible.

The sampling head (Fig. 4.11) consisted of a 5 cm long X 3 cm OD stainless steel cylinder with openings for the silica tube, the glass capillary tube and the gas exit tube. Although the cylinder was constructed in two parts, a 2.5 cm 'O' ring placed between the sections ensured that the unit was gas-tight. A 0.4 mm OD 'O' ring was also used to ensure a seal between the sampling head and the silica tube. In order to allow the glass capillary a certain amount of flexibility, it was secured in a Teflon plug inserted into the bottom of the sampling head.

4.2.7 Equipment Calibration

The extraction equipment was checked periodically to ensure that the solvent flowrate, oven temperature and system pressure were accurately displayed. The calibration procedures were performed in accordance with Section 5 of the Hewlett Packard 1081B service manual. The accuracy of the parameters are listed in Table 4.2.

TABLE 4.2 Errors associated with the various system parameters as determined experimentally.

parameter	error
oven temperature	<±2%
extractor pressure	±2%
CO_2 flowrate	<±3%
total CO_2 volume	<±0.5%

4.3 Materials

4.3.1 Carbon Dioxide

The carbon dioxide that was used in the experiments was obtained from pressurized steel cylinders supplied by Medigas Pacific (Appendix III). Each cylinder held 30 kg of carbon dioxide which could be withdrawn in liquid form through a siphon tube within the cylinder. The amount of carbon dioxide remaining in the cylinder at any time was determined by subtracting the tare weight of the cylinder (marked on the wall of the cylinder) from its actual weight. Approximately 200-300 h of system run time were obtained with each cylinder. The carbon dioxide specifications are listed in Table 4.3.

TABLE 4.3 Specifications of commercial siphon grade carbon dioxide

USP siphon grade carbon dioxide
>99% pure
<10 ppm CO
<1 ppm H_2S
<5 ppm NO_2
<0.3 ppm $COCl_2$(phosgene)
<5 ppm SO_2
<25 ppm H_2O

4.3.2 Seed Samples

Two varieties of Rapeseed are currently grown in Canada, the "Polish" species (*Brassica campestris*), and the "Argentine" species (*Brassica napus*). Genetic strains, or cultivars within these varieties are numerous (and all are

named eg. "Midas", "Torch", ,"Regent,"Candle"), due to continuous breeding programs designed to improve both the agronomics of the plant as well as its oil quality. Unlike the seed originally introduced into Canada in 1943, most of the rapeseed presently grown in Canada, contains only small amounts of erucic acid and glucosinolates. This modern version of the seed is termed "Canola".

One cultivar from each of the two varieties of Canola was used in this work. The first, a dark colored seed called "Regent", is a member of the Argentine species and was obtained from a commercial processor (CSP Foods, Appendix III). The second cultivar "Candle", is a member of the Polish variety and was obtained from the Food Science Department of the University of British Columbia.

4.3.3 Oil Samples

Four Canola oil samples were used in the experiments. Three of these samples, "crude", "acid degummed", and "refined" were obtained from CSP Foods Ltd. All were extracts of the "Regent" cultivar. The fourth sample was a commercially refined bleached and deodorized oil ("Scotchbuy") available in local supermarkets.

The crude oil sample was a mixture of the oil obtained from 'pressing' the seed and the hexane extract. The acid degummed oil was produced by subjecting the crude oil to an acid treatment procedure, during which phospholipid material is removed from the oil (sec. 1.2.3). The refined oil is an acid degummed product, essentially free of fatty acids, phosphatides

and proteinaceous and mucilagenous material.

4.3.4 Simple Triglycerides

Triacylglycerols, more commonly referred to as triglycerides, are glycerol esters derived from several different carboxylic acids. The number of different triglycerides is very large and many are commercially available. Three were obtained for this research project and used to represent the triglycerides found in Canola oil.

All of the triglycerides were obtained from Nu-Chek Prep Ltd. (Appendix III). The specifications for each are given in Table 4.4.

TABLE 4.4 Specifications of the triglyceride samples used during the experiments

name	acid	molecular formula	molecular weight	purity
tripalmitolein	C16:1	$C_{51}H_{92}O_6$	801	>99%
trilolein	C18:1	$C_{57}H_{104}O_6$	885	>99%
tri-11-eicosenoin	C20:1	$C_{63}H_{116}O_6$	969	>99%

When not in use, the triglycerides were stored at -20 °C.

4.4 Seed Treatment Methods Prior To Extraction

4.4.1 Introduction

The Canola seed used during the course of this work was usually treated prior to extraction in one or two ways. In one of the treatments the seed was physically ruptured by various techniques. In the other treatment the physically ruptured seed was extracted with a solvent to reduce its overall oil content. This material in turn, was used to provide data on oil solubility in the CO_2 as a function of seed-oil concentration.

4.4.2 Seed Crushing

The seed was crushed using a 20 cm mortar and pestle. Approximately 10 g of whole seed were placed into the mortar. Crushing of the seed was done by hand over a five minute period. The crushed seed material was free flowing and contained particles ranging from approximately 0.1 mm to 0.5 mm. For details see section 5.4.2.

4.4.3 Seed Chopping

Finely chopped seed material was produced by placing 50 g of whole seed into a 2L 'Osterizer blender', model "Cyclo-Trol-Ten" for 5 min on the "blend" setting. The seed material produced in this manner was more homogeneous than the crushed material. Individual particles ranged in size from about 0.05 mm to 0.1 mm (sec. 5.4.2).

4.4.4 Flaking And Cooking Of Seed

The flaked seed material was procured from a commercial seed processor (CSP). The material was produced by forcing whole seed through a series of roller mills. During the flaking process the seed is crushed and flattened (rupturing most of the seeds' cell walls) which renders the material more susceptible to solvent extraction. Typical thicknesses of the seed flakes range from 0.2 - 0.5 mm. After flaking, the seed is subjected to a short heating process (90 C, 0.5 h). This serves to inactivate certain undesirable enzymes and to enhance the extractability of the material (Teasdale and Mag, 1983). Seed treated by this method is referred to as having been"cooked". For a size distribution of the particles see Sec. 5.4.2.

4.4.5 Pressure Rupturing

Pressure rupturing is a technique which involves placing a biological material in a high pressure gas for an appropriate length of time (typically 60 min). During this time the pressurized gas penetrates the material. The pressure is then quickly released and the contained gas expands, rupturing the cells.

The procedure for preparing pressure ruptured seed was as follows. Samples of whole seed (typically 12 g) were placed into extraction vessel #3. The extractor was then placed in the HPLC oven for about 30 min and allowed to equilibrate at 55°C. The pump was then turned on for about 5 minutes, during which time the vessel pressure rose to 36.0 MPa. Because the restricter was closed, there was no flow of carbon dioxide through the vessel.

At the end of one hour, the top Swagelok fitting on the extractor was loosened thereby allowing the carbon dioxide to escape rapidly. The ruptured seed material was then removed from the vessel.

The pressure-ruptured seeds exhibited a wide variation in particle size ranging from 1 mm (whole seed) to 0.05 mm; the majority of the fragments was greater than 0.5 mm.

4.4.6 Partial Extraction Of Seed

The natural concentration of oil in Canola seed is about 0.6 g of oil/g oil-free seed. Certain experiments however called for seed material with an oil content less than this. Furthermore, it was required that these samples exhibit a range of concentrations and that the concentration of each be known. Two methods were used to produce these samples. The first method described is a standard solvent extraction procedure which utilized hexane as the extracting solvent. In the second method the seeds were extracted under high pressure using a stream of supercritical carbon dioxide.

4.4.6.1 Partial Extraction With Hexane

Twelve, ceramic, extraction thimbles (2 cm) were weighed to the nearest 0.001 g. Into each of the thimbles were placed 10 g of crushed seed material. All the thimbles were then transferred into a Goldfisch Extraction unit and extracted with hexane. After allowing the extraction to proceed for various lengths of time (depending upon the degree of extraction desired), the thimbles were removed (in pairs) and the seed material within

each thimble was dried overnight at 40 °C. The seed material in each thimble was then mixed and subjected to a total oil determination (Section 4.6.1) in order to establish the degree of extraction. The average value between pairs was used as the working oil concentration. In this manner samples were prepared ranging in oil concentrations from 0.05 g oil/ g oil-free seed to 0.5 g oil/ g oil-free seed.

4.4.6.2 Partial Extraction With Carbon Dioxide

The apparatus that was used to extract the seed material with CO_2 is described by Campbell (1983). It consisted of a 1 L temperature controlled, stainless steel vessel, an Aminco double ended diaphragm compressor, a restricter valve and a CO_2 source. During the extraction, CO_2 flowed from the source tank into the compressor where its pressure was raised from 6 MPa to 36 MPa. From here the CO_2 flowed through the heated autoclave (entering via the bottom and exiting through the top) and then through the pressure restricter valve. The system pressure was a function of the CO_2 flow rate and the restricter valve orifice size. A variable speed motor, magnetically coupled to a stirring mechanism within the autoclave allowed the vessel contents to be continuously mixed.

The extent of the extraction was determined by the length of time the seed material was exposed to the CO_2 gas stream. A typical extraction run was as follows: with the autoclave at working temperature (60°C), a sample (100 g) of crushed seed material was placed in the bottom of the vessel. The top of the

autoclave (and the stirring mechanism) were set in place and secured. The stirring rate was typically 120 rpm. The vessel was then flooded with CO_2 and the compressor activated. By adjusting the restricter valve, the pressure within the system could be maintained at 36.0 MPa for the required length of time. At the end of the run, the system was depressurized (over 1 h), and the seed material removed. To establish the degree of extraction which had taken place, the seed was subjected to a total oil determination (Sec. 4.6.1.). The samples produced in this manner had oil concentrations similar to those discussed in the previous section.

4.5 Experimental Extraction Procedures

4.5.1 Vessel Loading

4.5.1.1 Seed Material

At the beginning of each experiment, a weighed amount of seed material (normally 4 g for vessel #1, 7 g for vessel #2, 12 g for vessel #3 and 1.5 g for vessel #4) was placed in the extractor. Fine-spun glass wool was placed at both ends of the vessel to prevent small particles from entering the tubing. Before closing the top of the autoclave, both the extractor and the sealing surface were wiped free from dirt using a chloroform-wetted tissue. In addition, all fibres of glass wool protruding from the vessel were cut and removed. It was noted that even a single strand of the glass prevented the completion of the seal and could permanently destroy it.

4.5.1.2 Liquid Oil Samples

Before using the autoclaves with the liquid Canola oil or

the triglyceride samples, the vessels were first partially filled with fine (0.3mm) Ottawa sand. The liquid samples were then deposited on the Ottawa sand. This procedure was used for two reasons: 1) the liquid on a matrix of Ottawa sand presented a larger surface area available for mass transfer than would an equal mass of liquid in the empty vessel; 2) the liquid on the Ottawa sand could release dissolved CO_2 during the de-compression stage of the extraction experiments more effectively thereby reducing the risk of the liquid foaming out of the vessel.

The procedure by which the liquid samples were loaded into the vessels was as follows: a known weight of Ottawa sand--enough to approximately half-fill each vessel--was placed in the respective autoclave on top of a small plug of glass wool. The liquid sample, typically in the range of 1 to 2 g, was then pipetted on top of the beads and allowed to 'sink in' over a period of ten minutes. By performing the procedure in a glass test-tube, it was established that the method results in an even distribution of oil throughout the bed. The mass of liquid transferred was determined to the nearest 0.001 g by weighing the pipette before and after the liquid transfer. Before fitting the top of the vessel, another plug of glass wool was inserted. During extraction the CO_2 flow through the vessel was from bottom to top.

4.5.2 Equipment Startup

Approximately 2 h prior to the start of an experiment, the cooler and HPLC were switched on. The cooler was typically set to -20 °C. During this pre-extraction period the autoclave was washed with chloroform and then acetone. Additionally, the flow system downstream of the vessel, including the frits and the extract collection system, was flushed with about 20 mL of a 1/1 chloroform/methanol solution. In order to remove any last trace of solvents, the system was assembled and flushed with supercritical CO_2 for 1 h. Following the system flushing the vessel was placed in the oven and the feed and exit tubes were connected. The shut-off valve on the CO_2 cylinder was then opened and the restricter valve set to allow a carbon dioxide flow of approximately 50 mL/min at Standard Temperature and Pressure (STP). The temperature of the oven was set to the desired value (usually 55 °C) while the restricter valve, was usually set to 65°C. The system remained in this 'pre-run' state for approximately 30 min. By placing a temperature probe in the bed it was established that this period of time was sufficient for temperature equilibration. At the end of this period, pressure and flow setpoints were entered.

In the majority of experiments the system was required to operate in a 'pressure tracking' mode (Sec. 4.2.4). In order to ensure this, the desired experimental pressure was entered while the flow rate setpoint (usually 9.9 mL/min) was adjusted to greatly exceed the value which corresponded to this pressure.

The pump was next switched on by pressing the "pre-run"

button on the HPLC. Once the pump was activated, the chart recorder, which provided a permanent record of the CO_2 flow and pressure, was turned on and the coarse control on the restricter adjusted. This adjustment set the flow of carbon dioxide through the vessel at between 0.5 and 1.5 g /min.

4.5.3 Extract Sampling

The sampling procedure normally began immediately after the system flow stabilized, i.e. about 5 to 10 min after activating the pump.

Two sampling procedures were employed. In the first of these a piece of folded 10 cm filter paper was used to collect the oil. The mass of oil collected was determined by weighing the paper before and after using it. The amount of CO_2 used during each sampling period was determined from the digital flow gauge on the instrument.

The first sampling procedure was used in the initial stage of the project. This stage included the initial experiments in which the solubility of Canola oil in CO_2 was determined as a function of pressure and temperature. These experiments were repeated subsequently using the second sampling procedure.

In the second sampling procedure the apparatus shown in Fig. 4.12 was used. The method consisted of fitting, pre-weighed, 1.8 mL., glass vials on the sampling tube for varying amounts of time. From the 'before' and 'after' weights of each vial, the oil collected during that period could be determined. The volume of gaseous carbon dioxide that passed through each vial during the sampling period was determined using the wet

test meter. The corresponding mass of CO_2 was calculated from the CO_2 molar volume corresponding to the temperature of the wet test meter. As each vial was removed and replaced with a new one, a mark was made on the chart recorder. From the marks and known chart speed, the length of time that each bottle was exposed to the flow of gas could be determined, as well as the flowrate of the CO_2 during the sampling period.

4.5.4 Solubility Determinations

The average oil concentration in the CO_2 at the extractor outlet, at any interval during the extraction, can be determined from the mass of oil collected during the interval and the mass of CO_2 passed. If the oil concentration measurement is made during the initial linear portion of the extraction curve (under certain conditions), this measurement will represent the solubility of the oil in the CO_2 at that pressure and temperature. Two procedures were used to ensure that this concentration measurement did in fact represent the oil solubility.

In the first procedure, two separate extractions were performed on identical samples of seed under identical conditions of pressure, temperature and carbon dioxide flowrate. The only difference between the two extractions was that in the second extraction the length of the seed bed was increased. If the slope of the linear part of the second extraction curve was identical to the first, it indicated that the increased bed length did not increase the concentration of the oil in the CO_2 phase. This in turn suggested that the CO_2 was saturated and

that the slope of the curves could be used to calculate the oil solubility.

In the second procedure the extraction was stopped during the 'linear phase' and the bed of seed divided into sections and each section analyzed for its oil content. If the oil content of the section of seed nearest the extractor outlet was still equal to its original concentration, it indicated that the carbon dioxide was saturated prior to reaching this segment. This in turn is proof that the slope of the linear portion of the extraction curve represented the oil solubility.

4.5.5 Equipment Shutdown

At the completion of an experiment, the pump was turned off by setting the system into the "standby" condition and the shut-off valve on the CO_2 cylinder was closed. The system typically depressurized to ambient over a period of 1 h at which time the equipment was turned off and the extraction vessel was removed.

4.6 Analytical Procedures

4.6.1 Total Seed Oil Determinations

The total amount of oil within the seed was determined by placing pre-weighed amounts of the seed into cellulose extraction thimbles and extracting them with hexane for 8 h at 60°C as outlined in International Union of Pure and Applied Chemistry (IUPAC) Method I.B.2 (Paquot, 1979) The extracted seed samples were then removed and the solvent evaporated at 40°C for an additional 24 h in a small, air-convection oven. By subtracting the weight of the dried seed tissue from its weight

prior to extraction, the total amount of hexane extractable oil within each sample could be determined. At the same time the hexane extracts from the samples were evaporated at room temperature for 24 h and the collected oil determined from the before and after weight of the receiving flasks. Each of the extractions was done in triplicate. The final accepted value of oil in the seed was the arithmetic mean of the three.

4.6.2 Scanning Electron Microscopy

A number of examinations of Canola seed, in a crushed and whole state, were performed using the scanning electron microscope (SEM). The instrument used was an Etec Autoscan with a maximum resolution of 20 nm. The methods by which the seed samples were collected and prepared prior to their examination are described below.

4.6.2.1 Sample Collection

In order to investigate the seed material at different stages of extraction, samples of unextracted, partially extracted and fully extracted seed were first obtained. This was done in the following manner.

A 4 g sample of flaked seed was placed in the small autoclave and extracted with CO_2 at 36 MPa and 55 °C. Approximately halfway through the linear phase of the extraction, the experiment was stopped and the system allowed to depressurize slowly. The bed of seed was then divided into five equal sections with each section representing a different stage of the extraction.

4.6.2.2 Sample Preparation For SEM

All of the seed samples, whether whole or crushed, were first coated with an alloy of gold-palladium in a Technics Hummer (model 4) sputter coater. The maximum plating current which was used during the coating was 4 milliamperes. All of the samples were coated for three, 1 min intervals, which gave a coating thickness of approximately 2000 nm.

4.6.3 Seed-bed Sectioning Method

At the completion of certain experiments the contents of the extraction vessel were sectioned into several equal volume portions. This was done in the following manner. The lid of the vessel was removed and the glass wool on top of the seed material was pulled out. A 0.6 mm glass tube attached to a flexible Tygon hose, which in turn was connected to a 25 ml vacuum flask, was next inserted into the vessel. By applying a vacuum to the flask, the seed material could be removed from the vessel layer by layer without being mixed. A small plug of glass wool prevented the seed material from being swept into the main vacuum line.

4.6.4 Fatty Acid Analysis

The fatty acid analysis was performed in two steps. In the first step the fatty acid moieties were cleaved from the triglycerides and simultaneously converted to their methyl esters. In the second step, the methyl esters were identified and quantified using gas chromatography.

4.6.4.1 Transesterification

The fatty acid composition of triglycerides is one useful criterion for characterizing animal and vegetable oils (Ackman, 1983). Normally, triglycerides are first saponified and the resulting fatty acids converted to their methyl esters prior to GC analysis. By contrast, the transesterification procedure converts triglycerides directly into their component fatty acid methyl esters (Knapp, 1979).

The transesterification procedure used in this work is based on a method developed by Shehata et al.(1970). The reaction was carried out at room temperature using sodium methoxide($NaOCH_3$) in a single-phase mixture of methanol, petroleum ether and diethyl ether. When the reaction was complete, the mixture was forced to separate into two phases (by the addition of a small amount of water); the fatty acid methyl esters dissolve in the petroleum ether phase. The petroleum ether, and the dissolved methyl esters were then removed from the reaction vial by pipette. The specific details of the technique are as follows.

A 0.5 N solution of sodium methoxide (Fisher Scientific, Appendix III) in methanol was prepared by adding 0.675 g of the anhydrous powder to 25 mL of absolute methanol. To another 25 mL flask, containing 8.5 mL of anhydrous diethyl ether and 5.0 mL of petroleum ether, 12.5 mL of the sodium methoxide solution were added. The esterification reagent was prepared on a weekly basis and stored at -10°C in a capped flask. The esterification reaction and associated vessels were 1.8 mL borosilicate-glass

screw top vials equipped with Teflon septa. Disposable pipettes were used to transfer liquids between vials. All weights were determined to within ±0.001 g by using a Mettler PT-320 balance.

The esterification procedure was as follows: between 15 and 50 mg (1 to 3 drops) of the oil was quantitatively transferred to the reaction vial. This was done by weighing the oil-containing transfer pipette both before and after transfer. The esterification reagent (1 mL) was then added to the vial. The vial was capped, shaken to dissolve the oil, and allowed to stand for two minutes. After this time, the mixture was forced to separate into two phases by adding 0.5 mL of petroleum ether and one drop of water. The resulting mixture was then shaken for about 30 s to facilitate the transfer of the methyl esters into the petroleum ether phase. The reaction vial and its contents were then centrifuged for 10 min in order to remove any suspended sodium methoxide. After centrifuging, the top 2 mm of the petroleum ether layer, which contained the methyl esters, were transferred by pipette for further analysis to another 1.8 mL vial containing a few grains of anhydrous sodium sulphate (Na_2SO_4).

4.6.4.2 Gas Chromatographic Procedure

The fatty acid ester solution was analyzed using a Perkin-Elmer(PE) Sigma 2 gas chromatograph (GC), connected to a PE Sigma 10 data system. The esters were separated with a 6 ft. X 1/8 in. stainless steel column packed with SP-2330 on 100/120 mesh Chromosorb WAW. The column was obtained pre-packed from

Supelco Corp. (Appendix III). All GC analyses were performed in accordance with the conditions shown in Table 4.5.

TABLE 4.5 Gas chromatograph parameters for the fatty acid methyl ester analyses.

column	SP-2330 on 100/120 mesh Chromosorb WAW
column temperature	200 °C isothermal
detector temperature	250 °C
injector temperature	250 °C
carrier gas	helium
carrier gas flow	20 cm^3/min
sample size	1 μL
detector	flame ionization

Using the above column and conditions, baseline separation of each of the fatty acid esters could be obtained. The integration of the peaks was thus baseline to baseline.

4.6.4.3 Validation Procedure

In order to establish the effectiveness and precision of the esterification procedure, a mixture was prepared, consisting of equal weights of tripalmitolein, triolein, tri-11-eicosenoin and trierucin. The procedure described in the above section was used to esterify 25 mg and 75 mg of the mixture. The resultant esters were then analyzed by gas chromatography using previously determined response factors, and the results compared to calculated ratios. It was determined using this method that the analytical procedure worked equally well with each of the above triglycerides.

4.7 Phosphorus Analysis

4.7.1 Introduction

The procedure by which oil samples were analyzed for their phosholipid content was based on the work of Duck-Chong (1979). The method consists of two parts. In the first part the sample is ashed at high temperature in the presence of magnesium nitrate $Mg(NO_3)_2\ 6H_2O$. This step is required to convert the phospholipids to phosphate and to 'burn off' non-phospholipid organic material. In the second step the residual phosphate is determined using a standard colorimetric procedure and known concentration reference standards.

4.7.2 Digestion Procedure

Typically, 7 mg of each oil sample was ashed. This mass of oil was significantly more than that specified by Duck-Chong (1979). Consequently her method was modified to allow for the increased sample size. The modified ashing procedure was then tested using known concentration standards, consisting of a mixture of pure triglycerides and pure phosphatidyl choline (Avanti Polar-Lipids, Appendix III). The modified ashing procedure was as follows: a 7 mg oil sample was placed into the bottom of an acid-washed 8cm X 1cm Pyrex test tube using a 10μL pipette. The mass of oil transferred was determined to the nearest 0.01 mg by weighing the pipette before and after transfer using a Mettler H20T balance. Following this, 30μL of magnesium nitrate solution, 10%(w/v) $Mg(NO_3)_2\ 6H_2O$ in methanol were then added. The test tube and mixture were then gently

heated over a Bunsen burner flame. Digestion of the mixture was achieved by lowering the tube into the hottest region of the flame for several seconds. During this time the oil mixture charred and evaporated, coating the lower portion of the test tube. It was found that it was necessary to burn this black residue away since its presence interfered with the subsequent photometric technique. This was done by further heating the tube for several minutes. After digestion the tubes typically contained small quantities of white powder.

After allowing the tubes to cool for a few minutes, 1 mL of 1 M HCl was added. Each of the tubes was then covered with a glass marble to minimize evaporation and heated for 15 minutes in a water bath at 90-95 °C.

4.7.3 Phosphate Standards

Six standards containing from 0(blank) to 0.5μg of potassium phosphate (KH_2PO_4) were prepared according to the method of Duck-Chong(1979). These solutions were then used to prepare a phosphorus standard curve with which the oil samples were compared.

4.7.4 Photometric Procedure

The amount of phosphorus in the standards and the sample solutions was determined according to the method of Duck-Chong (1979). In this procedure the solutions are reacted with a malachite green-ammonium molybdate reagent. The amount of phosphate-dye complex formed during the reaction, which can be determined by measuring the absorbance of the solution at 650

nm, is an indication of the amount of phosphorus present.

In practice, the phosphorus content of each sample was determined by first measuring its absorbance and then comparing the absorbance with that of the standard solutions. A Beckman (model 124) spectrophotometer was used for all absorbance measurements.

4.7.5 Phospholipid Calculations

The procedure described above detects only the phosphorus present in the samples. In order to be able to calculate the amount of phospholipid present, the ratio between the phosphorus and the phospholipid must be known. In principle, this ratio can be calculated from the molecular weight of phosphorus and phospholipid. However, since many types of phospholipids occur in biological tissues, the ratio in practice is between the molecular weight of phosphorus and an 'average' molecular weight of phospholipids.

The average molecular weight of phospholipids in the Canola oil was calculated from the concentration and molecular weight of the major phospholipids known to occur in the oil (Table 4.6), i.e.

$$\text{mol wt}(x) = 0.48(781) + 0.2(873) + 0.09(769)/0.77 = 800$$

The ratio of phosphorus to phospholipid is 31/800 = 0.0386; i.e. 3.86wt% of phospholipid in Canola oil is phosphorus. Thus the mass of phospholipid in the oil can be estimated by multiplying the mass of phosphorus as determined by the

aforementioned procedure by 1/0.0386 or 25.9.

TABLE 4.6 Major phospholipid components of Canola oil (Sosulski et al., 1981).

phospholipid	mol form	mol wt	% occurrence
phosphatidyl choline	$C_{44}H_{80}O_8NP$	781	48
phosphatidyl innositol	$C_{47}H_{70}O_{13}P$	873	20
phosphatidyl ethanolamine	$C_{43}H_{80}O_8NP$	769	9

4.7.6 Detection Limits Of Procedure

The minimum amount of phosphorus which could be determined using the above procedure was found to be 0.05μg. Since the minimum oil sample size was 7 mg, the limits of detection were 0.05μg/7 mg or 7 ppm of phosphorus. This value corresponds to a minimum phospholipid detection limit of approximately 0.02%.

V. RESULTS AND DISCUSSION

5.1 Introduction

The present section is divided into three principal parts. In the first part, information about select physical aspects of the extraction process is presented. This information includes such items as the solubility of Canola oil in CO_2 as a function of system temperature and pressure, and the effectiveness of seed pre-treatment methods. Part Two deals with chemical aspects of the extraction process, chemical composition of CO_2 extracts of Canola seed and synthetic mixtures of pure triglycerides. In the third part the predictions made by the computer model are compared with experimental data and the validity of the model is discussed.

5.2 Oil Solubility as a Function of Pressure and Temperature

The solubility of Canola oil in CO_2 at different temperatures and pressures was determined from the corresponding extraction curves using the procedure outlined in Sec. 4.5.4.

All solubility experiments were repeated at least twice. Additionally, material balances were carried out on the extractions and closed. The data points shown on the figures are the average value obtained from the experiments. The error bars shown on the figures represent the maximum and minimum slopes of the lines which could be drawn through the initial portion of the extraction curves.

In Fig. 5.1 the solubility of Canola oil in CO_2 is plotted as a function of pressure at different temperatures. The figure

indicates that as the pressure of the CO_2 increases, the oil solubility also increases. In Fig.5.2 the solubility of the oil is plotted as a function of temperature, at different pressures. From this figure it is evident that the effect of temperature on the solubility of the oil in the CO_2 changes with pressure. At low pressures, the oil solubility decreases with increasing temperature, whereas at higher pressures the solubility curve exhibits a maximum. This temperature effect is not unusual and has been reported for napthalene dissolved in supercritical ethylene (Williams, 1981) and in CO_2 (de Filippi, 1982).

In Fig.5.3 the solubility of Canola oil is plotted as a function of CO_2 density, at different temperatures, while in Fig. 5.4 the solubility is plotted as a function of temperature at different CO_2 densities. These plots show that a simple monotonic relationship exists between oil solubility and CO_2 density. The complex relationship between oil solubility and pressure (at different temperatures) can be explained by referring to Fig. 5.5. As indicated in this figure, a rise in temperature, at constant pressure, leads to a decrease in CO_2 density. On the other hand, a rise in temperature also leads to an exponential increase in the vapor pressure of the oil (Formo, 1979; Peter and Brunner, 1978). Near the critical point of the CO_2 (Fig. 5.5), the density changes rapidly with temperature. A small temperature change in this region may lead to a large change in CO_2 density and a commensurate change in oil solubility.

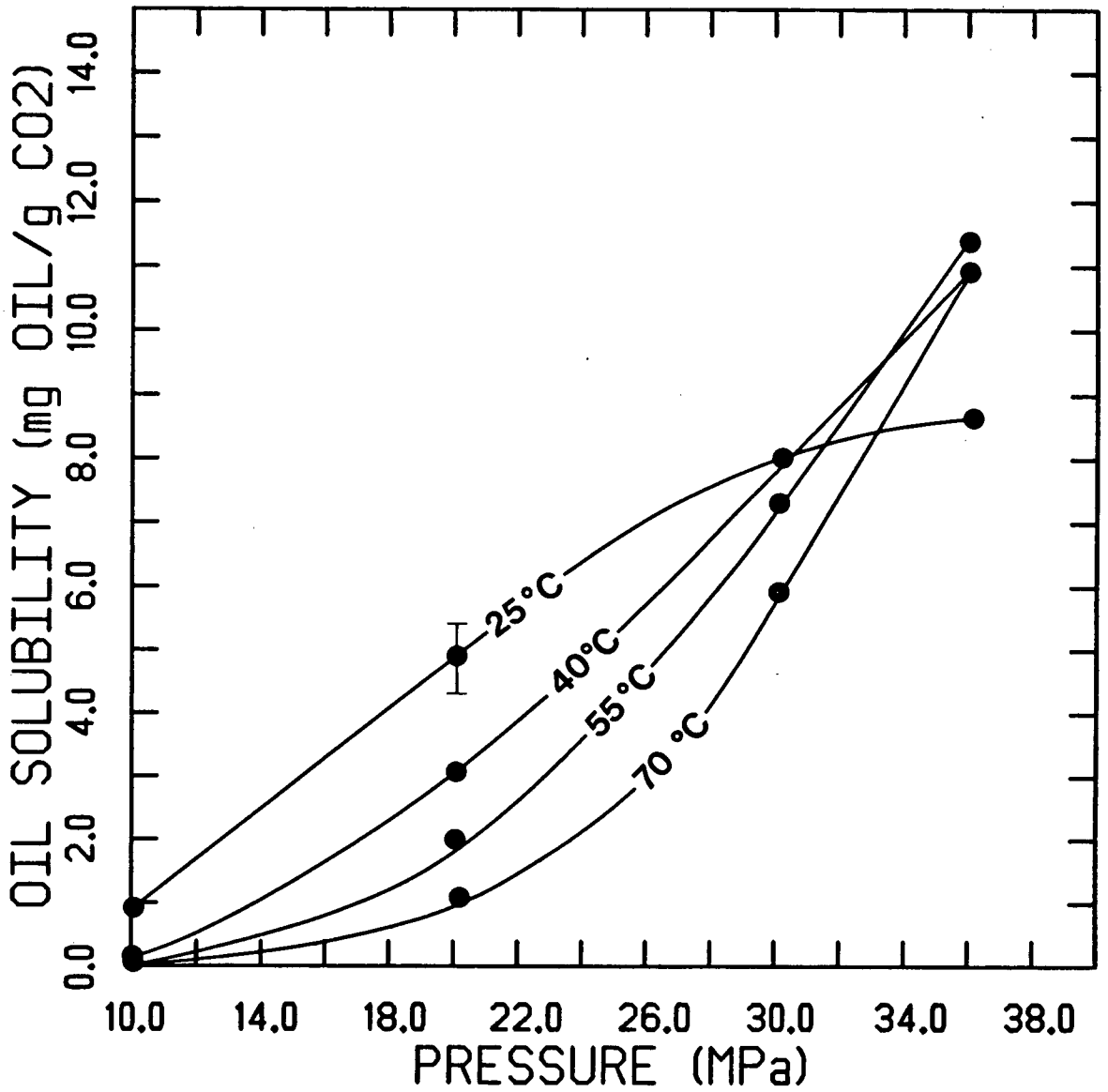

FIGURE 5.1 Solubility of Canola oil in CO_2 as a function of pressure at four temperatures. Conditions: vessel #2, CO_2 flow rate 0.7 g/min, 7 g flaked seed.

