THE EFFECT OF MOLECULAR STRUCTURE AND OPERATING CONDITIONS ON THE SOLUBILITY OF TRIGLYCERIDES IN SUPERCRITICAL CO₂

by

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Date: May, 1986
Abstract

In order to assess the feasibility of using supercritical fluid as a solvent for oil extraction in the oil and fat industry, basic information is required on oil solubility as a function of various system parameters. Such information would be useful in the design of extraction systems.

This research work studied the effects of temperature and pressure on the equilibrium solubilities of triglycerides in supercritical carbon dioxide which is considered one of the best solvents for supercritical fluid extraction. A series of experiments was carried out using a modified liquid chromatograph. Samples tested included pure simple triglycerides that are saturated and unsaturated, and triglyceride mixtures. Extraction experiments were extended to cocoa butter, palm kernel oil and their mixtures with various weight ratios. The effect of sample water content on the oil solubility was investigated using cocoa butter/water mixtures in different proportions.

The solubilities of simple triglycerides were found to depend strongly on pressure and temperature. Triglycerides with a longer carbon chain (C18) exhibit lower solubilities over the range of temperatures and pressures studied. Unsaturated triglycerides were more soluble than their saturated counterparts. Furthermore, for the saturated triglycerides, the solubilities varied inversely as their molecular weights.
Significant fractionation occurred during the extraction of simple triglyceride mixtures at 36 MPa and 55 °C. Results of tests on cocoa butter and palm kernel oil separately indicated that no fractionation had taken place during the extraction process. However, significant fractionation was again observed when the two oils were mixed.

Sample water content up to 50% by weight had negligible effect on the oil extractability and its equilibrium solubility in CO₂.
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Chapter 1

INTRODUCTION

1.1 GENERAL

The separation of oils or lipids and other liquid hydrocarbons from plant materials is a well developed technology. In the oils and fats industry, solvent extraction using n-hexane, a paraffinic petroleum fraction, is the most extensively used technique for recovering food grade oil from oilseeds such as soybeans, cottonseeds, canolas and sunflowerseeds. A serious disadvantage of n-hexane extraction is its extreme flammability. Rather elaborate precautions have been developed to avoid fire and explosion, but danger of severe accidents remains. In addition, its increasing cost and potential health problems has made it desirable for the industry to search for alternative extraction methods.

Recently, supercritical fluid extraction (SCFE), a novel kind of separation technique which does not use organic solvents has generated a great deal of interest. It has been shown that supercritical CO₂ can be efficient at extracting oil from different oil sources (Stahl et al.,1980; Hubert and Vitzthum,1978; deFillipi,1982, Mangold,1982 ;Friedrich and List,1982 and Fattori,1986). The use of SCFE for achieving fractionation of oils has also been reported (Anon,1981; Zosel,1978 ; Friedrich and Pryde,1984; Stahl et al.,1980). Although the technique is
quite complicated and requires sophisticated high pressure equipment, research into its use has grown almost exponentially over the last decade.

Supercritical fluid extraction is a technique that exploits the solvent power of supercritical fluids at temperatures and pressures near the critical point. In this region, slight changes in temperature and pressure can cause large changes in solvent density and thus dissolving power (Humphrey et al., 1984).

In the basic process of supercritical extraction, a substrate is brought into contact with a supercritical fluid, the oil/supercritical phase is isolated, and finally the isolated fluid phase is decompressed to the point where the solvent power of the gas is reduced and the dissolved material condenses as a solid or liquid. An extension of this process is the separation by successive extractions of a mixture of materials, using mild conditions to extract first the more volatile materials and then more severe conditions to extract the less volatile materials. The extent of an extraction can be controlled by the selection of an appropriate gas, by adjusting the temperature and pressure of the extraction, and by altering the ratio of substrate/gas in the extraction vessel.

SCFE is also known as 'dense gas extraction', 'supercritical gas extraction' or 'destruction'. It combines features of both distillation and liquid extraction. It is particularly effective for the isolation of substances of
relatively high molecular weight and relatively low polarity. The technique is similar to conventional solvent extraction in that the material to be extracted is 'washed' from the substrate using a suitable solvent, and yet distinct from the conventional method in that the solvent is not a liquid, but rather a fluid above its critical point.

In comparison with liquid solvents the supercritical fluid has high diffusivity, but low density and viscosity, thus allowing rapid extraction and phase separation (Williams, 1981). Moreover, when compared with the liquid-liquid processes, there is a greater flexibility in the selection of the operating parameters of temperature and pressure. One of the principal advantages over distillation is that separation can be accomplished at moderate temperatures and as a consequence the process can be applied to the recovery of heat-labile substances of low volatility.

In simple terms, this technique can be characterized by the following unique features which make it one of the most promising technologies for future use in the fats and oils industry (Friedrich and Pryde, 1984).

1. three supercritical fluid parameters, density, temperature and composition can be easily varied,
2. temperatures are usually close to the critical temperatures. High boiling point and/or heat sensitive components may be taken into the extracting phase at relatively low temperatures.
3. essentially complete separation of solvent/solute with
high solvent recovery can be accomplished by isothermal
decompression
4. compounds can be selectively dissolved by changing the
density of the fluids,
5. A great range of solvents may be employed, for example, 
carbon dioxide, ethane, ethylene and propane. The 
solvent power of an appropriate gas may be improved, if 
required, by addition of a third component called an 
entrainer, thus extending further the range of solvent 
characteristics available.
6. the solutes can be fractionated during the 
solvent/solute separation.

In order to assess the feasibility and merits of using 
supercritical fluid as a solvent in the oils and fats 
industry, basic information is required on oil solubility as 
a function of various system parameters. Since triglycerides 
are the major components of food grade oils, results 
obtained from experiments using pure triglycerides should 
lead to a better understanding of the more complex mixtures 
of oils. It has been shown that different triglycerides 
exhibit different solubilities in supercritical carbon 
dioxide (Fattori, 1986). Research on the relationship between 
a triglyceride molecular structure and its equilibrium 
solubility would provide a further view on the process. 
Also, changes in solubility and extract composition for 
triglyceride mixtures would provide basic information for 
use in the design of extraction/fractionation systems.
The effect of water content in the oil source materials also requires study. Since water content is an alterable parameter, understanding of its basic effects on the kinetics of extraction is important.

1.2 OBJECTIVES

The objectives of this research are:

1. To determine the equilibrium solubilities of pure simple triglycerides in supercritical CO₂ as a function of:
   a. temperature
   b. pressure

2. To determine the effect of triglyceride molecular structure on its solubility in supercritical CO₂ with emphasis on:
   a. carbon chain length of the fatty acids
   b. carbon chain saturation of the fatty acids

3. To determine the change of triglyceride fatty acid composition as a function of extraction time for simple two component triglyceride mixtures.

4. To evaluate the potential use of the effect of changes in pressure and/or temperature on fractionation of the supercritical triglyceride extracts.

5. To determine the effect of sample water content on the solubility of triglycerides in CO₂.

6. Extracting of palm kernel oil, cocoa butter and the mixture of both as the test materials to assess potential for extraction/fractionation.
2.1 **SUPERCRITICAL FLUID**

An understanding of the term supercritical fluid can be obtained by referring to the pressure-temperature phase diagram of a pure substance (Figure 2-1).

When a gas such as CO₂ or ethylene is compressed, some distinct changes in the physical properties and behavior are observed below a certain temperature \( T \), whereby saturated liquid and vapour can exist together. Below a certain volume for a given pressure the material must be a liquid, and if it is allowed to expand, all the given mass will be gasified at a particular volume. At temperature above \( T \), it is not possible to liquefy the gas no matter what pressure is applied. The temperature, \( T_c \), is termed the critical temperature of the gas.

Figure 2-2 is plotted using the reduced variables \( T_r \), \( P_r \), and \( \rho_r \). The supercritical fluid region for a pure compound is strictly defined as that region of temperatures and pressures greater than or equal to the critical temperature and critical pressure, respectively, of the compound (i.e., reduced pressures and temperatures greater than or equal to unity). Typically, the SCF region of interest is defined at conditions bounded approximately by \( 0.95 < T_r < 1.4 \) and \( P_r > 1.0 \). In this region, the fluid is highly compressible. For example, at a constant \( T_r \) of 1.10, increasing pressure from
Figure 2-1: Phase diagram for carbon dioxide showing the relationship of the supercritical state to the solid, liquid and vapour states. The critical point is designated as C and the triple point as TP.
Figure 2-2: Reduced pressure-density diagram for carbon dioxide. Supercritical fluid (SCF) and near-critical liquid regions are indicated. (Giddings et al., 1969).
$P_r = 1.0$ to $P_r > 1.0$ significantly increases the density from relatively low values to liquid-like densities. At higher $T_r$s, the pressure increase to produce an equivalent density increase become greater. This consideration sets the upper bound on temperature. At constant $P_r$ of 1.50, decreasing temperatures has a similar effect on density, and at higher reduced pressures, the density is less sensitive to temperature changes. In the vicinity of the critical point, large density changes can be produced with either relatively small pressure or temperature changes.

In addition to the unique solubility behavior of supercritical fluid, there are certain desirable physico-chemical properties which make it a good solvent.

As shown in Table 2-1, while supercritical fluids have a liquid-like density and hence solvent loading comparable to a liquid, the diffusivity and viscosity are intermediate to that of a liquid and gas. Therefore, the dense gas phase will manifest solubilities approaching those of the liquid phase, yet will penetrate faster and deeper into a solid matrix of natural substances to be extracted or would progress faster through a densely packed fixed bed or column.
TABLE 2-1 : TYPICAL VALUES OF VISCOSITY, DENSITY AND DIFFUSIVITY FOR LIQUID, GASEOUS AND SC-CO₂ (Newitt et al., 1956).

<table>
<thead>
<tr>
<th>Property</th>
<th>Gas</th>
<th>Liquid</th>
<th>Supercritical Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (kg m⁻³)</td>
<td>0.001</td>
<td>1.0</td>
<td>0.1-0.8</td>
</tr>
<tr>
<td>Viscosity (cP)</td>
<td>0.01</td>
<td>0.5-0.1</td>
<td>0.05-0.10</td>
</tr>
<tr>
<td>Diffusivity (mm² s⁻¹)</td>
<td>10</td>
<td>0.001</td>
<td>0.01-0.1</td>
</tr>
</tbody>
</table>

In general terms, it is suggested that the close proximity of molecules in the liquid phase imparts to the liquid certain solvent powers through the action of intermolecular forces. Solvent extraction can therefore be related to the density of the fluid.

