EFFECTS OF SUBSTRATE COMPOSITION ON CHOLESTEROL SOLUBILITY IN SUPERCRITICAL CARBON DIOXIDE

By

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Abstract

This research work investigated the effects of extraction temperature, pressure, entrainer type and concentration on the equilibrium solubilities of pure cholesterol and egg yolk lipids in supercritical carbon dioxide, and on the selectivity of supercritical carbon dioxide for cholesterol over other lipids present in egg yolk. The effects of one component in a mixture on the solubility of the other component of the mixture in supercritical carbon dioxide was also investigated. The samples tested included pure cholesterol, mixtures of cholesterol and triolein, and freeze-dried egg yolk. The temperature range and the pressure range investigated were 32 - 60 °C and 20 - 36 MPa. Methanol and ethanol were selected as entrainers and the concentration tested varied from 3 to 9% by weight.

The solubilities of all species were found to be functions of extraction temperature, pressure, entrainer type and concentration, and to increase with increasing pressure and entrainer concentration. The effect of temperature on solubilities depended on extraction pressure. The selectivity of supercritical carbon dioxide for cholesterol over other lipids was found to be dependent on extraction pressure, temperature, and entrainer type and concentration. The selectivity increased with an increase in temperature and decreased with increasing pressure over the range of 20 to 25 MPa and then increased as the pressure increased from 25 to 36 MPa. The addition of ethanol as an entrainer resulted in a greater enhancement in lipid solubility in SC CO_2 than the addition of methanol. Both alcohols also reduced the selectivity of SC CO_2 for cholesterol.

The optimum extraction conditions for removing cholesterol from egg yolk was

found to be 36MPa, 50°C and with no alcohol as an entrainer.

The effect of other components present in egg yolk on the solubility of cholesterol was found to depend on extraction pressure. At 20, 25, and 30MPa, the solubility of cholesterol was reduced 20 to 133 % over the temperature range of 32 - 60 °C due to the presence of other yolk lipids. However, the solubility of cholesterol was increased 25 to 72% at 36 MPa over the same temperature range.

The addition of triolein into cholesterol increased the solubility of cholesterol in supercritical CO_2 by a factor of 1.6 to 2.6 fold at 36 MPa and 40°C, however, the solubility of triolein itself was reduced by a factor of 1.8 - 2.2 fold. The total solubility of the mixture did not vary with the mixture composition significantly.

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1. INTRODUCTION

1.1 Supercritical Fluid Extraction (SFE) Process

The supercritical fluid extraction process is a separation technique which utilizes special properties of supercritical fluids - substances heated above their critical temperatures and compressed beyond their critical pressures - to extract some valuable compounds or remove impurities or harmful matter from multicomponent mixtures, such as flavours and aromas from hops, or caffeine from coffee.

The SFE process is based on the fact that the solvation capacity of a solvent is strongly dependent on its density which can be easily varied by changing the pressure or the temperature at which the process is carried out. The SFE process involves two stages: the extraction stage and the separation stage. In the extraction stage, a solvent in its supercritical state is brought into contact with a substrate matrix, dissolves some components of the substrate, and carries them until the solute laden solvent enters into the separation stage. In the separation stage, either the pressure is reduced or the temperature is altered to reduce the solvation capacity of the solvent. The solute is collected and the solvent is expelled or recycled.

The phase diagram of CO_2 (Figure 1.1) is used to depict the special properties of a supercritical fluid. In Figure 1.1, the sublimation line, fusion line and vaporization line divide the whole diagram into three regions representing three phases: solid, liquid, and vapour, respectively. Along the sublimation line the solid and vapour phases are in equilibrium, along the fusion line the solid and liquid phases are in equilibrium, along the

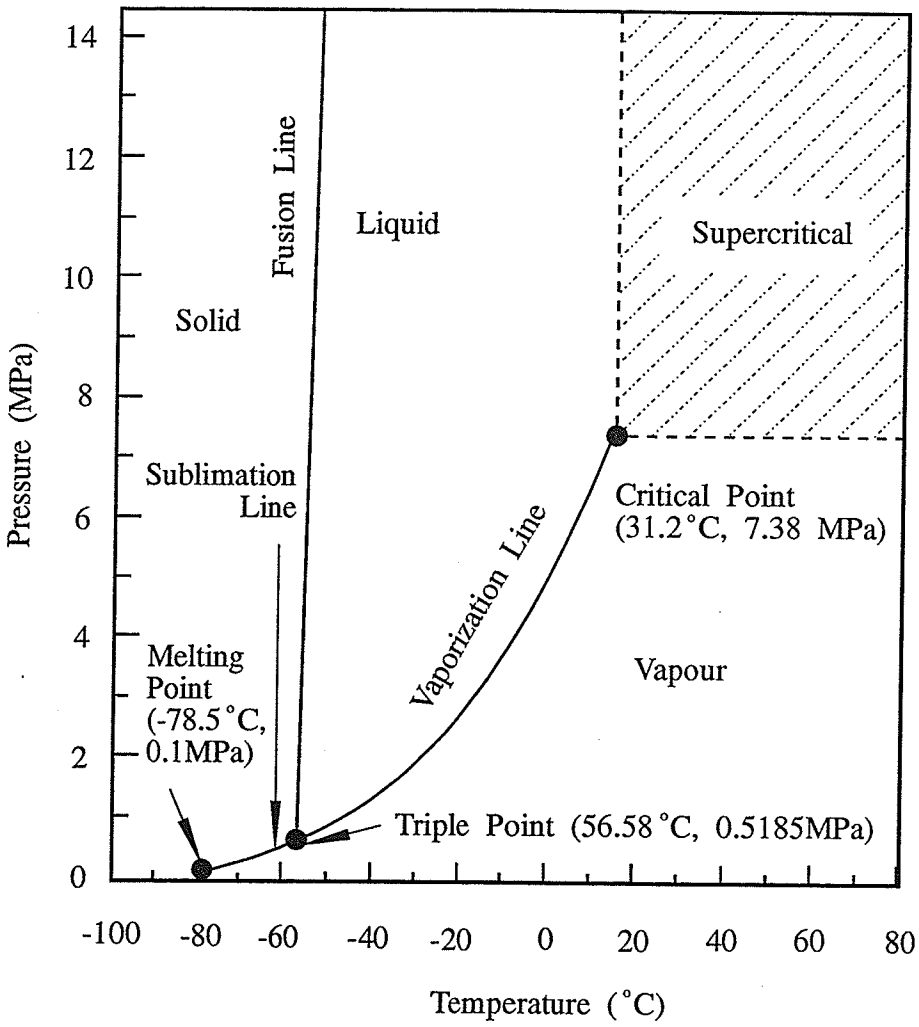

Figure 1.1 Phase diagram for carbon dioxide (according to the data from Angus et al. 1976).

vaporization line the liquid and vapour phases are in equilibrium. At the triple point, where the three lines intersect, all three phases are present in equilibrium. The vaporization line terminates at the critical point. Beyond the critical point, the vapour and liquid phases become identical and only a single phase is present. The single phase is now referred to as a supercritical fluid. Therefore, supercritical CO_2 has some characteristics of not only gaseous CO_2 but also liquid CO_2. When the pressure is increased, the density of supercritical CO_2 increases and approaches the density of liquid CO_2, while the viscosity of supercritical CO_2 still stays close to the viscosity of gaseous CO_2 (Table 1.1). The liquid-like density of supercritical CO_2 means that it has a higher solvation capacity for solutes than gaseous CO_2. The gas-like viscosity of supercritical CO_2 means that its molecular diffusivity is much higher than that of liquid CO_2. The high molecular diffusivity facilitates the mass transfer of solutes into supercritical CO_2. These properties of supercritical CO_2 are dependent on its temperature and pressure. The change in density and viscosity of CO_2 associated with pressure changes at 40 °C is detailed in Figure 1.2.