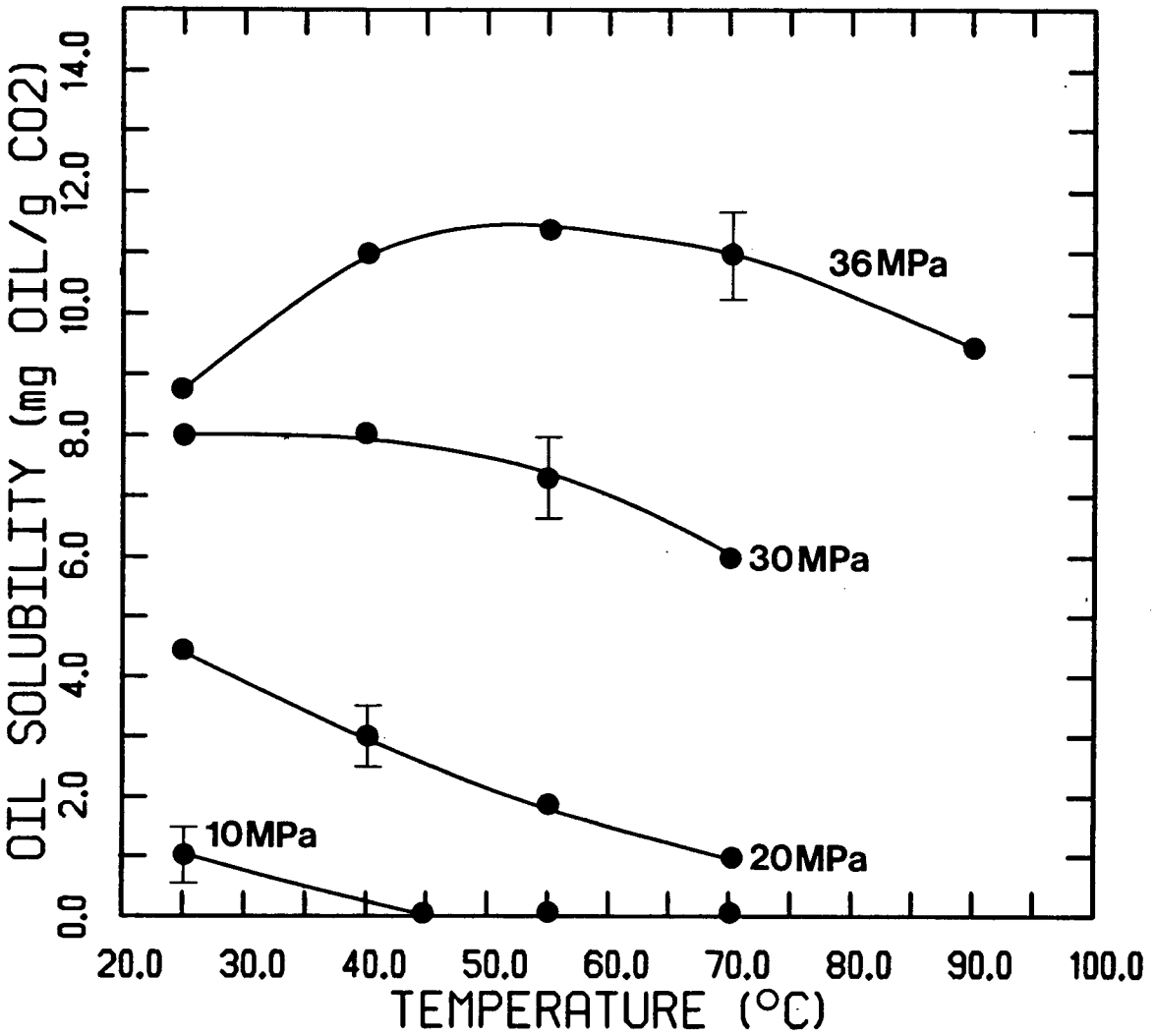

FIGURE 5.2 Solubility of Canola oil in CO_2 as a function of temperature at four pressures. Conditions: vessel #2, CO_2 flow rate 0.7 g/min, 7 g flaked seed.

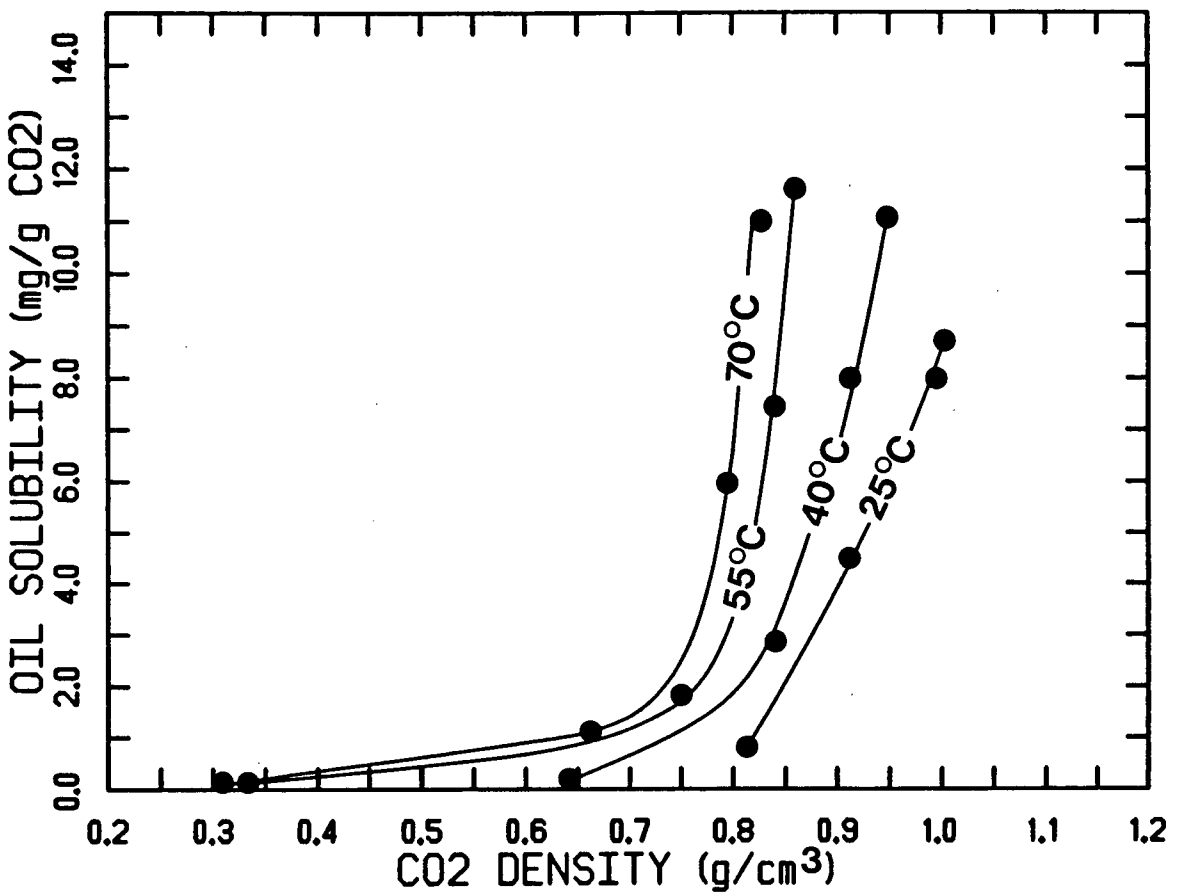

FIGURE 5.3 Solubility of Canola oil in CO_2 as a function CO_2 density at four temperatures. Conditions: vessel #2, CO_2 flow rate 0.7g/min, 7 g flaked seed.

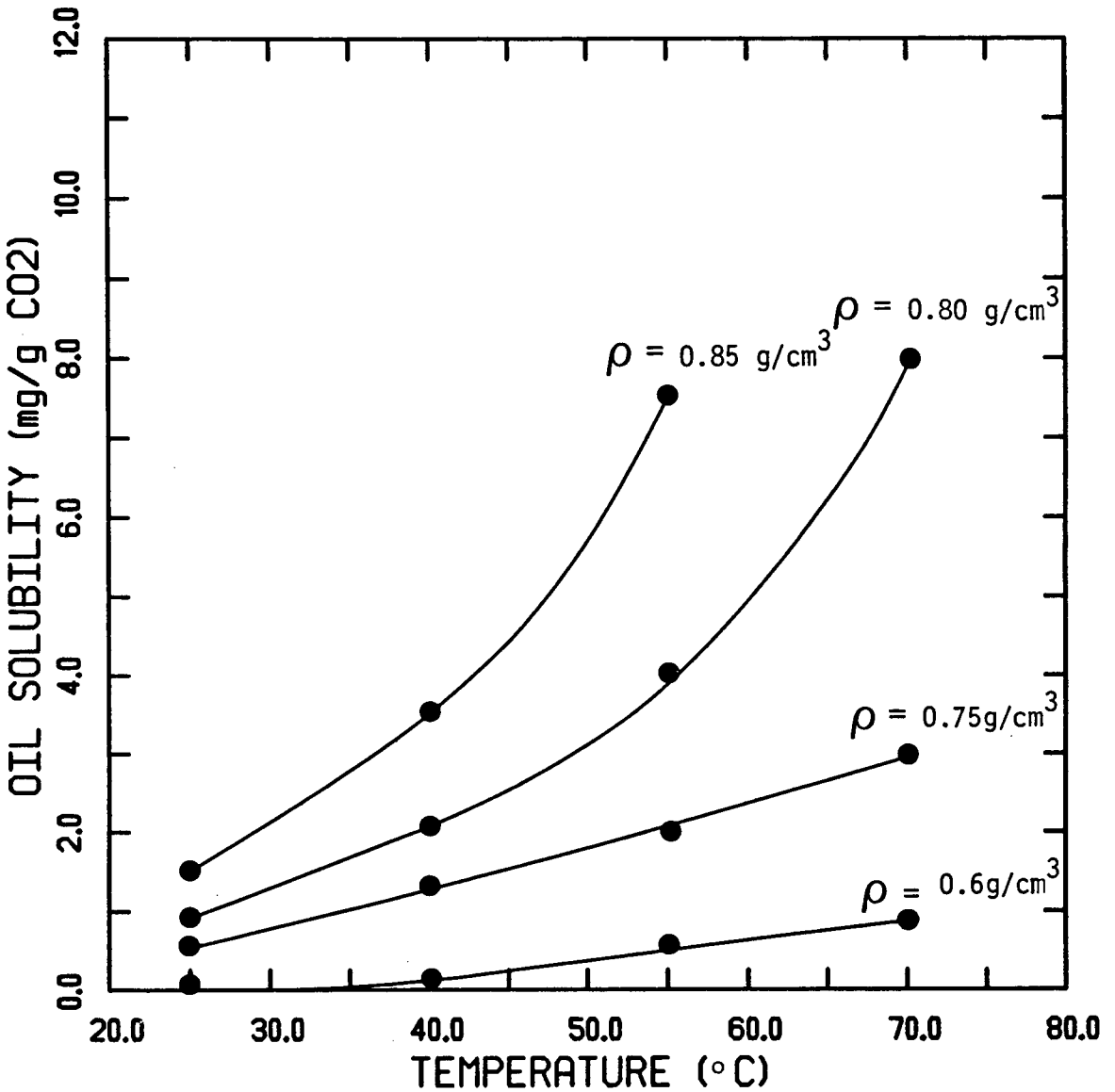

FIGURE 5.4 Solubilty of Canola oil in CO_2 as a function of temperature at different CO_2 densities. Conditions: vessel #2, CO_2 flow rate 0.7 g/min, 7 g flaked seed.

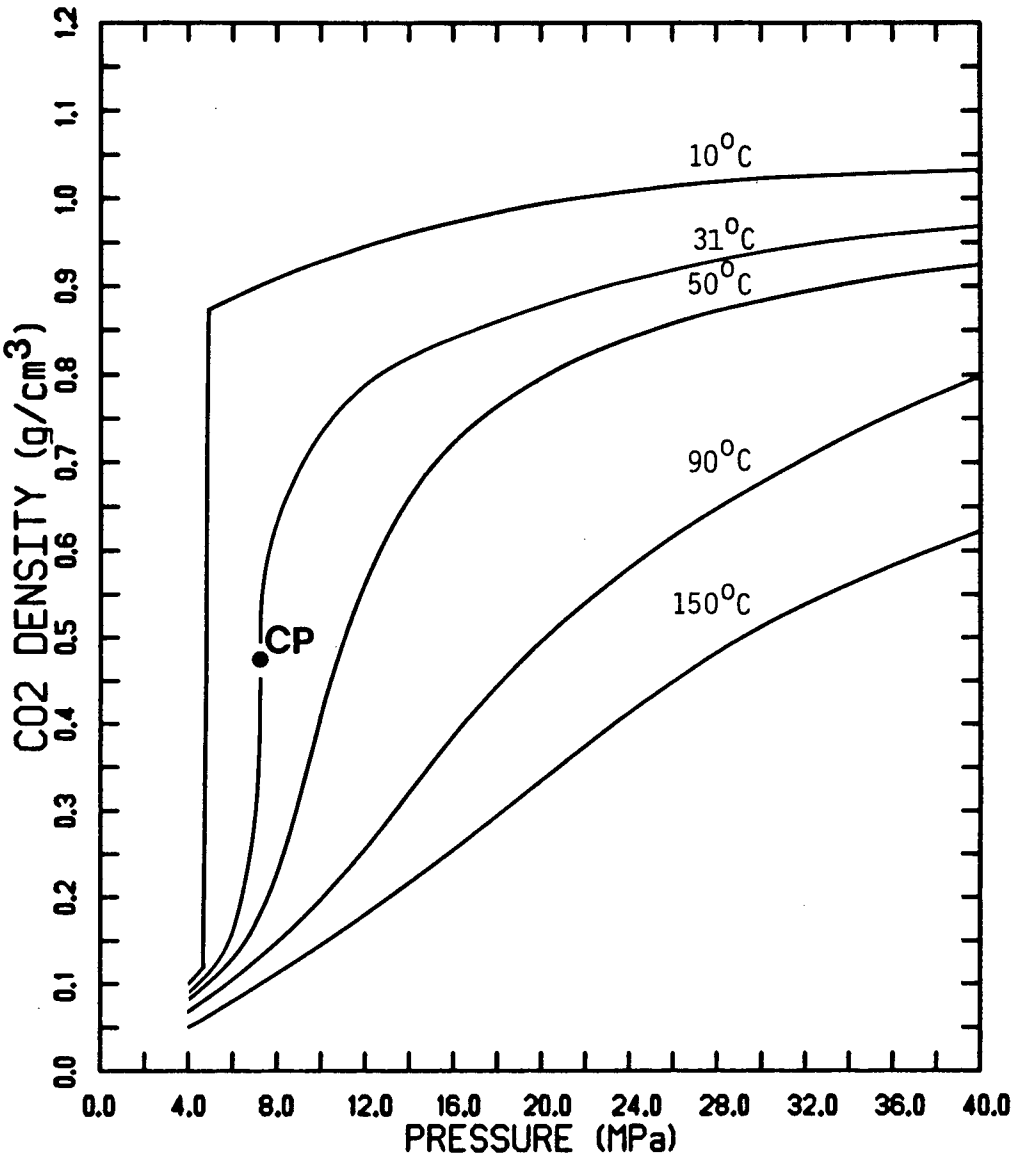

FIGURE 5.5 Density of carbon dioxide as a function of pressure at different temperatures. The critical point (CP) of the CO_2 is indicated on the diagram. (Newitt et al., 1956)

At higher pressures however, the same temperature change has a smaller effect on the fluid density. In this case, the increase in the vapor pressure of the oil may more than offset the decreased solvent capacity of the fluid due to its decreased density. The net effect results in an overall increase in solubility.

These results support the generally accepted 'rules' that: i) the solvent power of a supercritical fluid will increase with density at a given temperature and ii) the solvent power of a supercritical fluid increases with temperature at constant density (Brogle, 1982).

5.2.1 Practical Implications of Solubility Data

Figure 5.2 indicates that excellent separation of Canola oil and CO_2 solvent can be achieved by a simple pressure reduction. The figure also indicates that the pressure reduction need not be to atmospheric since the oils solubility in CO_2 at 10 MPa is >0.05%. This fact is significant since it indicates that, in a re-circulating extraction system, the costs of re-pressurizing the CO_2 could be reduced. Nonetheless, on a large scale, a separation based on pressure reduction may be expensive. In this case a separation based on a temperature change would be more desirable (Peter and Brunner, 1978).

The fact that the solvent power of a supercritical fluid changes with temperature at constant pressure can be of considerable practical importance. It indicates that, in some cases, it may be possible to effect a separation of solvent and solute merely by changing temperature.

However it seems unlikely that Canola oil can be economically separated from CO_2 solely by changing the temperature. As indicated above, the most effective separations of this kind involve the use of a supercritical solvent near its critical point. For CO_2 this would require pressures and temperatures approximately in the region of 7-10 MPa and 30-40°C, respectively. However, under these conditions the solvation capacity of CO_2 for Canola oil is too low to make such an extraction worthwhile.

A temperature-based separation at higher carbon dioxide pressure would probably also be impractical. For example, if the extractor operates at 55°C and 36 MPa and the separator operates at 25°C and 36MPa the oil solubility is only changed from 11 to 9 mg/g CO_2 (Fig 5.2). The recirculated carbon dioxide would thus enter the extraction chamber with a high oil content thereby lowering the driving force in the extraction and consequently the rate of extraction.

A separation based solely on temperature might, however, be possible provided a suitable entrainer can be found for the CO_2. Brunner and Peter (1982) have demonstrated such a separation using carbon dioxide charged with 10 wt% ethanol. At 50° C and 17.5 MPa, the solubility of Palm oil in this mixture was reported as approximately 8wt%. At the same pressure but at 90°C, the solubility was only 2wt%. This indicates that an effective separation of Palm oil from the solvent could be achieved with a temperature change of only 40 ° C. Since Palm oil is similar to Canola oil, it would be reasonable to assume

that similar separations could be performed with Canola oil. However, due to the limitations of the experimental equipment, extraction experiments using entrainers could not be performed.

5.3 Equilibrium Oil Concentration In Carbon Dioxide

The value of y* has previously been defined as the concentration of oil in the carbon dioxide solvent phase in equilibrium with seeds having an oil concentration x. In section 3.2 it was shown that the relationship between y* and x (y*=f{x}) was required to solve the mass balance equations (Eqs. 3.3, 3.4). Accordingly, two sets of experiments were conducted to provide information relating y* to x.

Extractions with CO_2 at 36 Mpa and 55° C were carried out on flaked seed which had been pre-extracted with either CO_2 (36 Mpa, 55° C) or hexane to the levels shown in Table 5.1.

TABLE 5.1 Concentration of oil in the partially extracted seed samples.

sample	g oil/g oil-free seed	
	CO_2 extracts	hexane extracts
1 unextracted seed	0.67	0.67
2	0.48	0.35
3	0.40	0.23
4	0.30	-
5	0.22	-

In Figs. 5.6 and 5.7 the value of the oil concentration in the carbon dioxide at the bed outlet is plotted as a function of the initial x value of the the seed for different CO_2 flow

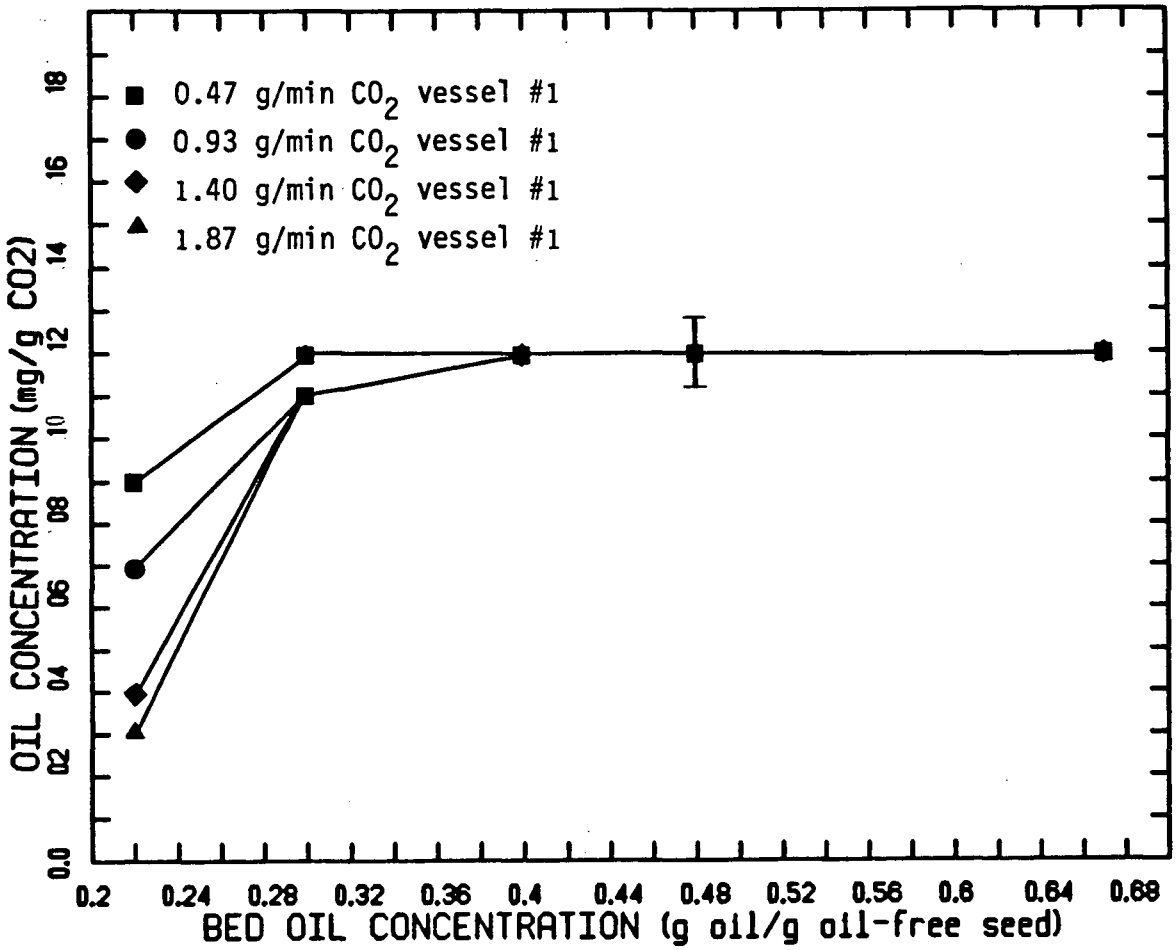

FIGURE 5.6 Oil concentration in the CO_2 phase at the extractor outlet for different seed-bed oil concentrations. The reduced oil concentration seed was prepared by partially extracting samples of flaked seed for different lengths of time with hexane. Conditions: vessel #1, pressure 36 Mpa, temperature 55°C, CO_2 flowrate as indicated on the Figure.

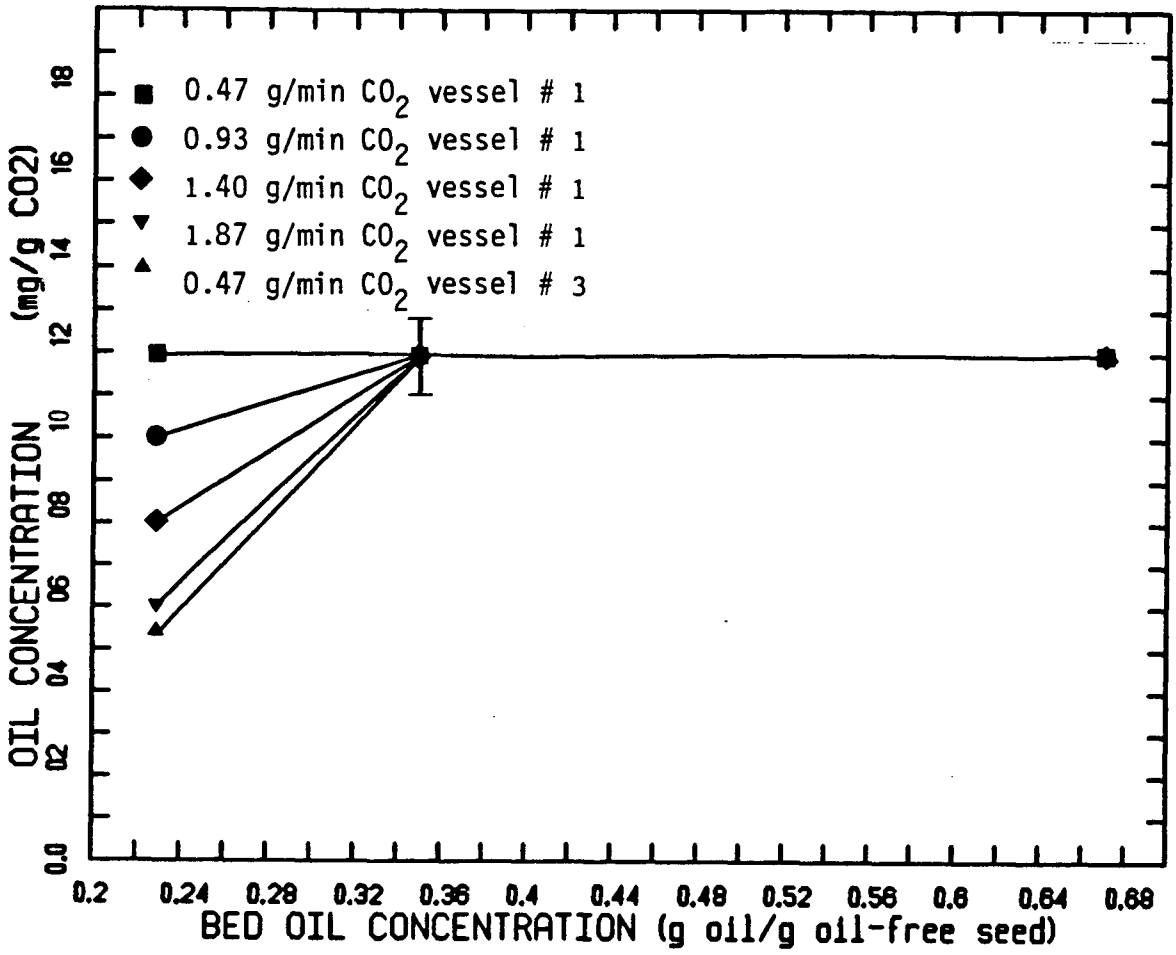

FIGURE 5.7 Oil concentration in the CO_2 phase at the extractor outlet for different seed-bed oil concentrations. The reduced oil concentration seed was prepared by partially extracting samples of flaked seed for different lengths of time using supercritical CO_2 at 36 MPa and 55 ° C. Conditions: pressure 36 MPa, temperature 55°C, CO_2 flow rate as indicated on the Figure.

rates. As can be seen from these figures, the value of y* was constant for the seed samples having oil contents in excess of about 0.4 g/g oil-free seed. For the seed samples having lower oil concentrations, it initially appeared as if y* depended on x. However, it can also be seen from these figures that, in this region, y* was a function of the carbon dioxide flowrate. As the flowrate decreased, the exit concentration increased thereby indicating that the carbon dioxide had not come to equilibrium with the oil in the bed.

From these experiments, it can be concluded that, over the range of seed oil concentrations used, y* is independent of x. This result is not unexpected. For y* to be dependent on the seed oil concentration, the oil and seed tissue would need to exhibit an attraction for each other at the molecular level, i.e. the oil would need to be bound to the seed. However, this is not the case. The oil in the intact seed exists as small oil droplets (Hofsten, 1970; Yiu et al., 1982) and in the crushed seed as a film on the surface of the seed particles.

Within the limitations of these experiments it appears that the seed tissue does not have a 'chemical affinity' for the oil and acts merely as an inert substrate. The relationship $y^*=f\{x\}$ can thus be represented by y^* = constant, where the constant corresponds to the oil solubility at a given temperature and pressure.

5.4 Effect of Seed Treatment

5.4.1 Seed Particle Size

In Table 5.2 the range of seed particle sizes resulting from the various treatment procedures is presented. The analysis was performed by passing the seed material through sieves of various mesh sizes and weighing the fraction associated with each tray.

TABLE 5.2 Distribution of seed particle sizes, for the different methods of seed treatment.

size range (mm)	percent of total mass					
	whole seed	crushed	finely chopped	flaked	cooked	exploded
< 0.149	-	0.8	8.6	0.2	0.2	-
0.149-0.589	-	41.3	70.8	19.2	12.6	-
0.590-0.850	-	30.2	15.5	24.8	24.3	-
0.851-1.00	1.1	12.1	1.5	11.5	22.8	0.4
1.00 -1.40	25.8	9.7	1.1	19.0	33.3	19.5
>1.40	73.1	5.9	2.4	25.2	6.7	80.1

5.4.2 Experimental Results

Initially, attempts were made to extract whole, unbroken Canola seeds with carbon dioxide at 36 MPa and 55 °C. Virtually no oil was recovered after extraction for 5 h at a carbon dioxide flow-rate of 0.8 g/min. This result agrees with previous reports. Othmer and Agarwal (1955), extracted whole and laterally sectioned soybeans with hexane for 166 h. It was found that less than 0.08% of the oil originally in the whole beans and less than 0.19% of oil in the half beans were extracted. This indicates that hexane is unable to penetrate and remove oil

from unbroken cells. Commercial Canola seed extraction is therefore preceded by cooking and flaking prior to extraction (Anjou, 1972; Beach,1983)

In Fig. 5.8 typical extraction curves are shown for seeds having undergone various pre-treatments. All extractions were carried out at 36 MPa, 55° C at a CO_2 flowrate of 0.7 g/min. In each case, a 4g sample was extracted. For comparative purposes the extraction curve of pure Canola oil on a bed of 0.3mm Ottawa sand is also shown. The amount of oil on the beads (1.6 g) was equivalent to the oil content of 4g of oilseed. It is evident from this figure that seed pre-treatment greatly affects the total quantity of oil removed from the seeds.