Supercritical solvents covering a wide range of extraction temperatures and varying considerably in size and polarity are available. Many of these are relatively inexpensive and abundant. Table 2-2 lists the critical pressures, critical temperatures and critical densities of these substances.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Critical temperature (K)</th>
<th>Critical pressure (MPa)</th>
<th>Critical density (g cm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td>191</td>
<td>4.60</td>
<td>0.162</td>
</tr>
<tr>
<td>Ethylene</td>
<td>282</td>
<td>5.03</td>
<td>0.218</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>304</td>
<td>7.38</td>
<td>0.468</td>
</tr>
<tr>
<td>Ethane</td>
<td>305</td>
<td>4.88</td>
<td>0.203</td>
</tr>
<tr>
<td>Propylene</td>
<td>365</td>
<td>4.62</td>
<td>0.233</td>
</tr>
<tr>
<td>Propane</td>
<td>370</td>
<td>4.62</td>
<td>0.217</td>
</tr>
<tr>
<td>Ammonia</td>
<td>406</td>
<td>11.3</td>
<td>0.235</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>467</td>
<td>3.64</td>
<td>0.265</td>
</tr>
<tr>
<td>n-Pentane</td>
<td>470</td>
<td>3.37</td>
<td>0.237</td>
</tr>
<tr>
<td>Acetone</td>
<td>508</td>
<td>4.70</td>
<td>0.278</td>
</tr>
<tr>
<td>Methanol</td>
<td>513</td>
<td>8.09</td>
<td>0.272</td>
</tr>
<tr>
<td>Benzene</td>
<td>562</td>
<td>4.89</td>
<td>0.302</td>
</tr>
<tr>
<td>Toluene</td>
<td>592</td>
<td>4.11</td>
<td>0.292</td>
</tr>
<tr>
<td>Pyridine</td>
<td>620</td>
<td>5.63</td>
<td>0.312</td>
</tr>
<tr>
<td>Water</td>
<td>647</td>
<td>22.0</td>
<td>0.322</td>
</tr>
</tbody>
</table>

$\text{CO}_2$ is considered one of the best solvents for supercritical fluid extraction for a number of reasons: In addition to its solvent properties and low critical temperature, $\text{CO}_2$ has the advantages of being neither flammable nor toxic, and is considered non-corrosive when in combination with moisture to the materials used in processing natural products. It is also inexpensive and readily available in large quantities and high purity (Hubert and Vitzthum, 1978).

The restriction of using a supercritical gas alone as the solvent is not necessary. Brunner and Peter (1982)
suggested that by using a suitable entrainer, the separation factor can be enhanced.

2.2 PREVIOUS WORKS

The phenomenon of supercritical fluid extraction was recognized over 100 years ago when the effects of pressure on the solubilities of potassium iodide in ethanol were observed (Hannay and Hogarth, 1879). Later, it was realized that the solvent power of supercritical gases could be involved in geological processes through the influence of water on rock formation, and of methane in petroleum formation and migration. In power stations the adoption of supercritical steam pressures led to the deposition of silica on the blades of steam turbines.

According to Williams (1981), the first proposal for the practical application of supercritical extraction was made in 1943 for the deasphalting of petroleum oils. Zhude (1958) in the U.S.S.R. described a similar scheme using supercritical propane, and demonstrated the fractionation of crude oil with supercritical methane, the extraction of ozocerite wax from ores, and the extraction of lanolin from wool grease.

Fundamental studies on SCFE were initiated by Zosel as early as 1962 in Germany. His work includes the extracting of lipids and other natural products with supercritical fluids (Zosel, 1976). In 1970, he discovered the decaffeination of green coffee with the pharmacologically
completely acceptable carbon dioxide which then has been developed into commercial scale (Zosel, 1978). Using this technique, the caffeine content of the bean is decreased from an initial value lying between 3% and 0.7% to 0.02%. It is found that the caffeine is selectively removed by the CO₂, no substances contributing to the aroma being lost.

Hubert and Vitzthum (1978) have demonstrated that lipids can be extracted from copra, sunflower seeds, soybeans and shell peanuts with CO₂ at pressures ranging from 28 to 35 MPa.

Paul and Wise (1971) published a comprehensive review of the principles of gas extraction. They discussed the physical basis in relation to distillation and extraction, and suggested possible areas of application.

A review of the available data for a number of supercritical fluid solvents was made by Irani and Funk (1977). They also compared the energy requirement for distillation versus supercritical fluid extraction, and concluded that energy savings are realized with supercritical fluid extraction if the process is operated at low gas compression ratios.

The first symposium devoted entirely to extraction with supercritical gases was held in Germany in 1978 (Wilke, 1978). At this symposium, the pioneer researchers from Germany presented papers on different aspects of supercritical fluid extraction. Zosel (1978) and Hubert and Vitzthum (1978) discussed the general principles of
supercritical fluid extraction and their practical aspects, including extensive experimental information on the extraction of natural products such as hops, caffeine, tobacco, and flavors, with a number of different supercritical solvents such as ethane, ethylene, CO₂ and H₂O. Although numerous solvents are presented, carbon dioxide is, by far, the most extensively used supercritical fluid solvent. Other topics discussed include the theoretical aspects of supercritical fluid extraction (Schneider, 1978), empirical methods for determining the solubilities of compounds in supercritical fluids (Stahl et al., 1978) and criteria for the design of a full-scale supercritical fluid extraction plant (Eggers, 1978).

After this symposium, many additional papers have appeared on both the practical and theoretical aspects of supercritical fluid extraction.

The review by Williams (1981) gives an indication of the breadth of separation problems to which supercritical fluid extraction has been applied. These problems include the decaffeination of coffee, extraction of hops, spices, and tobacco, fractionation of high boiling mixtures, deasphalting of heavy petroleum fractions, extraction of mineral deposits, and tertiary oil recovery with supercritical fluid extraction, especially with supercritical CO₂.

Paulaitis et al (1982) described a number of practical applications, and gave an extensive account of experimental
studies which include the area of transport properties of supercritical fluid solvents.

The recent review paper by McHugh et al. (1985) provided the most up to date review of the theoretical aspects and data related to SCFE, as well as the latest applications.

One of the most extensive studies was carried out in Germany by Stahl and co-workers (1978, 1980, 1983). They have developed a microextraction apparatus which they directly coupled to a thin layer chromatography for quickly screening the solubilities of a wide range of natural products in several supercritical fluid solvents. In the study, they established a number of variables which control the solubility of natural products in supercritical CO₂. They found that:

-- fractionation of condensed phases is possible if the mixture constituents exhibit large differences in vapour pressure, mass, or polarity.

-- low molar mass hydrocarbons and lipophilic organic compounds such as esters, ethers, and lactones are easily extractable;

-- hydroxyl and carboxyl groups on compounds make their extraction extremely difficult;

-- sugars and amino acids are not extractable.

As the knowledge of supercritical fluid extraction increases, its applications are becoming more diverse. Apart from the applications mentioned previously, other areas of potential application include: extraction in drug
manufacturing (McHugh et al., 1985), removing organics from waste water (Worthy, 1981), extraction of volatile aromatics from flowers and plants (Calame and Steiner, 1982), and different kinds of spices (Hubert and Vitzthum, 1978).

2.3 EFFECT OF PRESSURE AND TEMPERATURE

The system ethylene-\(p\)-iodochlorobenzene, Figure 2-3 illustrates the large enhancement in solubility that occurs at supercritical conditions (Williams, 1981).

Measurements were made at a temperature of 298 K, close to the critical temperature of ethylene, 282 K. At low pressures the gas phase concentration of the involatile solid was extremely small. Increasing the pressure to above 5 MPa, the critical pressure of ethylene, the solubility of the solid increased by three orders of magnitude.

Extraction pressures in the range of 10-40 MPa are generally necessary to achieve adequate densities. This pressure range has so far been investigated most frequently. The occurrence of further dissolution phenomena at higher pressures cannot be excluded, but their use would probably be rejected for economic reasons. The preferred temperature range for extraction with \(\text{CO}_2\) is approximately 35 - 80 °C.

The importance of carrying out the extraction near the critical temperature of the gas is illustrated in Figures 2-4, which show results obtained when phenanthrene was contacted at 313 K and 40 MPa with gases of different critical temperatures (Williams, 1981). The gases having
Figure 2-3: The large increase in the solubility of p.iodochlorobenzene in the supercritical ethylene ($T_c = 282$ K, $P_c = 5$ MPa) at 298 K brought about at elevated pressures (Williams, 1981).
Figure 2-4: The gas phase concentration of phenaphthelene in various supercritical gases at 313 K and 40 MPa, showing the importance of a close correspondence between the extraction temperature and the critical temperature of the gas (Williams, 1981).
critical temperatures much below the extraction temperature, including nitrogen (116 K), methane (191 K) and carbon tetrafluoride (222 K), did not extract the phenanthrene, whereas those with critical temperatures near the extraction temperature, namely ethylene (283 K), carbon dioxide (304 K) and ethane (305 K), proved effective solvents.

Tsekhanskaya et al. (1964) reported that the solubility of a substance in a supercritical fluid (e.g. naphthalene in compressed ethylene \( T_c = 9.21^\circ C; P_c = 5 \) MPa) changes with pressure at different temperatures, as seen in Figure 2-5. At moderate pressures, a rise in temperature caused a decrease in the solubility of the solid component. At higher pressures, a rise in temperature causes an increase in the solubility. This behavior can be explained in terms of the density of the gas. A rise in temperature at constant pressure leads, on the one hand, to a decrease in gas density and on the other, to an exponential increase in the vapour pressure of the solid. At the region slightly above the critical temperature, gas density at moderate pressures is lowered by a temperature rise to such an extent that the concentration of the solute in the supercritical phase decreases considerably. At high pressures the decrease in density caused by the temperature rise is so small that the increase in vapour pressure of the solid leads to a higher concentration in the supercritical phase. In a separate study by Brogle (1982), similar phenomena were observed. Hence the author suggested two general rules for
Figure 2-5: Solubility of naphthalene in supercritical ethylene as a function of temperature at different pressures (Ethylene : Tc = 282 K, Pc = 5 MPa). (Williams, 1981).
supercritical solvents:

--- Solvent power of a supercritical solvent increases with density at a given temperature.
--- Solvent power of a supercritical solvent increases with temperature at a given density.

2.4 EFFECT OF WATER CONTENT

Little work has been reported on the effect of water content on the supercritical fluid extraction process. It is an important parameter to be considered, as water is present in almost all natural substances.

The presence of water is essential in the extraction of nicotine. However, at the normal moisture content (10-13% by weight) of tobacco, supercritical CO₂ is a poor solvent for nicotine and tends to remove the aroma instead (Hubert and Vitzthum, 1978). For the extraction of nicotine in a single stage process, they found that it was necessary to increase the water content to at least 25%.

Zosel (1978) stated that coffee beans should be soaked using water prior to decaffeination with supercritical carbon dioxide.

During the extraction of soybean oil, Friedrich and Pryde (1984) reported that moisture contents between 3 and 15% had no significant effect on oil extractability and composition.
2.5 SCFE OF TRIGLYCERIDES

2.5.1 OILS AND FATS

Fats and oils consist predominantly of glyceryl esters of fatty acids (typically above 85% by weight), so-called triglycerides also referred to as triacylglycerols. Naturally, triglycerides exist in many different forms, with their fatty acid series ranging from C1 to C22 and higher. It is recognized that the triglyceride structure of the fats and oils is intimately related to the type and distribution of their fatty acids, and they are mainly mixtures of mixed rather than simple triglycerides (Sonntag, 1979).

A number of fats are prized for their individual flavors and structures, and are used with little or no purification. For example, cocoa butter has a specific arrangement of the fatty acid constituents in the glycerides that give it the unique flavor, texture and melting characteristics desirable for use in confectionary products, especially chocolate (Gutcho, 1979). Since cocoa butter is a relatively expensive natural product, for many years, attempts have been made to provide a product as its substitute.

It has been suggested that selective fractionation of other cheaper oils (such as palm kernel and palm oil) could produce some products that have similar properties of the specialty fats. Conventionally, fractionation of oils can be achieved by expensive vacuum distillation at fairly high
temperatures, or by crystalization methods where petroleum solvents are involved (Pike 1980).