Table 1.1 Typical values of density, diffusivity and viscosity for gas, liquid and supercritical carbon dioxide (Rizvi et al. 1986).

CO_2 State	Density (g/cm^3)	Diffusivity (cm^2/sec)	Viscosity (g/cm sec)
Gas			
P=1 atm T=15-30 °C	$(0.6\text{-}2)*10^{-3}$	0.1-0.4	$(1\text{-}3)*10^{-4}$
Liquid			
P=1 atm, T=15-30 °C	0.6-1.6	$(0.2\text{-}2)*10^{-5}$	$(0.2\text{-}3)*10^{-2}$
Supercritical			
P=Pc, T=Tc	0.2-0.5	$0.7*10^{-3}$	$(1\text{-}3)*10^{-4}$
P=4Pc, T=Tc	0.4-0.9	$0.2*10^{-3}$	$(3\text{-}9)*10^{-4}$

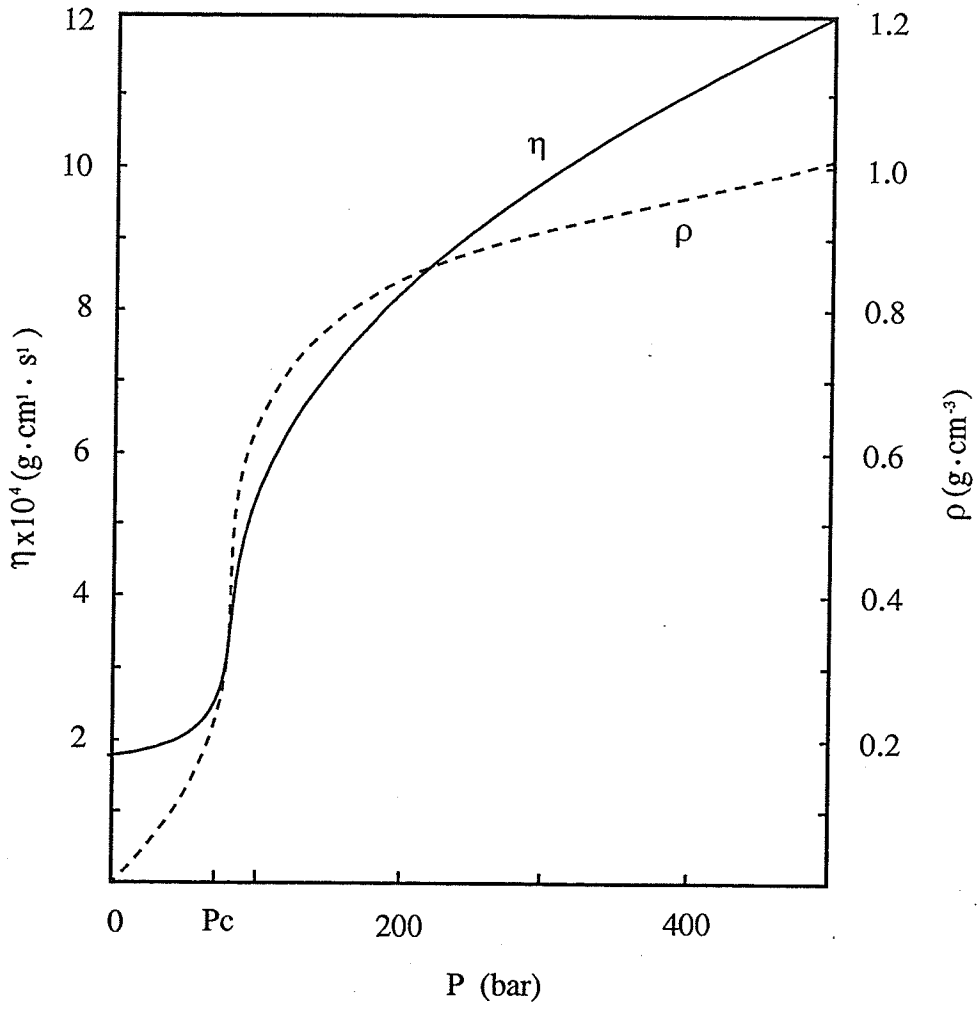

Figure 1.2 Density ρ, viscosity η for pure CO_2 as a function of pressure at 40 °C (reproduced from Schneider 1978).

A good example illustrating the change in solvation power associated with pressure change is the solubility of naphthalene in CO_2. The solubility of naphthalene in CO_2 at 55°C and 6.0 MPa is about 10.5 * 10^{-4} moles/mole CO_2. As the pressure of CO_2 is increased above the critical pressure of CO_2, carbon dioxide changes from gas state to supercritical state. The solubility of naphthalene in supercritical CO_2 rises steeply and approaches 500 * 10^{-4} moles/mole CO_2 when the pressure is at 25.0 MPa (Paul and Wise 1971). This indicates almost a fifty-fold increase in solubility.

1.2 Solvents Used in the SFE Process and Their Properties

Solvents which can be used in supercritical fluid extraction and their properties are listed in Table 1.2 (Ortmanis 1986). Most hydrocarbons have a relatively low critical pressure, but most are toxic and combustible and are rarely chosen as the solvent for a SFE process. Similar problems exist with oxygenated compounds. In addition, many solvents from both classes are liquids under atmospheric conditions, hampering their complete removal from the solute in the separation stage. Most food related SFE applications consequently concentrate on inorganic solvents such as carbon dioxide, nitrogen, ammonia, and water, and of these, carbon dioxide has been especially attractive for use in the food industry for a number of reasons. Carbon dioxide is nontoxic, nonflammable, environmentally acceptable, inexpensive and readily available in large quantities and high purity. The use of carbon dioxide as a supercritical fluid eliminates the need for other potentially harmful solvents.

Table 1.2 Solvents for supercritical fluid extraction and their critical parameters (Ortmanis 1986).

Fluid Type	Name	Critical T (°C)	Critical P (MPa)
Inorganic	Carbon dioxide	304.2	7.38
	Nitrogen	126.2	3.39
	Argon	150.8	4.87
	Nitrous oxide	309.6	7.24
	Ammonia	405.6	11.28
	Water	647.1	22.05
Hydrocarbons	Methane	190.5	4.60
	Ethane	305.4	4.86
	Ethylene	282.3	5.04
	Propane	369.8	4.25
	Pentane	469.7	3.37
	Benzene	562.2	4.90
	Toluene	591.8	4.10
Oxygenated compounds	Methanol	512.6	8.09
	Ethanol	513.9	6.14
	Acetone	508.1	4.70
	Ethyl Acetate	523.2	3.38
	Ethyl Ether	466.7	3.64

1.3 Features of the SFE Process

The supercritical fluid extraction process has the following advantages over conventional solvent extraction methods:

1. Supercritical fluids have a capacity to dissolve compounds which are barely soluble in conventional liquids or gases (Parkinson and Johnson 1989).

2. The extraction can be carried out at relatively low temperatures and used to extract hydrophobic and thermolabile materials with less denaturation.