In Fig. 5.9 the extraction data are plotted in a different form. The curves were generated by fitting a second order polynomial function to the data points in Fig. 5.8 and differentiating the function at selected positions. The results correspond to the oil concentration in the carbon dioxide at the outlet of the extractor (sec 3.1). As indicated in Fig. 5.8, the least effective pre-treatment procedure was the 'exploding' technique. After extracting this material for 5 h, less than 10% of its oil had been removed. The figure also illustrates that the carbon dioxide did not reach saturation. This seed pre-treatment technique differs from all of the others in this respect and indicates that the cellular disruption was only slight. Hence, either the CO_2, which was originally used to explode the seeds, did not penetrate to a significant depth and disrupted only the cells on the surface of the seeds or,

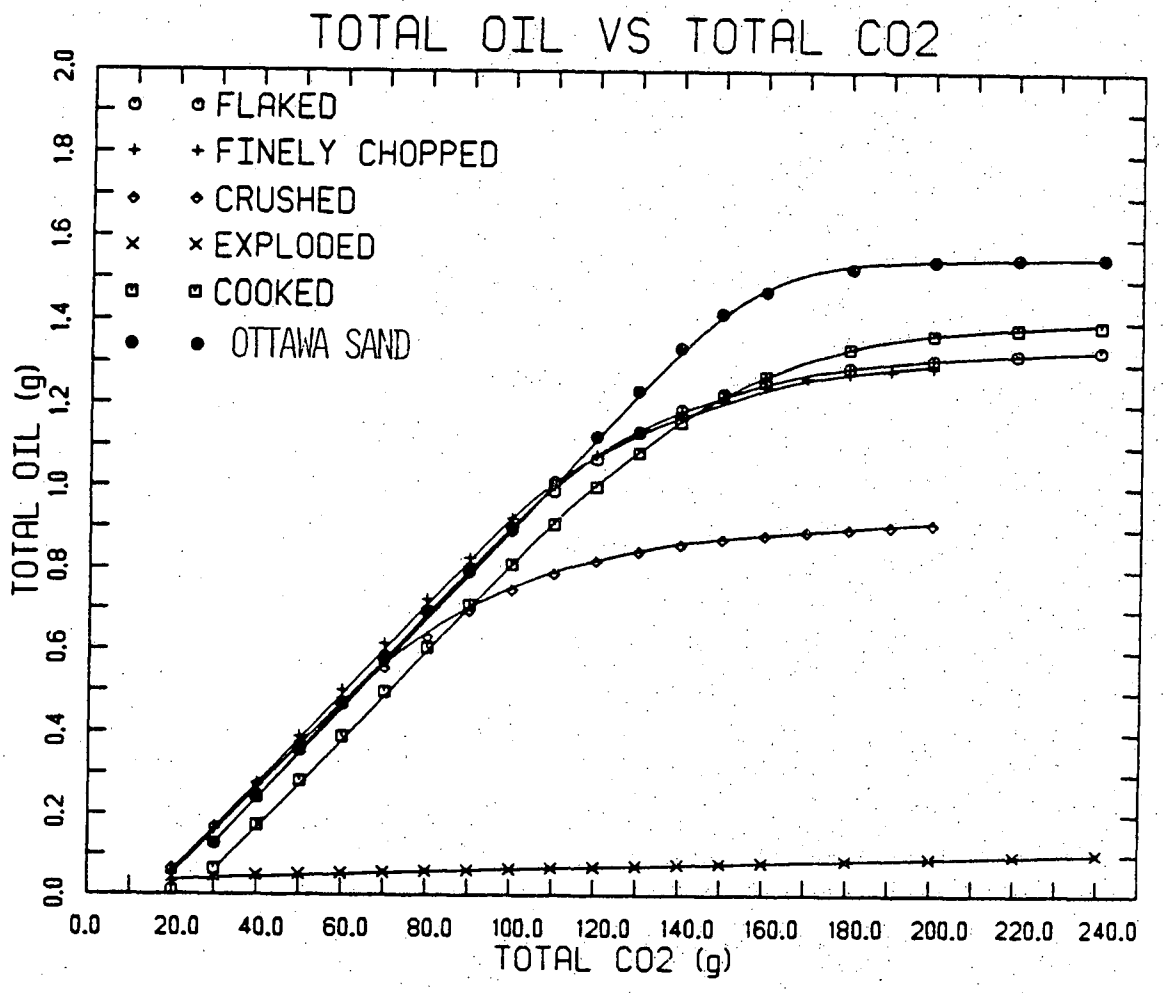

FIGURE 5.8 Extraction curves 4 g samples of Canola seed subjected to different pre-treatments. For comparison purposes the extraction curve for Canola oil on Ottawa sand is also shown. Conditions: vessel #1, pressure 36 MPa, temperature 55°C, CO_2 flow rate 0.8g/min.

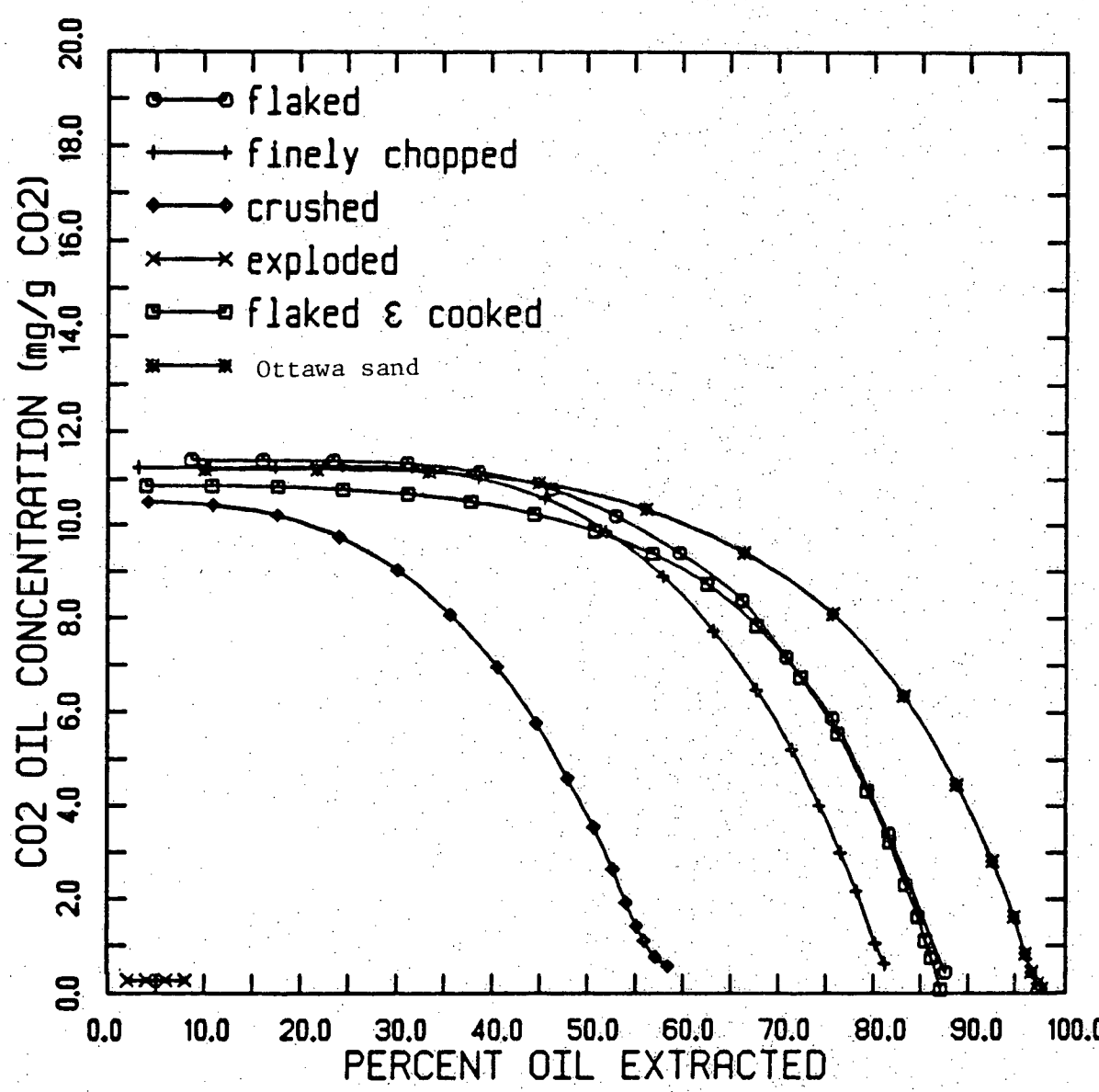

FIGURE 5.9 Transformed extraction curves for several Canola seed pre-extraction treatments. The Y axis of the graph represents the concentration of oil in the CO_2 at the extractor outlet.

alternatively the cell walls were sufficiently robust to withstand the large pressure differential generated during decompression.

The crushing procedure, although considerably more effective than the pressure-rupturing treatment, evidently left much of the seed intact as well. As indicated in Fig. 5.9, concentrations at the extractor outlet began to decline rapidly after approximately 10% of the oil had been removed. By the time 60% of the seed oil was removed, the oil concentration in the outlet carbon dioxide had decreased to less than 5% of its saturation value.

For the finely chopped seed, the outlet oil concentration began to decrease after about 35% of the oil had been extracted. The oil concentration in the carbon dioxide fell to 5% of its saturation value only after 80% of the seed oil had been removed. The flaked and cooked seeds had extraction characteristics similar to those of the finely chopped seed in the initial stage of the extraction. However, the amount of oil extracted from these samples exceeded 85% of total before the oil concentration of the extractor outlet fell to 5% of saturation. Although Beach,(1983) and Clandinin, (1981) have reported that cooked Canola seed releases oil more readily than uncooked seed, there was no difference between these two samples when extracted with CO_2.

As can be seen from Fig. 5.8, the Canola oil was most effectively removed from the glass beads. Over 95% of the oil in the bead matrix was removed before the outlet oil concentration

in the carbon dioxide fell to 5% of its saturation value. This result is not unexpected, since the beads are non-porous and all of the oil lies on the bead surface. Furthermore, the bead beds are composed of many open channels through which CO_2 can flow. By contrast, the oil in beds of crushed or flaked seed may be trapped in regions between particles impervious to the CO_2 or within intact cells. Much of the oil contained within these regions is not exposed to the moving stream of carbon dioxide and is only transferred out of these channels by the slow process of diffusion.

The difference in the extraction characteristics of flaked and finely chopped seed may also be due to the different flow patterns in the beds. Unlike the flaked seed, the finely chopped seed consists predominantly of very small particles. The passages between these particles are consequently also small and the resistance to flow through the network is high. In such a case the carbon dioxide would flow predominantly through a small number of large channels which probably exist in the bed as well. This channeling effect is undesirable since only a small fraction of the bed would be exposed to the moving stream of CO_2.

From these experiments it can be concluded that supercritical CO_2 has the ability to extract as much oil from Canola seed as hexane and that the current commercial pretreatment processes are also suitable for supercritical CO_2 extraction process.

5.5 Scanning Electron Microscopy of Seed Particles

In section 3.2.3 a mechanism was proposed to account for the extraction characteristics of Canola seed (Fig. 3.4). One of the assumptions of the mechanism was that the crushed seed, in its unextracted state, was covered with a layer of oil which gradually became depleted during the extraction process. In order to determine whether this assumption was valid, samples of unextracted crushed seed and crushed seed at various stages of carbon dioxide extraction were examined under the scanning electron microscope (SEM). The method by which these samples were obtained was described in Sec. 4.6.2.

Fig. 5.10 is an SEM photograph of a typical seed particle prior to extraction. The presence of an unbroken layer of oil on the surface of the seed fragment is suggested by the particle's amorphous, globular, liquid-like appearance and the lack of any fine cellular detail.

In Fig. 5.11 an SEM photograph of a partially extracted seed particle is shown. Regions with fine cellular details are visible thereby indicating an absence of oil. In Fig. 5.12 a 'fully extracted' seed particle is shown. In this figure the cellular structure of the seed is clearly visible and the amorphous globular regions, which were present in the previous two photographs, are completely absent. This photograph suggests that the surface of the fully extracted seed particle is oil-free.

These photographs generally support the extraction mechanism suggested in Sec. 3.4 and indicate that the seed

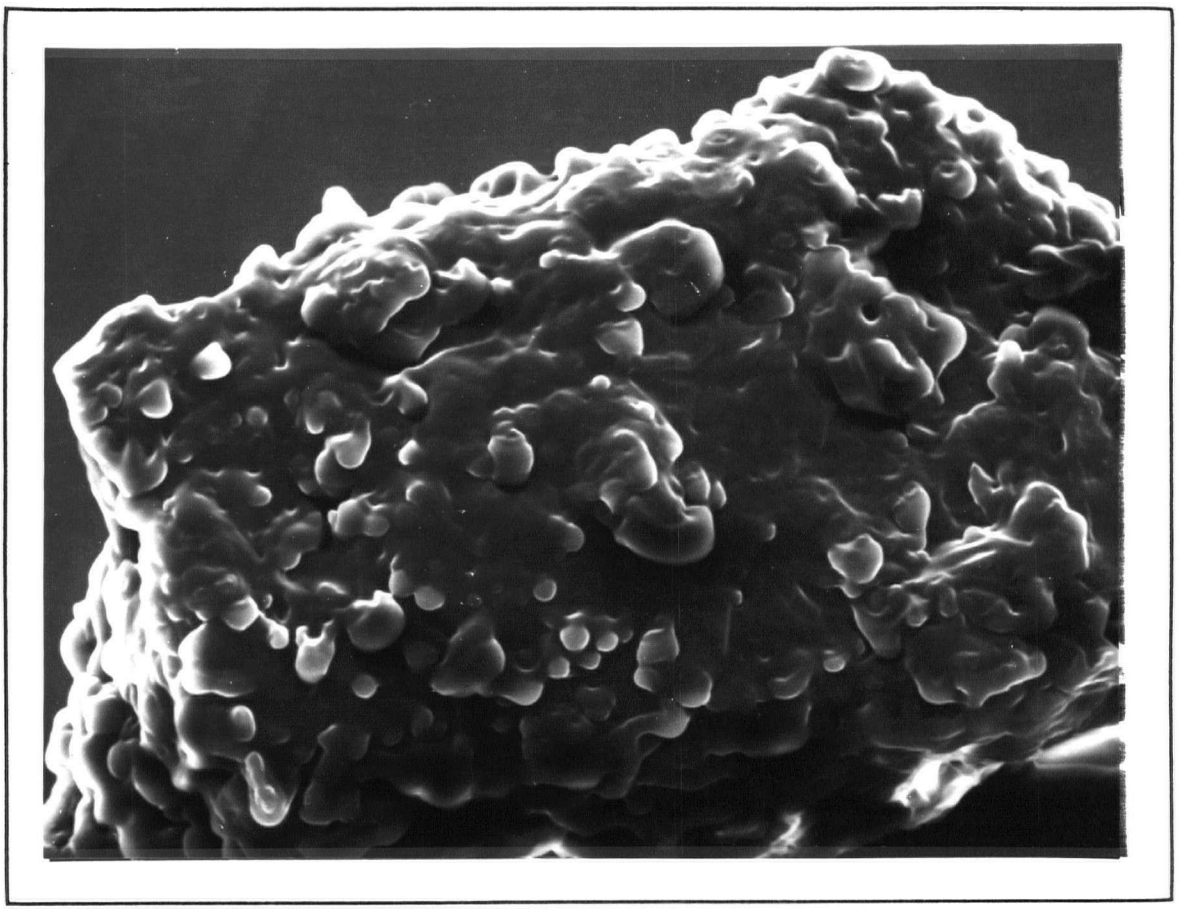

FIGURE 5.10 Scanning electron micrograph of a fragment of flaked Canola seed prior to extraction. (Mag. 620X, 20 Kv, Au-Pd)

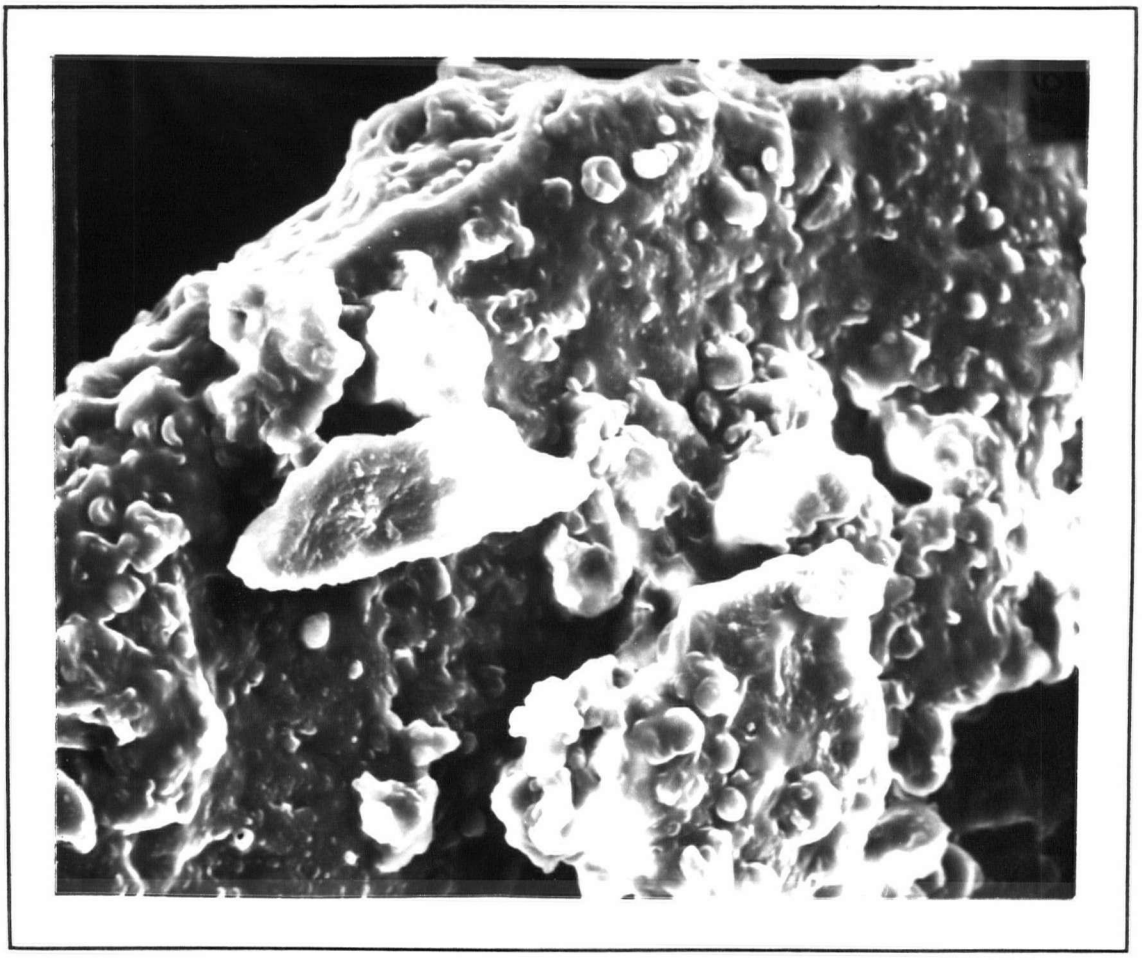

FIGURE 5.11 Scanning electron micrograph of a fragment of flaked Canola seed after partial extraction with CO_2 at 36 MPa and 55°C (Mag. 620X, 20 Kv, Au-Pd). The seed fragment was extracted for approximately 15 minutes at a CO_2 flow rate of 0.5g/min.

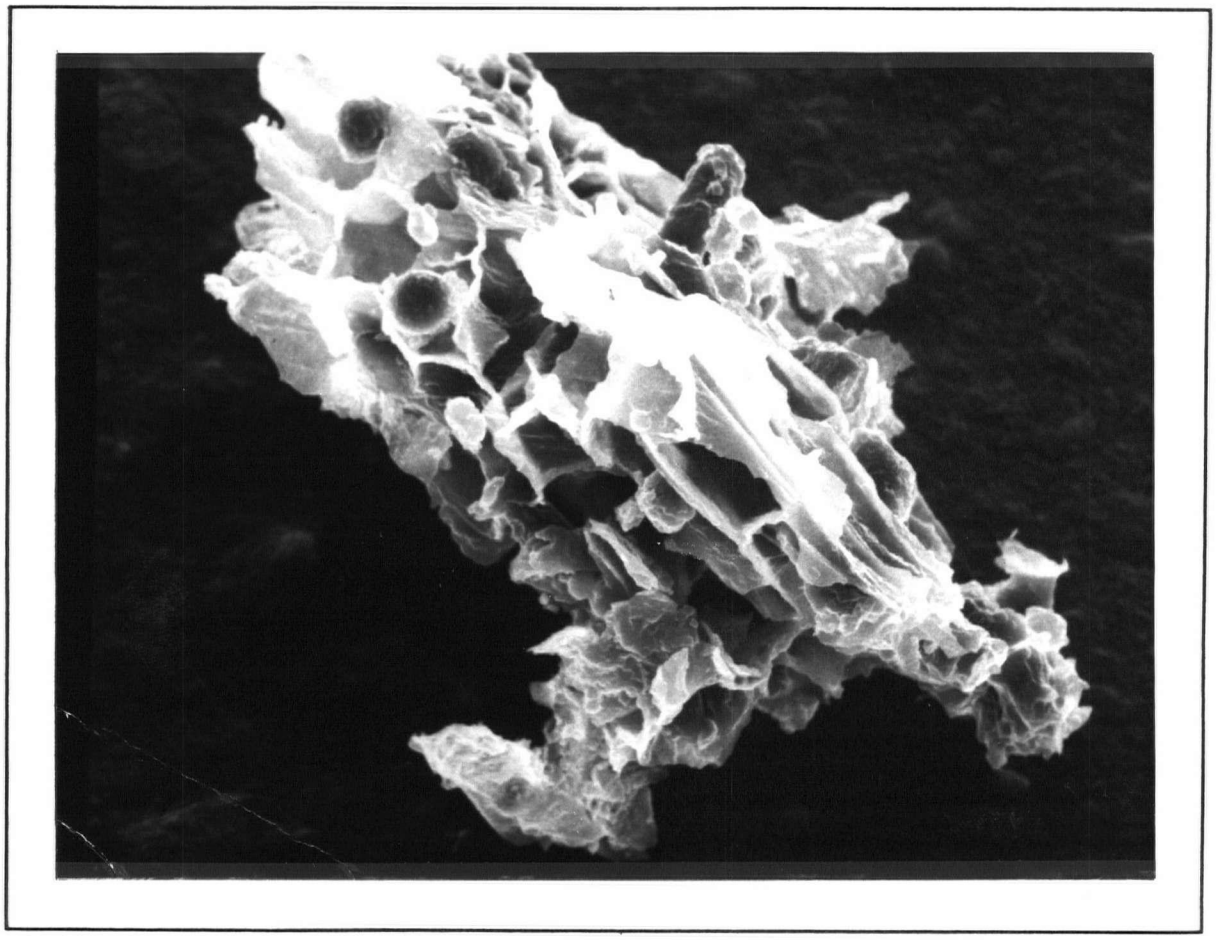

FIGURE 5.12 Scanning electron micrograph of a fragment of flaked Canola seed after being 'fully' extracted with CO_2 at 36 MPa and 55 °C. (Mag. 780X, 20Kv, Au-Pd). The seed fragment was extracted for approximately 2 h at a CO_2 flow rate of 0.5g/min.

particles are initially covered with a layer of oil which gradually becomes depleted as the extraction proceeds.

5.6 Fatty Acid Composition of Extracts

5.6.1 Introduction

One of the objectives of this study was to determine the composition of the supercritical carbon dioxide extracts as a function of extraction time. Additionally, these extracts were compared with those obtained by conventional hexane extraction

5.6.2 Fatty Acid Ester Response Factors

Conflicting information exists as to whether or not flame ionization detectors (FID) respond with equal magnitude to equal masses of fatty acid esters (Ackman and Sipos, 1964). Since this is an important consideration, the first step in this investigation consisted of determining the FID response factors for the relevant fatty acid (FA) esters.

TABLE 5.3 FID response factors for fatty acid methyl esters relative to methyl palmitate.

fatty acid ester	carbon number	response factor
methyl myristate	C14:0	1.000
methyl palmitate	C16:0	1.000
methyl palmitoleate	C16:1	1.000
methyl stearate	C18:0	1.000
methyl oleate	C18:1	1.000
methyl linoleate	C18:2	1.074
methyl linolenate	C18:3	1.000
methyl arachidate	C20:0	0.999
methyl eicosenoate	C20:1	1.000
methyl behenate	C22:0	0.991
methyl erucate	C22:1	0.999
methyl lignocerate	C24:0	1.000

The analytical standards used for the above were obtained from Supelco Corp (Appendix III). In Table 5.3 the response factors determined for several FA esters is presented. All subsequent calculations, which apply to the fatty acid esters, were obtained using the above response factors.

5.6.3 Fatty Acid Composition of Canola Oil

The various triglycerides which make up Canola oil contain both saturated and unsaturated FA moieties ranging in carbon number from 14 to 24. The fatty acid profile can thus be used as a means of characterizing the oil and is often the measure by which oil from different varieties of Canola seed are compared (Table 5.4; Ackman, 1983).

In Table 5.5 the fatty acid compositions of Canola oil from four sources are shown. The CO_2 extract listed in the table was obtained by extracting flaked Canola seed at 36 MPa and 55°C. The hexane extract was produced from flaked seed using hexane at 55°C. The refined and crude oils were obtained from CSP Foods and are described in Sec. 4.3.4.

TABLE 5.4 Fatty acid composition of triglycerides in Canola oil obtained from three varieties of Canola seed (Ackman, 1983).

Variety	16:0 %	16:1 %	18:0 %	18:1 %	18:2 %	18:3 %	20:0 %	20:1 %	22:0 %	22:1 %
Andor	3.9	0.2	1.3	58.2	21.6	12.1	0.5	1.6	0.4	0.0
Tobin	3.8	0.1	1.2	58.6	24.0	10.3	0.6	1.0	0.1	0.3
Jet Neuf	4.9	0.4	1.4	56.4	24.2	10.5	0.7	1.2	0.3	0.0

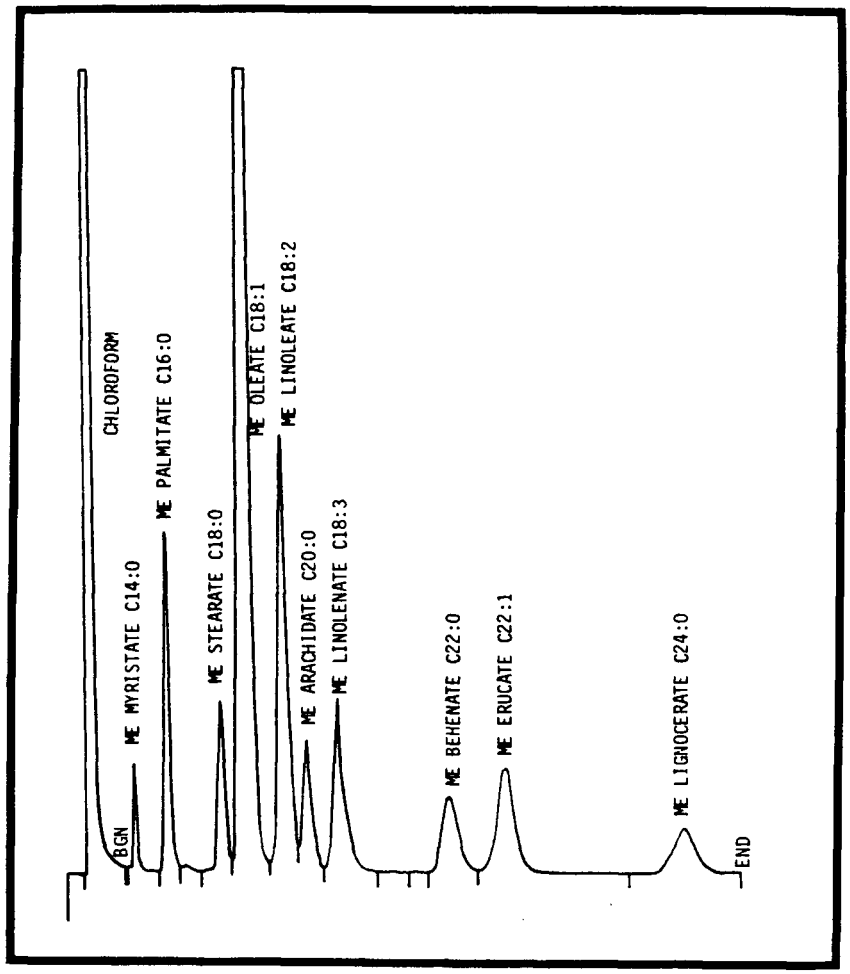

FIGURE 5.13 Chromatogram of the fatty acid methyl esters in an esterified sample of a typical CO_2 extract of Canola seed (36 MPa, 55°C, flow 0.7 g/min). Analysis conditions: Column- SP-2330 on 100/120 mesh chromosorb WAW; detector(FID) and injector temp. 250°C; isothermal 200°C.

A typical chromatogram showing the elution sequence of each FA ester and the peak resolution is presented in Fig. 5.13.

TABLE 5.5 Fatty acid composition of the triglycerides in four samples of Canola oil. For a description of each sample refer to text. The fatty acids C20:1 and C18:3 were not resolved using the chromatographic procedure.

Canola oil	14:0 %	16:0 %	18:0 %	18:1 %	18:2 %	20:1 18:3 %	20:0 %	22:0 %	22:1 %	24:0 %
refined oil	0.2	3.9	1.8	58.0	22.0	11.0	0.8	0.8	1.3	0.3
CSP crude	0.2	4.4	1.8	58.2	21.5	10.9	0.7	0.3	1.0	0.2
CO2 extract	0.1	4.7	2.0	56.9	21.9	11.2	0.8	0.5	0.6	0.3
hexane extract	0.2	4.8	2.1	57.4	22.0	11.0	0.9	0.4	1.0	0.2
absolute error ±	0.1	0.2	0.2	1.4	0.3	0.2	0.2	0.2	0.2	0.2

The identity of each major peak in the chromatogram was obtained initially by comparing retention times with analytical standards and was later confirmed using GC/mass spectrometer techniques. The absolute error in each case is the sum of the total measurement error involved with the integration of the chromatographic peaks and the error associated with the analytical standards. Details of the error analysis appear in Appendix II. It can be seen from Table 5.5 that the hexane and carbon dioxide extracts of the same seed are quantitatively identical in all of the fatty acids with the exception of erucic acid (C22:1). In the hexane extract this fatty acid appeared in

a slightly higher concentration.

5.6.4 Fatty Acid Composition of Carbon Dioxide Extracts

In the first experiment 4.2 g of cooked CSP seed were extracted at 36.0 MPa, 55 °C, at a CO_2 flowrate of 0.7g/min. Seven consecutive extract fractions were collected (Fig. 5.14).