2.5.2 **CO₂ EXTRACTION TRIGLYCERIDES**

Although no commercial supercritical-CO₂ oil extraction plants are known to be in operation, recent intensive studies of this technique on various oilseeds show its potential.

One of the first intensive studies dealing with oilseed extraction using SC-CO₂ was undertaken by Stahl et al. (1980). The authors have described a semi-batch extraction system and a method of measuring the equilibrium solubility of oil in CO₂. Various parameters influencing the extraction process were studied. These include: temperature, pressure, solvent flowrate and oilseeds pre-treatment. The physical properties (such as color, taste and odour) and chemical properties (such as major and minor constituents and oxidative stability) were also studied.

Similar studies were also carried out by some researchers in North America. Friedrich and co-workers (Friedrich and List, 1982; List and Friedrich, 1985; Christianson et al, 1984, Friedrich and Pryde, 1984), worked on soybeans, cottonseeds and corn germ. Fattori (1986) studied the extraction of canola oil. In most cases, CO₂ was used as the solvent.

The solubility of oil, as influenced by operating parameters and oil composition are the main concerns of this
work. Figure 2-6, 2-7 and 2-8, show the solubilities of various seed oils as a function of temperature and pressure as determined by different authors. As can be seen, in all cases, the solubility varies with temperature and pressure.

In general, the solubility increases with increasing pressure. For example, canola oil (Figure 2-6), at 55 °C the solubility increases from 2 mg/g CO₂ at 20 MPa to 12 mg/g CO₂ at 36 MPa. However, the change in solubility with temperature is different at different pressure levels for most oils. Typically, the solubility isotherms cross at the intermediate pressures. This crossover of solubility curves has been explained in terms of the interaction between the densities of CO₂ and the vapour pressure of the oils (Peter and Brunner, 1978; Friedrich and Pryde, 1984; Brogle, 1982 and Fattori, 1986). Generally, an increase in temperature causes an increase in vapor pressure of the solute which means an increase of solute solubility in solvent. On the other hand, the temperature increase will also cause a decrease in CO₂ density which means a decrease of its solvent capacity. As can be seen in Figure 2-9, the density of CO₂ changes most rapidly with temperature at pressures near the critical region. Hence, in this region, the density effect due to temperature changes is much more pronounced than that of the vapor pressure effect, whereas at higher pressures, the density/temperature change is relatively small. Hence, the vapor pressure effect predominates.
Figure 2-6: Solubility of Canola oil in CO₂ as a function of pressure at various temperature (Fattori, 1986).
Figure 2-7: Effects of temperature and pressure on the solubility of soybean oil in supercritical CO$_2$ (Friedrich and Pryde, 1984).
Figure 2-8: Solubility of Rapeseed and Soybean oils in CO₂ as a function of pressure at 20°C and 40°C (Stahl et al., 1980; Bunzenberger et al., 1984).
Figure 2-9: Density of carbon dioxide as a function of pressure at different temperatures. The critical point is designated as CP (Vukalovich and Altunin, 1968).
It is often not an easy task to predict which effect predominates under certain condition for different substances without experimental data. As can be seen from Figures 2-6 to 2-8, the crossover occurs at different pressures and temperatures for different oils.

The range of solubility data varies among the different oils. For example, Figure 2-8 shows that soybean oil is more soluble than rapeseed oil under similar extraction conditions. This difference could be due to the difference in the components of the oils. Rapeseed oil contains a higher percentage of erucic acid (C22:1) than soybean oil. As suggested by Peter and Brunner (1978), an oil having triglycerides of higher molecular weight would be expected to exhibit a lower solubility in CO₂. Fattori (1986) has also reported similar results for pure triglycerides (Figure 2-10).

2.5.3 SUPERCritical FRACTIONATION

Many high molecular weight oils consists of a wide range of homologous members. Because the oils exhibit low vapor pressure characteristics, they are difficult to fractionate either by high vacuum distillation or by solvent crystallization. Moreover, these oils possess components that resemble different structures or functionalities but exhibit the same, or nearly the same, vapor pressure. Thus distillation can not purify such materials. In addition, liquid solvent extraction is generally ineffective for
Figure 2-10: Extraction curves for pure tripalmitolein (C16:1), triolein (C18:1) and tri-11-eicosenoin (C20:1). Extraction conditions: 36 MPa and 55°C. (Fattori, 1986).
removing such impurities or for fractionating the oils because the solvent power of a liquid which can dissolve such an oil is so great that it can not be readily differentiated by molecular weight. Because the solvation power of supercritical fluids can be controlled to a close degree by appropriate selection of pressure, and because many high-molecular oils can be dissolved in a variety of common supercritical fluids, fractionation of these oils by high-pressure gases has been accomplished (Zosel, 1978; Stahl and Quirin, 1983; Brunner and Peter, 1982).

In general, if suitable P/T combinations are selected it is possible to achieve some degree of fractionation during the separation (Hubert and Vitzthum 1978).

The phenomenon of fractionation was first reported by Zosel (1978), who described a procedure to separate a mixture of \( \rho \)-olefins. The initial mixture was made up of \( \mathrm{C}_{16} \), \( \mathrm{C}_{18} \) and \( \mathrm{C}_{20} \) olefins with 17, 17 and 34 % volume respectively. Analysis of the various fractions of the extract showed that separation had been accomplished. He reported that one fraction containing about 25 % of the \( \mathrm{C}_{16} \) olefin had a purity of over 95 %. He applied the procedure to separate cod-liver oil which consists of a multicomponent mixture of triglycerides that are too involatile to separate by conventional distillation methods. Figure 2-11 shows the changes in saponification values and iodine numbers of each extract fraction during the course of extraction. The saponification value and iodine number are associated with
Figure 2-11: Saponification number and iodine number of the supercritical extracts of cod-liver oil at different stages of an extraction (Zosel, 1978).
the molecular weight and degree of unsaturation respectively.

Later, Peter and Brunner (1978) demonstrated the separation of the monoglycerides from a mixture of oleic acid glycerides using a countercurrent process with CO\textsubscript{2} as the solvent. They managed to concentrate the monoglyceride content of the extract to 95-100\% from a mixture containing about 45 \% monoglycerides.

Panzner et al. (1980) has reported some data on separation of glycerides using the supercritical extraction technique with an entrainer. Extractions were carried out at low pressures (8 - 10 MPa), with entrainers such as carbon tetrachloride and hexane being used to improve the solubility. His results showed that when using mixtures of carbon dioxide and carbon tetrachloride to separate mixtures of glycerides (mono-oleate 40 \%, dioleate 50 \% and trioleate 10 \%), the trioleate made up 75-99 \% by weight of the extract. With hexane as an entrainer, he was able to separate trioleate of 98-99 \% purity from dioleate and mono-oleate. He concluded that the solubility of trioleate in CO\textsubscript{2} can be increased substantially by suitable adjustment of the concentration of an entrainer such as hexane in the CO\textsubscript{2}.

It has also been found that butter fat can be fractionated using supercritical CO\textsubscript{2} (Mangold, 1982). This process yields a fraction that contains twice as much triglyceride of short-chain fatty acids as the starting
materials. Similar results have been reported (Anon, 1981) that supercritical CO₂ was extremely effective as a specific fractionation solvent for coconut oil and cocoa butter. In the coconut oil trial, 20 grams of oil were separated into five fractions, each of which was analysed by sensory evaluation and gas chromatography. Lactones, low molecular weight fatty acids which give coconut oil its familiar flavor, were effectively isolated in the first two fractions. The same trends were observed with cocoa butter. The cocoa flavor and aromatic volatiles were isolated in the early fractions. No information on the fatty acid composition of the extracted oils was given by the author.

Stahl and co-workers (1980,1983) have reported extensively on the study of supercritical fractionation. It is interesting to point out the two different findings from their studies. In their first paper, they stated that fractionation of condensed phases is possible if the mixture constituents exhibit large differences in vapor pressure, mass, or polarity (Stahl et al., 1980). In their later studies, Stahl and Quirin (1983) measured the solubilities of tetracyclic steriods which differed in molecular structures but had practically the same vapour pressure at the system operating temperature. They found that carboxyl groups on the steriods, such as bile acids, rendered the steriods virtually insoluble in the supercritical CO₂, while carbonyl groups had little effect on steriod solubilities. In this study, however, the differences in masses and melting points
of the steroids had no direct influence on the solubility behavior. Hence, they argued that supercritical fluid extraction constitutes a new separation and fractionation technique which does not depend on differences in the mixture components' vapour pressure and which exhibits selectively for certain classes of compounds.

Brogle (1982) has proposed a few practical examples for fractionated extraction:

--- fractionation of spice extraction into flavor fraction (essentially oil) and taste fraction.

--- fractionation of fatty oils into first fraction rich in flavor and free fatty acids, a second fraction of mostly glycerides and a third fraction rich in waxes and pigments.

--- fractionation of fatty oils into fractions rich in mono-, di- or triglycerides respectively.

In a recent study by Fattori (1986), the author found that the degree of fractionation of canola oils using supercritical CO₂ was not as pronounced as previously reported (Anon, 1981). However, he was able to fractionate a mixture of pure simple triglycerides (C16:1, C18:1 and C20:1), with the lighter fraction appearing in the early periods of the process, and the heavier fraction was concentrated in the later stages (Figure 2-12). He suggested that the canola oil was made up of a pool of homologous mixed triglycerides which were very similar in molecular mass, even though the distribution of fatty acids among them
Figure 2-12: Mass fraction of the supercritical CO$_2$ extracts of a mixture of three simple triglycerides at different stages of the extraction. Extraction conditions: 36 MPa and 55°C. (Fattori, 1986).
might be different. Hence, these triglycerides might have approximately the same solubility in supercritical CO$_2$ and might explain the relatively constant solubility observed throughout the entire extraction process. For the mixture of pure simple triglycerides, each component has a different molecular mass and thus a distinct solubility behavior. This resulted in changes in the overall solubility rate depending on the composition of the mixture.
3.1 EXPERIMENTAL EQUIPMENT

In this research, all extraction experiments were carried out using a modified Hewlett-Packard 1081B High Performance Liquid Chromatograph (HPLC). Basically, the system consists of four major components: the HPLC, extraction vessels, flow restrictor and sampling unit. Details on the modifications to the HPLC are reported by Fattori (1986).

3.1.1 HEWLETT-PACKARD LIQUID CHROMATOGRAPH

The HPLC is a microprocessor controlled instrument incorporating a reciprocal diaphragm pump and a solvent flow system. Flow rates can be selected between 0 to 9.9 ml/min in increments of 0.01 ml/min. Pressure can be selected from 0 to 40 MPa, with 0.1 MPa increments. The chromatograph also incorporates an oven with operating temperatures varying from 25°C to 99°C (with 0.1°C increments). The different process parameters can be precisely controlled within the allowable variation limits for this work: ±0.03 ml/min for the flowrate; ±0.2 MPa for the pressure and ±1.0°C for the oven temperature.

There is no direct manual control of these parameters, as they can only be set through the microprocessor. The

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'Sources for all equipments and suppliers are detailed in Appendix I.'
system will automatically shut off the pump if the maximum pressure is exceeded. Again, Fattori (1986) gives a detailed description on the control mechanism of pressure and flowrate.

### 3.1.2 EXTRACTION VESSELS

Extraction vessels (autoclaves) were used to accommodate the materials to be extracted. These vessels, made of 316 stainless steel, can withstand the very high operating pressure often encountered. Three extraction vessels of different sizes were used for this work. The size specifications for each of the extraction vessels are listed in Table 3-1.