3. The separation of the dissolved substances from the solvent can be obtained by simply changing pressure or temperature.

4. Supercritical fluid extraction does not need a posttreatment for purification such as the

removal of the solvent residue and yields purer products. (Ikushima et al. 1988; Larson and King 1986; Parkinson and Johnson 1989).

5. The extraction selectivity can be altered by changing the solvent density through various temperature and pressure combinations (Parkinson and Johnson 1989); or by adding an appropriate entrainer into the supercritical fluid.

6. If nontoxic solvents, such as carbon dioxide, are used in the SFE process, there is no harmful residue left in the extract.

The SFE process is, however, not perfect for all separations. It requires high pressures and expensive equipment and is limited to batch processing for solid feedstock. The capital cost of a supercritical fluid extraction plant is at least 50% more than that for a conventional extraction plant (Basta and McQueen 1985). There is still a lack of data on which to base designs of supercritical fluid extraction (Willson 1985). The solubilities of most substances in pure supercritical solvents are generally lower than their solubilities in conventional liquid solvents (Brogle 1982; Stahl and Quirin 1983; Willson 1985).

The low solubility can sometimes be improved through the addition of an entrainer or cosolvent. The addition of some amount of entrainer serves to modify the solvation behaviour of the supercritical fluid and to allow it to be 'tailored' for a specific extraction (Brunner and Peter 1982). Continued research is therefore necessary to establish the proper niche for supercritical fluid extraction among separation technologies.

1.4 Research Objectives and Hypotheses

To assess the feasibility of separating cholesterol from egg yolk with a SFE

process, information related to the following areas is required:

(1) how solvent pressure and temperature affect the total solubility of egg yolk lipids, the solubilities of egg yolk cholesterol and triglycerides in supercritical (SC) CO_2.

(2) how solvent pressure and temperature affect the extraction selectivity of SC CO_2 for cholesterol over triglycerides.

(3) how entrainer concentration and type affect the total solubility of egg yolk lipids, the solubilities of egg yolk cholesterol and triglycerides in SC CO_2.

(4) how entrainer concentration and type alter the extraction selectivity of SC CO_2 for cholesterol over triglycerides and phospholipids.

(5) how the extract composition varies with extraction time when entrained SC CO_2 is used as solvent.

(6) how the presence of a SC CO_2 soluble component affects the solubility of cholesterol.

The research reported here is focused on three aspects. First, the solubility of pure cholesterol in SC CO_2 and alcohol entrained SC CO_2 had to be measured. Second, the total solubility of egg yolk lipids, the solubilities of egg yolk cholesterol and triglycerides in SC CO_2 had to be determined as a function of temperature and pressure. Third, the total solubility of egg yolk lipids, the solubilities of egg yolk cholesterol, triglycerides, and phospholipids in entrained SC CO_2 had to be determined over a given range of entrainer concentration. All these values are important for determining the optimum extraction conditions for separating cholesterol from egg yolk.

The specific objectives of the research were:

(1) to measure the solubility of pure cholesterol in SC CO_2 and in alcohol entrained SC

CO_2.

(2) to investigate the effect of composition of binary mixtures (cholesterol and triolein) on component solubilities and the effect of one compound present in the mixture on the solubility of the other compound.

(3) to investigate the effect of other components present in egg yolk powder on the solubility of cholesterol in SC CO_2.

(4) to investigate the variation with temperature and pressure of the extraction selectivity of SC CO_2 for cholesterol over triglycerides.

(5) to investigate how entrainer concentration and type affect the total solubility of egg yolk lipids, the solubilities of cholesterol, triglycerides and phospholipids in mixed SC CO_2.

(6) to investigate how entrainer type and concentration alter the extraction selectivity of SC CO_2 for cholesterol over other lipids in egg yolk.

The research was carried out in four stages. In the first stage, the solubility of pure cholesterol in SC CO_2 and SC CO_2-ethanol mixtures was measured in the temperature range of 32 - 60 °C and the pressure range of 20 - 36 MPa. In the second stage, the extraction of the mixture of cholesterol and triolein was carried out at 40°C and 36 MPa. In the third stage, the extraction of freeze-dried egg yolk was performed with supercritical CO_2 in the temperature range of 32 - 60 °C and pressure range of 20 - 36 MPa, and the extracts were collected for further analysis. In the final stage, the extraction of freeze-dried egg yolk was carried out at 40°C and 36 MPa with alcohol entrained SC CO_2. The entrainer concentration ranged from 3 to 10% by weight.

The research was directed at testing the following hypotheses:

(1) the solubility of pure cholesterol in SC CO_2 increases with extraction pressure, temperature, and entrainer concentration.

(2) for cholesterol and triolein mixtures, the solubility of each component in SC CO_2 is enhanced due to the presence of the other component in the mixtures.

(3) the solubility of cholesterol in SC CO_2 increases with an increase in triolein content in the mixtures.

(4) the solubility of a mixture of cholesterol and triolein can be predicted if the composition of the mixture is known.

(5) the overall solubility of egg yolk lipids in SC CO_2 increases as the pressure increases and decreases as the extraction temperature increases; the solubilities of egg yolk cholesterol and triglycerides increase as the extraction pressure increases and decreases when the extraction temperature increases.

(6) the solubility of cholesterol in SC CO_2 is reduced due to the presence of other components in egg yolk.

(7) the extraction selectivity of SC CO_2 for cholesterol over triglycerides is independent of pressure or temperature.

(8) the overall solubility of egg yolk lipids and the solubilities of egg yolk cholesterol, triglycerides, and phospholipids in SC CO_2 and entrainer mixture increase when entrainer concentration increases.

(9) the extraction selectivity of alcohol entrained SC CO_2 for cholesterol over triglycerides and phospholipids is affected by entrainer type and concentration.

2. LITERATURE REVIEW

2.1 Development of the SFE Process

The phenomenon of supercritical fluid extraction was first observed by Hannay and Hogarth over one hundred years ago when potassium iodide was dissolved in supercritical ethanol and was precipitated on reducing the pressure (as cited in Rizvi et al 1986; Balaban 1989). Buchner (1906) subsequently reported that the solubility of organic materials with low volatility in supercritical carbon dioxide were orders of magnitude higher than in carbon dioxide vapour. However, the SFE process had not found commercial applications before the 1940s probably due to the difficulty of understanding the supercritical phenomenon, the cheap organic solvents for conventional extraction methods and the high cost of high pressure equipment for the SFE process at that time.

The first proposal for the practical application of the SFE process was made to deasphalting petroleum oil in 1943 (Williams 1981). In the 1950's the Residuum Oil Supercritical Extraction process was developed for the removal of lighter products from the residue of commercial distillation of crude oil (as cited in Basta 1984).

In the meantime, the use of supercritical fluids including carbon dioxide for extracting compounds of high molecular weight was suggested in some patents. The same principle was recommended for extracting fats and oils, yet practical applications were not described (Stahl et al. 1980).

In 1962, Zosel began his study of supercritical gas extraction at the Max Planck Institute as a result of investigating a complaint from a licensee about the quality of the

product of an organic synthesis. His procedure led to the removal of α-olefin contaminants from the reaction product by dissolving them in supercritical ethylene. From that time on, the idea of extracting lipids and other natural products with liquid or supercritical CO_2 has gained new impetus (Williams 1981).