TABLE 5.6 Fatty acid composition of sequential carbon dioxide extracts of CSP cooked Canola seed. Conditions: vessel #1, pressure 36 MPa, temperature 55°C, flowrate 0.7 g/min.

fatty acid ester	extract number 1	2	3	4	5	6	7	error %
C14:0 %	0.1	0.1	0.1	0.1	0.1	0.1	0.2	± 0.1
C16:0 %	4.7	5.1	5.2	5.1	4.6	4.1	3.4	± 0.2
C18:0 %	2.0	1.9	1.8	1.9	2.0	2.2	2.5	± 0.2
C18:1 %	56.9	57.3	57.3	57.9	58.8	59.6	58.5	± 1.4
C18:2 %	21.9	22.6	22.7	22.4	21.8	20.8	19.6	± 0.3
C20:0 %	0.8	0.6	0.6	0.6	0.7	0.9	1.3	± 0.2
C20:1 + C18:3 %	11.2	11.2	11.3	11.1	10.9	10.6	10.7	± 0.2
C22:0 %	0.5	0.3	0.3	0.3	0.3	0.4	1.0	± 0.2
C22:1 %	0.6	0.6	0.5	0.5	0.6	0.9	2.5	± 0.2
C24:0 %	0.3	0.2	0.2	0.1	0.1	0.2	0.6	± 0.2

In Table 5.6 the FA ester composition of each extract is provided while in Fig. 5.15 this information appears in graphical form. As can be seen, only small variations occur in the oil composition during the extraction. In the final extract sample, which was obtained after about 80% of the oil had been removed from the seedbed, the proportion of the heavier FA esters (C20-C24) was higher than in the previous fractions. In an effort to verify this and to collect extracts after longer times, the experiment was repeated under

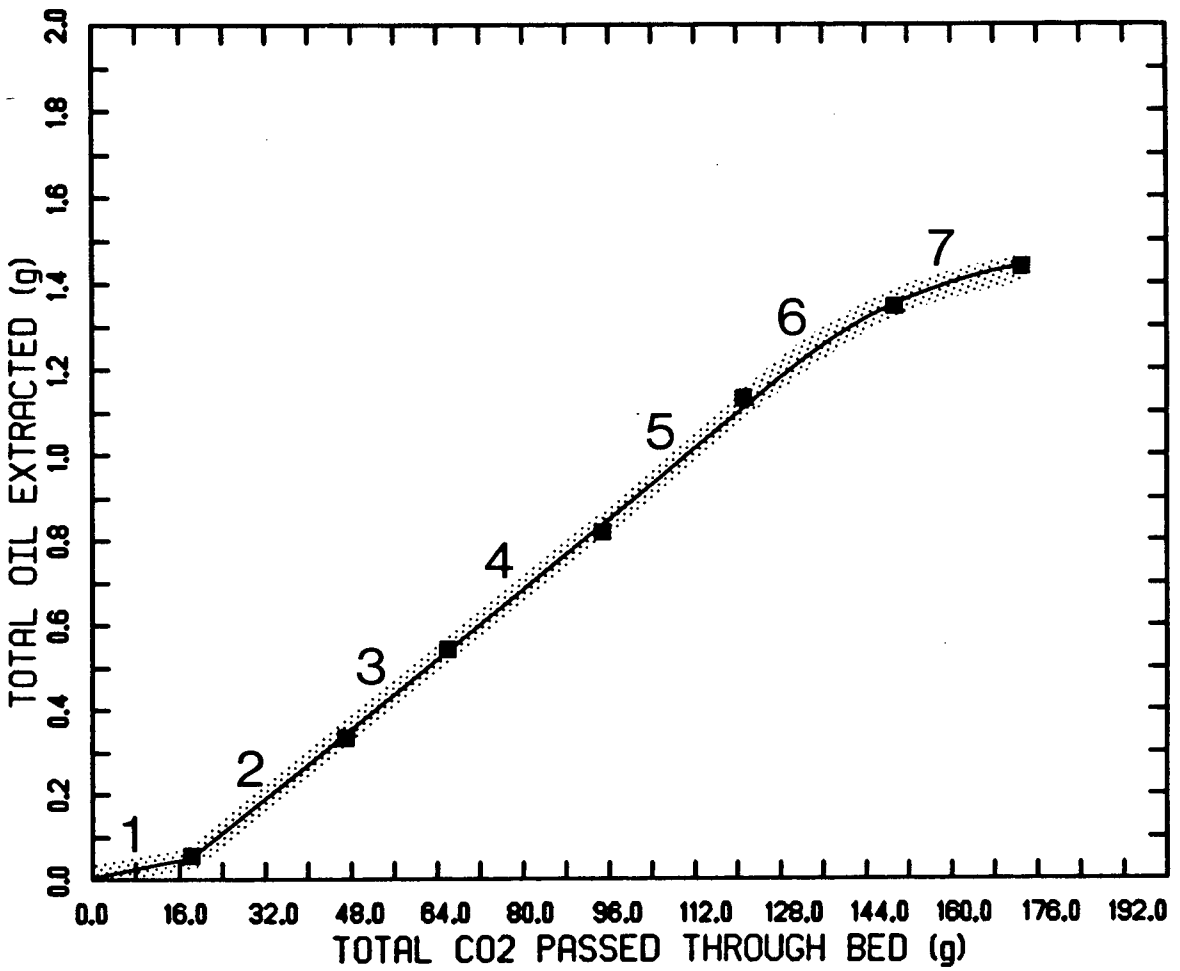

FIGURE 5.14 Extraction curve for a 4.2 g sample of commercially cooked Canola seed. The extraction was carried out at 36 MPa and 55 °C at a CO_2 flowrate of 0.7 g/min. The numbered intervals on the curve indicate the regions over which oil samples were collected for fatty acid analysis.

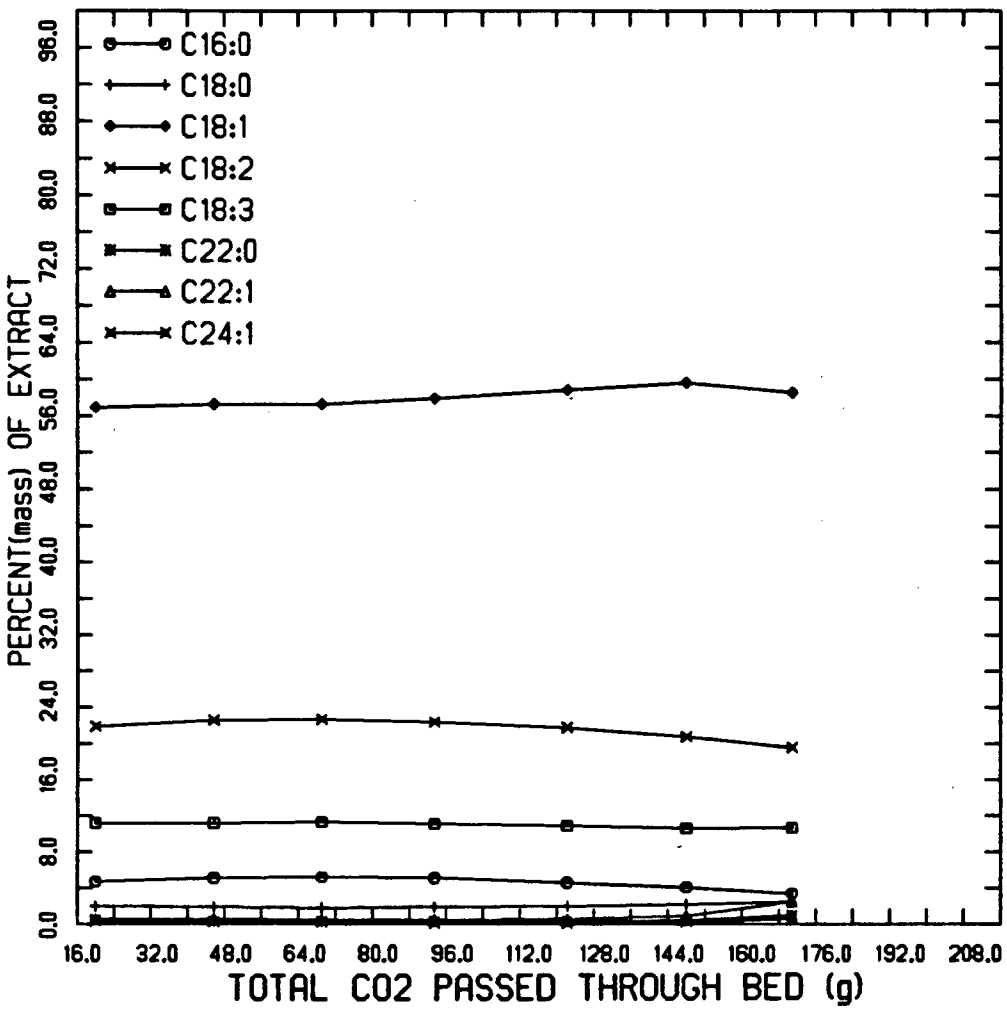

FIGURE 5.15 Fatty acid composition of the extracts indicated in Fig. 5.14.

identical conditions. At the completion of this second experiment the restricter valve was also washed with $CHCl_3$ and the oil analyzed. In Fig. 5.16 the extraction curve is shown along with the positions where the extracts were collected for analysis. In this case the last extract was obtained after 90% of the oil had been removed from the sample. In Table 5.7 the composition of the various extracts is listed while in Fig. 5.17 this information appears in graphical form.

TABLE 5.7 Fatty acid composition of sequential carbon dioxide extracts of CSP flaked and cooked Canola seed. The analysis of oil extracts obtained from the restricter valve is also provided. Conditions: vessel #1, pressure 36 MPa, temperature 55°C, flowrate 0.7g/min.

fatty acid ester	extract number 1	4	7	oil from valve	error %
C14:0 %	0.1	0.1	<0.1	<0.1	± 0.1
C16:0 %	5.4	5.1	3.3	4.5	± 0.2
C18:0 %	1.9	1.9	2.7	2.8	± 0.2
C18:1 %	56.5	56.9	55.2	54.5	± 1.4
C18:2 %	23.0	21.5	19.9	19.8	± 0.3
C20:0 %	0.5	0.5	1.1	1.3	± 0.2
C20:1 + C18:3 %	11.3	11.4	11.3	10.9	± 0.2
C22:0 %	0.3	0.3	1.3	1.4	± 0.2
C22:1 %	0.6	0.6	4.3	4.3	± 0.2
C24:0 %	0.1	0.1	0.9	0.9	± 0.2

Extracts 1 and 2, which were obtained early and midway through the extraction, were similar in composition to the previous extracts. The proportion of the heavier FA esters was significantly higher in the last extract and in the oil collected from the restricter valve.

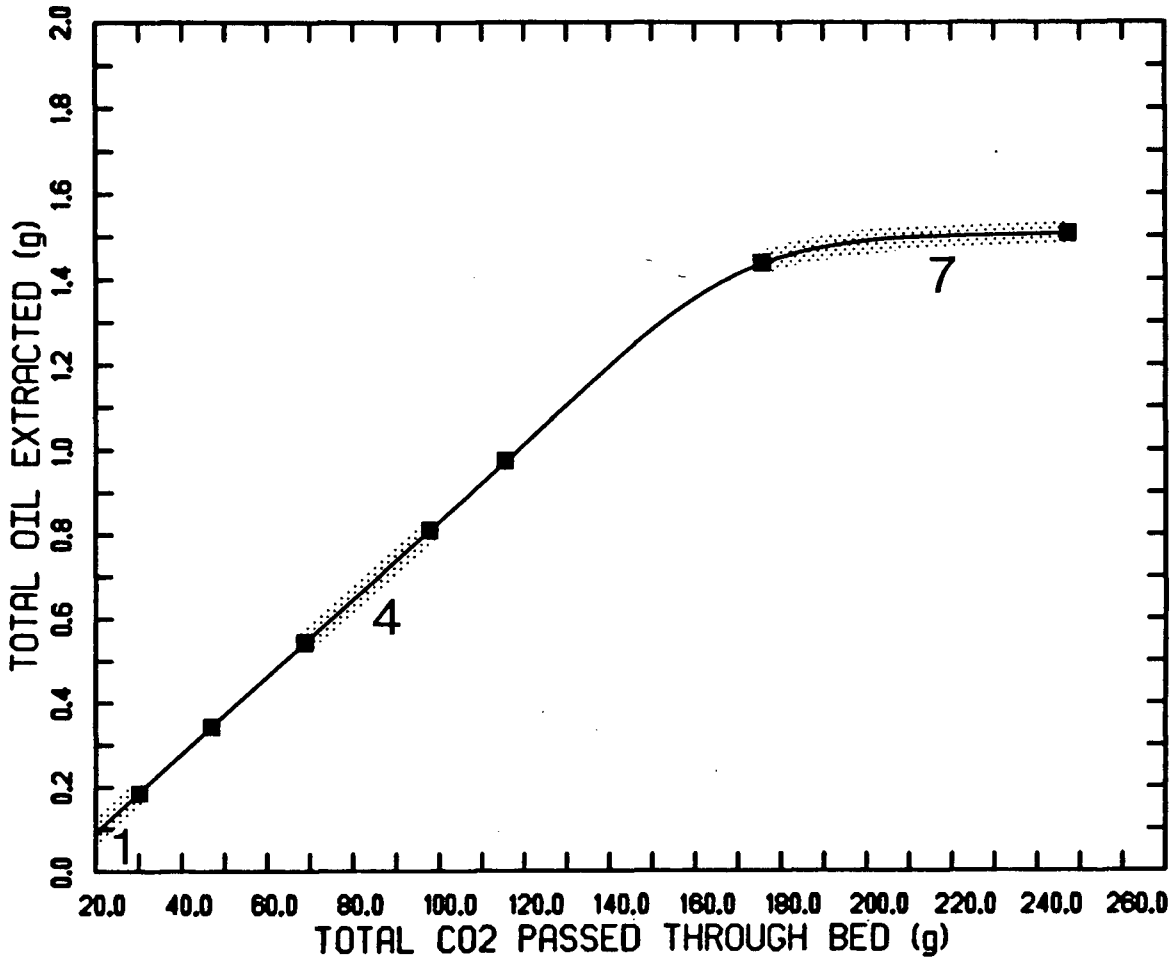

FIGURE 5.16 Extraction curve for a 4g sample of commercially cooked Canola seed. Conditions: 36 MPa, 55°C, CO_2 flow rate 0.7 g/min. The dotted areas on the curve represent the regions over which samples were collected for fatty acid analysis.

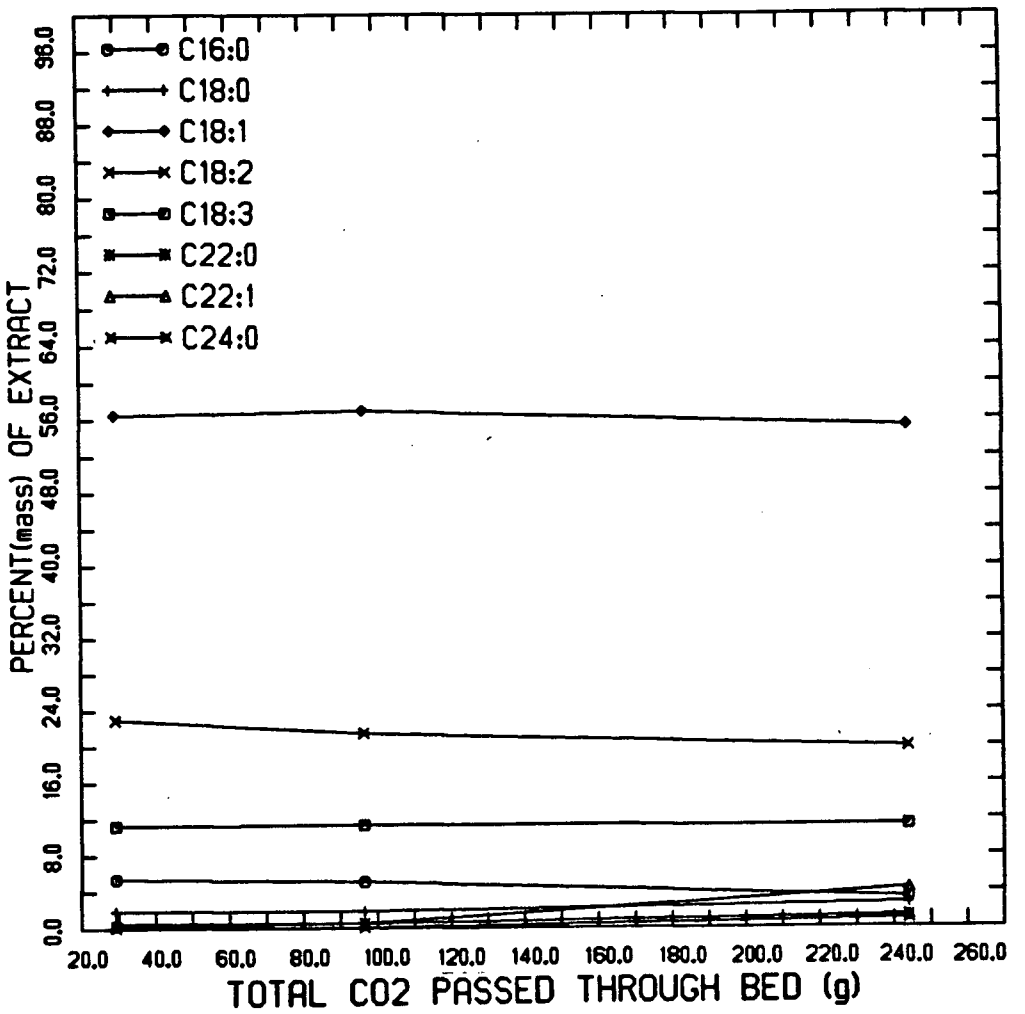

FIGURE 5.17 Fatty acid composition of the Canola seed-CO_2 extracts indicated in FIG. 5.16.

It is of interest to note that the concentration of the C24:0 FA in the final extract fraction, exceeds 0.2%, i.e. the maximum specified for edible Low Erucic Acid Rapeseed oil (LEAR) by the Codex Alimentarius Commission (Ackman, 1983). The concentration of fatty acids in the final fraction does however, conform to the specifications of the Canada Agricultural Products Standards Act (Boulter, 1983).

A possible reason why the composition of the oil extracts remains constant during most of the extraction, may be obtained by considering the distribution of triglycerides which appear in the Canola oil. In Table 5.8 the triglyceride carbon number is listed for two samples of Canola oil. The table indicates that a large proportion of the oil is composed of triglycerides having 55 or 57 carbons. Consequently the MW range of the triglycerides in the oil will be small.

TABLE 5.8 Profile by carbon number of the various triglycerides present in Canola oil from two different sources (Ackman, 1983).

seed sample		triglyceride carbon number								
		C51	C53	C55	C57	C59	C61	C63	C65	C67
1	%	2	6	18	61	7	2	1	1	-
2	%	3	4	18	70	5	1	<1	-	-

Since the various triglycerides are also very similar in chemical nature, it might be expected that they would exhibit similar solubilities in the carbon dioxide. Consequently, a large portion of the triglycerides will likely be extracted at

the same rate and the composition of the extract remains constant for much of the extraction. However, towards the end of the extraction, the small amounts of higher molecular weight triglycerides would be expected to constitute a larger fraction of the CO_2 extracts since their mole-fractions in the residual oil are higher.

5.6.5 Fatty Acid Composition of Hexane Extracts

In an effort to determine whether the hexane extracts obtained with cooked Canola seed were similar to the CO_2 extracts, the above experiments were repeated using hexane at 55°C and 1.5MPa in place of CO_2. At the completion of the extraction, each collection vial was heated to 55 °C for two hours to facilitate the removal of the hexane from the extracts. Figure 5.18 is a plot of the total oil collected during the experiment vs the total amount of hexane used. Indicated on the figure are the regions over which each of the extracts was collected. In Table 5.9 the fatty acid composition of each extract is presented while in Fig. 5.19 this information appears in graphical form. It is evident that the FA composition of the hexane extracts is similar to that of the carbon dioxide extracts(Tables 5.6 and 5.7). It is worth noting however that the late hexane extracts, unlike the corresponding CO_2 extracts, do not show an increased proportion of the high molecular weight (MW) fatty acids. One explanation for this could be that both high and low molecular weight triglycerides are equally soluble in the hexane and therefore neither is preferentially removed.

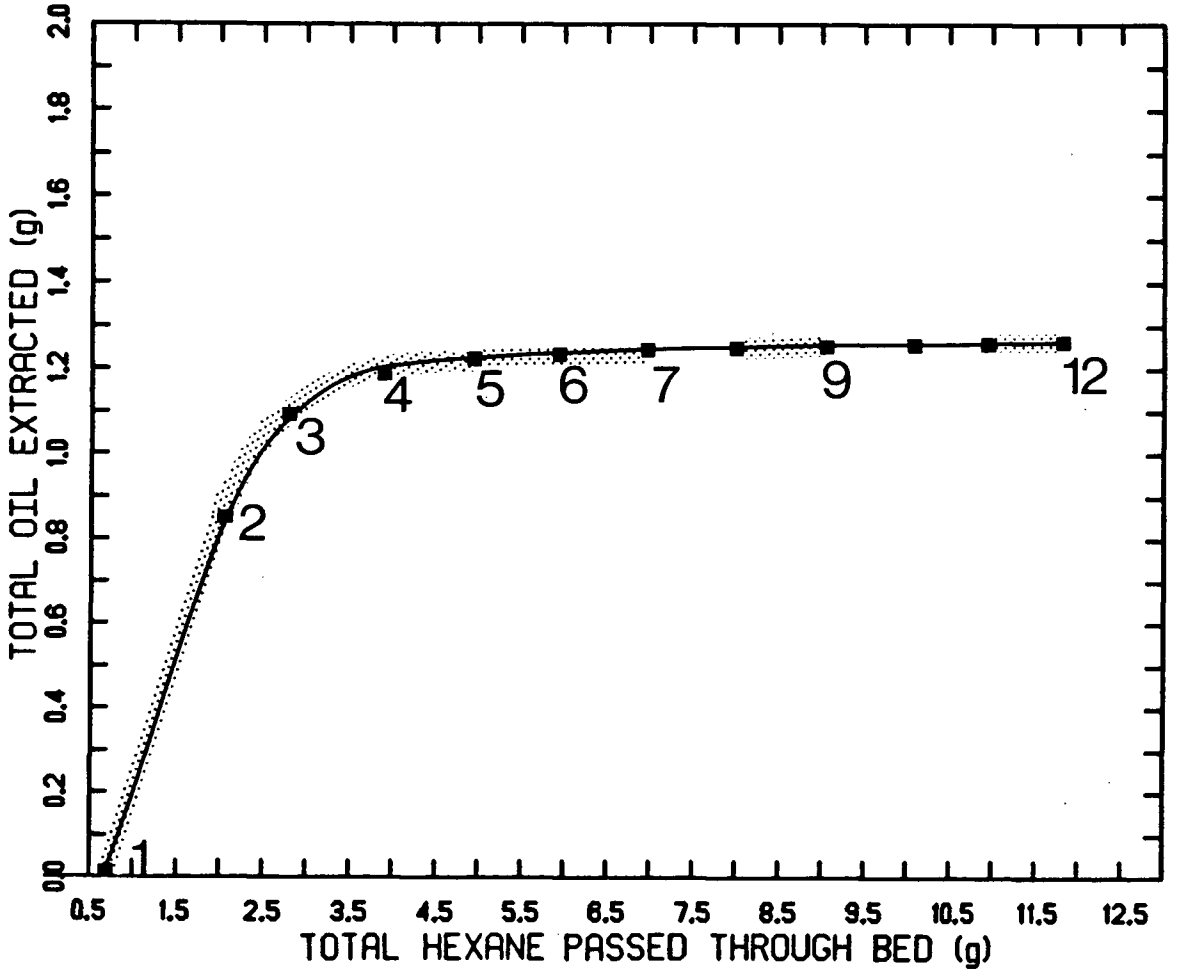

FIGURE 5.18 Extraction curve for a 4g sample of cooked Canola seed. The extraction was carried out using hexane at 1.5 MPa and 55 ° C. The dotted areas on the curve represent the regions over which samples were collected for fatty acid analysis.

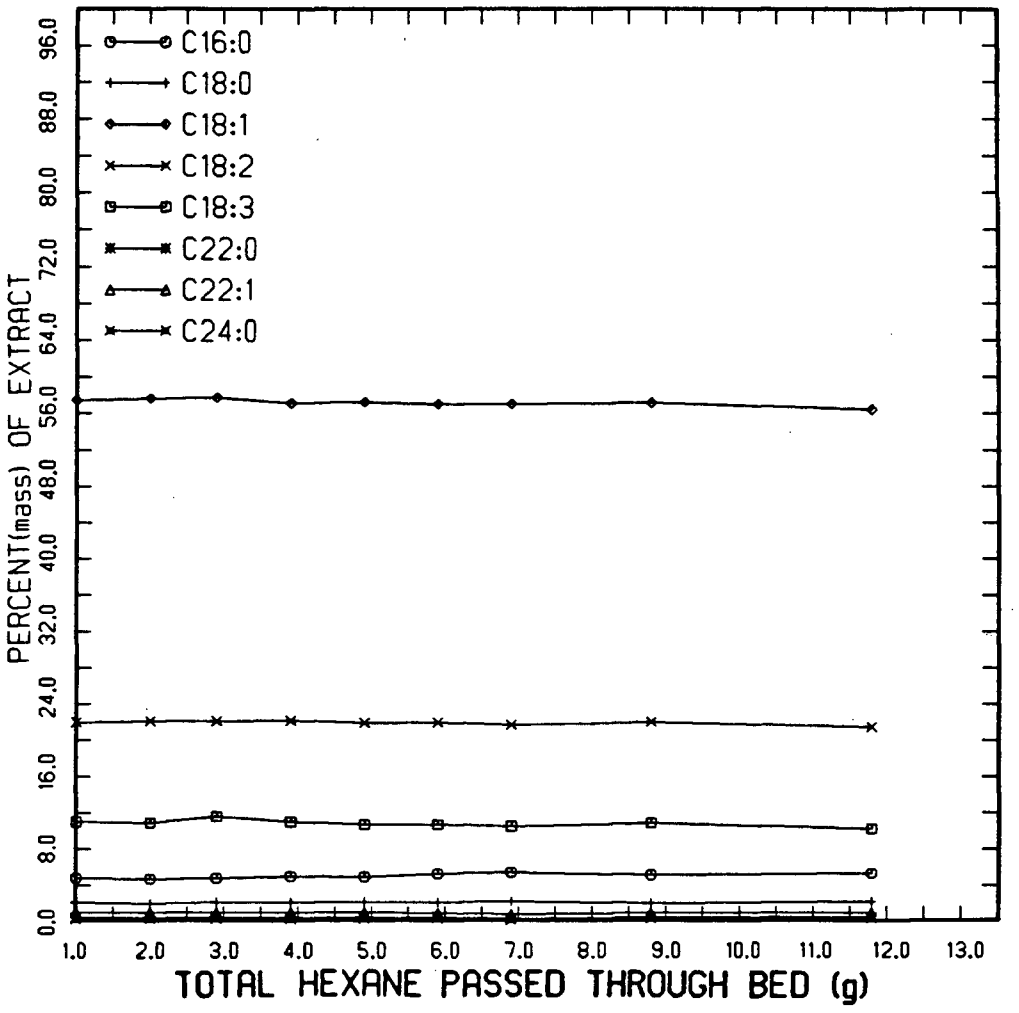

FIGURE 5.19 Fatty acid composition of the Canola seed hexane extracts indicated in Fig. 5.18.

TABLE 5.9 Fatty acid composition of the sequential hexane extract of CSP cooked Canola seed. Conditions: vessel #1, pressure 1.5 MP temperature 55°C, flowrate 0.7g/min.

fatty acid ester	hexane extract number									
	1	2	3	4	5	6	7	9	12	error %
C14:0 %	0.2	0.2	0.2	0.2	0.5	0.6	1.0	0.5	2.0	± 0.2
C16:0 %	4.8	4.7	4.8	5.0	4.9	5.3	5.4	5.1	5.3	± 0.2
C18:0 %	2.1	2.0	2.1	2.1	2.1	2.1	2.2	2.0	2.1	± 0.2
C18:1 %	57.4	57.6	57.7	57.1	57.2	57.0	57.0	57.1	56.4	± 1.4
C18:2 %	22.0	22.1	22.1	22.2	21.9	22.0	21.7	22.0	21.4	± 0.2
C20:0 %	0.9	0.8	0.9	0.9	1.0	0.9	0.9	0.9	0.9	± 0.2
C20:1 + C18:3 %	11.0	10.9	11.6	11.0	10.7	10.7	10.5	10.8	10.2	± 0.2
C22:0 %	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.4	± 0.2
C22:1 %	1.0	1.0	1.0	1.0	1.0	0.9	0.8	0.9	0.9	± 0.2
C24:0 %	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	± 0.2

5.7 CO_2 Extracts of Simple Triglycerides

5.7.1 Introduction

The previous section demonstrated that very little fractionation of the Canola oil took place during the extraction process and suggested that the reason for this was the similarity of the triglycerides which make up the oil. In order to provide further insight into the extraction process, a simple mixture of pure triglycerides was extracted. Since each triglyceride was composed of a unique fatty acid, the chromatographic procedures described in section 4.6.4 could be used to 'follow' the triglyceride composition of each extract fraction. Using this procedure, it was possible to study how the pure triglycerides interact with each other during the

extraction process. From this information it was possible to suggest a method for calculating the solubility of other vegetable oils in CO_2, based on their fatty acid composition.

Two sets of experiments were conducted using the pure triglycerides, tripalmitolein (C16:1), triolein (C18:1), and trieicosenoin (C20:1). In the first set the solubility of each triglyceride in CO_2 at 55 °C and at 36.0 MPa was determined. In the second set a mixture of these three compounds was prepared and extracted using carbon dioxide at the above conditions. The extracts from this second set of experiments were collected sequentially and subsequently analyzed for triglyceride composition. During the experiments vessel #1 was used. The CO_2 flowrate in all cases was 0.7 g/min.