**TABLE 3-1: DIMENSIONS OF THE EXTRACTION VESSELS.**

<table>
<thead>
<tr>
<th>Vessel number</th>
<th>Inside Diameter (cm)</th>
<th>Inside Length (cm)</th>
<th>Vessel Volume (cm³)</th>
<th>Vessel Wall Thickness (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.27</td>
<td>8.2</td>
<td>10.4</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>1.27</td>
<td>11.4</td>
<td>14.4</td>
<td>0.6</td>
</tr>
<tr>
<td>3</td>
<td>2.54</td>
<td>8.2</td>
<td>41.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Figure 3-1 is a machine drawing of vessel no.1. The extraction vessel (autoclave) resembles a thick walled tube with a flanged top. The cap of each vessel is a separate piece which can be removed upon loading the material, and is tightly secured to the body of the vessel by eight stainless steel cap screws after filling. The cap was fabricated with
Figure 3-1: Cross sectional view of extraction vessel #1. All measurements shown are in cm, except where indicated.
a machined sealing ridge which will sit on the flat sealing surface of the vessel. The seals were designed to be leak proof. The bolt size for vessel 1 is 10.6 mm, and 6.4 mm for the larger vessels. Each of the 10.6mm bolts was tightened to 60 cm-kgs. The 6.4mm bolts were tightened to 120 cm-kgs.

The bottom and top of the vessels are fitted with 3.2 x 1.6 mm Swagelok NPT fittings. Solvent flow-line connections to and from the vessels are made via these fittings.

3.1.3 FLOW RESTRICTER

The restricter is located in the HPLC oven and mounted in a heated aluminum block to prevent freezing up during the rapid expansion of the depressuring CO₂/solute mixture. The operation of the extraction system is affected by the characteristics of the flow restricter which serves to maintain the pressure in the extractor at the desired value. A variety of flow restricters were assessed. The Parker MV-200 metering valve, used in the earlier work, could adequately control the CO₂ flowrate and maintain the desired pressure. However, the performance drifted after a number of operations because the seal gradually wore off. Another restricter tested was a Nupro 'R3A' series externally adjustable pressure relief valve, and it was found to have a large dead volume in addition to the leak problem. Eventually a Rheodyne Model 7037 pressure relief valve was found to be the most stable for this type of material. It has a very small internal volume and allows pressure setting
to be adjusted over a range of 7 to 48 MPa. The flow stream only comes in contact with 316 stainless steel. Figure 3-2 shows an exploded view of the valve. It consists of a spring-loaded diaphragm which is sealed against the cap around its periphery and which seats against a polished central seat machined in the cap. This geometry forms an annular channel surrounding the central seat. The volume contained in the annular channel is approximately 6 μl. Four spring washers are used to exert a force on the diaphragm to seal it against the vent seat. The adjustment of spring force is accomplished by a differential thread arrangement. When the fluid pressure exceeds the relief setting, the fluid lifts the diaphragm off the seat and exposes the vent post where the fluid is continuously flushed out.

3.1.4 SAMPLING UNIT

After passing through the restricter, carbon dioxide within the system changes from the supercritical to the gaseous state. Accompanying this change of state is a large decrease in the solvation capacity of the carbon dioxide. Compounds such as oils, that are soluble in the CO₂ on the upstream side of the restricter, precipitate downstream. The sampling unit allowed the separation and collection of the oil droplets thus formed.

The sampling system enables sequential oil samples to be collected and the carbon dioxide to be measured by a wet test meter. The system (Figure 3-3), consisted of a 30 mm
Figure 3-2: Exploded view of Model 7037 pressure relief valve.
Figure 3-3: Cross sectional view of the sampling unit. All measurements shown are in mm, except where indicated.
long section of 1.6 mm O.D. stainless steel tubing connected to the restricter valve using a Swagelock adaptor.

A 50 mm section of 1.6mm O.D. stainless steel tubing was connected to the opposite end of the union and directed through a separator. The separator resembled a 25 mm long x 15 mm O.D. brass cylinder with a 1.5mm diameter channel for the inlet/outlet tubing down the centre, a 6.0 mm channel for gas exit at one side and a threaded fitting for the sample vial at the bottom. An O-ring was used for the sample vial fitting, and Swagelok fittings were used for the other connections to ensure a seal. The two-phase gas and entrained oil mixture flowed through the inlet tubing into the sample vial where the oil was deposited. The oil-free CO₂ then passed from the vial into the gas exit channel along a 1 m rubber tubing, 6.4mm in diameter. The tubing was channeled through the oven wall and then connected to the wet test meter where the flow rate of oil-free CO₂ was measured and the CO₂ vented.

3.2 SOLVENT FLOWPATH

In brief the operation of the system (schematically shown in Figure 3-4), was as follows.

Liquid carbon dioxide from a storage cylinder passes through the shutoff valve, a Nupro 7 μm sintered filter and then into the cooled diaphragm pump head. The cooled liquid CO₂ then flows through a pressure-flow monitoring device into the HPLC oven. Here its temperature is brought to the
Figure 3-4: Schematic diagram of the experimental supercritical fluid extraction system.
desired value by passing through a 7 m stainless steel (1.6mm diameter) tube. At this stage, carbon dioxide in its supercritical state is ready to enter the extraction vessel where solute is dissolved. The CO₂ leaving the vessel travels through two 2μm frits to a flow restricter where pressure is reduced to near atmospheric. The CO₂ and oils separate into two phases, and flow into the sampling unit where the oil is collected. The gaseous CO₂ is directed through a wet gas-meter and finally vented to the atmosphere.

All of the tubing within the system is 0.1 mm ID stainless steel and the connections were made using 1.6mm stainless steel Swagelok fittings.

3.3 EXPERIMENTAL EXTRACTION PROCEDURES

3.3.1 OPERATING CONDITIONS

3.3.1.1 Pressure
Four levels were selected at 15, 25, 30 and 36 MPa. They range between the critical pressure of CO₂ (7.3 MPa) and the maximum operating pressure of the HPLC. Density of CO₂ (and hence its solvent power) changes rapidly within this pressure range.

3.3.1.2 Temperature
Four levels were selected at 25, 35, 55 and 75 °C. The minimum operating temperature of the HPLC oven is
25°C and is below the critical temperature of CO₂, while the other temperatures chosen are above the critical temperature.

3.3.1.3 CO₂ Flowrate

It has been shown that the equilibrium solubility of oil in CO₂ is independent of the flowrate of CO₂ passing through the extraction bed (Fattori, 1986; Stahl et al., 1980). Hence, the flowrate of liquid CO₂ passing through the pump head was set at 1.0 ml/min for all experiments in this work.

3.3.2 VESEL LOADING PROCEDURE

3.3.2.1 Liquid Samples

The vessels were first partially filled with fine (0.5 mm) glass beads. The liquid triglyceride samples were then deposited on the glass beads. This procedure was used for two reasons:

--- the liquid on a matrix of glass beads presented a larger surface area available for mass transfer than would an equal mass of liquid in the empty vessel;

--- dissolved CO₂ could be released more effectively during the decompression stage of the extraction experiments thereby reducing the risk of the liquid foaming out of the vessel.

Details of the vessel loading procedure are outlined below: Glass beads - enough to approximately
half-fill each vessel - were placed in the autoclave on top of a small plug of glass wool. The liquid sample, equivalent to about 7% of the mass of the beads, 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 a relatively even distribution of oil throughout the bed. The mass of liquid was determined to the nearest 0.001 g by weighing the pipette before and after the liquid oil transfer. Before fitting the top of the vessel, another plug of glass wool was inserted. During extraction CO₂ flowed through the vessel from bottom to top.

3.3.2.2 Solid Samples

The vessel was first filled with fine (0.5 mm) glass beads up to 1/3 of its volume. A weighed amount of solid fat (about 2 to 4 g) was placed in the vessel on top of the bead bed. Fine-spun glass wool was placed at both ends of the vessel for the same purpose mentioned before. The sealing surfaces were then cleaned with a chloroform-wetted tissue. After sealing, the autoclave was placed in the oven at a temperature above the melting point of the fat for 1/2 hour to ensure that all the solid fat melted and flowed into the matrix of the glass beads. All of the solid fats were extracted at temperatures above their melting points.
3.3.3 PRE-EXTRACTION CLEANING

Before each extraction was performed, the flow system downstream of the extraction vessel including the filters, the restricter valve and the sampling unit, was flushed with 1:1 chloroform/methanol solution, to clean up any materials left behind from the previous run.

The HPLC was always on its standby mode when not in operation. The cooler was switched on approximately two hours prior to the start of an experiment. Typically, it was set at -20°C. The pre-extraction cleaning routine began when the pump head temperature shown on the HPLC display board stabilized at around -5°C. The system was assembled with the extraction vessel filled with approximately 1:1 chloroform/methanol solution. The shut-off valve of the CO₂ cylinder was opened and the restricter valve was set to allow the CO₂ to flow through freely. The solution in the vessel was first forced through the downstream line and discharged. Thence, the CO₂ was allowed to flush through the system continuously for one hour. Finally, the CO₂ was shut off and the emptied vessel was removed.

3.3.4 EQUIPMENT STARTUP

Following the cleaning procedure, the system was reassembled with another extraction vessel filled with the extracting materials (typically oil and beads for this work). The oven temperature was first set to the desired value (the operating temperature) and the heater on the
restricter valve set at 75°C. The system was allowed to stabilize for about 30 minutes after which time the set values of flowrate (typically 1.0 ml/min) and pressure (40 MPa) were entered.

The pump was activated by pressing the 'pre-run' button on the HPLC. Within 5 to 10 seconds, the flow reached the set value, while the pressure continued to build up within the system. By adjusting the restricter valve, the pressure eventually reached and stabilized at the desired operating value.

3.3.5 EXTRACT SAMPLING

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

Before the extraction began, 10 to 30 empty 1.8 ml glass vials were weighed. The number of vials used depended on the number of data points needed. During the sampling procedure, a vial of known weight was fitted to the sampling head and oil collected for set periods of time. The vial and its contents were removed and re-weighed, and a new pre-weighed vial attached to the collector. The quantity of gaseous CO₂ that passed through the vial during that sampling period was determined using the wet test gas meter and the corresponding mass of CO₂ calculated from its molar volume with reference to the temperature indicated on the gas meter. The exact sampling time could be obtained from the
chart recorder. With this information, the flowrate of CO₂ passing through the extraction vessel was calculated.

3.3.6 EQUIPMENT SHUTDOWN

At the completion of an experiment, the pump was turned off by setting the system back to the standby mode. The CO₂ supply was shut off, the heaters were turned off, and the system allowed to depressurize. Normally, the system pressure would drop to 0.1 MPa within 1 to 2 hours, after which the vessel was removed from the system.

3.3.7 SOLUBILITY DETERMINATION

The average oil concentration in CO₂ at any interval during the extraction was determined from the mass of oil collected and the mass of CO₂ that passed through during that interval.