Paul and Wise (1971) published the first comprehensive review of the principles of gas extraction, in which they discussed the physical basis in relation to distillation and extraction and suggested possible areas of application. The intensive research on the use of the SFE process in the food industry started at about that time. Many patents have been granted on the supercritical fluid extractions of nicotine from tobacco, recovery of coffee oil from roasted coffee, decaffeination of crude coffee, deodorization of plant oil, and extraction of spices and hops (Roselius et al. 1972a, b; 1973a, b; Vitzthum and Hubert 1972, 1973, 1976; Vitzthum et al. 1975, 1976; Zosel 1971, 1972, 1974, 1975).

Irani and Funk (1977) made a further review describing the advances made in thermodynamic analysis, experimental data and process identification and developments in the intervening six years. In the same year, the 6th AIRAPT International High Pressure Conference was held in Boulder, Colorado, including three sessions on fluid phase equilibria and separation processes at high pressure.

In 1978, the first symposium devoted entirely to extraction with supercritical fluids was held in Germany. At the symposium, the pioneer researchers from Germany presented papers on different aspects of supercritical fluid extraction such as the general principles of supercritical fluid extraction (Zosel 1978; Hubert and Vitzthum 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 extraction plant (Eggers 1978).

In 1979, the first large scale production plant, which uses SFE to remove caffeine from green coffee beans, was built by HAG A.G. (West Germany) and was put into use. The caffeine content in beans can be decreased from the initial range of 0.7% - 3% to a content as low as 0.02% by the process.

In the 1980's, the SFE process was being used extensively to extract vegetable oils from oil seeds such as soybean, sunflower seed, canola, and rapeseed (Stahl et al. 1980; Bulley et al. 1984; Friedrich 1984; Eggers et al. 1985; List and Friedrich 1985; Yamaguchi et al. 1986; Fattori et al. 1988; Eggers and Sievers 1989; Temelli 1992), to extract pigments from paprika, annatto seed, and leaf protein concentrates (Haeffner and Coenen 1986; Manabe et al. 1987; Chao et al. 1991b; Degnan et al. 1991; Favati et al. 1988); to concentrate natural flavour (Vidal et al. 1989); to remove cholesterol from egg yolk, beef, fish oil, and dairy products (Froning et al. 1990; Yun et al. 1991; Chrastil 1982; Wong and Johnston 1986; Pasin et al. 1991; Chao et al. 1991a; Shishkura et al. 1986, Bulley and Labay 1991); and to purify fatty acids and vitamins (Ikushima et al. 1989; Artz and Sauer 1992).

In 1991, the second international symposium on supercritical fluids was held in Boston, Massacheusetts. A wide variety of topics on the applications and fundamentals of supercritical fluids were discussed. New applications of supercritical fluids which were not presented at the first symposium appeared as well. Twenty-four percent of the authors were from industry indicating their increasing interest in the developing technology. Part

of the motivation to develop this method is due to increased governmental regulation of common industrial solvents, stricter pollution control, and increased performance demands on food products.

One of the recent active areas of research is the supercritical CO_2 extraction of cholesterol and other lipids from egg yolk, dairy products, and other foods of animal origin.

2.2 Effect of Extraction Conditions

2.2.1 Effect of Extraction Pressure and Temperature on Solubility and Selectivity

The solubility of peanut oil and rapeseed oil in SC CO_2 as a function of pressure for different temperatures is shown in Figure 2.1 (Goodrum and Kilgo 1989). Figure 2.2 shows the changes of oil solubility in supercritical CO_2 and N_2O with pressure at three temperatures. For a fixed temperature, the oil solubility increases with an increase in pressure. The rate of increase is greater at higher temperature. This has also been reported by other researchers (Mangold 1983; Christianson et al. 1984; Friedrich and Pryde 1984; Friedrich et al. 1982; Johnston and Eckert 1981; Stahl et al. 1980; Taniguchi et al. 1985; Chimowitz and Pennisi 1986; Eggers et al. 1985). However, the selection of the operating pressure range is still limited by two main factors: the critical pressure of the solvent and the cost of high pressure equipment. The most common pressure range reported in the literature is from 10 to 40 MPa.

The effect of temperature on solubility is more difficult to predict due to two competing factors existing in the extraction system. On one hand, as temperature

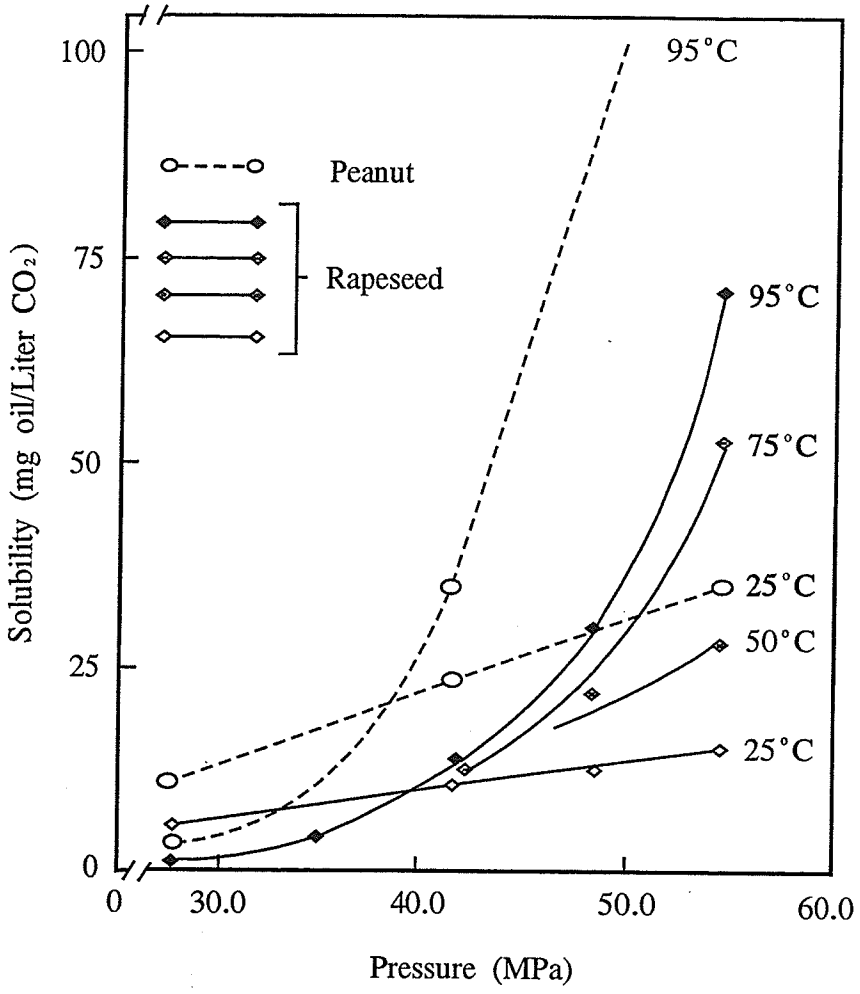

Figure 2.1 Solubility isotherms for peanut oil and rapeseed oil as a function of pressure. Extraction conditions: particle size 0.97mm; moisture 6%; flow rate of CO_2 40 L/min (reproduced from Goodrum and Kilgo 1989).