5.7.2 Solubility of Single Triglycerides

The solubility of each triglyceride in CO_2 was determined by extracting it from a matrix of Ottawa sand. Figure 5.20 shows the extraction curves for each of the three triglycerides. The linear portion of the curves indicate that saturation was achieved during the extraction. The results from these experiments show that the solubility of the triglycerides decreased with increasing molecular weight (Fig. 5.21). When the solubility is plotted against molecular weight on a semi-logarithmic scale (Fig. 5.22), a straight line relationship is observed. Since the three triglycerides are part of a homologous series this behaviour is not unexpected. A similar solubility

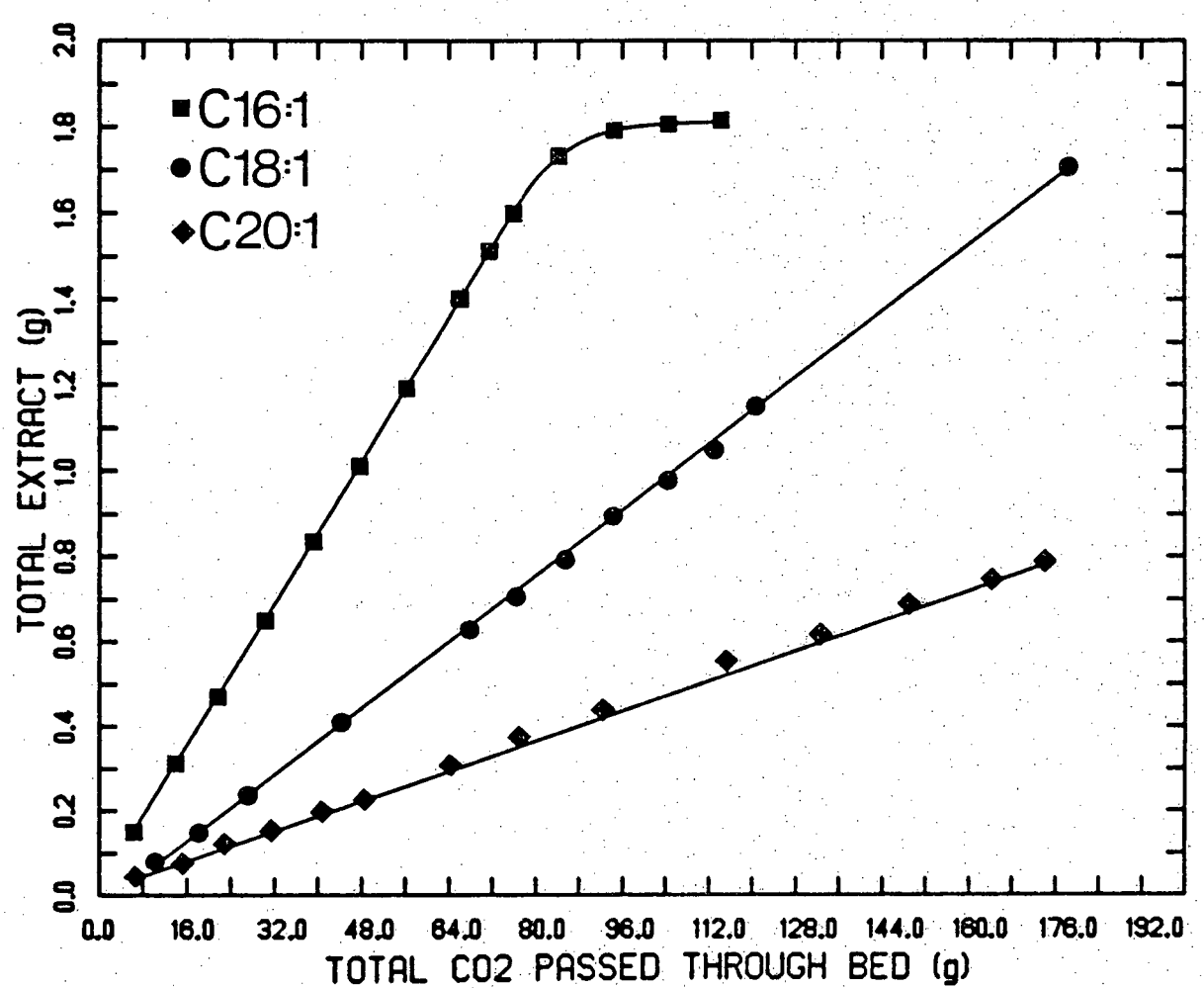

FIGURE 5.20 Extraction curves for pure-tripalmitolein (C16:1), triolein (C18:1) and tri-11-eicosenoin (C20:1). The extractions were performed from Ottawa sand using CO_2 at 36 MPa and 55 °C. Conditions: vessel #1, CO_2 flow rate 0.7 g/min.

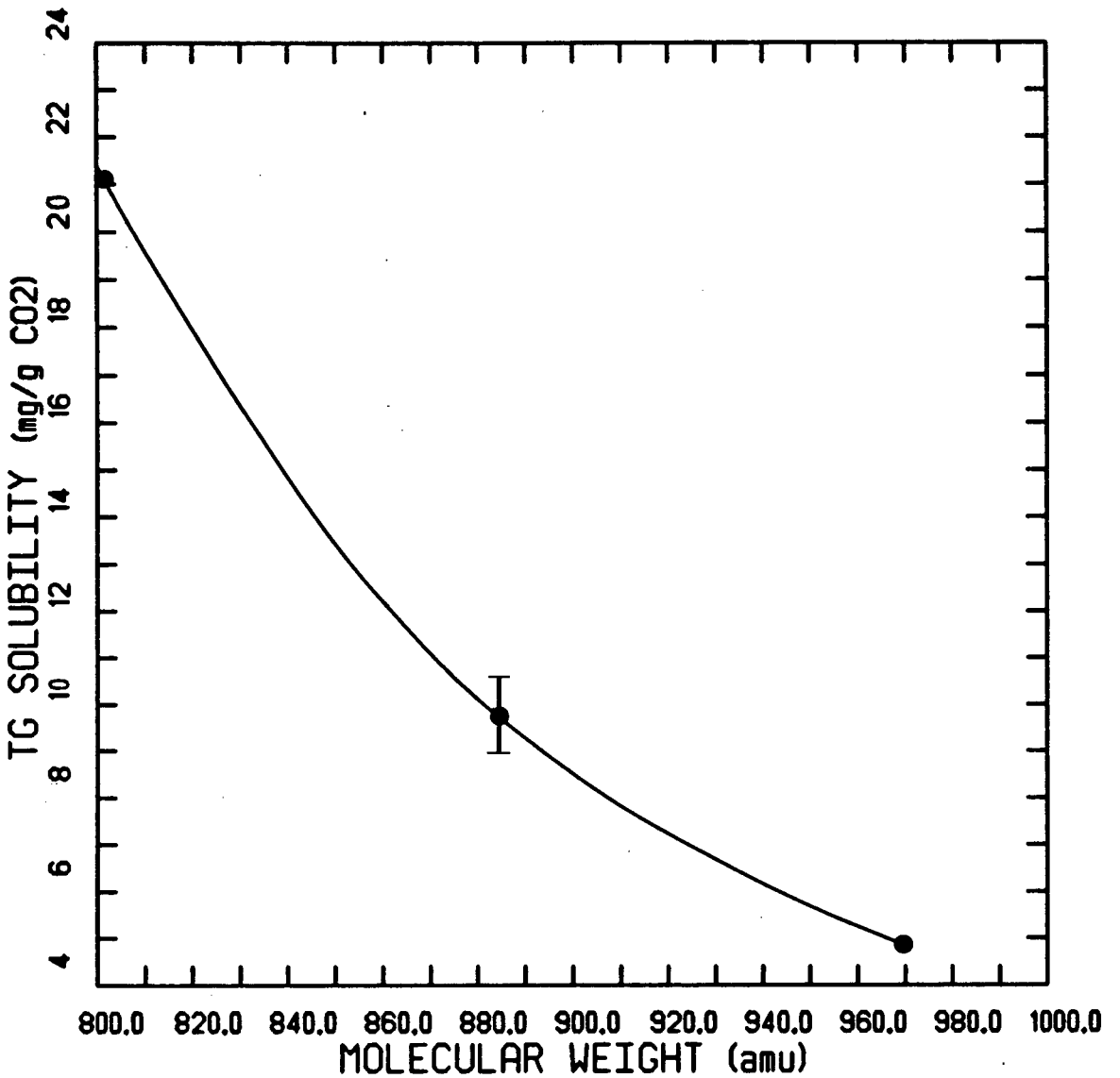

FIGURE 5.21 Solubility of the three triglycerides indicated in Fig. 5.20 as a function of their molecular weight. The error bar shown is representative of all points.

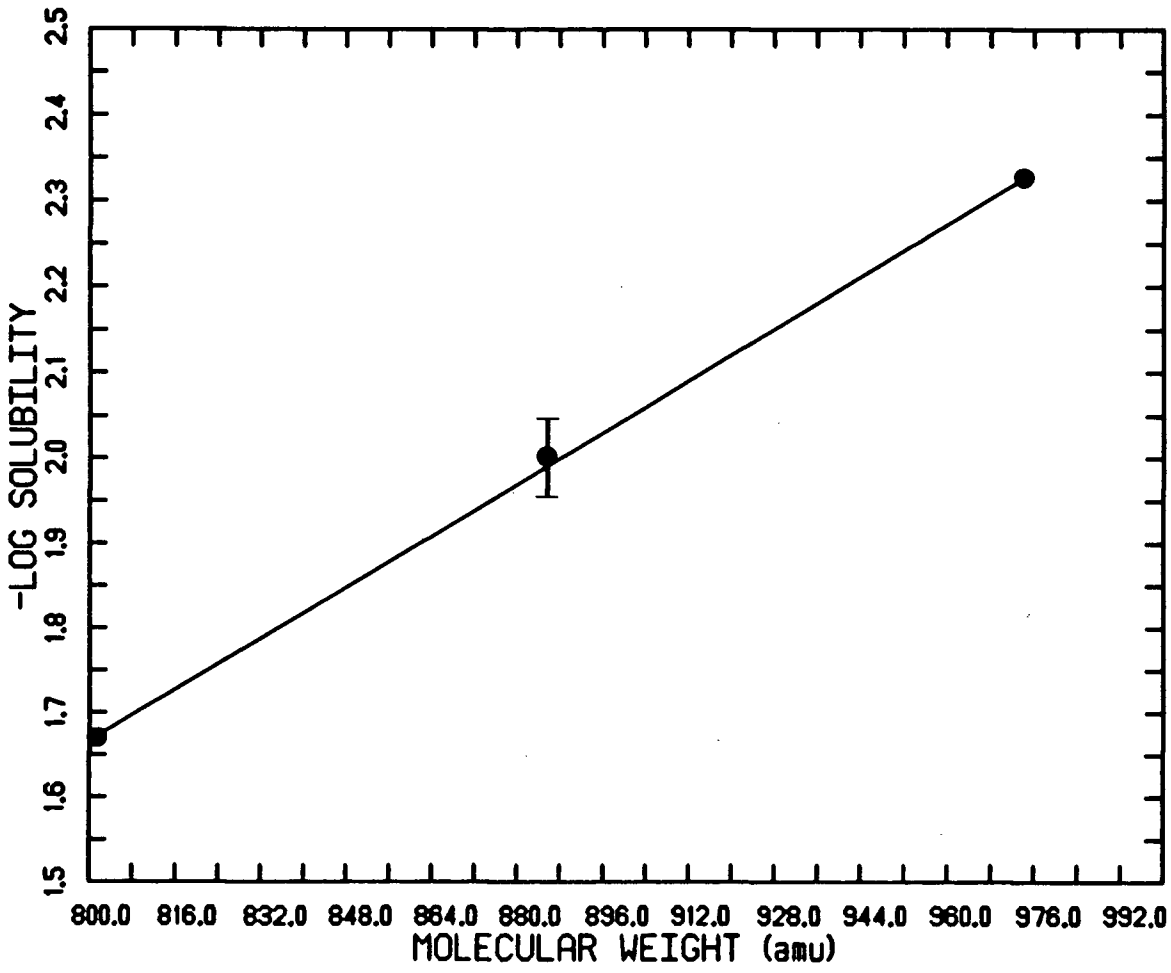

FIGURE 5.22 The negative logarithm of the solubility of the three triglycerides indicated in Fig. 5.20 as a function of their molecular weight. A typical error bar is indicated.

effect has been observed indirectly when triglycerides are dissolved in liquids. If a homologous series of triglycerides is eluted through an HPLC column it is found that a linear relationship exists between the number of carbon atoms in the molecule and the logarithm of the volume of solvent required for the passage of the triglyceride through the column(Hersloff et al., 1979; Plattner et al., 1977). This elution volume in turn is proportional to the solubility of the triglyceride in the eluting solvent

5.7.3 Solubility of a Triglyceride Mixture

Figure 5.23 is the extraction curve for a mixture of triglycerides in the proportions shown in Table 5.10.

TABLE 5.10 Mass fraction and mole fraction of components in the triglyceride(TG) mixture.

TG	molecular weight	mass fraction	mole-fraction
C16:1	801	0.330	0.366
C18:1	885	0.331	0.331
C20:1	970	0.330	0.302

From the graph it is evident that the concentration of extract in the carbon dioxide, measured at the extractor outlet, continuously declined during the course of the extraction. The sequentially collected extracts from this experiment were subsequently analyzed using the transesterification/GC procedure outlined in Sec 4.6.4. By using this analysis, it was possible to determine the mass fraction of each triglyceride present.

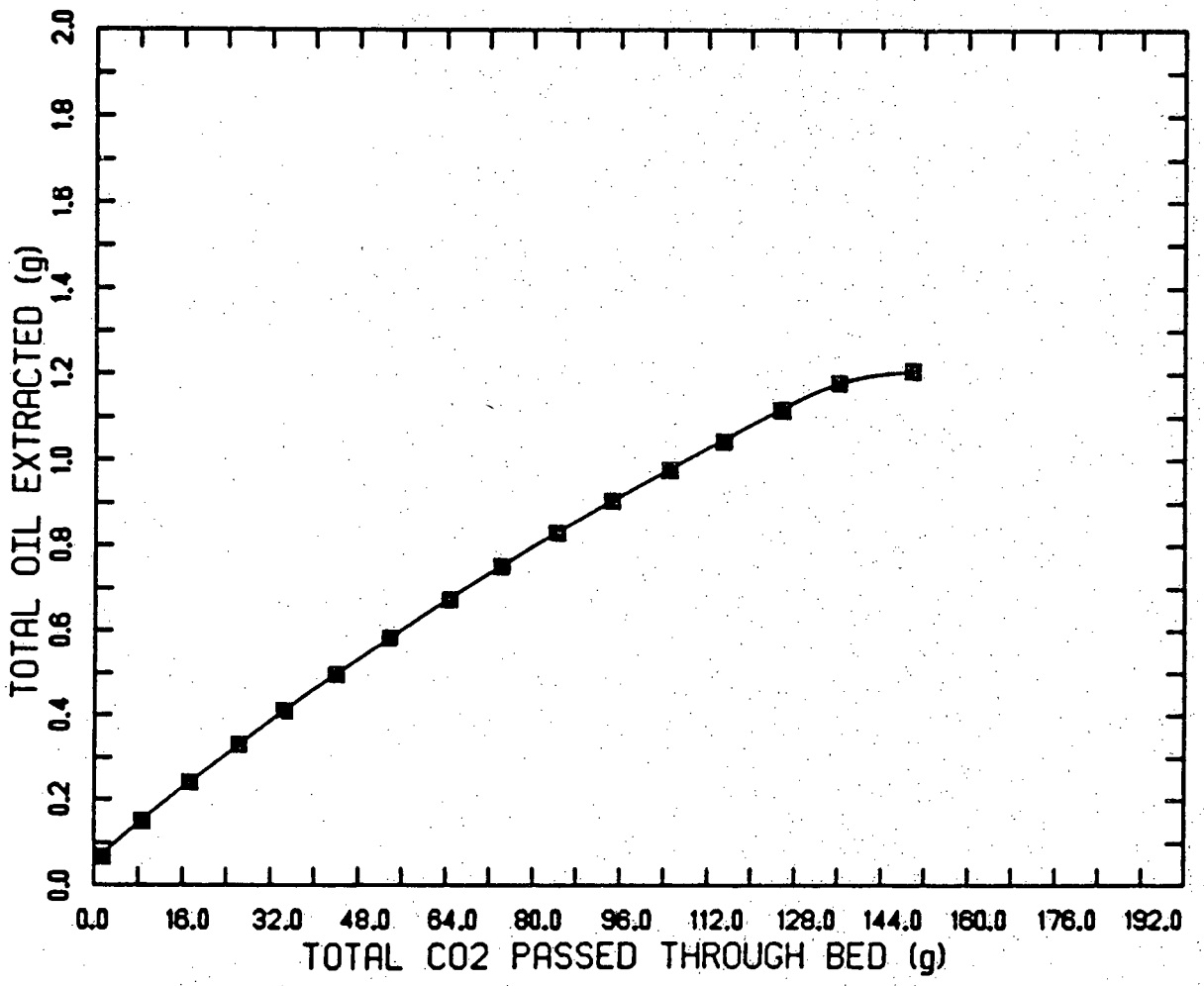

FIGURE 5.23 Extraction curves for an equal weight mixture of tripalmitolein, triolein and tri-11-eicosenoin. The extraction of the mixture was performed from Ottawa sand using CO_2 at 36 MPa, 55 °C at a flow rate of 0.7 g/min.

from the information it is evident that the composition of these extracts changed continuously (Table 5.11, Fig. 5.24). The initial extracts contained a high percentage of the lighter C16:1 triglyceride while the final extracts were composed predominantly of the C20:1 triglyceride. Hence it must be assumed that the composition of the triglyceride phase within the extractor must also have changed continuously. Since the solubilities of the triglycerides are considerably different from one another, it is thus possible that saturation can be achieved within the extractor while the overall rate of extraction continuously changes. For a mixture composed of compounds with widely differing solubilities (eg. the triglyceride mixture) a constant rate of extraction would only be expected if the molar ratio of each component in the mixture remained constant throughout the course of the extraction.

From these experiments it is evident that some fractionation of the simple mixture of triglycerides occurred during extraction with supercritical carbon dioxide. It is also evident that the degree of fractionation depends on the solubility differences that exist among the triglycerides in the mixture. It would be expected therefore, that the degree to which Canola oil could be fractionated would also depend on the solubilities of the individual oil triglycerides in the carbon dioxide.

Since Canola oil contains numerous different triglycerides (Persmark, 1972), many of which would probably exhibit similar solubilities in CO_2, it seems unlikely that simultaneous

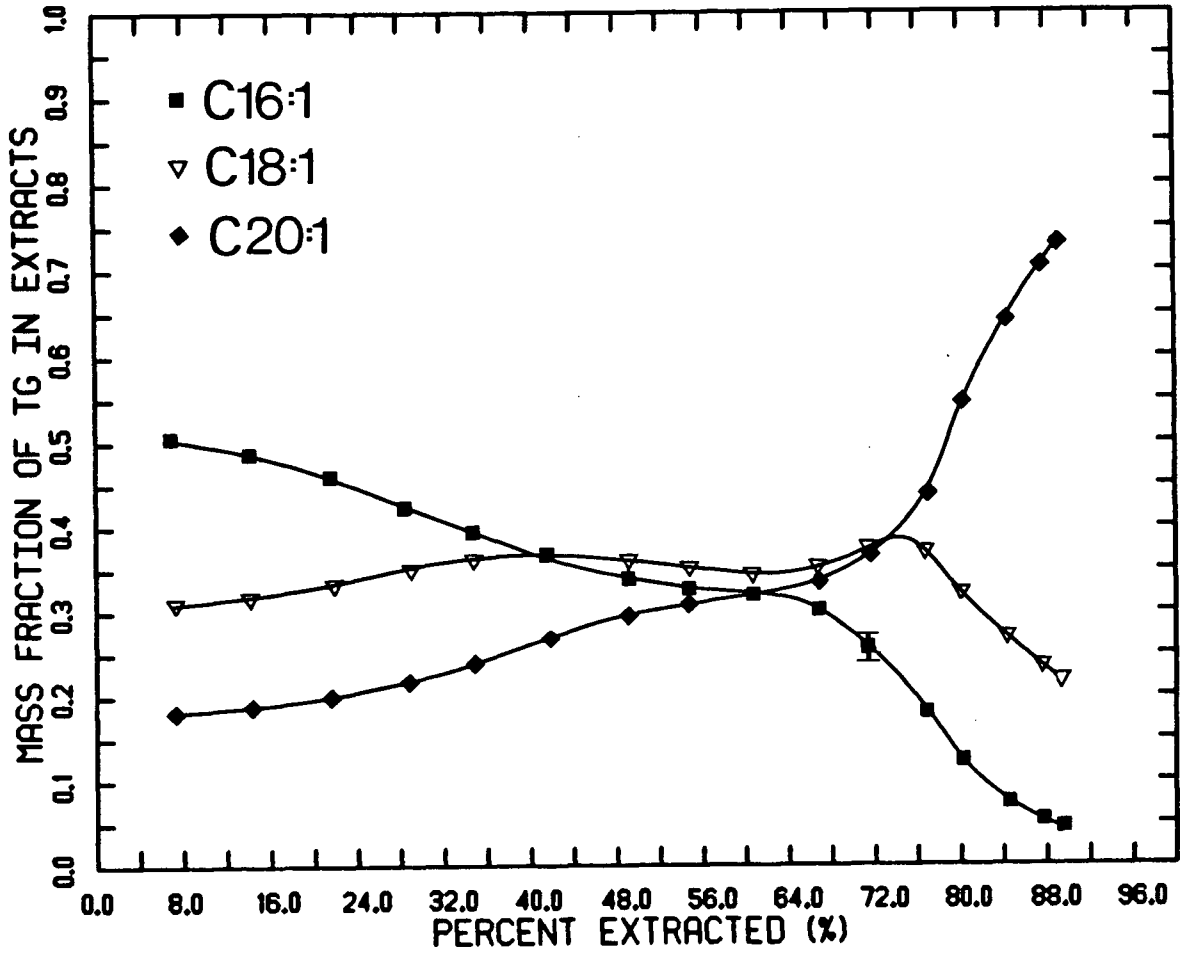

FIGURE 5.24 Mass fraction of the CO_2 extracts of the triglyceride mixture at different stages of the extraction. The composition of the extracts was determined using the transesterification-GC procedure. Each set of points represents an amount of oil corresponding to the points on Fig. 5.23. Conditions: vessel #1, pressure 36 MPa, temperature 55°C, CO_2 flow rate 0.7 g/min.

extraction and fractionation of the oil could be achieved using only supercritical carbon dioxide. If an entrainer is used with the CO_2, as suggested by Brunner and Peter (1982), a more efficient extraction and a better separation might result. The determination of the solvent mixture conditions under which such a separation might be obtained would, however, be a formidable task.

TABLE 5.11 Mass fraction of C16:1, C18:1, and C20:1 triglycerides at each data point as determined with the transesterification procedure. Mass fractions are reported with an error of ± 0.005. Conditions: vessel #1, pressure 36 MPa, temperature 55°C, flow rate 0.7 g/min.

total CO_2 (g)	cumulative mass of extract(g)	total extract %	C16:1 extract %	C18:1 extract %	C20:1 extract %
8.3	0.020	7.2	50.6	31.0	17.7
16.2	0.019	14.6	48.7	32.1	18.5
25.4	0.288	21.7	45.8	33.6	19.9
34.3	0.378	28.5	42.1	35.3	22.0
43.3	0.462	34.8	39.1	36.3	24.2
54.6	0.560	42.2	35.7	36.4	27.1
65.2	0.652	49.1	33.6	35.9	29.8
74.3	0.733	55.2	33.3	35.4	30.9
83.4	0.811	61.1	32.5	34.8	32.0
92.4	0.886	66.8	30.5	35.5	33.6
101.3	0.949	71.5	25.3	37.3	36.6
112.4	1.022	77.0	18.2	37.5	43.8
121.1	1.072	80.1	12.0	32.7	54.8
130.0	1.121	84.5	8.3	27.4	63.8
139.0	1.164	87.8	5.8	23.4	70.2
149.2	1.176	88.6	4.9	22.1	72.7

5.7.4 Triglyceride Solubility Interactions in Carbon Dioxide

When a supercritical fluid exists in equilibrium with a liquid mixture, it may be possible to write an equation which describes the total solubility of the mixture in terms of the solubilities of the pure components of the mixture. eg.

$$St = xaSa + xbSb \quad . \quad . \quad . \quad . \quad . \quad [5.1]$$

where St = solubility of the mixture in the supercritical CO_2 phase

Sa,b = solubility of the pure substance in the CO_2 phase

In an effort to determine whether the solubility of the triglyceride mixture could be described by this equation, the calculated and measured concentration values of each component in the CO_2 extract were compared (Table 5.12). The calculated composition of the extract was determined by multiplying the solubility of each triglyceride by its mole-fraction in the mixture. As the results indicate, both the calculated and observed values are within a few percent of each other. The system therefore behaves in an ideal manner. This result is not unexpected for a homologous series since molecular interactions between homologs are comparable to the interactions between molecules of a pure substance.

TABLE 5.12 Comparison of calculated and experimental composition of the CO_2 extract of a triglyceride mixture. Conditions: vessel #1, pressure 36MPa, temperature 55°C, flow rate 0.7 g/min.

TG	TG solubility g/gCO_2	oil-phase TG mole-fraction	calculated composition g/gCO_2	measured composition g/gCO_2
C16:1	0.021	0.366	0.0077	0.0072
C18:1	0.010	0.331	0.0033	0.0034
C20:1	0.005	0.302	0.0015	0.0016

5.7.5 Prediction of Oil Solubility in Carbon Dioxide

Theoretically it is possible to calculate the extract composition and total solubility of a particular oil in CO_2 provided two types of information are known: i) the identity and proportion of the fatty acids which comprise the triglycerides of the oil, and ii) the solubilities in the carbon dioxide of the various triglycerides which arise from the component fatty acids. A method to predict solubility would be valuable since gathering the same information experimentally is costly and time consuming. A calculation based on fatty acid composition would be particularly valuable since this information already exists for many seed oils.

The calculation requires, in addition to the above information, two assumptions:

1) the distribution of the fatty acids among the various triglycerides is random

2) the solution of the triglycerides in the carbon dioxide is ideal

There is some evidence that the first assumption may be valid.

There is some evidence that the first assumption may be valid. In a recent study by Merritt et al. (1982), the triglyceride composition of several oils could be predicted accurately from the known proportion of fatty acids present in the oil. One of the assumptions of this study was that the acids were distributed randomly.

It is not known whether the second assumption is valid. However, some support for it was provided in the previous section, where it was shown that the concentration of triglycerides in supercritical carbon dioxide could be calculated based on the their mole-fractions in the mixture.

An example of how the oil-solubility calculation would be performed is as follows:

If a given oil is comprised of n fatty acids in proportions $p_1, p_2 \ldots pn$, then from the multinomial distribution it can be calculated that the probability of the occurrence of a particular triglyceride is:

$$\text{prob (FFF)} = \left(\frac{3!}{x_1!\, x_2! \cdots x_n!} \right) p_1^{x_1} p_2^{x_2} \cdots p_n^{x_n} \quad . \quad [5.2]$$

where x represents the number of occurances of a fatty acid in a particular triglyceride. For example, consider a hypothetical oil composed of palmititc (C16), oleic (C18) and eicosenoic (C20) acids in the proportion 0.1, 0.8 and 0.1 respectively.

The probability of finding a triglyceride with the composition C16-C16-C18 would be:

$$\text{prob (P P O)} = \left(\frac{3!}{2!\ 1!\ 0!} \right) 0.1^2\ 0.8^1 = 0.024$$

The complete distribution of triglycerides in the hypothetical oil calculated using Eq. 5.4 is listed in Table 5.13.

If the second assumption made earlier is also valid, then the total solubility of the oil in the CO_2 becomes the sum of the individual triglycerides solubilities multiplied by their respective mole-fractions (x) in the mixture, i.e.

$$\text{sol(mix)} = \sum x_i\ (S_i) \quad . \quad . \quad . \quad [5.3]$$

If we make an additional assumption that the overiding factor dictating triglyceride solubility is molecular weight then triglycerides 2 and 6, 3 and 7, and 4 and 8, in Table 5.13, will have similar solubilities. From the solubility data presented in Fig. 5.22 the solubility of the hypothetical oil can be calculated, using Eq. 5.5, to be 0.011 g oil/g CO_2 at 36.0 MPa and 55 °C.

In order to more fully evaluate the above method for predicting oil solubilities, more research needs to be done. This research would involve making synthetic mixtures of triglyceride oils and comparing the solubility of the mixtures in the CO_2, as determined experimentally, with the solubilities calculated using Eqs. 5.4 and 5.5.

TABLE 5.13 calculated mole-fraction concentration of triglyceride in an oil composed of three fatty acids in the molar ratio: C16(P) 0.1, C18(O) 0.8, and C20(E) 0.1

number	fatty acid makeup	molecular weight	mole-fraction
1	PPP	801	0.001
2	POO,OPO	857	0.192
3	POE,OEP,OPE	885	0.048
4	PEE,EPE	913	0.003
5	PPO,POP	829	0.024
6	PPE,PEP	857	0.003
7	OOO	885	0.512
8	OEO,OOE	913	0.192
9	OEE,EOE	941	0.024
10	EEE	969	0.001

5.8 Phosphorus Content Of Oils

5.8.1 Introduction

When Canola seed is crushed, phospholipid components, which appear naturally in the seed's cell membranes, are released. These phospholipids are subsequently dissolved by the seeds storage lipids (seed oil). As a result, the crude oil obtained by expelling the Canola seed contains 1.5 to 3wt% phospholipid (Teasdale and Mag, 1983). In the conventional hexane extraction process these phospholipid gums are removed along with the seed oil. Since these gums are undesirable in the finished oil product, they are removed during the oil refining process and added back to the Canola meal (Anjou, 1972). There was some indication that CO_2 would not extract phospholipid gums from the crushed seed (Friedrich and List, 1982). Accordingly, experiments were conducted to investigate this, by using the procedure described in Sec. 4.7.

In the first set of experiments, flaked and cooked Canola seed were extracted with carbon dioxide. Samples of the oil extract were collected at different periods and subsequently analyzed for their phosphorus content. As well, at the termination of the extraction, the system was dismantled and the stainless steel filters and restricter valve were analyzed to determine if any phospholipid had been deposited on them.

In the second set of experiments, a quantity of pure crude Canola oil with a known phospholipid concentration was placed on Ottawa sand and extracted under the same pressure and temperature conditions as above. At the conclusion of the experiment the total amount of phospholipid remaining in the extraction vessel was determined. By subtracting this value from the initial value, the total amount of phospholipid extracted could be determined.

5.8.2 Phosophorus in Commercially Produced Canola Oil

Prior to determining the phosphorus content of the CO_2 extracts of Canola seed, the phosphorus content of commercially produced crude and refined Canola oil was studied.

Since the phosphorus content of the commercial crude oil was known to be high, it was diluted using $CHCl_3$ prior to analysis. The dilution was necessary in order to keep the samples within the range of the analytical procedure. The dilution factor was such that 10 μL of solution contained 0.498 mg of oil. The results (Table 5.14) indicate that the concentration of phospholipid in the oil was about 1.87 ± 0.09%

The commercially refined oil, considered to be a finished

product, was known to contain little or no phosphorus and consequently was used without dilution. As indicated in Table 5.15 no phosphorus was detected in the samples.

TABLE 5.14 Phospholipid content of commercial unrefined Canola oil. Absorbance readings (Abs) are in absolute values.

sample number	sample size mg	Abs	Phospho-lipid %
1	0. 498	0.345	1.86
2	0. 498	0.348	1.88
3	0. 498	0.346	1.87
4	0. 249	0.170	1.83
5	0. 249	0.171	1.84
6	0. 249	0.178	1.92

TABLE 5.15 Phospholipid content of refined and bleached commercial Canola oil. Absorbance (Abs) readings are in absolute units.

sample number	sample size mg	Abs	Phospho-lipid %
1	7.52	0.002	<0.01
2	7.35	0.000	<0.01
3	7.39	0.010	<0.01
4	7.49	0.002	<0.01
5	7.48	0.008	<0.01
6	7.45	0.002	<0.01

5.8.3 Phosphorus In Carbon Dioxide Extracts Of Canola Seed

In this experiment, the phosphorus content of the CO_2 extracts of commercially flaked and cooked Canola seed was investigated. The sample size was 4g and the CO_2 extraction was carried out at 36 MPa and 55°C using extraction vessel #1. The CO_2 flow rate was 0.7 g/min. The samples obtained for analysis were collected over five intervals during the extraction (Fig.

5.25). The results of the analysis are given in Table 5.16.

TABLE 5.16 Phospholipid content of CO2 extracts of cooked Canola seed. Absorbance (Abs) readings are in absolute units.

sample number	sample size mg	Abs	Phosholipid %
1	7.26	0.007	<0.01
2	6.87	0.005	<0.01
3	7.13	0.010	<0.01
4	7.20	0.024	<0.01
5	7.26	0.028	<0.01

At the completion of each experiment the extraction system was taken apart and the frits and restricter valve were washed with a mixture of chloroform and methanol. The washings were then made up to 2.0 mL using chloroform and four 50μL samples of the washings were analyzed for phosphorus. No phosphorus was detected in any of the washings.