If the accumulative mass of oil is plotted against the corresponding mass of CO₂ at certain operating conditions, an extraction curve as shown in Figure 3-5 can be obtained. The slope of the initial linear portion of the curve represents the saturated concentration of oil in CO₂ (equilibrium solubility) at the operating conditions. All the experiments were performed at least twice under the same conditions (pressure, temperature and CO₂ flowrate).
Figure 3-5: Extraction curve of cocoa butter fat using carbon dioxide. Extraction conditions: 36 MPa and 55°C.
3.4 MATERIALS

3.4.1 CARBON DIOXIDE

Carbon dioxide was obtained from pressurized steel cylinders supplied by Medigas Pacific. Each cylinder held 30 kg of carbon dioxide which could be withdrawn in liquid form through an eductor 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 from its actual weight. 200-300 hours of system run time could be obtained with each cylinder. Specifications of commercial siphon grade carbon dioxide are listed in Table 3-2.

TABLE 3-2: SPECIFICATIONS OF COMMERCIAL SIPHON GRADE CARBON DIOXIDE.

<table>
<thead>
<tr>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>99% PURE</td>
</tr>
<tr>
<td>&lt;10 ppm CO</td>
</tr>
<tr>
<td>&lt;1 ppm H₂S</td>
</tr>
<tr>
<td>&lt;5 ppm NO₂</td>
</tr>
<tr>
<td>&lt;0.3 ppm COCl₂ (phosgene)</td>
</tr>
<tr>
<td>&lt;5 ppm SO₂</td>
</tr>
<tr>
<td>&lt;25 ppm H₂O</td>
</tr>
</tbody>
</table>

3.4.2 PURE TRIGLYCERIDES

Triglycerides are glycerol esters derived from several different carboxylic acids. Many triglycerides are commercially available. Five were obtained for this research project. They were selected to represent many of the
commonly occurring triglycerides in vegetable oils (Sonntag, 1979).

All of the triglycerides were obtained from Sigma Chemical Co. Ltd. The specifications for each are given below (Table 3-3). When not in use, the triglycerides were stored at -20°C.

**TABLE 3-3: SPECIFICATIONS OF THE TRIGLYCERIDE SAMPLES USED DURING THE EXPERIMENTS.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Carbon Number</th>
<th>Melting Point</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimyristin</td>
<td>C14:0</td>
<td>56.5</td>
<td>C₄₅H₈₆O₆</td>
<td>723.19</td>
<td>99 %</td>
</tr>
<tr>
<td>Tripalmitin</td>
<td>C16:0</td>
<td>66.4</td>
<td>C₅₁H₉₈O₆</td>
<td>807.35</td>
<td>99 %</td>
</tr>
<tr>
<td>Tristearin</td>
<td>C18:0</td>
<td>71.5</td>
<td>C₅₇H₁₁₀O₆</td>
<td>891.51</td>
<td>99 %</td>
</tr>
<tr>
<td>Triolein</td>
<td>C18:1</td>
<td>4.9</td>
<td>C₅₇H₁₀₂O₂</td>
<td>885.47</td>
<td>99 %</td>
</tr>
<tr>
<td>Trilinolein</td>
<td>C18:2</td>
<td>12.9</td>
<td>C₅₇H₁₀₂O₂</td>
<td>879.50</td>
<td>99 %</td>
</tr>
</tbody>
</table>

3.4.3 OIL SAMPLES

Cocoa butter and palm kernel oil were used in this work. Both oils were provided by the Weston Research Centre. When not in use, they were also kept refrigerated at -20°C.

3.5 FATTY ACID ANALYSIS

Two steps were involved. In the first step the fatty acid chains were cleaved from the triglycerides and simultaneously converted to their methyl esters. In the second step, the methyl esters were identified and quantified using a gas chromatograph.
3.5.1 TRANSESTERIFICATION

The method adopted was based on that developed by Shehata et al. (1970).

The esterifying reagents are made up of 12.5 ml of 0.5N sodium methoxide in methanol solution, 8.5 ml of anhydrous diethyl ether and 5.0 ml of petroleum ether, which formed a single phase at room temperature. The 0.5N solution of sodium methoxide in methanol was prepared by adding 0.675 g of the anhydrous sodium methoxide powder to 5 ml of absolute methanol. This reagent was stored at -20 °C in a cap flask, and the maximum storage time was two weeks. All of the chemicals were obtained from BDH Chemicals.

A small amount (usually 1 to 3 drops) of oil, melted if necessary, was transferred to a 1.8 ml borosilicate-glass screw capped vial equipped with Teflon septor. Approximately 1 ml of the esterifying reagent was added to the vial using a disposable pasteur pipette. The vial was capped, then rotated and shaken gently to ensure complete mixing. The reaction mixture was allowed to stand at room temperature for two minutes, after which the mixture was diluted with about 0.5 ml of petroleum ether, and separated into two phases when a drop of water was introduced. The resulting mixture was shaken for about 30 seconds to facilitate the transfer of the methyl esters into the petroleum ether phase. The vial and its contents were then centrifuged for 10 minutes in order to remove any suspended sodium methoxide. Thereafter, the top mm of the petroleum ether
layer, which contained the methyl esters, was transferred by a pipette to another 1.8 ml vial. The solution was ready for injection into a gas chromatograph.

3.5.2 GAS CHROMATOGRAPHIC PROCEDURE

3.5.2.1 Gas Chromatographic Conditions

The fatty acid ester solution was analysed using a Perkin-Elmer (PE) Sigma gas chromatography (GC), connected to a SP4290 Integrator. The GC was equipped with a Hydrogen Flame Ionization Detector (FID) and fitted with a 1.83 m x 3.2 mm stainless steel column packed with SP-2330 on 100/120 mesh Chromosorb WAW. The column was obtained pre-packed from Supelco Corp. All GC analyses were performed in accordance with the conditions shown in Table 3-4.

| TABLE 3-4: GAS CHROMATOGRAPHIC PARAMETERS FOR THE FATTY ACID METHYL ESTER ANALYSES. |
|-----------------------------------|---------------------------------|
| Column                           | SP-2330 on 100/120 mesh Chromosorb WAW |
| Detector                         | Flame ionization                |
| Detector gas                     | Air and Hydrogen                |
| Carrier gas                      | helium                          |
| Carrier gas flow                 | 20 cm³/min                      |
| Initial temperature             | 160 °C                          |
| Initial time                     | 1.5 s                           |
| Initial rate                     | 20°C/s                          |
| Final temperature               | 200 °C                          |
| Final time                       | 5 s                             |
| Sample size                      | 1 μl                            |
3.5.2.2 Peak Identification and Quantification Procedure

The standard fatty acid methyl esters used for the retention time and response factor information were obtained from Sigma Chemical Co. Ltd.

The methyl esters were identified by their corresponding retention time they stay in the column before eluted. Two standard mixtures of pure methyl esters were used to obtain retention time information. The standards were diluted to a concentration of 100mg/25ml petroleum ether, before injecting into the GC which was set at the conditions mentioned above. The retention times obtained for the various methyl esters are shown in Table 3-5.

Weight response factor (WRF) was used to correct the discrepancy between the peak areas on the chromatogram (i.e. the response of FID) and the actual weight percents (Ackmans and Sipos, 1964). The values of WRF are device dependent. Hence, calibration of WRFs was performed. The integrator SP4905 used in this work has a programme to calculate the WRF.

Table 3-5 shows the Hydrogen FID weight response factors for various fatty acid methyl esters used in this research. The response factors were calculated with reference to methyl palmitate (C16:0). Each value was an average of values obtained from ten separate injections.
TABLE 3-5: RETENTION TIMES AND RESPONSE FACTORS FOR FATTY ACID METHYL ESTERS RELATIVE TO METHYL PALMITATE (C16:0).

<table>
<thead>
<tr>
<th>Fatty Acid Methyl Ester</th>
<th>Carbon Number</th>
<th>Response Factor</th>
<th>Retention Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl Caprylate</td>
<td>C8:0</td>
<td>0.872</td>
<td>0.80</td>
</tr>
<tr>
<td>Methyl Caprate</td>
<td>C10:0</td>
<td>0.873</td>
<td>1.25</td>
</tr>
<tr>
<td>Methyl Laurate</td>
<td>C12:0</td>
<td>0.910</td>
<td>2.08</td>
</tr>
<tr>
<td>Methyl Myristate</td>
<td>C14:0</td>
<td>0.997</td>
<td>3.10</td>
</tr>
<tr>
<td>Methyl Palmitate</td>
<td>C16:0</td>
<td>1.000</td>
<td>4.10</td>
</tr>
<tr>
<td>Methyl Stearate</td>
<td>C18:0</td>
<td>1.083</td>
<td>5.35</td>
</tr>
<tr>
<td>Methyl Oleate</td>
<td>C18:1</td>
<td>1.011</td>
<td>5.75</td>
</tr>
<tr>
<td>Methyl Linoleate</td>
<td>C18:2</td>
<td>1.117</td>
<td>6.45</td>
</tr>
<tr>
<td>Methyl Linolenate</td>
<td>C18:3</td>
<td>1.229</td>
<td>7.45</td>
</tr>
<tr>
<td>Methyl Arachidate</td>
<td>C20:0</td>
<td>1.092</td>
<td>7.15</td>
</tr>
</tbody>
</table>
4.1 CO\textsubscript{2} EXTRACTION OF PURE TRIGLYCERIDE

In this study, five pure simple triglycerides were extracted using supercritical CO\textsubscript{2} at different levels of pressure and temperature. In each case, the solubility of triglyceride in CO\textsubscript{2} was determined from the corresponding extraction curve using the procedure outlined in Section 3.3.7.

The general solubility characteristics of triglycerides as a function of pressure are shown in Figure 4-1. The solubilities for all of the triglycerides increase with increasing pressure at 75 °C. Trimyristin (C\textsubscript{14:0}), the lightest compound, has the highest solubility, followed by tripalmitin (C\textsubscript{16:0}), the next lightest, and then the C\textsubscript{18} compounds. The effect of fatty acid unsaturation on solubility (i.e. C\textsubscript{18:0}, C\textsubscript{18:1} and C\textsubscript{18:2}) is also shown and will be discussed in more detail in the following sections.

4.1.1 TRIGLYCERIDE SOLUBILITY AS A FUNCTION OF TEMPERATURE AND PRESSURE

Figure 4-2 and Figure 4-3 show the effect of temperature and pressure on the equilibrium solubilities of triolein (C\textsubscript{18:1}) and trilinolein (C\textsubscript{18:2}) respectively. At constant temperature, the solubility of both triglycerides increases with increasing pressure over the range studied.
Figure 4-1: Solubility of various simple triglycerides in CO$_2$ as a function of pressure at 75°C.
Figure 4-2: Solubility of triolein (C18:1) in CO₂ as a function of pressure at four different temperatures.
Figure 4-3: Solubility of trilinolein (C18:2) in CO₂ as a function of pressure at four different temperatures.
However, this increase is different at different temperatures. At the lower temperatures, 25°C and 35°C, the solubility increases almost linearly with pressure, and at 55°C and 75°C, the relationship becomes non-linear, whereby the slope of the solubility curve is seen to increase with pressure. Hence, at low pressure, the oil solubility is higher at lower temperatures, whereas at higher pressures, the reverse phenomenon was observed. For example, at 15 MPa, the solubility of triolein (C18:1) (Figure 4-2) is 2.0 mg/g CO₂ at 25°C and 0.3 mg/g CO₂ at 55°C. As pressure increases, the solubility increases, however, the solubility/pressure gradient at 55°C is larger than that at 25°C. The solubility isotherms cross when the pressure reaches 29 MPa. For any pressure greater than this value, the solubility at higher temperature is greater than that at lower temperature. This crossover of the solubility curve at different temperatures has been reported by other authors (Zosel, 1978; Peter and Brunner, 1978; deFillipi, 1982; Friedrich and Pryde, 1984 and Fattori, 1986).