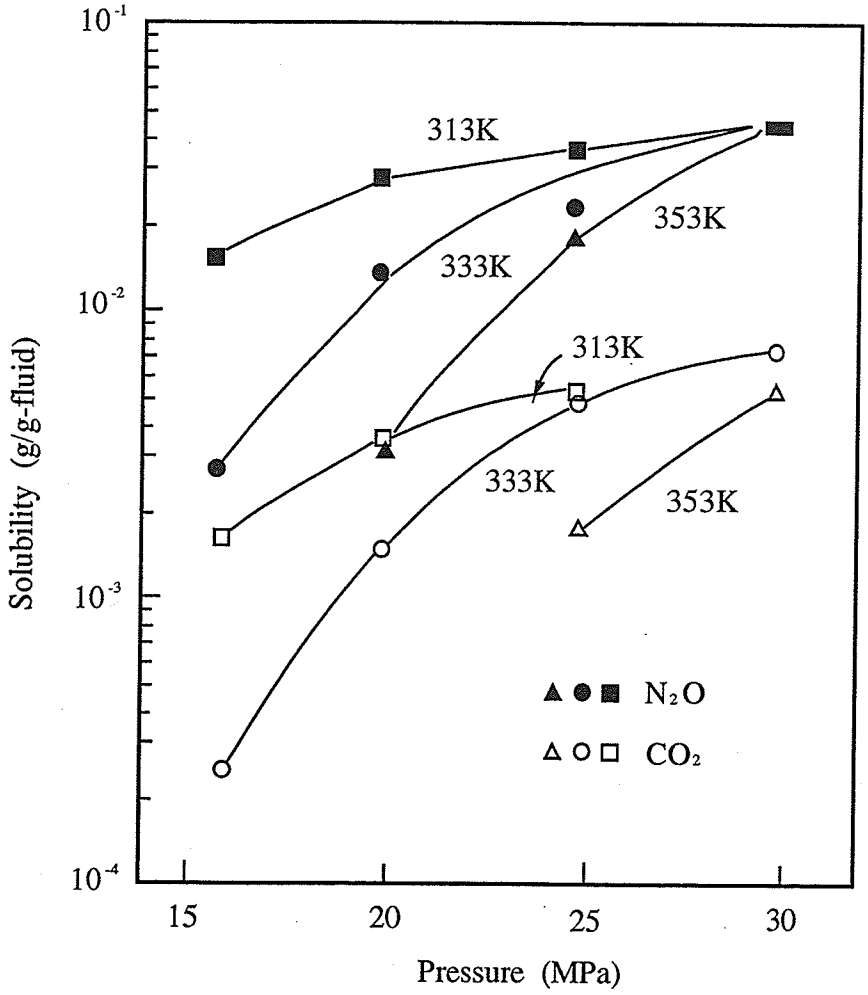

Figure 2.2 Effect of pressure on oil solubility in supercritical CO_2 and N_2O at 313, 333, and 353°K (reproduced from Sakaki et al. 1990).

increases, the vapour pressure of solute increases which tends to increase the solubility. On the other hand, CO_2 density decreases with increasing temperature which tends to decrease the solubility (Nilsson et al. 1988). When the former predominates, the solubility increases with the increase in temperature. When the latter predominates, the solubility decreases with the increase in temperature. When the effects caused by the two factors are in balance, the solubility in supercritical fluid stays constant. These phenomena can be seen from Figure 2.3 and Figure 2.4 and have been reported by Temelli et al. (1988), Robey and Sunder (1984), and Taniguchi et al. (1985).

Although solubility is related to the extraction efficiency, the selectivity of a solvent for a component of interest in a multicomponent mixture is more significant than the solubility. The selectivity of a solvent for a component in a multicomponent mixture reflects the separation possibility of the component from the other components in the mixture by the SFE process. The effects of temperature and pressure on the selectivity of a solvent have been reported. Dobbs and Johnston (1987) stated that the selectivity of pure CO_2 for a component in a mixture of organic solids is related primarily to the solute vapour pressures and only secondarily to intermolecular forces in the supercritical phase. In their 2-aminobenzoic acid-anthracene-CO_2 system, the selectivity of CO_2 for 2 aminobenzoic acid over anthracene at 35°C was insensitive to pressure over a range of 12 - 35 MPa with or without co-solvent present. Kosal et al. (1992) also reported that in their physical mixture systems of progesterone and testosterone, testosterone and cholesterol, and progesterone and cholesterol, the selectivities did not change with pressure at high temperatures. On the other hand, Temelli et al. (1988) has shown that the

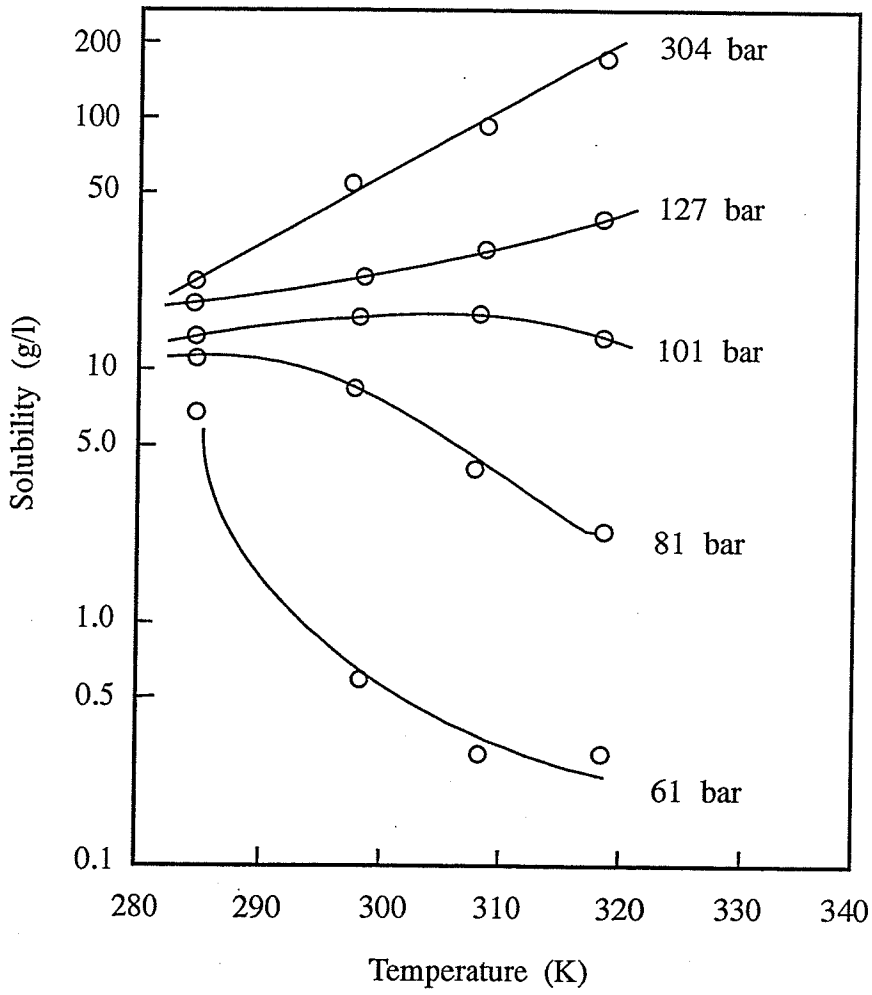

Figure 2.3 Solubility of naphthalene in compressed ethylene as a function of temperature at various pressures (reproduced from Peter and Brunner 1978).