5.8.4 Phosphorus in Carbon Dioxide Extracts of Canola Oil

At the start of the experiment 1.030 g of crude oil was introduced into extraction vessel #1 which had previously been filled with 0.3 mm Ottawa sand and packed at both ends with glass wool. From the known concentration of phospholipid in the oil (Table 5.14) it was determined that the total phospholipid within the vessel was 0.0193 g, which corresponds to a total phosphorus mass of 749 μg. After extracting the oil with CO_2 (flow rate 0.7g/min) at 55 °C and 36.0 MPa for 3.8 h, the glass wool and Ottawa sand were each washed several times

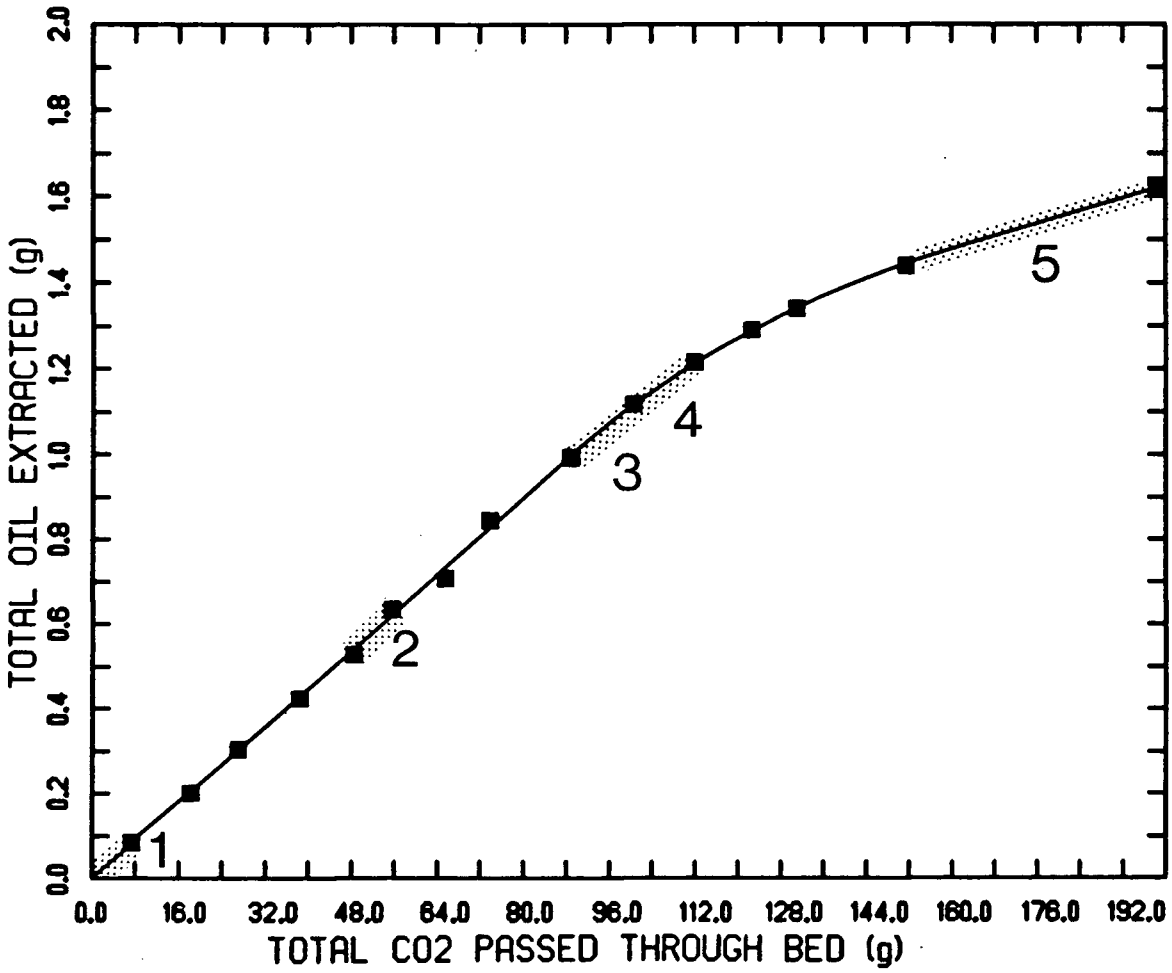

FIGURE 5.25 Extraction curve for a 4g sample of cooked Canola seed showing the intervals (dotted) over which samples of oil were collected for phosphorus analysis. Conditions: pressure 36 MPa, temperature 55°C, CO_2 flow rate 0.7 g/min.

with chloroform and the washings made up to 50 mL and 25 mL respectively. Phosphorus determinations were then carried out on each washing. The results from these analyses indicated that at the end of the experiment the glass wool held approximately 497 μg of phosphorus and the Ottawa sand 246 μg for a total of 743 μg. Within the limits of error of the experiment, the supercritical CO_2 did not extract any phosphorus from the vessel. The detailed results from this experiment are summarized in Table 5.17.

TABLE 5.17 Phosphorus analysis of chloroform washings of the glass wool and Ottawa sand after extraction. Absorbance (abs) is shown in absolute units.

sample size μL	sample	Abs	phosphorus content μg	total phosphorus μg
50	wool	0.450	0.470	
10	wool	0.100	0.105	497
20	sand	0.207	0.215	
10	sand	0.085	0.089	246

These results indicate that under the conditions tested and within the limits of the analytical procedure, supercritical carbon dioxide does not extract phospholipid material from the crushed Canola seed. These findings are similar to those reported in the literature dealing with SFE of corn germ (Christianson et al., 1984) and cottonseed (List et al., 1983). In these reports the maximum phosphorus content of the extracted oils were 5 ppm and 1 ppm, respectively. In both of these studies the oils were extracted using CO2 at 50 °C and 54 MPa.

In two additional studies, which dealt with the application

of SFE to soybean meal, it was found that small amounts of phosphorus did appear in the oil extracts. In the first of these studies (Friedrich and List, 1982) it was found that the extract obtained by using CO_2 at 34 MPa and 50 °C, had a phosphorus concentration of 60 ppm. In the second study (List et al., 1982), it was found that the oil produced by using CO_2 at 54 MPa and 50 ° C, had a phosphorus concentration of 45 ppm.

The differences in the phosphorus contents of the supercritical extracts from the various seeds cannot be accounted for on the basis of phospholipid concentration in the different meals since all have approximately the same value (Sonntag, 1979). However, a possible reason for the differences may be that the phospholipids in each of the seeds are of different polarities. Since it is known that a compound's solubility in a supercritical fluid is strongly dependent on the compound's polarity, it would be expected that different polarity phospholipids would also exhibit different solubilities in the CO_2 (Zosel, 1978).

The fact that CO_2 does not remove the phospholipids from the Canola meal is significant. Conventional hexane extracted oil requires an acid de-gumming step to reduce the phosphorus levels in the oil to 5-10 ppm (Appelqvist, 1971). Since the oil produced using supercritical carbon dioxide has a phosphorus content in this region to begin with, it would not require acid de-gumming. An extraction process based on supercritical extraction would in this respect be simpler and less expensive than the corresponding hexane process.

5.9 Computer Simulation Of The Extraction Process

5.9.1 Introduction

Extraction from a fixed bed is a semi-batch process and can be mathematically described by differential Eqs. 3.3 and 3.4 which were introduced earlier. To solve this system of differential equations a computer program was developed. The solution to the equations provided by the computer gave both the solvent and seed phase oil concentration profiles along the seed bed as a function of time. An additional program was used to generate the process extraction curves from the obtained concentration profiles. Using the mathematical model, which is described in chapter 3, it was possible to determine approximate values of the overall mass transfer coefficients for the extraction process at different solvent velocities.

5.9.2 Results

In Figs. 5.26-5.33 the extraction curves generated by the computer are plotted along with the corresponding experimental data for crushed, finely chopped and flaked seed. The experimental data shown in Fig. 5.26 were obtained from the extractor #4 (p.58) containing 1.5 g of seed. Figs. 5.26-5.31 represent experimental data which was collected using extraction vessel #1 containing approximately 4 g of seed while Figs. 5.32 and 5.33 represent data collected with extraction vessel #3 containing 12 g samples of seed. The close agreement between the experimental data and the computer generated data suggests that the model was able to simulate the physical extraction process reasonably well.

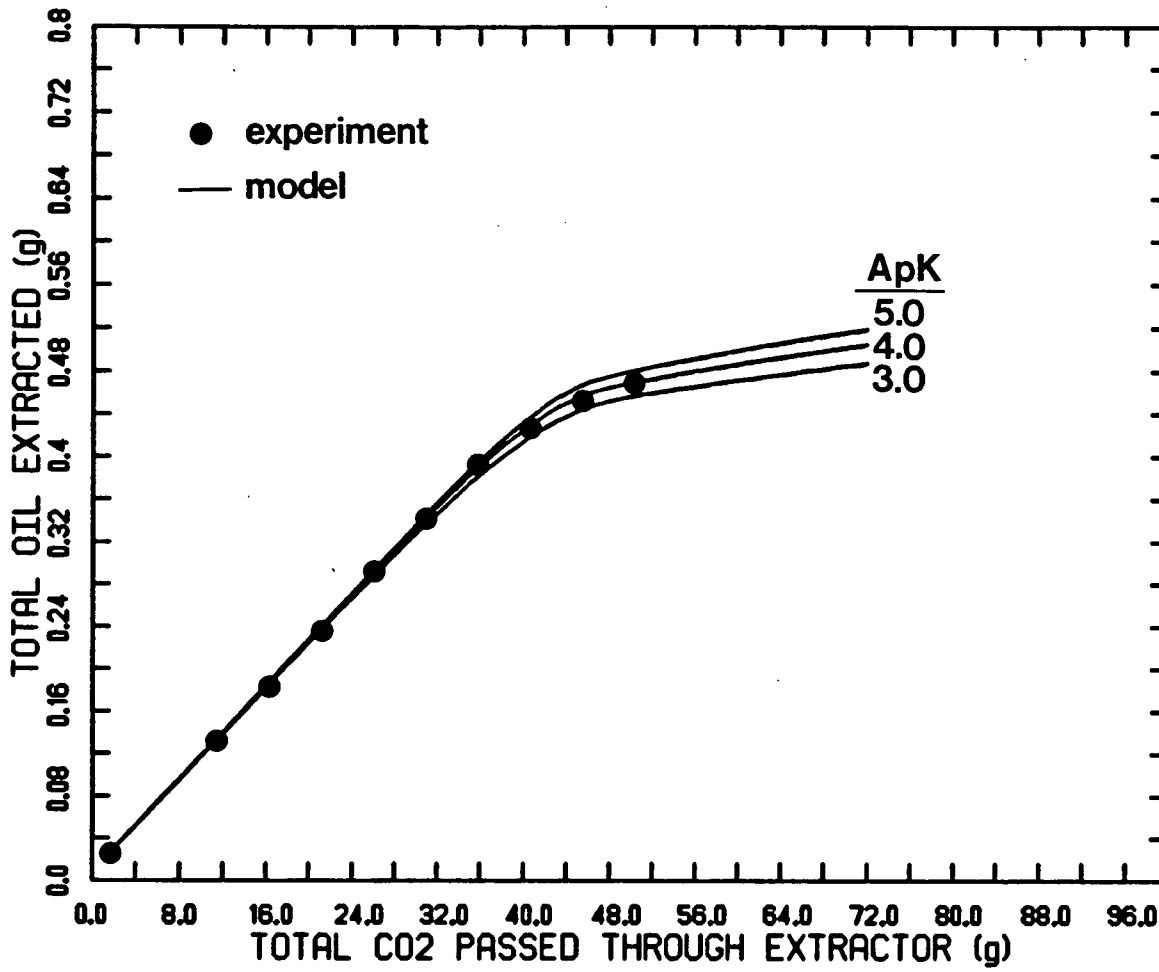

FIGURE 5.26 Extraction curve obtained by passing CO_2 at 55°C and 36 MPa through extraction vessel #4 containing 1.5 g of crushed seed. The CO_2 flow rate was 1.6 g/min which corresponds to an interstitial velocity of 16.7 cm/min. The computed extraction curves were calculated using three different values of ApK.

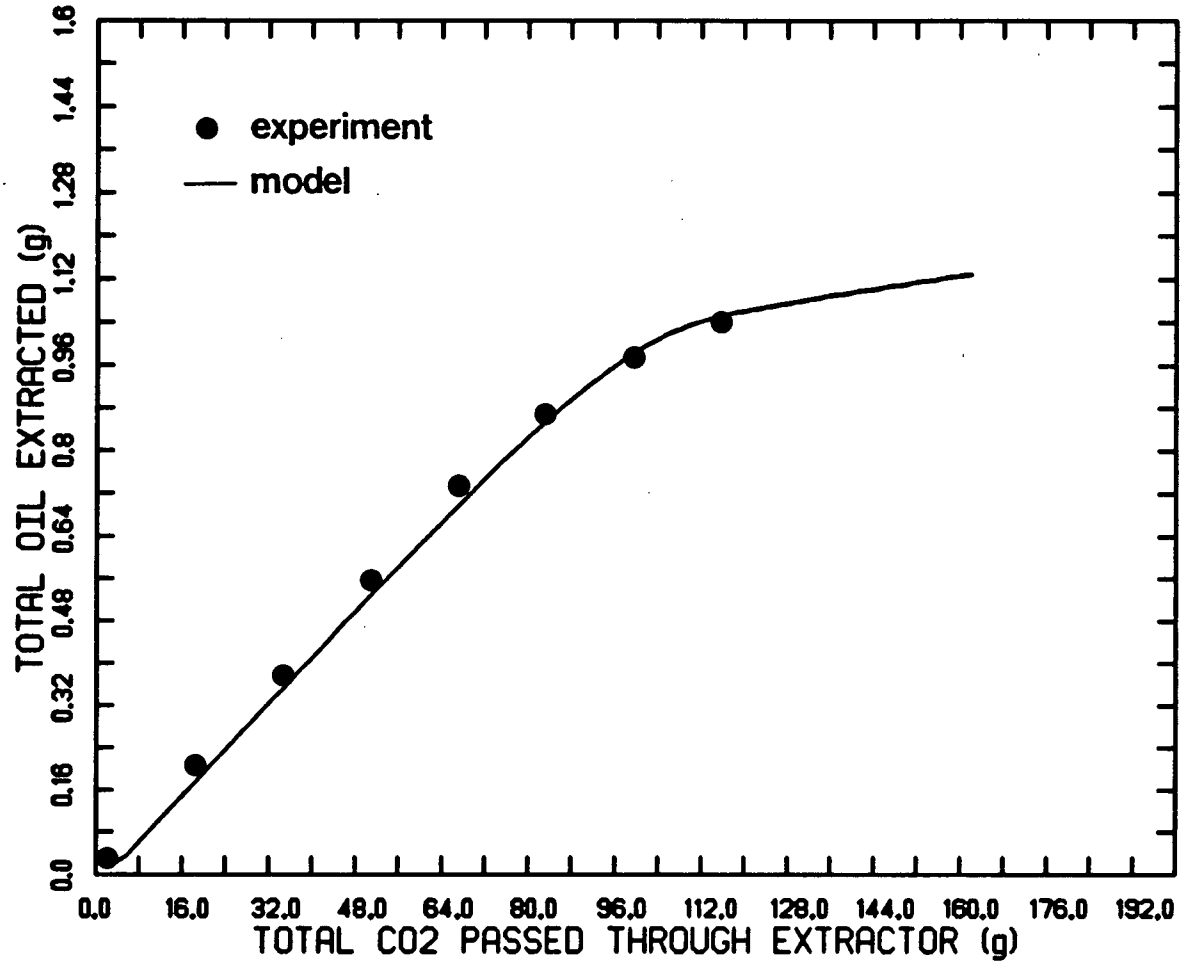

FIGURE 5.27 Extraction curve obtained by passing CO_2 at 55°C and 36 MPa, through extraction vessel #1 containing 4.0 g of crushed seed. The CO_2 flow rate was 2.7 g/min which corresponds to an interstitial velocity of 3.9 cm/min. The computed extraction curve was calculated using an ApK value of 2.0 gCO_2/cm^3min.

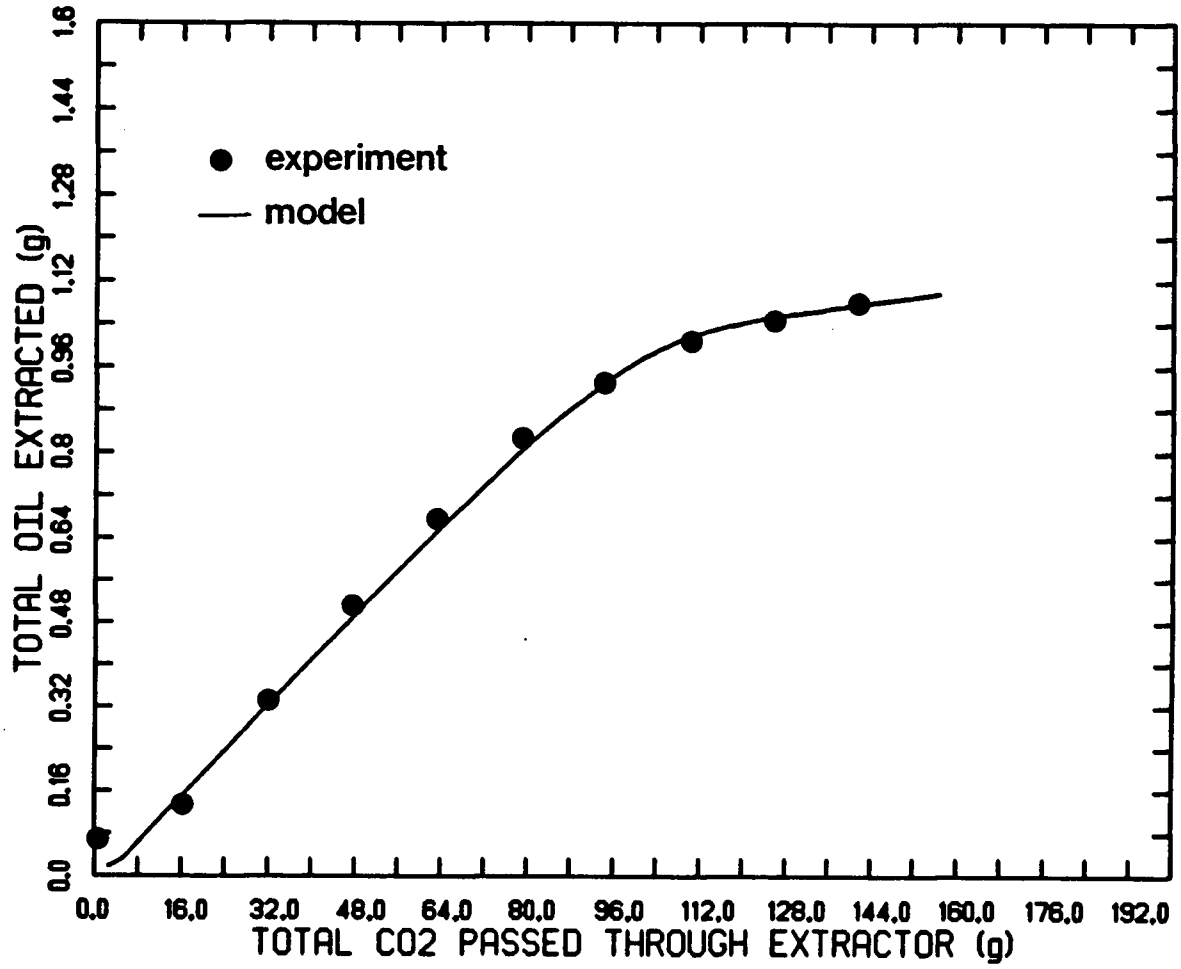

FIGURE 5.28 Extraction curve obtained by passing CO_2 at 55 °C and 36 MPa, through extraction vessel #1 containing 4.0 g of crushed seed. The CO_2 flow rate was 2.6 g/min which corresponds to an interstitial velocity of 3.8 cm/min. The computed curve was calculated using an ApK value of 1.8 gCO_2/cm^3min.

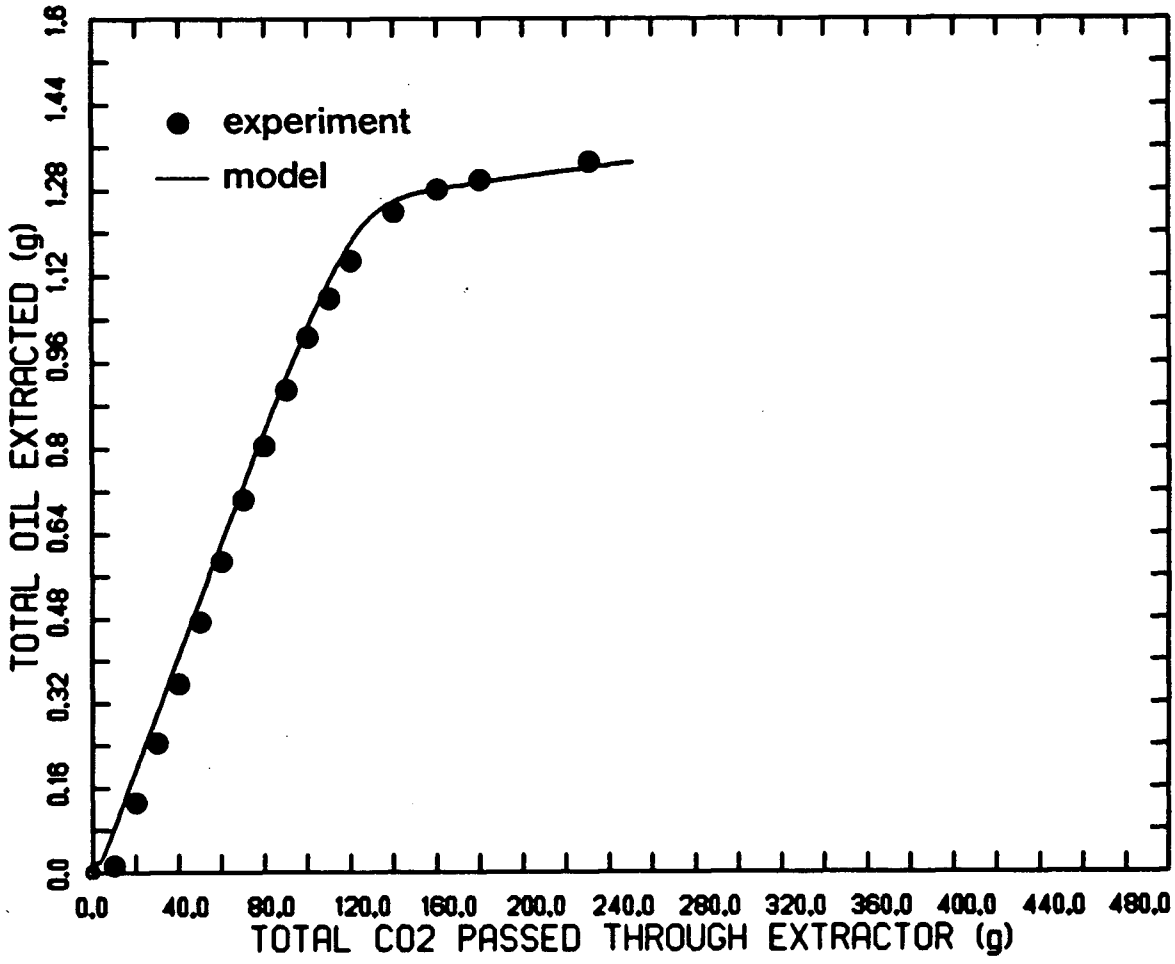

FIGURE 5.29 Extraction curve obtained by passing CO_2 at 55 °C and 36 MPa, through extraction vessel #1 containing 3.8 g of commercially flaked seed. The CO_2 flow rate was 1.7 g/min which corresponds to an interstitial velocity of 2.5 cm/min. The computed extraction curve was calculated using an ApK value of 1.3 gCO_2/cm^3min.

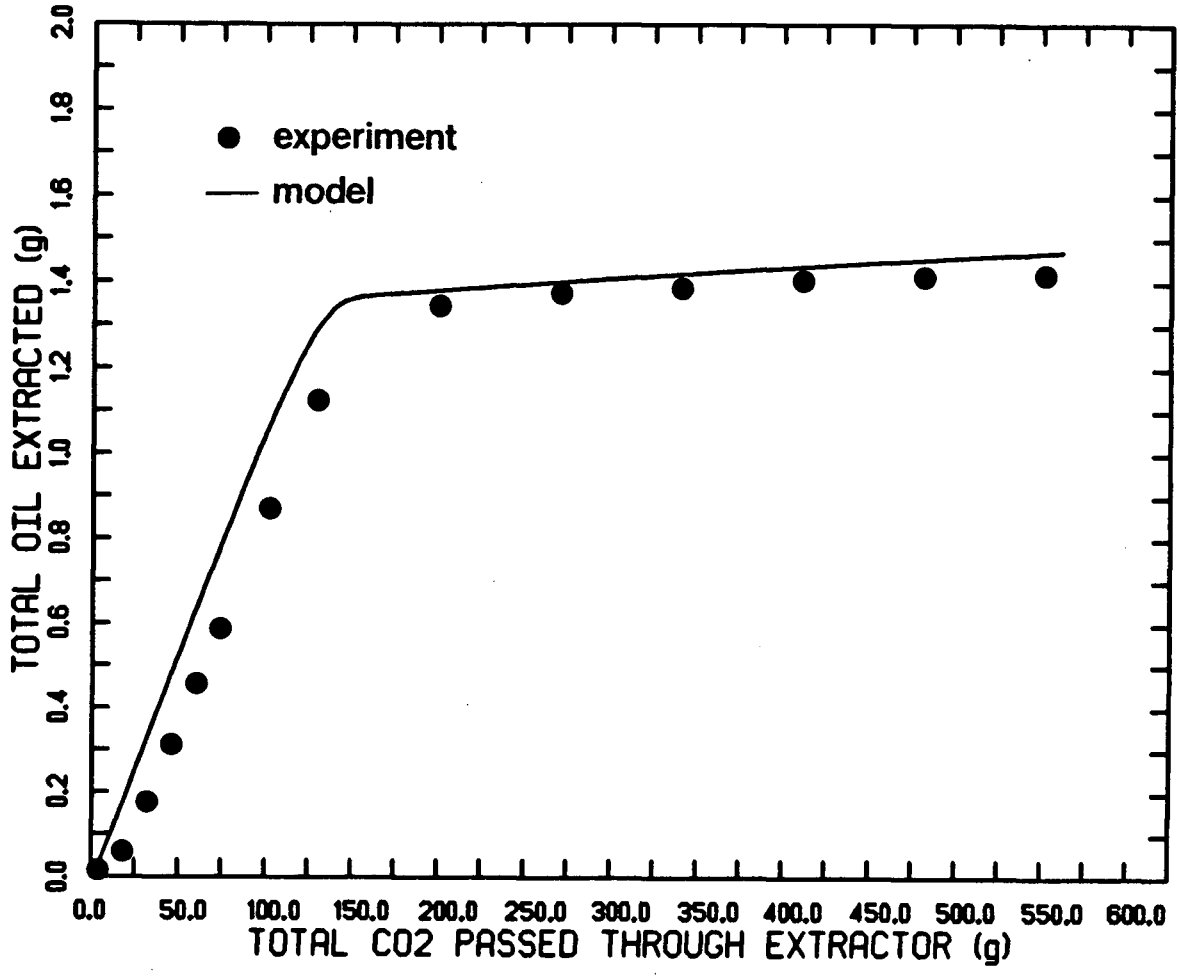

FIGURE 5.30 Extraction curve obtained by passing CO_2 at 55 °C and 36 MPa, through extraction vessel #1 containing 4.0 g of finely chopped seed. The CO_2 flow rate was 2.3 g/min which corresponds to an interstitial velocity of 3.5 cm/min. The computed extraction curve was calculated using an ApK value of 2.0 gCO_2/cm^3min.

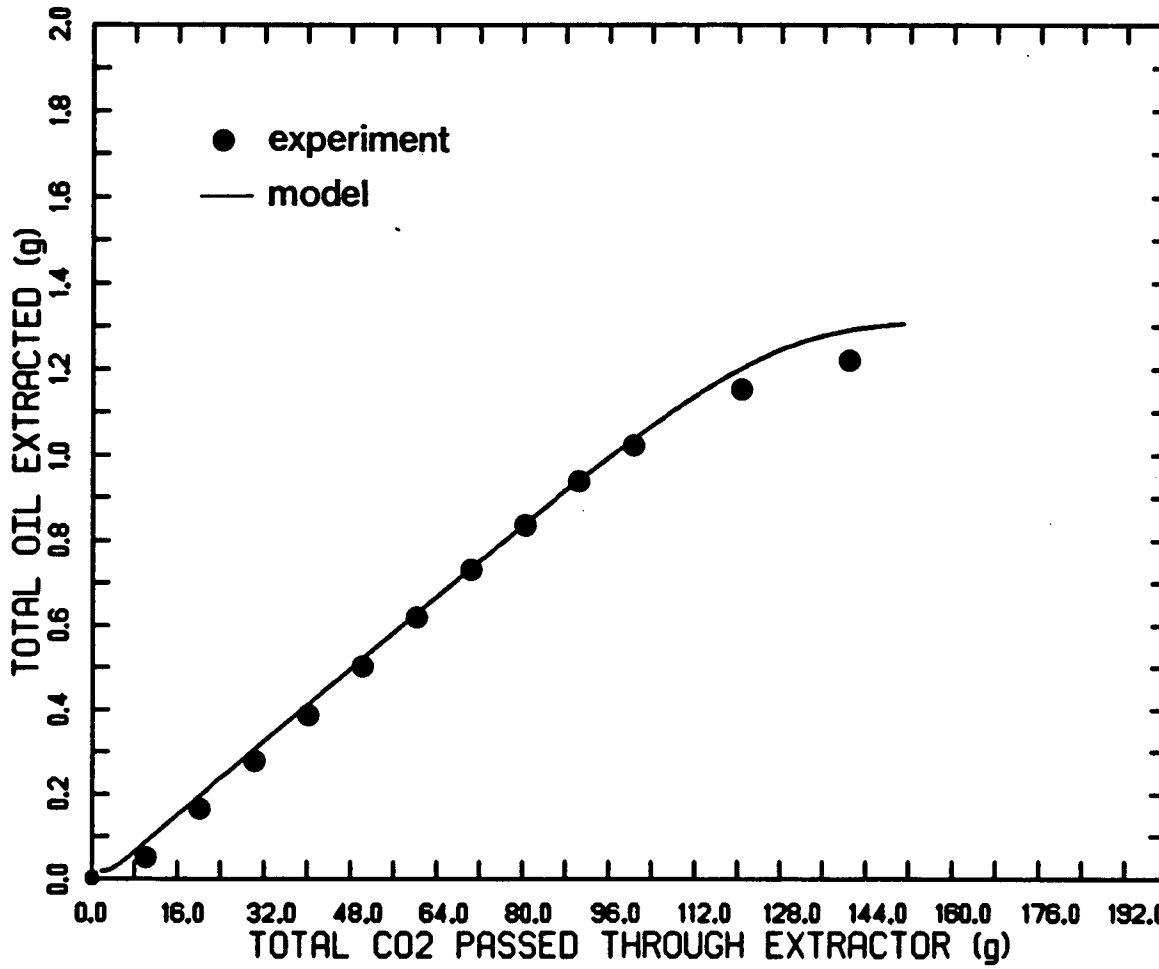

FIGURE 5.31 Extraction curve obtained by passing CO_2 at 55 °C and 36 MPa, through extraction vessel #1 containing 4.0 g of finely chopped seed. The CO_2 flow rate was 1.7 g/min which corresponds to an interstitial velocity of 2.5 cm/min. The computed extraction curve was calculated using an ApK value of 1.5 gCO_2/cm^3min.