If the solubility is expressed as a function of the density of CO₂ (which in turn is a function of temperature and pressure), a simpler relationship can be obtained (Figure 4-4, 4-5 and Figure 4-6, 4-7). For any density, the solubility increases with temperature; likewise at any temperature the solubility increases with increasing density. These phenomena follow the two general rules proposed by Brogle (1982), which have been briefly discussed
Figure 4-4: Solubility of triolein (C18:1) in CO₂ as a function of density of CO₂ at various temperatures.
Figure 4-5: Solubility of trilinolein (C18:2) in CO₂ as a function of density at various temperatures.
Figure 4-6: Solubility of triolein (C18:1) in CO₂ as a function of temperature at four CO₂ densities.
Figure 4-7: Solubility of trilinolein (C18:2) in CO₂ as a function of temperature at four CO₂ densities.
in Section 2-2. The complex relationship between solubility and pressure at different temperatures can be explained by referring to Figure 4-8. In general, a rise in temperature at constant pressure leads to a decrease in CO₂ density. On the other hand, a rise in temperature also leads to an exponential increase in the vapour pressure of the oils in the supercritical phase. An increase of the vapour pressure of the solute in the solvent phase results in an increase in the concentration of the solute in the solvent (Brunner and Peter, 1978). Near the critical point of CO₂, the density changes rapidly with temperature below 80 °C. A small change in this region may lead to a large change in CO₂ density and a commensurate change in oil solubility. At higher pressures, however, the same temperature change has a smaller effect on the fluid density. In this case, the increase in vapour pressure of the solute may more than offset the decrease in solvation capacity of the fluid due to its decreased density. The net effect is an overall increase in solubility.

In general, the extraction of most triglycerides should be carried out at a pressure greater than 15 MPa. For the triglycerides studied, the solubilities are relatively low at pressure below 15 MPa, ranging from 0.1 mg/g CO₂ to 2.0 mg/g CO₂. The steep curves between 15 and 36 MPa show that more efficient separation can be achieved by decompression. For example, for an extraction of triolein, a pressure drop from 36 MPa (solubility = 10.5 mg/g CO₂) to 15 MPa
Figure 4-8: Density of carbon dioxide as a function of pressure at different temperatures. The critical point is designated as CP. (Newritt, 1956; Vukalovich and Altunin, 1968).
(solubility = 0.5 mg/g CO₂) at 55 °C will yield about 95% of the dissolved oil.

Separation can also be achieved by a temperature change while holding pressure constant. However, this method is more difficult due to the non-linear variation of solubility with temperature at different pressures. Under certain conditions, a temperature rise is required but other cases may call for the opposite action. Considering trilinolein (Figure 4-3), with a temperature rise from 25 °C (6.5 mg/g CO₂) to 75 °C (1.5 mg/g CO₂) at 15 MPa, about 75% of the dissolved oil can be recovered. If the same temperature change is applied at 36 MPa, virtually no oil can be collected.

4.1.2 TRIGLYCERIDE SOLUBILITY AS A FUNCTION OF MOLECULAR WEIGHT (OR CARBON CHAIN LENGTH)

Solubility curves of three saturated simple triglycerides; trimyristin (C₁₄:0), tripalmitin (C₁₆:0) and tristearin (C₁₈:0) as determined at 75 °C and various pressures (15, 25, 30 and 36 MPa) are presented in Figure 4-9.

At the lower pressure of 15 MPa, the three compounds exhibit essentially the same solubility in CO₂. As pressure rises, the solubility of each compound has increased substantially. Trimyristin (C₁₄:0), lightest among the group, exhibits the greatest increase in solubility over the range of pressures studied. When pressure reached 36 MPa, the solubility of trimyristin is 40 mg/g CO₂, compared to
Figure 4-9: Solubility of trimyristin (C14:0), tripalmitin (C16:0) and tristearin (C18:0) in CO₂ as a function of pressure at 75°C.
16.5 mg/g CO₂ for tripalmitin and 7.5 mg/g CO₂ for tristearin.

It is apparent that triglyceride solubility varies inversely with molecular weight. This effect was expected as these triglycerides belong to a homologous series. They possess similar chemical properties such as polarity and molecular configuration, but their volatility in the solvent phase decreases with increasing molecular weight (Sonntag, 1979; Arnold et al., 1963). As shown, the lightest triglyceride has the higher solubility in the supercritical fluid and this supports the findings of Brunner and Peter (1978).

The relatively large variation in solubility between these triglycerides indicates that fractionation might be used to facilitate separation of a mixture during the extraction (Stahl and Quirin, 1983).

When the solubilities of these pure triglycerides (C₁₄:0, C₁₆:0 and C₁₈:0) are plotted as a function of their respective molecular weights on a semi-logarithmic scale (Figure 4-10), a straight line relationship is observed when the pressure is held constant. This implies that the same percentage increase in solubility results from an equal increase in molecular weight.

Fattori (1986) reported on a series of triglycerides having a single bond: tripalmitolein (C₁₆:1), triolein (C₁₈:1) and tri-11-eicosenoin (C₂₀:1). The solubility of each was determined at 36 MPa and 55 °C, and are shown in Figure
Figure 4-10: The negative logarithm of the solubility of the three triglycerides in CO$_2$ as a function of their molecular weights.
4-11 for comparison. The difference in slopes for the two lines could be due to the difference in operating temperatures, 55 °C verses 75 °C, or to the difference in degree of unsaturation for the fatty acids within the two groups of triglycerides, one being saturated and the other having one single bond.

Similar solubility effect has also been observed by Plattner et al. (1977). The authors found that when a homologous series of saturated triglycerides were eluted through an HPLC column, a linear relationship existed 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.

It is therefore possible to predict the solubility of other saturated triglycerides (such as trilaurin and tricaprin) from the relationship established for the conditions studied.

4.1.3 EFFECT OF DEGREE OF FATTY ACIDS SATURATION ON THE SOLUBILITY OF PURE TRIGLYCERIDES

Figure 4-12 shows the change in equilibrium solubility with pressure at 75°C for three simple triglycerides namely, tristearin (C18:0), triolein (C18:1) and trilinolen (C18:2), having the same number of carbons and different degrees of unsaturation. The addition of one double bond to each of the fatty acids resulted in a 40% increase in the equilibrium solubility at 30 MPa and a 38% increase at 36
Figure 4-11: The negative logarithm of the solubility of two groups of triglycerides in CO₂ as a function of their molecular weights.
Figure 4-12: Solubility of tristearin (C18:0), triolein (C18:1) and trilinolein (C18:2) in CO$_2$ as a function of pressures at 75°C.
MPa. However with the second double bond incorporated in each of the fatty acids, there was no significant difference in the equilibrium solubilities for pressures from 15 to 36 MPa. The closeness between the solubilities of trioelin and trilinolein shows that separation of these triglycerides using supercritical fluid extraction technique can hardly be achieved.

The linear relationship developed earlier between the molecular weight and the negative logarithm of the solubility does not apply in this case. The triglycerides will experience a significant change in both the chemical and physical properties (Formo, 1979) when a double bond is added to each of the fatty acid chains. The molecular configuration of the fatty acid chains is different and there is an increase in the polarity around the chain (Arnold et al., 1963). It has been reported that polar compounds such as phospholipids are virtually insoluble in supercritical CO₂ (Friedrich and List, 1982; Fattori, 1986). However, in this study, the more polar monosaturated triolein was found to be more soluble in CO₂ than the less polar tristearin.

The effect of the molecular configuration change due to the double bonds on the solubility could not be inferred from the limited observations derived from this study.
4.2 CO₂ Extractions of Triglyceride Mixtures

Extractions were performed on the mixture of trimyristin (C14:0) and triolein (C18:1) in the approximate weight ratio of 25:75, 50:50 and 75:25. The extraction curves shown in Figures 4-13, 4-14 and 4-15, illustrate that the amount of oil removed per unit mass of CO₂ passed through the bed changed continuously during the course of the extraction. It is possible that saturation had been achieved within the extractor during the initial part of the extraction since only a small fraction of triglycerides had been extracted at this stage. The continuous change of the extraction rate (or the equilibrium solubility) could possibly result from the changing composition of the oil in the vessel. Since trimyristin (C14:0) and triolein (C18:1) are relatively different in solubility at this pressure and temperature (40 mg/g CO₂ and 10 mg/g CO₂ respectively), it is very likely that fractionation was occurring during the extraction. To evaluate this observation, the extracts were collected for fatty acid analysis. The fatty acid profiles from these experiments are shown in Table 4-1.
Figure 4-13: Extraction curve for the mixture of trimyristin (C14:0) and triolein (C18:1) in the weight ratio of 75:25. The extraction was performed at 36 MPa and 75°C.
Figure 4-14: Extraction curve for the mixture of trimyristin (C14:0) and triolein (C18:1) in the weight ratio of 50:50. The extraction was performed at 36 MPa and 75°C.
Figure 4-15: Extraction curve for the mixture of trimyristin (C14:0) and triolein (C18:1) in the weight ratio of 25:75. The extraction was performed at 36 MPa and 75°C.
<table>
<thead>
<tr>
<th>(A)</th>
<th>Mass Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Acid</td>
<td>C14:0</td>
</tr>
<tr>
<td>Initial Mixture</td>
<td>72.7</td>
</tr>
<tr>
<td>Extraction Number</td>
<td>1</td>
</tr>
<tr>
<td>(see Figure 4-13)</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>34.5</td>
</tr>
<tr>
<td>4</td>
<td>19.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(B)</th>
<th>Mass Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Acid</td>
<td>C14:0</td>
</tr>
<tr>
<td>Initial Mixture</td>
<td>53.5</td>
</tr>
<tr>
<td>Extraction Number</td>
<td>1</td>
</tr>
<tr>
<td>(see Figure 4-14)</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>17.2</td>
</tr>
<tr>
<td>4</td>
<td>7.8</td>
</tr>
</tbody>
</table>
It can be seen that fractionation did occur during the extraction. In all cases, the initial extracts were significantly richer in C14:0, the lighter triglyceride, and the later extracts consist predominantly of triolein (C18:1).

Fattori (1986) has reported that the molecular interactions between a homologous series of monounsaturated triglycerides are very similar to those between the molecules of a pure substance, and hence the mixture of these triglycerides represented an ideal solution. The author was able to predict the solubility of the mixture using a modified version of Roult's equation:

\[
\text{Sol}_{\text{mix}} = \sum \text{mol}_i \times \text{sol}_i
\]

where \( \text{Sol}_{\text{mix}} \) is the solubility of the mixture in the supercritical CO\(_2\) phase; \( \text{sol}_i \) is solubility of the pure substance in the supercritical CO\(_2\) phase and \( \text{mol}_i \) is mole fraction of the pure substance in the mixture.

In this study, however, the mixture of trimyrisrin (C14:0) and triolein (C18:1) did not appear to behave like...
the ideal solution mentioned. This result was not unexpected since the molecular interaction between the saturated triglyceride and monounsaturated triglyceride could be significant due to their differences in polarity.