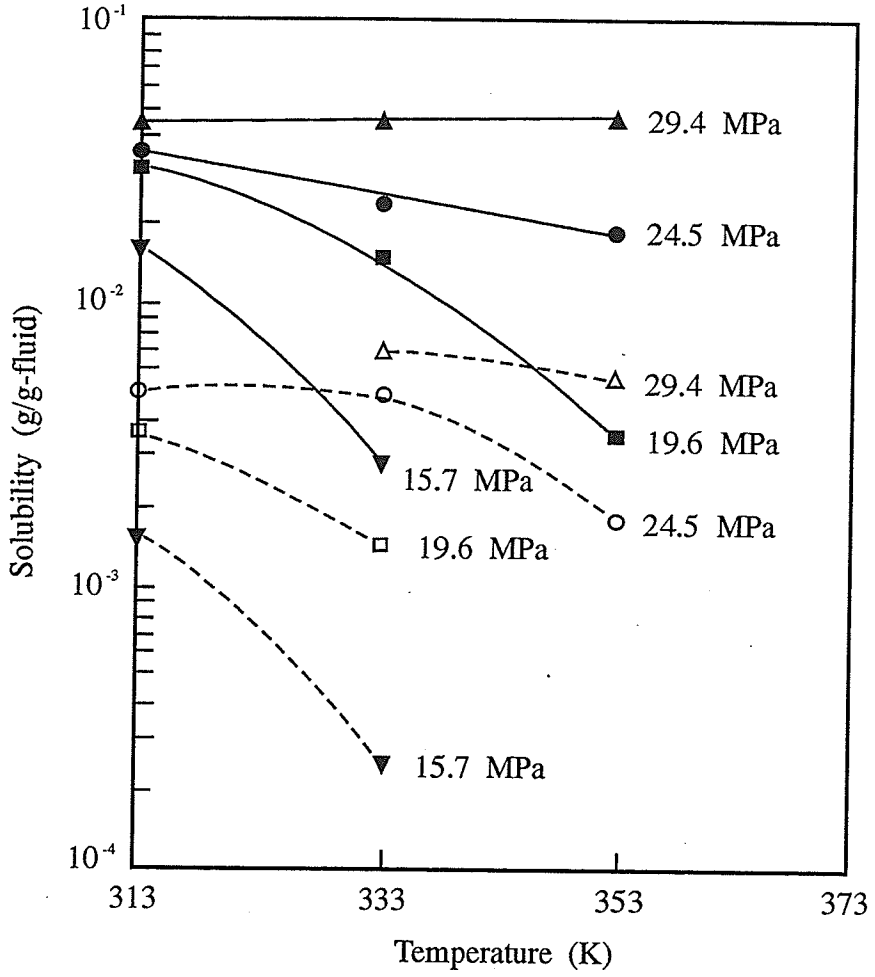

Figure 2.4 Effect of temperature on oil solubility in supercritical CO_2 and N_2O at 15.7, 19.6, 24.5, and 29.4 MPa. The dashed curves are for CO_2 and the solid curves are for N_2O (reproduced from Sakaki et al. 1990).

selectivity of supercritical CO_2 for a component in a natural synthetic mixture can be altered with pressure and temperature. In the citrus oil processing by the SFE process, they observed that the extract composition changed with pressure at different temperatures, as seen in Figure 2.5. The weight percent of oxygenates decreased as the temperature was increased. However, this decrease was not statistically significant for temperatures of 40, 50, and 60°C at pressures of 8.3, 9.7 and 11.0 MPa. The weight percent of oxygenated compounds in the extract was significantly lower at 70°C than that at other temperatures. At constant temperature, the percentage of oxygenates reached a minimum at 9.7 MPa, but this minimum was not significantly lower than those at 8.3 and 11.0 MPa. A pressure of 12.4 MPa gave the highest weight percent of oxygenates which was statistically significant at all temperatures. As a result, the conditions that gave the lowest amount of oxygenated compounds was 70°C and 9.7 MPa. Since the purpose of the process is to remove the terpene hydrocarbons from citrus oil and leave oxygenated compounds behind, 70 °C and 9.7 MPa represent the optimum operating conditions for citrus oil processing.

2.2.2 Effect of Entrainer on Solubility and Selectivity

Several studies have demonstrated that the solubility of compounds of interest in SC CO_2 can be greatly enhanced and the selectivity of SC CO_2 for compounds in a mixture can be changed by the addition of an entrainer (Dobbs et al. 1987, Dobbs and Johnston 1987; Larson and King 1986; Brunner and Peter 1982; Schaeffer et al. 1988; Wong and Johnston 1986; Li and Hartland 1991; Ikushima et al. 1985; Hardardottir and

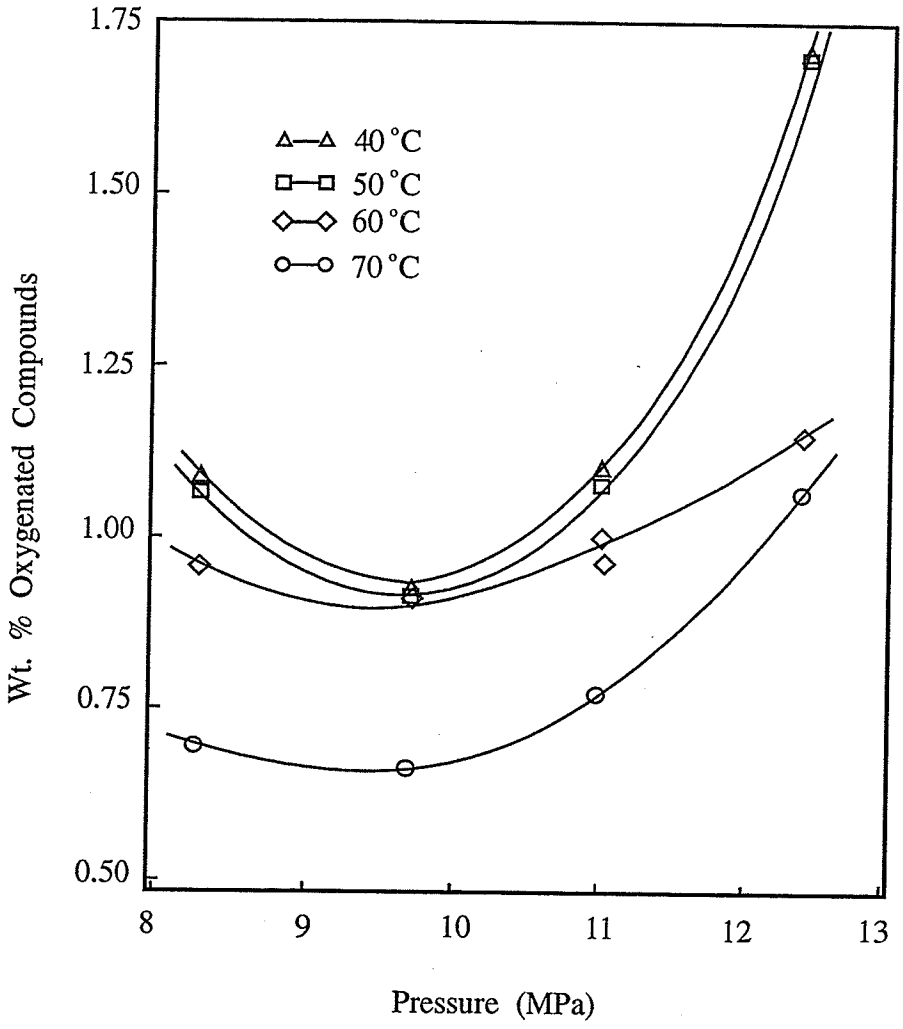

Figure 2.5 Weight percent of oxygenated compounds in the extract as a function of pressure at different temperatures (reproduced from Timelli et al. 1988).