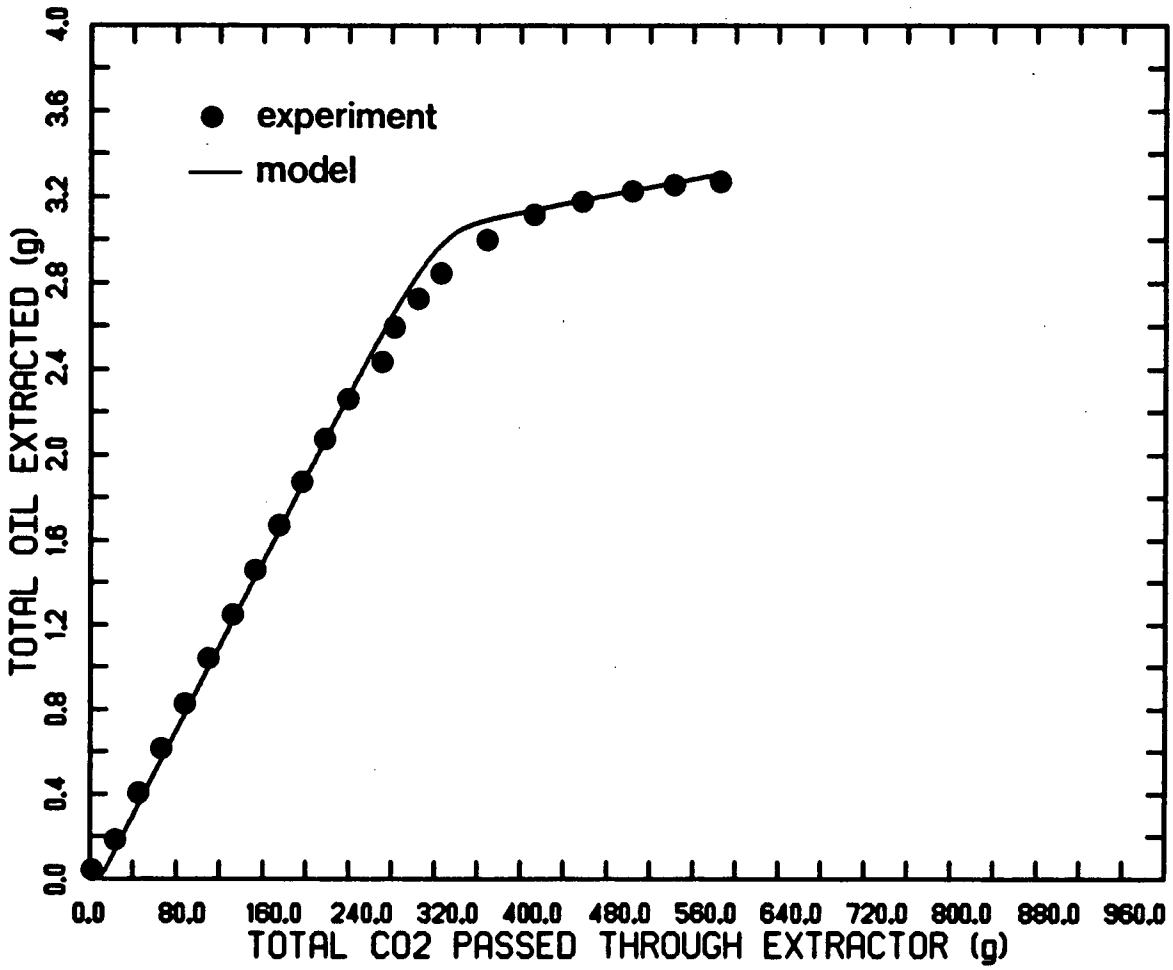

FIGURE 5.32 Extraction curve obtained by passing CO_2 at 55 °C and 36 MPa, through extraction vessel #3, containing 12.0 g of crushed seed. The CO_2 flow rate was 1.4 g/min which corresponds to an interstitial velocity of 0.5 cm/min. The corresponding extraction curve generated by the computer an ApK value of 0.6 gCO_2/cm^3min.

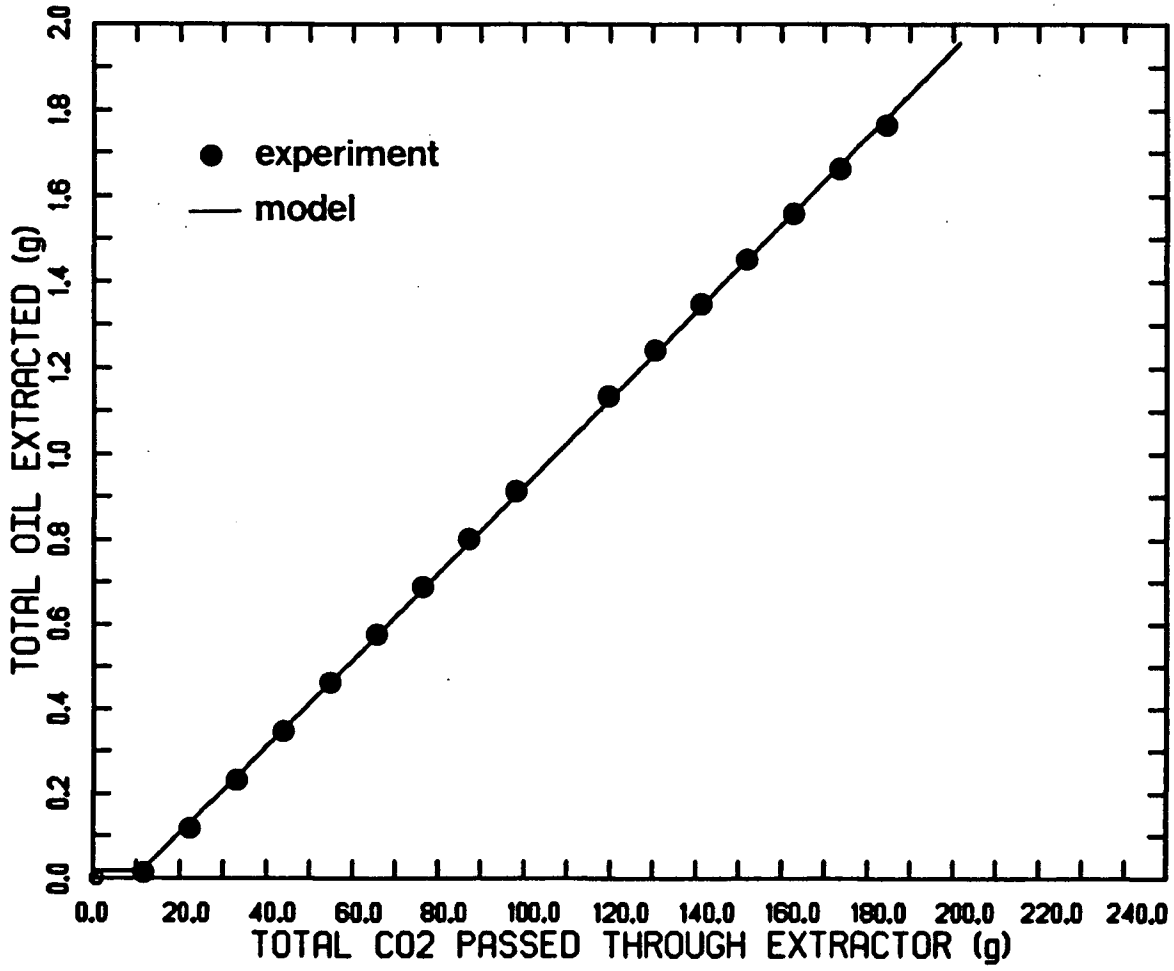

FIGURE 5.33 Extraction curve obtained by passing CO_2 at 55 °C and 36 MPa, through extraction vessel #3 containing 12.0 g of crushed seed. The CO_2 flow rate was 0.7 g/min which corresponds to an interstitial velocity of 0.3 cm/min. The computed extraction curve was calculated using an ApK value of 0.4 gCO_2/cm^3min.

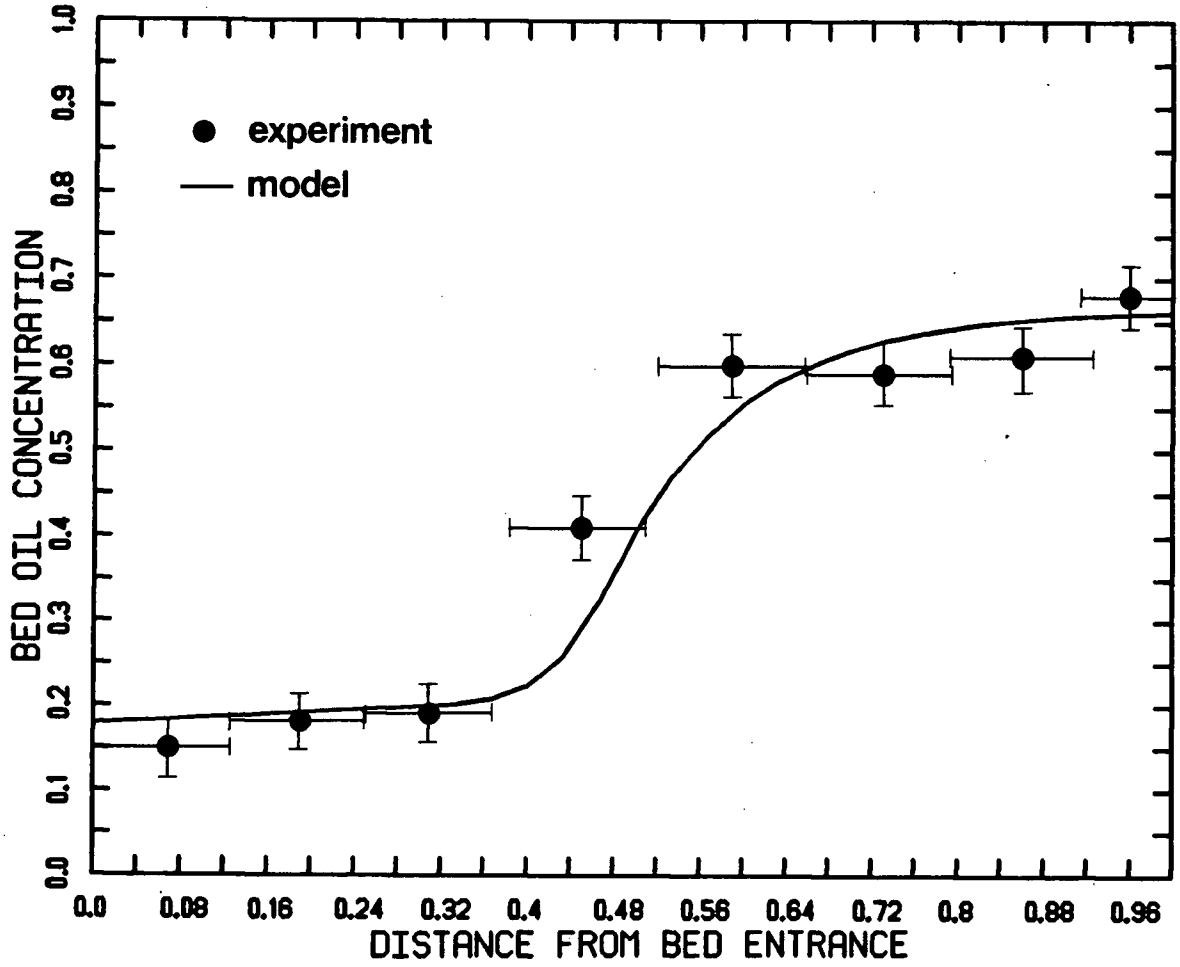

FIGURE 5.34 Oil concentration in the seeds (g oil/g oil-free seed) as a function of normalized distance from the bed entrance after 240 min. The conditions correspond to those shown in Fig. 5.33

Experimental data concerning the oil concentration in the seed phase at different points along the bed were obtained by terminating an experiment prematurely, while the extraction curve was still linear, sectioning the the bed of seeds and then analyzing each section for its oil content using hexane extraction (Sec. 4.6.1). The extraction curve for this experiment is shown in Fig. 5.33. In Fig. 5.34 the results from this analysis are presented along with the corresponding values generated by the computer. The large errors which appear in the horizontal direction of each data point were due to the inability of precisely determining the position which the seed sections originally occupied in the bed. As indicated in the figure, the agreement between the experimental data and the computer data is good.

Computed values for oil concentrations in the seed- and solvent-phases are shown in Figs. 5.35 and 5.36. In order to generate the data for these figures, the parameters in the model were set so that the model simulated extraction from the smallest extractor, packed with 1.5 g of seed. The nearly constant value of the seed oil concentration in the extractor at t=45 min represents the situation where the 'readily accessible' oil on the surface of the seed particles has been depleted. At this stage of the extraction, nearly all oil removal comes from the interior of the seed tissue and from regions not exposed to the flow of moving solvent , i.e. between the fragments of seed.

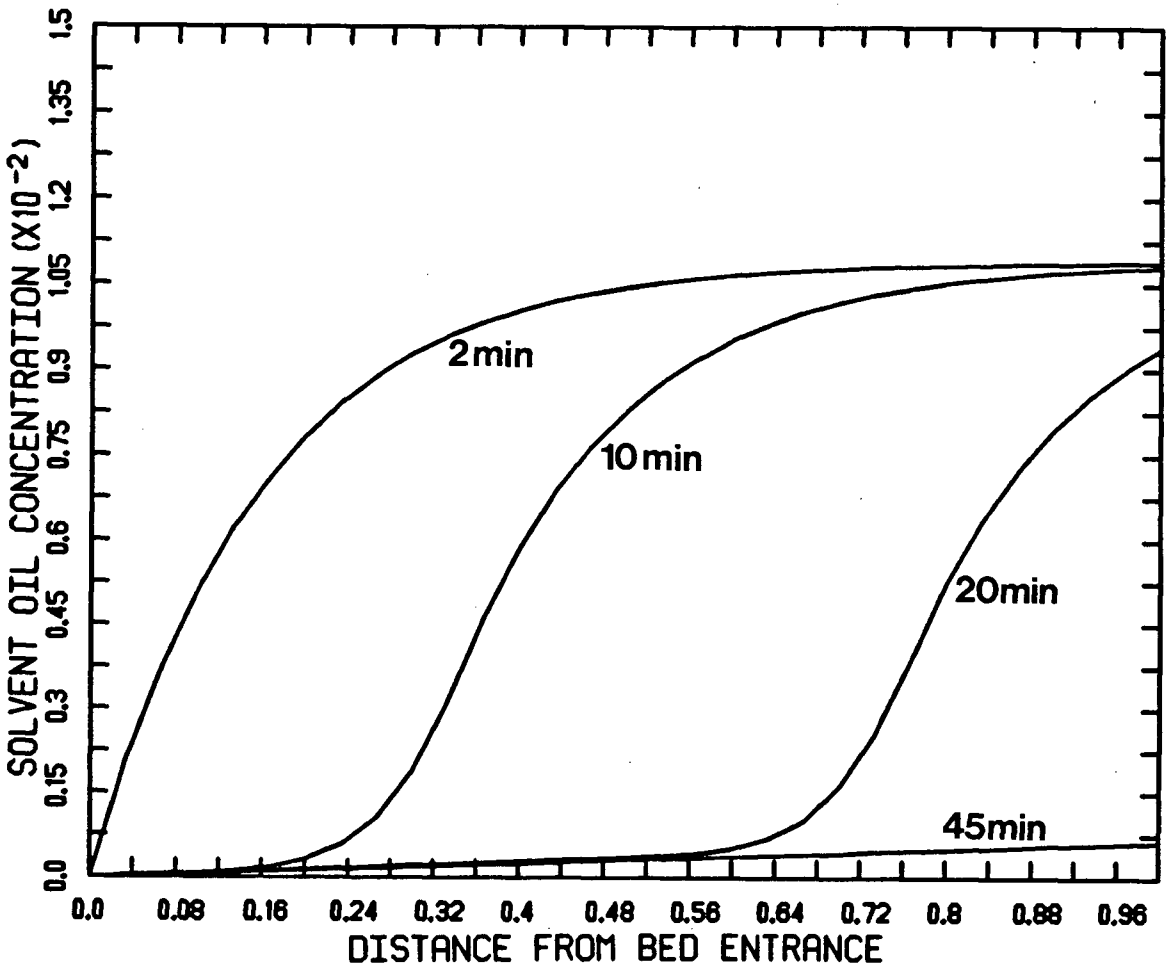

FIGURE 5.35 Oil concentration in the solvent-phase (g oil/g CO_2) as a function of normalized distance from the bed entrance at four different times. The conditions correspond to those shown in Fig 5.26.

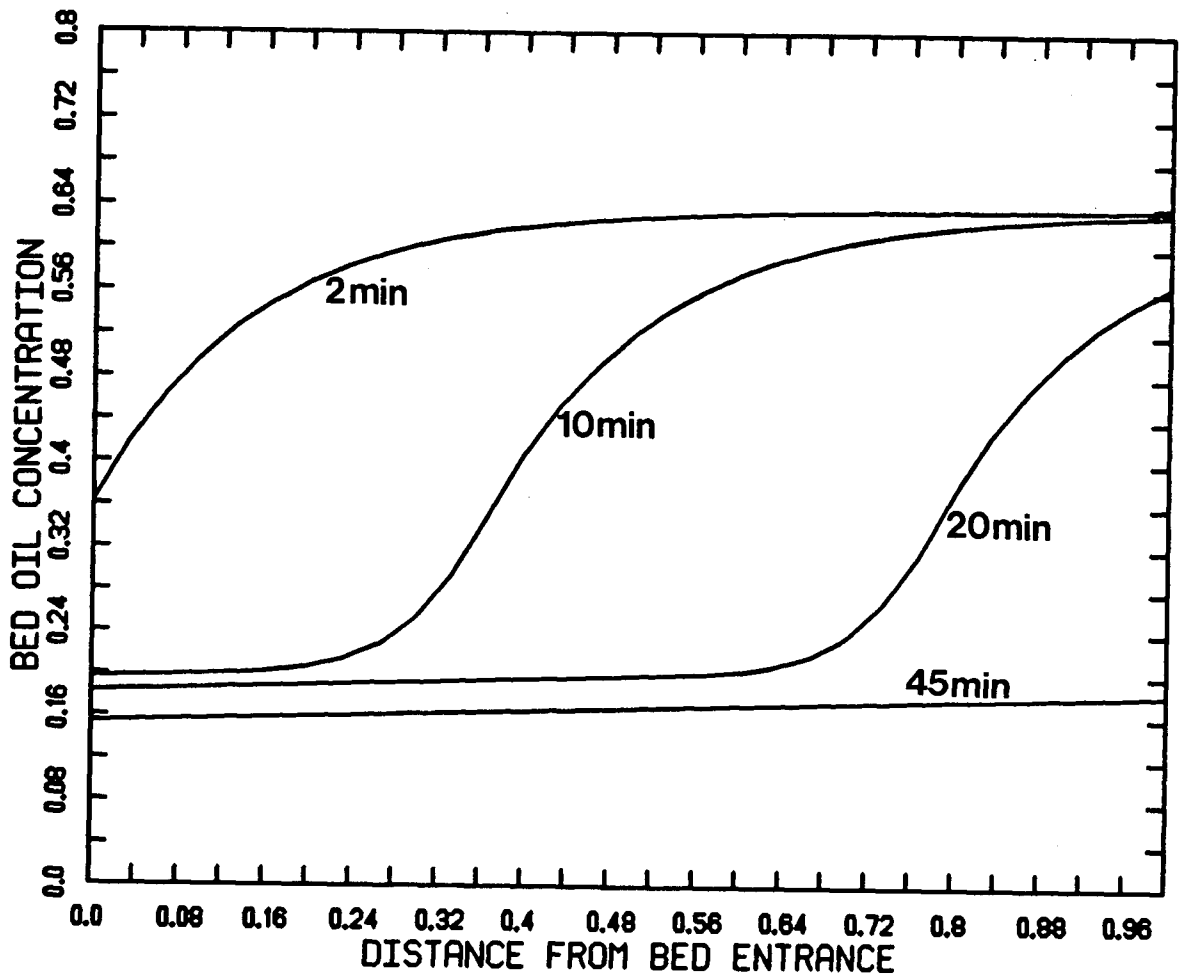

FIGURE 5.36 Oil concentration in the seed-phase (g oil/g oil-free seed) as a function of normalized distance from the bed entrance at four different times. The conditions correspond to those shown in Fig. 5.26.

As indicated by Fig. 5.35, when the extraction has proceeded to this stage (t=45 min) the solvent moves through the bed without becoming saturated with oil. The solvent- and seed-phase oil concentrations from the above simulation are also plotted as a function of both time and bed position in Figs. 5.37 and 5.38.

The values of the overall volumetric mass transfer coefficient (ApK) for the extraction experiments shown in Figs. 5.26-5.33 were also determined. This was done by running the program with different values of ApK and matching the computed and experimental curves. The vertical bars in Fig. 5.39 represent the range of ApK values which, when used in the model, produced extraction curves that matched the corresponding experimental curve reasonably well. The accepted value of ApK was taken to be the mean of the maximum and minimum of these values.

All of the computer-generated curves were matched with the experimental data by 'eye'. An example of how the value of ApK affects the simulated extraction curve is shown in Fig. 5.26. In this figure the extraction curves are shown for three different values of ApK together with the experimental data. It can be seen that the model predictions are relatively sensitive to the ApK values; a change in the ApK value by ± 25% significantly affects the shape of the extraction curve.

Figure 5.39 is a -log plot of ApK values versus the log of solvent interstitial velocity. The figure indicates that the value of ApK decreases with decreasing interstitial velocity as expected.

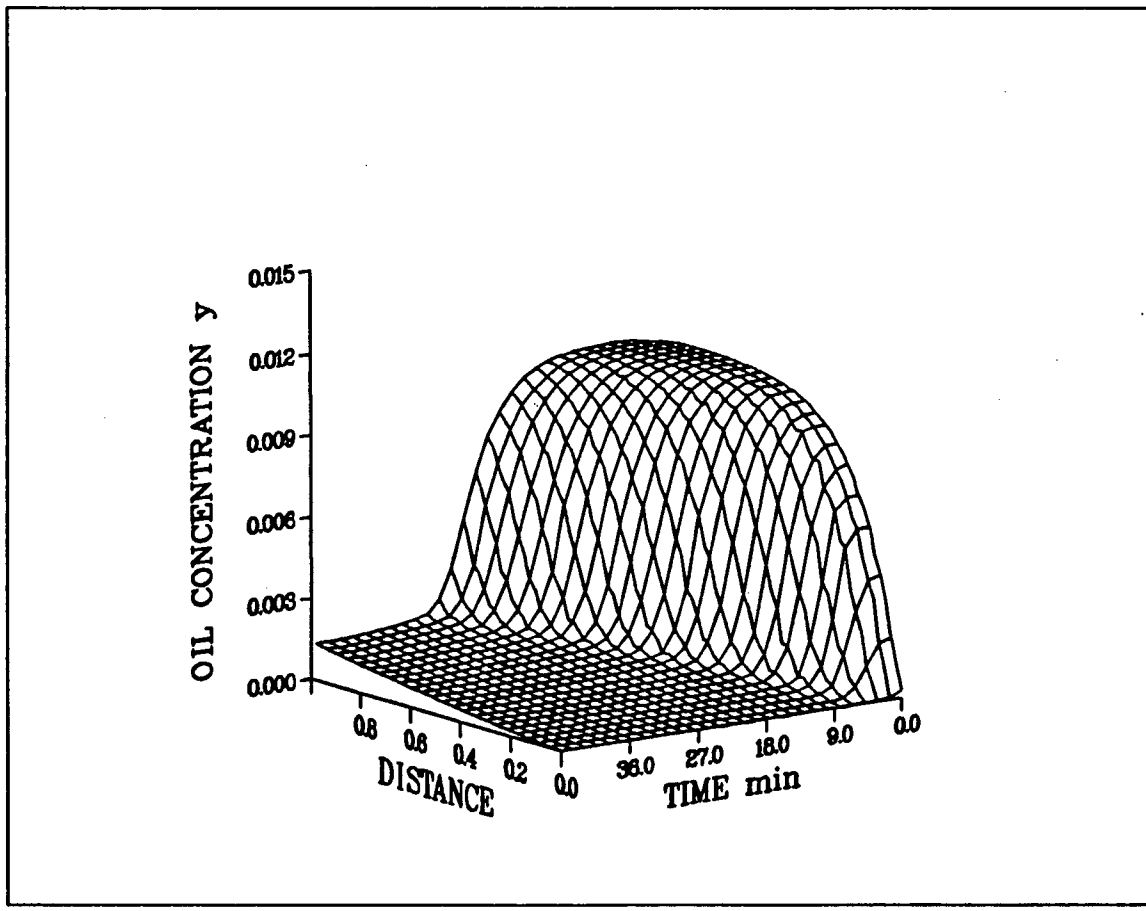

FIGURE 5.37 Oil concentration in the solvent phase (g oil/g CO21) as a function of normalized distance from the bed entrance and time. The conditions correspond to those shown in Fig. 5.26.

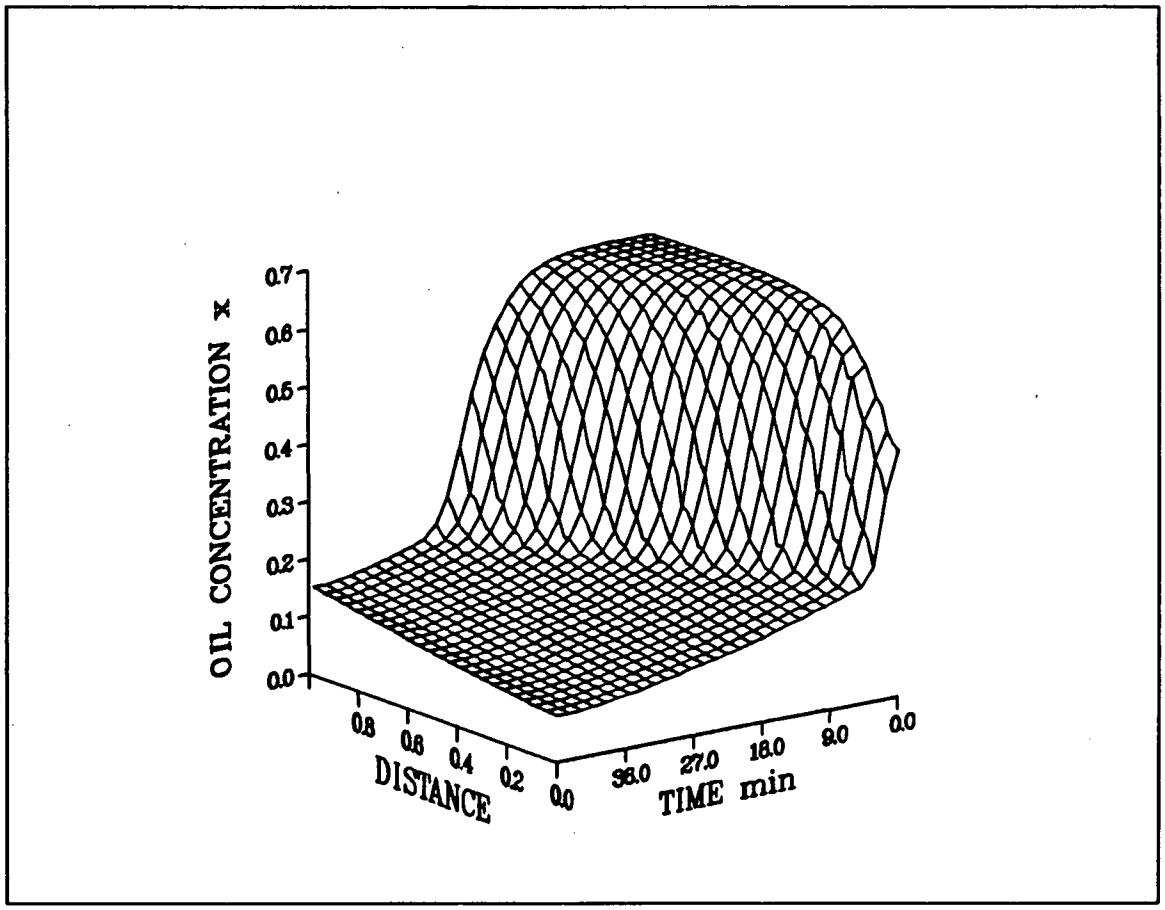

FIGURE 5.38 Oil concentration in the seed phase (g oil/g oil-free seed) as a function of normalized distance from the bed entrance and time. The conditions correspond to those in Fig. 5.26.

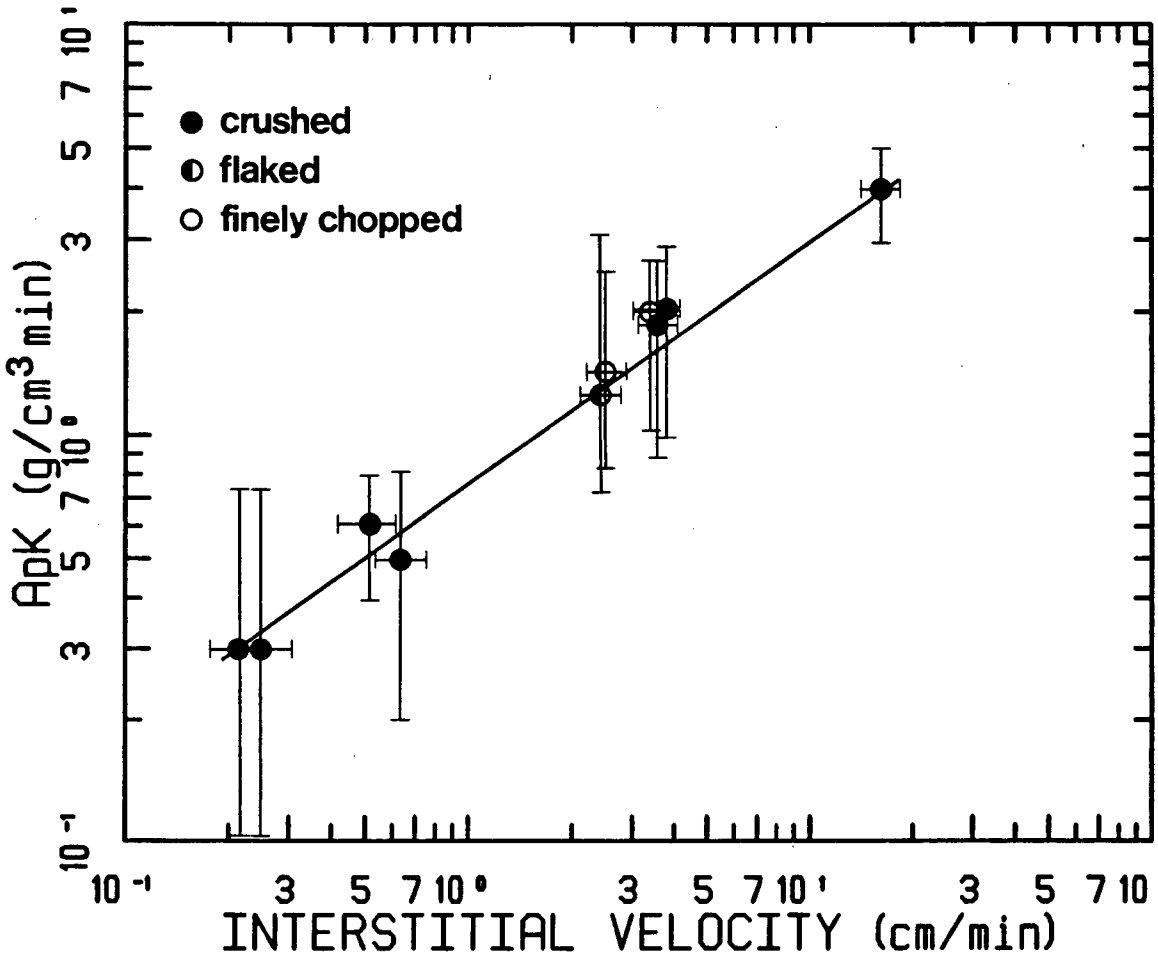

FIGURE 5.39 Volumetric mass transfer coefficients (ApK) as a function of interstitial velocity (v).