4.3 EXTRACTION OF COCOA BUTTER AND PALM KERNEL OIL

The CO$_2$ extraction of simple triglycerides have been discussed so far. Similar extraction experiments were extended to the more complex mixtures of triglycerides such as those found in palm kernel oil and cocoa butter. In this section, the fatty acid composition of each oil was first analysed, then the results of the CO$_2$ extraction experiments are discussed.

4.3.1 FATTY ACID COMPOSITION OF PALM KERNEL OIL AND COCOA BUTTER

From fatty acid analysis experiments, the fatty acid compositions of cocoa butter and palm kernel oil were obtained and are tabulated in Tables 4-2 and 4-3. They compare favorably with values reported by Sonntag (1979).
TABLE 4-2: FATTY ACID COMPOSITION OF COCOA BUTTER (Sonntag, 1979).

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Weight Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cheok (1986)</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.3</td>
</tr>
<tr>
<td>C16:0</td>
<td>23.2</td>
</tr>
<tr>
<td>C18:0</td>
<td>35.3</td>
</tr>
<tr>
<td>C18:1</td>
<td>36.8</td>
</tr>
<tr>
<td>C18:2</td>
<td>2.7</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.5</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.4</td>
</tr>
</tbody>
</table>

TABLE 4-3: FATTY ACID COMPOSITION OF PALM KERNEL OIL (Sonntag, 1979).

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Weight Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cheok (1986)</td>
</tr>
<tr>
<td>C8:0</td>
<td>3.0</td>
</tr>
<tr>
<td>C10:0</td>
<td>2.9</td>
</tr>
<tr>
<td>C12:0</td>
<td>42.7</td>
</tr>
<tr>
<td>C14:0</td>
<td>15.5</td>
</tr>
<tr>
<td>C16:0</td>
<td>10.8</td>
</tr>
<tr>
<td>C18:0</td>
<td>6.1</td>
</tr>
<tr>
<td>C18:1</td>
<td>15.8</td>
</tr>
<tr>
<td>C18:2</td>
<td>2.8</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.3</td>
</tr>
</tbody>
</table>
4.3.2 CO₂ EXTRACTION OF PURE OILS

Figure 4-16 demonstrates that the solubility of cocoa butter in CO₂ as a function of temperature and pressure followed a similar pattern as that reported previously for pure C18 triglycerides. The solubility ranges from a trace quantity of 0.2 mg/g CO₂ (at 15 MPa and 75 °C) to the maximum of 10 mg/g CO₂ (at 36 MPa and 55 °C). This solubility range is close to that of the pure C18 triglycerides studied and is not surprising since cocoa butter contains a high proportion of C18 fatty acids.

In another set of experiments, the solubility of palm kernel oil in CO₂ was found to be higher than that of cocoa butter (Figure 4-17). As palm kernel oil contains a high proportion of triglycerides that contain C12:0 and C14:0, the marked increase in solubility was to be expected.

In order to examine whether fractionation would occur during the extraction of a mixture of triglycerides such as those found in palm kernel oil and cocoa butter, selected extract fractions of each oil were collected for fatty acid analysis.
Figure 4-16: Solubility of cocoa butter in CO$_2$ as a function of pressure at various temperatures.
Figure 4-17: Solubility of palm kernel oil in CO$_2$ as a function of pressure at various temperatures.
TABLE 4-4: FATTY ACID COMPOSITION OF CO₂ EXTRACTS OF COCOA BUTTER. EXTRATION CONDITIONS: 36 MPa AND 55 °C.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Extraction Number 1</th>
<th>2</th>
<th>3</th>
<th>Residual oil left in vessel</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁₄:₀ %</td>
<td>0.27</td>
<td>0.14</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>C₁₆:₀ %</td>
<td>26.1</td>
<td>24.1</td>
<td>23.4</td>
<td>23.6</td>
</tr>
<tr>
<td>C₁₈:₀ %</td>
<td>34.4</td>
<td>35.4</td>
<td>36.0</td>
<td>35.4</td>
</tr>
<tr>
<td>C₁₈:₁ %</td>
<td>36.4</td>
<td>35.8</td>
<td>35.1</td>
<td>35.2</td>
</tr>
<tr>
<td>C₁₈:₂ %</td>
<td>2.1</td>
<td>3.7</td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>C₁₈:₃ %</td>
<td>0.27</td>
<td>0.96</td>
<td>1.18</td>
<td>1.23</td>
</tr>
<tr>
<td>C₂₀:₀ %</td>
<td>0.46</td>
<td>0.95</td>
<td>1.01</td>
<td>1.21</td>
</tr>
</tbody>
</table>

TABLE 4-5: FATTY ACID COMPOSITION OF CO₂ EXTRACTS OF PALM KERNEL OIL. EXTRATION CONDITIONS: 36 MPa AND 55 °C.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Extraction Number 1</th>
<th>2</th>
<th>3</th>
<th>Residual oil left in vessel</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₈:₀ %</td>
<td>3.91</td>
<td>3.27</td>
<td>2.01</td>
<td>1.19</td>
</tr>
<tr>
<td>C₁₀:₀ %</td>
<td>3.76</td>
<td>3.02</td>
<td>1.74</td>
<td>1.06</td>
</tr>
<tr>
<td>C₁₂:₀ %</td>
<td>45.9</td>
<td>44.0</td>
<td>43.2</td>
<td>41.5</td>
</tr>
<tr>
<td>C₁₄:₀ %</td>
<td>17.5</td>
<td>17.8</td>
<td>16.2</td>
<td>16.1</td>
</tr>
<tr>
<td>C₁₆:₀ %</td>
<td>9.22</td>
<td>9.91</td>
<td>11.1</td>
<td>12.1</td>
</tr>
<tr>
<td>C₁₈:₀ %</td>
<td>4.72</td>
<td>5.89</td>
<td>7.17</td>
<td>7.90</td>
</tr>
<tr>
<td>C₁₈:₁ %</td>
<td>14.0</td>
<td>14.8</td>
<td>16.1</td>
<td>17.0</td>
</tr>
<tr>
<td>C₁₈:₂ %</td>
<td>0.92</td>
<td>1.14</td>
<td>1.56</td>
<td>1.99</td>
</tr>
<tr>
<td>C₁₈:₃ %</td>
<td>0.09</td>
<td>0.10</td>
<td>0.59</td>
<td>0.66</td>
</tr>
<tr>
<td>C₂₀:₀ %</td>
<td>0.08</td>
<td>0.09</td>
<td>0.41</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Table 4-4 and 4-5 list the fatty acid composition of each extract. It is noticed that only small variations occur...
Figure 4-18: Extraction curve for pure cocoa butter. Extraction conditions: 36 MPa and 55°C.
Figure 4-19: Extraction curve for pure palm kernel oil. Extraction conditions: 36 MPa and 55°C.
in the fatty acid composition of each extract during each stage of the extraction process. In both cases, the oil extracts collected at the initial stage were slightly richer in lighter fatty acids (e.g. C12:0 in palm kernel oil and C16:0 in cocoa butter), while those obtained from the latter stages show a slight increase in heavier fatty acids (e.g. C18:1 in palm kernel oil and C18:2 in cocoa butter).

The small variation of the fatty acid composition could be due to the type of fatty acid distribution in the triglycerides of the oils. The triglycerides present in cocoa butter (Table 4-6) having 55 and 75 carbon atoms constitute a large portion of the oil, being 61% and 31% by weight respectively.

<table>
<thead>
<tr>
<th>Triglyceride</th>
<th>Carbon Number</th>
<th>Weight Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmito-distearin</td>
<td>55</td>
<td>2</td>
</tr>
<tr>
<td>Oleo-dipalmitin</td>
<td>53</td>
<td>6</td>
</tr>
<tr>
<td>Oleo-palmito-stearin</td>
<td>55</td>
<td>52</td>
</tr>
<tr>
<td>Oleo-distearin</td>
<td>57</td>
<td>19</td>
</tr>
<tr>
<td>Palmito-diolein</td>
<td>55</td>
<td>9</td>
</tr>
<tr>
<td>Stearo-diolein</td>
<td>57</td>
<td>12</td>
</tr>
</tbody>
</table>

TABLE 4-6 : TRIGLYCERIDE COMPOSITION OF COCOA BUTTER. (Hilditch and William, 1964)

These triglycerides, though different in molecular structure, have very similar molecular weights. It is very likely that they exhibit similar solubilities in CO₂. As a result, the majority of the triglycerides would be extracted
at the same rate and consequently the composition of the extract remains constant for much of the extraction.

However, the behaviour exhibited by palm kernel oil is more difficult to rationalize. The range of molecular weights of the triglycerides present in this oil (Table 4-7) is wider with carbon numbers ranging from 37 to 49.

TABLE 4-7: TRIGLYCERIDE COMPOSITION OF PALM KERNEL OIL. (Hilditch and William, 1964).

<table>
<thead>
<tr>
<th>Triglyceride</th>
<th>Carbon Number</th>
<th>Weight Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capro-dilaurin</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Capro-lauro-myristin</td>
<td>39</td>
<td>5</td>
</tr>
<tr>
<td>Trilaurin</td>
<td>39</td>
<td>1</td>
</tr>
<tr>
<td>Capro-lauro-palmitin</td>
<td>45</td>
<td>3</td>
</tr>
<tr>
<td>Dilauro-myristin</td>
<td>41</td>
<td>27</td>
</tr>
<tr>
<td>Dilauro-palmitin</td>
<td>43</td>
<td>8</td>
</tr>
<tr>
<td>Lauro-myristo-palmitin</td>
<td>45</td>
<td>7</td>
</tr>
<tr>
<td>Capro-lauro-olein</td>
<td>43</td>
<td>12</td>
</tr>
<tr>
<td>Dilauro-olein</td>
<td>45</td>
<td>6</td>
</tr>
<tr>
<td>Lauro-myristo-olein</td>
<td>47</td>
<td>11</td>
</tr>
<tr>
<td>Lauro-palmito-olein</td>
<td>49</td>
<td>10</td>
</tr>
</tbody>
</table>

Quite unexpectedly, in this study, the extraction rate was found to be relatively constant for much of the extraction process (Figure 4-19). This observation could be the result of an interaction between the three different fatty acid moieties within each of the mixed triglycerides affecting the solubility and there may also be an interaction between the different mixed triglycerides in the palm kernel oil resulting in changes in the solubilities of the oils in CO₂. Research on the effect of carbon chain
length and degree of fatty acid saturation in mixed triglycerides on oil solubility would help to separate the two possible explanations.

4.3.3 CO₂ EXTRACTION OF OIL MIXTURES

Mixtures of cocoa butter and palm kernel oil (weight ratio 75:25, 50:50 and 25:75) were extracted with CO₂ at 55°C and 36 MPa. Like the results obtained with mixture of pure triglycerides, the extraction curves (Figures 4-20, 4-21 and 4-22) indicate that the amount of oil removed per unit mass of CO₂ diminishes gradually with time, in contrast to the linear pattern obtained with pure oils. Again, the results indicate that the solubility of the mixture is changing with time over the course of the extraction.

The extracts from these experiments were also collected at different stages of the extraction for analysis of their fatty acid composition.
Figure 4-20: Extraction curve for the mixture of cocoa butter and palm kernel oil in the weight ratio of 75:25. Extraction conditions: 36 MPa and 55°C.
Figure 4-21: Extraction curve for the mixture of cocoa butter and palm kernel oil in the weight ratio of 50:50. Extraction conditions: 36 MPa and 55°C.
Figure 4-22: Extraction curve for the mixture of cocoa butter and palm kernel oil in the weight ratio of 25:75. Extraction conditions: 36 MPa and 55°C.
TABLE 4-8 FATTY ACID COMPOSITION OF THE CO₂ EXTRACTS OF THE MIXTURE OF COCOA BUTTER AND PALM KERNEL OIL IN THE APPROXIMATE WEIGHT RATIO OF 75:25 (A), 50:50 (B) AND 25:75 (C). EXTRACTION CONDITIONS: 36 MPa AND 55 °C.