Kinsella 1988; McNally and Wheeler 1988; Cygnarowicz-Provost et al. 1991, Kim et al. 1985, and Bulley and Labay, 1991).

2.2.2.1 Effect of Entrainer on Solubility

Larson and King (1986) reported that the solubility of mevinolin increased from 0.04 wt% for the pure supercritical CO_2 to 0.45 wt% for the supercritical CO_2 mixed with 5 wt% methanol at 38 MPa and 40°C. The steroid, L-636-028, which had a solubility of about 0.59 wt% in pure CO_2, exhibited a solubility of 5.9 wt% when dissolved in a CO_2 mixture with 3% methanol. These results illustrate how the use of entrainers can greatly improve the solvation capability of SC CO_2 for certain compounds.

The solubility enhancement induced by the addition of an entrainer into SC CO_2 is due to the increase in the density of the solvent mixture and certain specific chemical interactions between solute and entrainer, such as hydrogen bonding and Lewis acid-base interactions (Dobbs et al. 1987; Dobbs and Johnston 1987; VanAlsten et al. 1984; and Schmitt and Reid 1986). The entrainer type, concentration and the property of the solute of interest are the determinants to the enhancement magnitude of solubility. Figure 2.6 shows 2-aminobenzoic acid solubility enhancement induced by the addition of methanol or acetone with 3.5 mol% into SC CO_2. Methanol induced a higher solubility enhancement for 2-aminobenzoic acid than acetone. This result can be explained qualitatively by using the dispersion and acid-base solubility parameters of methanol, acetone and 2-aminobenzoic acid. Methanol and acetone have almost equivalent dispersion solubility parameters ($\delta^D = 7.2$ $(cal/cm^3)^{1/2}$ for acetone, $\delta^D = 6.8$ $(cal/cm^3)^{1/2}$ for

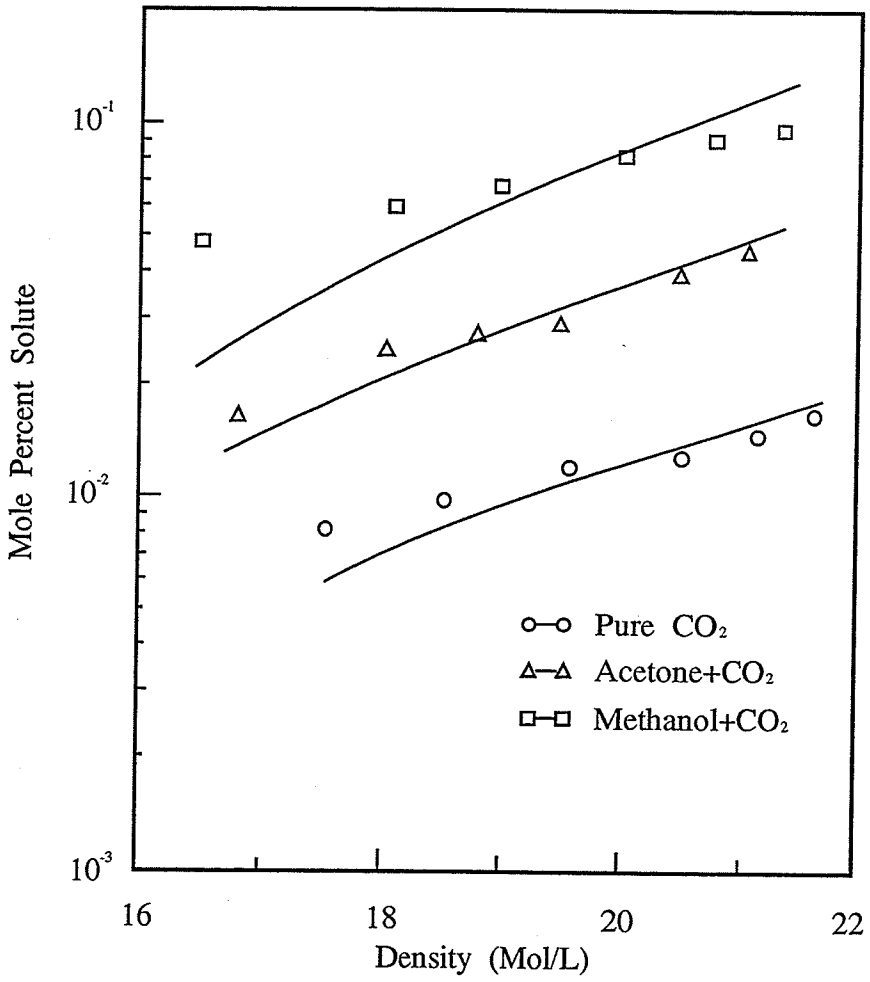

Figure 2.6 Solubility of 2-aminobenzoic acid in carbon dioxide with 3.5 mol% cosolvent at 35 °C (reproduced from Dobbs et al. 1987).

methanol), but methanol has significant acidity (δ^A = 8.3 (cal/cm^3) $^{1/2}$) as well as basicity (δ^B = 8.3 (cal/cm^3) $^{1/2}$), acetone is less basic (δ^B = 3.0 (cal/cm^3) $^{1/2}$) than methanol and is not acidic (δ^A = 0). 2-aminobenzoic acid can form acid-base complexes with methanol and only has weak acid-base interaction with acetone.

Figure 2.7 shows the effect of entrainer (n-octane) concentration on the solubility of phenanthrene in SC CO_2. The solubility of phenanthrene increases with an increase in n-octane concentration. The concentration of entrainer can not be unlimitedly increased to enhance the solubility of a solute. Dobbs et al. (1987) observed that when methanol concentration exceeded 9 mol%, a liquid phase occurred in the view cell.

McNally and Wheeler (1988) investigated the effects of entrainer type and concentration on the extraction efficiency of diuron, linuron and 3,4-dichloroaniline from Sassafras oil, where methanol, ethanol and acetonitrile were examined as entrainers. The general trend of greater extraction efficiency with higher entrainer concentration was observed. Methanol and acetonitrile induced the same solubility enhancement for diuron in SC CO_2. The solubility of linuron was enhanced more by ethanol than methanol. The authors suggested that the polarity of the mixture must be optimized to match the polarity of the solute.