No significant difference was observed between the values of ApK for the flaked and finely-chopped seed.

The slope of the line in Fig. 5.39 was found to be 0.54 ± 0.2. The value of ApK may thus be related to the interstitial velocity of the solvent by the following equation:

$$ApK = 0.75v^{0.54} \quad . \quad . \quad . \quad . \quad . \quad . \quad [5.4]$$

where v represents the interstitial velocity of the solvent in [cm/min]. The units for ApK in this case are [gCO_2/cm^3 min] The value of exponent (0.54) is reasonable and falls between the value determined for mass transfer in liquids (0.33) (Wilson and Geankoplis, 1966) and mass transfer in gases (0.6) (Wakao et al., 1976) for Reynolds numbers between 0.0016 and 55. The flowrates used in the Canola experiments represented values of Reynolds numbers from 0.24 to 14.

The close agreement between the experimental data and the data generated by computer, indicates that the model simulates the extraction process reasonably well. This in turn suggests that the values of ApK as evaluated using the model are probably reliable. These values can be expected to be applicable to a scaled-up version of the extraction process, provided the process- and flow-conditions in the scaled up version are closely similar to those used in the experimental extractors.

VI. CONCLUSIONS

This study has demonstrated that supercritical CO_2, under the appropriate conditions, can be an effective solvent for extracting oil from Canola seed. However, the maximum solubility of Canola oil in CO_2, under the conditions studied, was low compared with oil solubilty in hexane. Nonetheless, the degree to which the seeds could be extracted was similar for both CO_2 and hexane. Additionally, the oil produced by the CO_2 extraction process contained less impurities than the hexane extracted oil these results suggest that the CO_2 extraction process could be a viable alternative to the conventional hexane extraction process. In order to further evaluate this possibility, a pilot-plant scale version of the extraction system should be built and operated. The mathematical model presented herein should be of value in the design and operation of such a system.

The major findings of this study can be summarized as follows:

1) Over the range of pressures and temperatures studied, the maximum solubility of Canola oil in CO_2 (1.1wt%) was observed at 36 MPa and 55°C.

2) The equilibrium concentration of oil in the supercritical CO_2 was independent of the oil concentration in the seeds.

3) The total amount of oil recoverable from the seeds, using CO_2, was dependent upon the method of seed treatment prior to extraction. For commercially flaked seed, this value was comparable to the amount of oil recoverable by conventional hexane extraction.

4) The fatty acid composition of the CO_2 extracted oil was constant for most of the extraction. The oil extracts obtained very late in the extraction, however, tended to have slightly higher concentrations of heavier (C22, C24) fatty acids.

5) The CO_2 extracted oil was essentially free (< 7ppm) from phosphorus and, in this regard, comparable to commercially refined Canola oil.

6) Experimental and computed oil concentration profiles and extraction rates were in good agreement.

7) The overall volumetric mass transfer coefficient for the extraction process at 36 MPa and 55°C was correlated with solvent interstitial velocity by the equation:

$$ApK = 0.75\ v^{0.54}$$

VII. RECOMMENDATIONS

The following recommendations are suggested for future research.

1) A broader comparison between the quality of CO_2- and hexane-extracted oil should be made. The comparison should include the tests which are routinely used in industry to establish oil quality eg. Free fatty acid content, color, chromatographic refining loss.

2) The effectiveness of using an entrainer such as ethanol or acetone in conjunction with the CO_2 should be studied with the aim of enhancing the oils solubility in the CO_2 and providing a means by which the oil could be separated from the CO_2 solely by a temperature change.

3) The validity of the method for calculating triglyceride solubilities in the CO_2 (Sec. 5.7.5) should be established by extracting known composition oils and comparing predicted and observed oil solubilities.

4) The validity of the extraction model as discussed in Chapter 3 should be established over a wider range of operating conditions and the values of x_1 and x_2 (Chap. 3) should be obtained for each different type of seed pre-treatment

5) A routine should be incorporated into the computer program which would calculate the best fit value of ApK based on the experimental data.

6) An economic assessment of the CO_2 extraction process should be made; if the economic analysis seems favorable, a pilot-plant scale extraction system should be built and operated with the aim of gathering the engineering data needed for a full-scale CO_2 extraction plant.

NOTATION

A -cross sectional area of extractor [m^2]

Ap -surface area for mass transfer per unit volume [m^2/m^3]

ApK -overall volumetric mass transfer coefficient [$kgCO_2/m^3s$]

H -height of seed bed [m]

h -distance along extractor [m]

K -overall mass transfer coefficient [$kgCO_2/m^3s$]

m_e -mass of oil extracted from seed bed [kg]

m_o -mass of oil in seed bed before extraction [kg]

$\dot{m}$ -mass flowrate of solvent [kg/s]

S_t -total solubility of a mixture in CO_2 [kg/kg CO_2]

$s_{a,b}$ -solubility of pure components a,b in CO_2 [kg/kg CO_2]

t -time [s]

U -superficial solvent velocity [m/s]

v -interstitial solvent velocity [m/s]

x -seed oil concentration [kg oil/kg oil-free seed]

x_o -initial seed oil concentration
[kg oil/kg oil-free seed]

x_1 -seed oil concentration at which bare oil-free surfaces appear [kg oil/kg oil-free seed]

x_2 - seed oil concentration at which surface oil is depleted [kg oil/kg oil-free seed]

x_3 - the concentration of oil in the intact seed tissue which cannot be extracted by CO_2 [kg oil/kg oil-free seed]

$x_{a,b}$ -mole fraction of components a,b in solution [dimensionless]

y -concentration of oil in CO_2 phase [kg oil/kg CO_2]

y_o -concentration of oil in CO_2 solvent at extractor outlet [kg oil/kg CO_2]

y* -concentration of oil in the solvent in equilibrium with seeds having oil concentration x

δh -height of an element of extractor [m]

ϵ -seed bed voids [dimensionless]

ρ -density of CO_2 [kg/m^3]

ρ_s -density of oil-free seeds [kg/m^3]

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APPENDIX I

Errors Associated With The Fatty Acid Analysis

It is assumed that the transesterification procedure is quantitative and that the reagent reacts non-specifically with all the fatty acid moieties present in the oil, irrespective of their carbon number or their position within the triglyceride (Knapp, 1979). Additionally, since the fatty acid composition of the oil samples was determined only in a relative manner, there are no errors associated with sample preparation. Two sources of error are however significant: i) measurement error associated with the chromatograph, ii) accuracy of original analytical standards upon which response factors are based.

Measurement error

In order to assess the first source of error, a standard mixture (Supelco Rapeseed Oil Mixture-CT) of fatty acid esters was analyzed five times over a period of days. The results of these analyses and the measurement errors associated with the technique are listed in Table 1.

TABLE 1. Composition of Supelco CT fatty acid ester mixture as determined by chromatograph. Values are in weight %.

ester	sample 1	sample 2	sample 3	sample 4	sample 5	standard deviation	standar error
C14:0	1.21	1.03	1.05	1.02	1.07	0.08	0.1
C16:0	4.46	4.12	4.23	4.13	4.14	0.14	0.1
C18:0	3.10	2.99	3.02	3.02	3.02	0.01	0.1
C18:1	60.23	59.95	60.11	59.70	59.76	0.22	0.2
C18:2	11.55	11.41	11.61	11.50	11.53	0.07	0.1
C20:0	3.00	3.12	3.07	3.13	3.13	0.06	0.1
C20:1+C18:3	6.03	6.02	6.09	6.05	6.05	0.03	0.1
C22:0	2.91	3.12	2.97	3.13	3.09	0.10	0.1
C22:1	4.87	5.24	4.97	5.24	5.18	0.17	0.2
C24:0	2.76	2.99	2.86	3.05	3.00	0.12	0.1

Quantitative Standards

In order to determine the GC response factors for the fatty acid esters, Supelco GLC standard mixtures (GLC-10, GLC-40, GLC-50 and GLC-60) were used. The standard error associated with these mixtures, as specified by Supelco, was ±0.5%. For a mixture containing 4 compounds this translated to 2% relative error for each component.

Each measurement reported by the GC contains both of these errors. For the high concentration fatty acid esters (C18:1, C18:2, C18:3), the error associated with the accuracy of the response factors predominates, while for the remaining esters, the measurement error associated with the instrumental techniques is be the most important source of error. In Table 2 a summary of the errors is listed.

TABLE 2. Errors associated with measurement technique and response factors. All values are in Weight %.

fatty acid ester	measurement standard error	response factor error	total absolute error
C14:0	0.1	0.02	0.1
C16:0	0.1	0.08	0.2
C18:0	0.1	0.06	0.2
C18:1	0.2	1.20	1.4
C18:2	0.1	0.23	0.3
C20:0	0.1	0.06	0.2
C20:1 + C18:3	0.1	0.12	0.2
C22:0	0.1	0.06	0.2
C22:1	0.1	0.10	0.2
C24:0	0.1	0.06	0.2

APPENDIX II

The program which was used to generate the concentration profiles can be roughly divided into three sections. In the first section, values are assigned to a number of variables associated with the problem. These variables were of two types: those which could be measured directly, and those which could not be measured directly and for which values had to be assumed. Among the first type of variable were extractor volume, oil solubility in the solvent, seed packing density, seed weight, solvent flow rate, solvent density and the initial seed oil concentration. The second type of variable included the seed oil concentration at which bare oil-free seed surfaces first appear (described in Chap. 3), the density of oil-free seed tissue and the volumetric mass transfer coefficients for the extraction process.

In the second section of the program the various parameters used in the model are scaled in order to obtain results which can be compared with those obtained from the experimental extractors. This was necessary because the main program solves the mass balance equations for a unit volume extractor.

The third section of the program is essentially a series of subroutines which numerically solve the set of differential equations (Eqs. 3.4, 3.5). This package of subroutines was developed at the Argonne National Laboratory (Hyman, 1976), and was made available through the UBC computer centre under the library name "MOL1D".

In addition to the main program a second program was used to produce the process extraction curves. The program generated

the curves from the oil concentration profiles provided by the main program.

Also included are the programs which were used to generate the three-dimensional oil concentration profiles of the solvent and seed phases respectively.

```
C     MODEL OF EXTRACTION FROM A FIXED BED
C
      IMPLICIT REAL*8(A - H,O - Z)
      REAL*8 MFLOW
      DIMENSION UZ(2,101), XM(101), MORD(2,3), TOUT(401)
      COMMON /MOLPLT/ XAL, NPLT, MG, UAL, XAXIS(2), UAXIS(2,2)
      COMMON BEPS, RHOG, RHOS, XC1, XC2, XC3, Y2, F, C, D, E, V
      COMMON YEQ, CON1, CON2, RHOGV, VBEPS, DX, NPTS
C
C     BEPS -  BED VOIDAGE [-]
C     RHOG -  DENSITY OF CO2 PHASE [g/cm3]
C     RHOS -  DENSITY OF OIL-FREE SEED PHASE [g/cm3]
C     XC2  -  OIL CONCENTRATION CONSTANT [g OIL/g OIL-FREE SEED]
C     XC1  -  OIL CONCENTRATION CONSTANT [g OIL/g OIL-FREE SEED]
C     XC3  -  OIL CONCENTRATION CONSTANT [g OIL/g OIL-FREE SEED]
C     Y2   -  MAXIMUM VALUE OF ApK [g CO2/cm3min]
C     F    -  RATIO OF ApK(at XC2) to ApK(at XC1)
C     VOLU -  VOLUME OF EXPERIMENTAL EXTRACTION BED
C     AREA -  CROSS-SECTIONAL AREA OF EXPERIMENTAL EXTRACTOR [cm2]
C     YEQ  -  SOLUBILITY OF THE OIL IN THE CO2 [g oil/g CO2]
C     CON1 -  SPECIFIC VOLUME OF CO2 [cm3/g]
C     CON2 -  SPECIFIC VOLUME OF SEED [cm3/g]
C     APK  -  VOLUMETRIC MASS TRANSFER COEFFICIENT [g CO2/cm3min]
C     BPACK-  OIL-FREE SEED BED PACKING DENSITY [g/cm3]
C     TRES -  RESIDENCE TIME IN EXTRACTOR [min]
C     SEED -  MASS OF SEED IN EXPERIMENTAL EXTRACTOR [g]
C     OILC -  CONCENTRATION OF OIL IN THE WHOLE SEED  [g oil/g seed]
C     EOIL -  EFFECTIVE OIL CONCENTRATION ie CONCENTRATION OF OIL IN
C             SEED, EXTRACTABLE BY CO2 [g oil/g seed]
C     RFLOW-  EXPERIMENTAL CO2 MASS FLOWRATE [g/min]
C     SUPER-  EXPERIMENTAL SUPERFICIAL CO2 VELOCITY [CM/MIN]
C     VELOC-  EXPERIMENTAL CO2 INTERSTITIAL VELOCITY [CM/MIN]
C     MFLOW-  CO2 MASS FLOWRATE -UNIT VOLUME EXTRACTOR [G/MIN]
C     V    -  SUPERFICIAL CO2 VELOCITY -UNIT VOL EXTRACTOR [CM/MIN]
C     VBEPS-  INTERSTICIAL CO2 VELOCITY- UNIT VOL EXTRACTOR [CM/MIN]
C
C
C    SET UP EXTRACTION PARAMETERS
C
C
      SEED =  1.46D0
      BEPS =  0.61D0
      RFLOW = 1.600D0
      RHOS = 0.8D0
      RHOG = 0.88D0
      YEQ = 0.011D0
      Y2 = 2.00D0
      F =  30.0D0
C   REASONABLE VALUES OF XC2 RANGE  FROM 0.25 TO 0.67
      XC2 = 0.35D0
C   REASONABLE VALUES OF XC1 RANGE  FROM 0.35 TO 0.0
      XC1 = 0.20D0
C   CANOLA SEED VARIES IN OILC FROM 0.35 - 0.45
      OILC = 0.40D0
C   REASONABLE VALUES OF XC3 RANGE FROM 0.0 TO 0.05
      XC3 = 0.02D0
      EOIL = (OILC - (OILC*XC3))
      BPACK = RHOS*(1-BEPS)
C   CROSS SECTIONAL AREA OF EXTRACTOR #3
C      XAREA = 5.067D0
```

```
C  CROSS SECTIONAL AREA OF EXTRACTOR #1 AND #2
C     XAREA = 1.267D0
C  CROSS SECTIONAL AREA OF EXTRACTOR #4
     XAREA = 0.178D0
C
C  CALCULATE FLOWRATES, VELOCITIES, VOLUMES AND OTHER CONSTANTS
C
     C = (Y2/(XC2 - XC1)) * (1.D0-1.D0/F)
     D = Y2 - C * XC2
     E = Y2 / (XC1*F)
     MFLOW = (BPACK/(SEED*(1 - EOIL))) * RFLOW
     VOLU = SEED * (1 - EOIL) / BPACK
     AREA = 1.0000D0
     V = MFLOW / (RHOG*AREA)
     CON1 = 1.D0 / (BEPS*RHOG)
     CON2 = 1.D0 / ((1.D0-BEPS)*RHOS)
     RHOGV = RHOG * V
     VBEPS = V / BEPS
     TRES = BEPS / (MFLOW/RHOG)
     SUPER = (RFLOW/RHOG) / XAREA
     VELOC = SUPER / BEPS
C
     WRITE (7,10) C, D, E
  10 FORMAT ('C = ', F6.3, 2X, 'D = ', F7.3, 2X, 'E = ', F5.3)
     WRITE (7,20) BEPS
  20 FORMAT ('EXPERIMENT BED VOIDAGE = ', F5.3)
     WRITE (7,30) V
  30 FORMAT ('MODEL SUPERFICIAL VEL (CM/MIN) = ', F5.3)
     WRITE (7,40) TRES
  40 FORMAT ('EXTRACTOR RESIDENCE TIME (MIN) = ', F7.3)
     WRITE (7,50) VBEPS
  50 FORMAT ('MODEL INTERSTITIAL VEL (CM/MIN) =', F5.3)
     WRITE (7,60) MFLOW
  60 FORMAT ('MODEL MASS FLOW (G/MIN) = ', F5.3, 4X)
     WRITE (7,70) VOLU
  70 FORMAT ('EXPERIMENT VOLUME OF BED (CM3) = ', F7.3)
     WRITE (7,80) VELOC
  80 FORMAT ('EXPERIMENT INTERSTITIAL VELOC (CM/MIN) = ', F7.3)
     WRITE (7,90) SUPER
  90 FORMAT ('EXPERIMENT SUPERFICIAL VELOC (CM/MIN) = ', F7.3)
C
C     START OF MOL1D PROGRAM
C
     NPDE = 2
C     'NPTS' SPECIFIES THE NUMBER OF SPACE SECTIONS THAT THE BED
C     IS DIVIDED INTO
     NPTS = 31
     KEQN = 4
     KBC = 2
     DO 100 I = 1, NPDE
       MORD(I,1) = 0
       MORD(I,2) = 0
 100 MORD(I,3) = 0
     MORD(2,1) = 0
     METH = 20
     EPS = 1.D-4
     TINT = 0.D0
C   'TLAST' IS THE ENDTIME (IN MIN) OF THE EXTRACTION
     TLAST =  45.D0
     MOUT = 1
```

```
      KMOL = 0
C    THE NUMBER OF ITERATIONS OF THE DO-LOOP MUST EQUAL 'TLAST'
      DO 110 I = 1,  45
  110 TOUT(I) = I * 1.0D0
C
C      DEFINE INITIAL CONDITIONS
C
      DX = 1.D0 / DFLOAT(NPTS - 1)
      DO 120 I = 1, NPTS
        XM(I) = DFLOAT(I - 1) * DX
        UZ(1,I) = 0.D0
        UZ(2,I) = EOIL / (1 - EOIL)
  120 CONTINUE
C
      CALL MOL1D(NPDE, NPTS, KEQN, KBC, METH, EPS, MORD, TINT, TLAST,
     1     MOUT, TOUT, UZ, XM, KMOL)
      STOP
      END
      SUBROUTINE FUNC(F, U, UX, UXX, T, XM, IX, NPDE)
      IMPLICIT REAL*8(A - H,O - Z)
      DIMENSION F(NPDE), U(NPDE), UX(NPDE), UXX(NPDE)
C
C      CALCULATE FLUX FUNCTION, IF IT EXISTS
C
      RETURN
      END
      SUBROUTINE BNDRY(T, UL, AL, BL, CL, UR, AR, BR, CR, NPDE)
      IMPLICIT REAL*8(A - H,O - Z)
      DIMENSION UL(NPDE), AL(NPDE), BL(NPDE), CL(NPDE), UR(NPDE),
     1          AR(NPDE), BR(NPDE), CR(NPDE)
C
C      SET BOUNDARY CONDITIONS
C
      AL(1) = 1.D0
      RETURN
      END
      SUBROUTINE PDE(UT, U, UX, UXX, FX, T, XM, IX, NPDE)
      IMPLICIT REAL*8(A - H,O - Z)
      DIMENSION U(NPDE,1), UT(NPDE,1), UX(NPDE,1), UXX(NPDE,1)
      DIMENSION FX(NPDE,1), XM(1)
      LOGICAL F1, F2
      COMMON BEPS, RHOG, RHOS, XC1, XC2, XC3, Y2, F, C, D, E, V, YEQ, CON1,
     1       CON2, RHOGV, VBEPS, DX, NPTS
C
C      CALCULATE TIME DERIVATIVES
C
      F1 = .FALSE.
      F2 = .FALSE.
      DERV = 0.D0
      DO 40 I = 1, NPTS
        IF (U(2,I) .LE. XC2) GO TO 10
        APK = C * XC2 + D
        GO TO 30
   10   IF (U(2,I) .LE. XC1) GO TO 20
        APK = C * U(2,I) + D
        F2 = .TRUE.
        GO TO 30
   20   APK = E * U(2,I)
        F1 = .TRUE.
   30   CONTINUE
```

```
      IF (T .EQ. 0.D0) APK = 0.D0
      IF (XM(I) .GT. VBEPS*T) APK = 0.D0
      IF (I .NE. 0) DERV = (U(1,I) - U(1,I - 1)) / DX
      UT(1,I) = CON1 * (-RHOGV*DERV + APK*(YEQ - U(1,I)))
      UT(2,I) = -CON2 * APK * (YEQ - U(1,I))
40 CONTINUE
   IX = NPTS
   RETURN
   END
```

Listing of SUM at 14:04:27 on DEC 18, 1985 for CCid=DUST Page 1

```
 1            REAL*8 TIME,ULAST,U,SUM,SEED,BPACK,MFLOW,RFLOW,CMASS
 2            SEED =  1.46D0
 3            RFLOW = 1.600D0
 4            BPACK = 0.3150D0
 5            SUM = 0.018D0
 6            ULAST = 0.00D0
 7            MFLOW = (BPACK/(SEED*0.63))*RFLOW
 8      1     READ(4,200,END = 2)TIME
 9      200   FORMAT(22X,F5.1)
10            READ(4,300)U
11      300   FORMAT(36(/),15X,E11.4)
12            UA = (U + ULAST)/2
13            SUM = SUM + (UA*MFLOW*((SEED*0.63)/BPACK))
14            CMASS = TIME*RFLOW
15            WRITE(5,400)CMASS,SUM
16      400   FORMAT( 'TOTAL CO2(G)',2X,F5.1,6X,'TOTAL OIL G',F10.5)
17            WRITE(6,500)CMASS,SUM
18      500   FORMAT(2X,F5.1,6X,F10.5)
19            ULAST = U
20            GOTO 1
21      2     STOP
22            END
```

```
C THIS PROGRAM CREATES A 3D PLOT FROM SCATTERED DATA
C POINTS. THE INPUT DATA MUST BE IN THE FROMAT OF X,Y,Z.
C THE INPUT DATA MUST BE IN THE FOLLOWING RANGE: X (0-1.0)
C Y (0-45), Z (0-0.015)
      REAL X(1395),Y(1395),Z(1395),ZMAT(48,29)
      DIMENSION WSPC(8000),IWORK(4900)
C
C SET PLOTTING DEVICE
C
      CALL DSPDEV('PLOT')
      CALL BGNPL(0)
C
C SET AXIS PARAMETERS AND ALPHABETS
C
      CALL ZAXANG(90.0)
      CALL COMPLX
      CALL BASALF('L/CSTD')
      CALL MIXALF('STANDARD')
      CALL HEIGHT(0.30)
C CALL HEIGHT SETS LETTERSIZE. THE AXES LABELS
C WILL BE SET TO THE SIZE (IN INCHES)
C THAT HAS BEEN SPECIFIED IN BRACKETS.
C THE AXES NUMBERS ARE SET TO 5/7 OF
C THIS VALUE. THE TITLE IS SET TO 1.5 TIMES
C THIS VALUE.
C
C DEFINE 3D WORK AREA AND AXES
C
      CALL PAGE(14.,11.)
C
C CALL PAGE ALLOWS THE USER TO SPECIFY THE OUTPUT SIZE (INCH)
C IF "$" FOLLOWS THE TITLES THE PROGRAM AUTOMATICALLY
C CALCULATES THE STRING LENGTH
C
C      CALL TITL3D('(SOLVENT PHASE OIL)$',100,8.,
C     *8.)
      CALL TITL3D('()$',100,8.,8)
C
C THE LAST VALUES HERE SPECIFY THE RATIO OF THE XAXIS
C YAXIS AND ZAXIS RESPECTIVELY
C
      CALL AXES3D('(DISTANCE)$',100,'(TIME) MIN$',100,
     1'(OIL CONCENTRATION) Y$',100,1.0,1.0,1.0)
C
C DEFINE VIEWPOINT AND PROVIDE AXES VALUES
C THE DISTANCE THAT THE VIEW IS TAKEN FROM IS IN USER
C SUPPLIED UNITS eg IF THE AXIS IS 10 UNITS AND THE
C REQUESTED VEIW IS 30, IT WILL BE THREE TIMES AXIS LENGTH
C
      CALL VIEW(-29.0,1000.0,0.120)
C
C GRAF3D ALLOWS USER TO GIVE COORDINATES TO GRAPH
C IN X,Y,Z, FIRST NUMB=ORIGIN, SECOND=LABEL STEPS
C AND THIRD=MAX VALUE
      CALL GRAF3D(0.0,0.2,1.00,0.0,9.0,45.0,0.0,0.003,0.015)
C
C READ DATA
C
      DO 10 I=1,1395
      READ(4,1) X(I), Y(I), Z(I)
```

```
    1 FORMAT(2X,F5.3,5X,F5.2,4X,F7.5)

   10 CONTINUE
      NP=1500
      CALL BGNMAT(48,29)
      CALL GETMAT(X,Y,Z,1395,IWORK)
      CALL ENDMAT(ZMAT,IWORK)
      CALL SURMAT(ZMAT,2,48,1,29,WSPC)

C
C STOP
C
      CALL ENDPL(0)
      CALL DONEPL
      STOP
      END
```

```
C THIS PROGRAM CREATES A 3D PLOT FROM SCATTERED DATA
C POINTS (X,Y,Z)
C THE INPUT DATA MUST BE IN THE
C FOLLOWING RANGE: X (0-1.0), Y (0-45), Z (0-0.7)
C X IN THIS CASE REPRESENTS THE NORMALIZED DISTANCE FROM
C THE BED ENTRANCE; Y THE TOTAL TIME OF THE EXTRACTION; AND
C Z THE CONCENTRATION OF OIL IN THE BED
      REAL X(1395),Y(1395),Z(1395),ZMAT(48,29)
      DIMENSION WSPC(5000),IWORK(4000)
C
C SET PLOTTING DEVICE
C
      CALL DSPDEV('PLOT')
      CALL BGNPL(0)
C
C SET AXIS PARAMETERS AND ALPHABETS
C
      CALL ZAXANG(90.0)
      CALL COMPLX
      CALL BASALF('L/CSTD')
      CALL MIXALF('STANDARD')
      CALL HEIGHT(0.3)
C
C DEFINE 3D WORK AREA AND AXES
C
      CALL PAGE(14.,11.)
C
C CALL PAGE ALLOWS THE USER TO SPECIFY THE OUTPUT SIZE (INCH)
C IF "$" FOLLOWS THE TITLES THE PROGRAM AUTOMATICALLY
C CALCULATES THE STRING LENGTH
C
C      CALL TITL3D('(BED OIL PROFILE)$',100,8.,
C     *8.)
      CALL TITL3D('( )$',100,8.,8)
C
C THE LAST VALUES HERE SPECIFY THE RATIO OF THE XAXIS
C YAXIS AND ZAXIS RESPECTIVELY
C
      CALL AXES3D('(DISTANCE)$',100,'(TIME) MIN$',100,
     1'(OIL CONCENTRATION) X$',100,1.0,1.0,1.0)
C
C DEFINE VIEWPOINT AND PROVIDE AXES VALUES
C THE DISTANCE THAT THE VIEW IS TAKEN FROM IS IN USER
C SUPPLIED UNITS eg IF THE AXIS IS 10 UNITS AND THE
C REQUESTED VEIW IS 30, IT WILL BE THREE TIMES AXIS LENGTH
C
      CALL VIEW(-29.0,1000.0, 7.0)
C
C GRAF3D ALLOWS USER TO GIVE COORDINATES TO GRAPH
C IN X,Y,Z, FIRST NUMB=ORIGIN, SECOND=LABEL STEPS
C AND THIRD=MAX VALUE
      CALL GRAF3D(0.0,0.2,1.0,0.0, 9.0,45.0,0.0,0.1,0.7)
C
C READ DATA
C
      DO 10 I=1,1395
      READ(4,1) X(I), Y(I), Z(I)
    1 FORMAT( 2X,F5.3,4X,F6.2,4X,F6.4)

   10 CONTINUE
```

```
      NP=1450
      CALL BGNMAT(48,29)
      CALL GETMAT(X,Y,Z,1395,IWORK)
      CALL ENDMAT(ZMAT,IWORK)
      CALL SURMAT(ZMAT,2,48,1,29,WSPC)

C
C STOP
C
      CALL ENDPL(0)
      CALL DONEPL
      STOP
      END
```

APPENDIX III

List of Material Suppliers	Items
Avanti Polar-Lipids Inc. 2421 High Bluff Road, Birmingham, AL. 33216. (205) 822-9162	phospholipid standards
Chromalux Canada Ltd. 2455 Beta St., Burnaby, B.C. (604) 294-1801	cartridge heaters
Chromatographic Specialties Ltd. 300 Laurier Blvd, Brockville, Ont. (613) 342-4678	1/16" stainless steel tubing
Columbia Valve and Fitting Ltd. 380 E. Esplanade, Vancouver, B.C. (604) 986-5251	valves, fittings
CSP Foods Ltd. Box 580, Nipawin, Sask. (306) 862-4686	Canola seed and oil samples
Fisher Scientific Inc. 196 W. 3rd Ave., Vancouver, B.C. (604) 291-8866	Esterification chemicals
Fleck Bros. Ltd. 110 Alexander St., Vancouver, B.C. (604) 684-8131	refrigeration insulation tubing
Hewlett-Packard Inc. 10691 Shellbridge Way, Richmond, B.C. (604) 270-2277	HPLC, supportive hardware
Hyseco Fluid Systems Ltd. 145 W. 2nd Ave., Vancouver, B.C. (604) 879-8851	Parker MV-200s valve
Intek Electronics Ltd. 10-8385 St. George St., Vancouver, B.C. (604) 324-6831	RCA, Teledyne electronic components
Intertechnology Ltd. Richmond, B.C. (604)	pressure transducer
Medigas Pacific Ltd. 6841 Palm Ave., Burnaby, B.C. (604) 438-5276	carbon dioxide

Nu-Chek Prep Ltd. P.O. Box 295, Elysian, MN. 56028 (507) 267-4689	triglyceride samples
RAE Industrial Electronics Ltd. 3455 Gardner Crt., Burnaby, B.C. (604) 291-8866	temperature transducers
Supelco, Inc. Supelco Park, Bellefonte, PA. 16823-0048 (814) 359-3446	GC analytical standards, GC columns
Surrey Fluid and Power Ltd. 203-13395-76th ave., Surrey, B.C. (604) 594-3461	Parker MV-200s regulating Valve