(A) Extraction Number (see Figure 4-20)

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Initial Mixture</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₈:₀</td>
<td>0.62</td>
<td>2.38</td>
<td>1.53</td>
<td>0.29</td>
<td>0.18</td>
</tr>
<tr>
<td>C₁₀:₀</td>
<td>0.67</td>
<td>1.98</td>
<td>1.43</td>
<td>0.29</td>
<td>0.20</td>
</tr>
<tr>
<td>C₁₂:₀</td>
<td>12.2</td>
<td>25.2</td>
<td>20.0</td>
<td>5.55</td>
<td>3.92</td>
</tr>
<tr>
<td>C₁₄:₀</td>
<td>5.96</td>
<td>7.88</td>
<td>7.26</td>
<td>2.46</td>
<td>2.09</td>
</tr>
<tr>
<td>C₁₆:₀</td>
<td>20.3</td>
<td>17.7</td>
<td>19.8</td>
<td>24.5</td>
<td>22.8</td>
</tr>
<tr>
<td>C₁₈:₀</td>
<td>24.2</td>
<td>17.9</td>
<td>20.5</td>
<td>29.2</td>
<td>33.1</td>
</tr>
<tr>
<td>C₁₈:₁</td>
<td>30.5</td>
<td>23.2</td>
<td>25.6</td>
<td>33.4</td>
<td>33.7</td>
</tr>
<tr>
<td>C₁₈:₂</td>
<td>4.24</td>
<td>3.01</td>
<td>3.21</td>
<td>3.33</td>
<td>3.14</td>
</tr>
<tr>
<td>C₁₈:₃</td>
<td>0.31</td>
<td>0.22</td>
<td>0.21</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>C₂₀:₀</td>
<td>1.01</td>
<td>0.51</td>
<td>0.58</td>
<td>0.72</td>
<td>0.77</td>
</tr>
</tbody>
</table>

(B) Extraction Number (see Figure 4-21)

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Initial Mixture</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₈:₀</td>
<td>1.71</td>
<td>3.74</td>
<td>2.46</td>
<td>0.51</td>
<td>0.07</td>
</tr>
<tr>
<td>C₁₀:₀</td>
<td>1.55</td>
<td>3.14</td>
<td>2.42</td>
<td>0.60</td>
<td>0.09</td>
</tr>
<tr>
<td>C₁₂:₀</td>
<td>22.4</td>
<td>39.4</td>
<td>35.6</td>
<td>11.6</td>
<td>2.58</td>
</tr>
<tr>
<td>C₁₄:₀</td>
<td>8.28</td>
<td>11.9</td>
<td>12.4</td>
<td>6.28</td>
<td>2.01</td>
</tr>
<tr>
<td>C₁₆:₀</td>
<td>17.8</td>
<td>12.0</td>
<td>13.7</td>
<td>22.3</td>
<td>23.8</td>
</tr>
<tr>
<td>C₁₈:₀</td>
<td>19.3</td>
<td>10.3</td>
<td>11.4</td>
<td>21.6</td>
<td>31.7</td>
</tr>
<tr>
<td>C₁₈:₁</td>
<td>25.4</td>
<td>16.5</td>
<td>19.2</td>
<td>31.9</td>
<td>35.2</td>
</tr>
<tr>
<td>C₁₈:₂</td>
<td>3.37</td>
<td>2.47</td>
<td>2.61</td>
<td>4.34</td>
<td>3.88</td>
</tr>
<tr>
<td>C₁₈:₃</td>
<td>0.14</td>
<td>0.15</td>
<td>0.01</td>
<td>0.17</td>
<td>0.03</td>
</tr>
<tr>
<td>C₂₀:₀</td>
<td>0.67</td>
<td>0.40</td>
<td>0.24</td>
<td>0.55</td>
<td>0.77</td>
</tr>
</tbody>
</table>
From the information displayed in Table 4-8, it is evident that at the early extraction stages the composition contained a high percentage of the lighter fatty acids, such as C10:0, C12:0 and C14:0 which are the major constituents of palm kernel oil. On the other hand, all the later extracts seem to be enriched with heavier fatty acids (carbon number above 14) found in cocoa butter, with the exception of C18:3 and C20:0. This phenomenon implies that some fractionation has taken place during extraction of the cocoa butter and palm kernel oil mixtures which are composed of compounds with widely different solubilities.

### 4.4 EFFECT OF WATER CONTENT ON OIL SOLUBILITIES

Biotechnology is providing opportunities for the mass biological production of many chemical compounds. Since most of these systems operate in an aqueous medium, it would be desirable to be able to extract the products directly from
the liquid medium.

One of the objectives of this research therefore was to examine the effect of sample water content on triglyceride solubility. Cocoa butter/water mixtures in different proportions were extracted with CO$_2$ at 36 MPa, 55 °C and a CO$_2$ flowrate of 1.0 ml/min.

In the first series of extractions using a cocoa/water (90:10) mixture, the solubility data obtained were quite inconsistent and not reproducible. Upon passing through the mixture, CO$_2$ did not appear to be in contact with oil droplets long enough to attain a state of equilibrium. An alternative method was attempted. Two extracting vessels were connected in series and used to extract the oil/water mixture. This arrangement in effect lengthens the extraction bed, and provides a longer oil/CO$_2$ contact time and a more effective mass transfer rate.

The solubility data generated for cocoa butter/water mixtures with up to 50 % water content is shown in Table 4-9.
TABLE 4-9: SOLUBILITY OF COCOA BUTTER IN \textit{CO}_2 AT VARIOUS WATER CONTENTS. (EXTRACTION CONDITIONS: 36 MPa, 55 °C and 1.0 ml \textit{CO}_2/min.)

<table>
<thead>
<tr>
<th>Water Content (Weight %)</th>
<th>Solubility (mg oil/g \textit{CO}_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.3</td>
</tr>
<tr>
<td>10</td>
<td>10.0</td>
</tr>
<tr>
<td>20</td>
<td>9.78</td>
</tr>
<tr>
<td>30</td>
<td>10.3</td>
</tr>
<tr>
<td>40</td>
<td>10.1</td>
</tr>
<tr>
<td>50</td>
<td>9.91</td>
</tr>
</tbody>
</table>

The water content from 0 to 50 % by weight had no appreciable effect on the equilibrium solubilities of cocoa butter in supercritical \textit{CO}_2. The results from experiments using samples with water content higher than this concentration were not reproducible (i.e. a saturation curve could not be obtained) probably due to the poor mass transfer rate between the oil and \textit{CO}_2. Extraction of sample at this very high water content is impossible with this equipment without further modifications.

An extraction curve for 50:50 mass fraction cocoa butter/water mixture at 36 MPa and 55 °C is shown in Figure 4-23. Extraction was carried out for 4.5 hours and about 92% of the oil was recovered from the oil/water mixture at the end of the process.

As long as the oil/\textit{CO}_2 contact time is sufficient, a water content up to 50 % had little effect on the oil
Figure 4-23: Extraction curve for a mixture of 50:50 (wt %) of cocoa butter/water mixture. Extraction conditions: 36 MPa and 55°C.
extractability and the equilibrium solubility of oil in CO₂. The mass transfer rate can be improved by using longer extraction bed or smaller CO₂ flowrate. The optimum moisture content of the sample subjected to extraction should be determined from a feasibility study of the drying process of the sample prior to extraction and the design of the extraction equipment.
The findings of this study can be summarized as follows:

1. The solubility of triglycerides in supercritical CO$_2$ is a direct function of the CO$_2$ density and the extraction temperature. Results confirmed previous studies that solubility did increase with increasing temperature at constant density, and with increasing CO$_2$ density at constant temperature. The extraction of most triglycerides should be carried out at pressure greater than 15 MPa.

2. There exists a linear relationship between the molecular weights of a group of saturated triglycerides and the logarithm of their solubilities in CO$_2$. The solubility of any saturated triglyceride in the same homologous series may therefore be predicted from this relation.

3. For the C18 triglycerides studied, the presence of one double bond on each of the fatty acid chains caused a significant increase in the solubility of the triglyceride in CO$_2$. However, the addition of a second double bond to each of the fatty acid chains of the triglyceride showed no further increase in solubility.

4. In the extraction of the mixture of trimyristin and triolein, significant fractionation occurred during the process; the initial extracts were mainly the lighter trimyristin while the extracts at the later stage...
consist predominantly of the heavier triolein. The mixture did not behave like an ideal solution. It is believed that the interactions between the saturated and unsaturated triglycerides are significant.

5. The equilibrium solubility of palm kernel oil in CO$_2$ was found to be higher than that of cocoa butter, as the palm kernel oil consists mainly of the lighter C12 and C14 triglycerides while cocoa butter is rich in the heavier C18 compounds. No significant change in fatty acid composition of the extracts was observed at various stages of extraction when each oil was extracted separately with SC-CO$_2$. However, when the mixture of these oils was extracted, significant fractionation was observed.

6. The water content of a sample from 0 to 50 % by weight had little effect on the oil extractability and the equilibrium oil solubility in the SC-CO$_2$. 
Chapter 6

RECOMMENDATIONS

The following recommendations are suggested for future research:

1. The effect of double bonds on the fatty acid chains on the extraction process should be studied further using other triglycerides with different carbon numbers and at other temperatures.

2. A study on the supercritical CO₂ extraction of mixed triglycerides will provide useful information on the interaction between the fatty acid chains (with different carbon chain length and degree of unsaturation) of the triglyceride in the supercritical phase.

3. The role of entrainers such as ethanol or acetone in the supercritical CO₂ extraction should be investigated in light of their ability to enhance the oil solubility and fractionation.

4. A feasibility study on the optimum moisture content of an aqueous oil source prior to supercritical fluid extraction should be carried out.
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<th>List of Material Suppliers</th>
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<td>BDH Chemicals</td>
<td>Esterification chemicals</td>
</tr>
<tr>
<td>Chromatographic Specialties Ltd.</td>
<td>1/16&quot; stainless steel tube</td>
</tr>
<tr>
<td>Columbia Valve and Fitting Ltd.</td>
<td>Swagelok fittings, Nupro valve</td>
</tr>
<tr>
<td>Hewlett-Packard Inc.</td>
<td>HPLC, 2µm frits</td>
</tr>
<tr>
<td>Hyseco Fluid Systems Ltd.</td>
<td>Parker MV-200s valve</td>
</tr>
<tr>
<td>Malkin and Pinton Industrial Supplies</td>
<td>stainless steel bolts</td>
</tr>
<tr>
<td>Medigas Pacific Ltd.</td>
<td>carbon dioxide, air, helium and hydrogen</td>
</tr>
<tr>
<td>Rheodyne, Inc.</td>
<td>Rheodyne valve</td>
</tr>
<tr>
<td>Sigma Chemicals Ltd.</td>
<td>triglycerides, GC standards</td>
</tr>
<tr>
<td>Spectrex Limited</td>
<td>0.5 mm glass beads</td>
</tr>
</tbody>
</table>