2.2.2.2 Effect of Entrainer on Selectivity

A little work has been reported on the effect of entrainer on the selectivity of a solvent for a component. Dobbs et al. (1987) reported that the addition of only 3.5 mol% methanol in SC CO_2 increased the solubility of 2-amino benzoic acid by 620%, benzoic

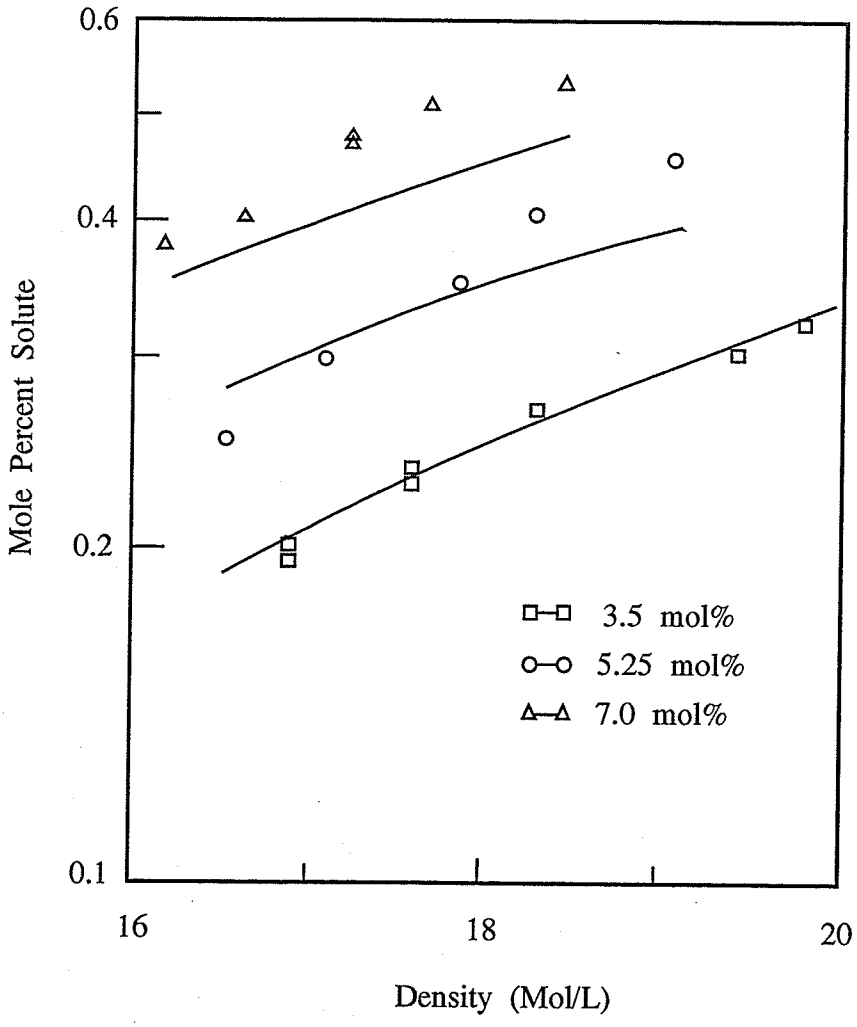

Figure 2.7 Solubility of phenanthrene in carbon dioxide with n-octane cosolvent at 35 °C (reproduced from Dobbs et al. 1986).

acid by 270%, 2-naphthol by 35%, hexamethyl benzene by 10% at 35°C and the CO_2 density of 20.5 mol/L. This result demonstrates that the addition of a small amount of methanol to supercritical CO_2 could change the selectivity of supercritical CO_2 for components in the sample mixture based on chemical functionality of compounds of similar volatility.

In another paper Dobbs and Johnston (1987) reported that the selectivity for 2-aminobenzoic acid over anthracene was only 2.0 at 350 bar. However, the addition of only 3.5 mol% methanol into SC CO_2 increased the selectivity from 2.0 to 10 at 120 bar. The selectivity change caused by the addition of an entrainer is due to polar forces, hydrogen bonding, or other specific chemical forces (Dobbs and Johnston 1987).

2.3 Supercritical Fluid Extraction of Cholesterol from Various Natural Products

Shishikura et al. (1986) attempted to modify butter oil by lowering its cholesterol level and improving its spreadability using SC CO_2 extraction.

First, the authors examined the effects of pressure, temperature and pre-treatments of butter on the extraction efficiency for butter oils and cholesterol concentrations of extracted oils. In the pressure range of 13 to 30 MPa at 40°C, the solubility of dehydrated butter oil increased linearly with an increase in pressure. The solubility of "as is" butter oil increased with the increase in pressure over the range of 13 to 20 MPa in a manner similar to that for dehydrated butter oil, but for pressures over 20 MPa, the solubility of butter oil decreased with an increase in pressure. No explanation was given for this particular phenomenon.

The cholesterol concentration of the extracted oil from both dehydrated and "as is" butter oil at various pressures and 40°C increased with increasing pressure and reached the maximum at 15 MPa; and then decreased with continued increase in pressure. This result indicates that the selectivity for cholesterol was changing with pressure at constant temperature.

The cholesterol concentrations of the extracted oils from "as is" butter were always higher than those from dehydrated butter oil throughout the pressure range examined. The authors suggested that the moisture in "as is" butter oil improves the selectivity for cholesterol by functioning as an entrainer. From the above experimental results, they concluded that the optimum extraction conditions for the highest selectivity for cholesterol over triglycerides was the extraction of "as is" butter at 15 MPa and 40°C.

In another set of experiments, the authors performed the extraction of "as is" butter at 15 MPa and 40°C. They found that the cholesterol concentration of the residual oil could be reduced to the level of 0.028%, but that the residual oil would no longer "melt in the mouth" and was almost flavourless. It may be possible to reduce cholesterol to a less extent and still retain the special "melt in the mouth" properties, but such experiments were not reported.

To further separate the cholesterol from the butter oil extracts, the authors applied a column of silica gel in the extractor. The cholesterol level of the extracted oil was reduced to 0.073%, and the flavour of the extracted oil was excellent and almost equal to that of the original butter oil.

Hardardottir and Kinsella (1988) explored the removal of lipids and cholesterol

from fish muscle using SC CO_2 and ethanol entrained SC CO_2 over the pressure range of 2000 to 5000 psig (13.8 to 34.5 MPa) at the temperatures of 40°C and 50°C. Their work was focused on investigating the effect of extraction pressure, temperature, time, and sample size on the extent of lipid removal. In the extractions with SC CO_2, a total of 3900 g of CO_2 was pumped through 5 g of sample at a flow rate of 460 ml/h. In the extractions with SC CO_2/ethanol, a total of 2600 g of CO_2 and 280 g of ethanol were passed through 10 g of sample at a flow rate of 460 ml CO_2 + 60 ml ethanol/h. They observed that extraction pressure had little effect on the total amount of lipid removed from fish muscle in the pressure range examined. The extraction at high temperature (50°C) removed slightly more lipids from the trout muscle than the extraction at low temperature (40°C). Increasing the extraction time from 3 h to 9 h increased the lipid removal from the muscle, but the increased magnitude of lipid removal was not mentioned. The addition of ethanol as an entrainer (10% by weight) increased the total lipid removal from the fish muscle from 78% to 97%, and cholesterol removal from 97% to 99% at all the pressures used.

Froning et al. (1990) performed the SC CO_2 extraction of spray dried egg yolk under the four different combinations of pressure and temperature, 163atm/40°C (16MPa/40°C), 238atm/45°C (23.5MPa/45°C), 306atm/45°C (30.2MPa/45°C), and 374atm/55°C (36.9MPa/55°C) to investigate the effects of SC CO_2 extraction on egg yolk composition and functionality. The flow rate of CO_2 was between 5 to 10 standard L/min. A total of 45±1 g CO_2 for each gram of sample was passed through the extractor. The composition of the residual egg yolk and the cholesterol content at each extraction

condition are shown in Figures 2.8 and 2.9. Total lipid content and cholesterol content were significantly reduced with increasing extraction temperature and pressure as compared to the control. At the highest temperature and pressure, 36 percent of the total lipids and approximately two-thirds of the cholesterol were removed.

The authors examined the effects of SC CO_2 extraction on egg yolk functionality in terms of emulsion stability of mayonnaise, sponge cake volume, and Hunterlab colour values. Emulsion stability of mayonnaise was significantly affected only at 374atm/55°C (36.9MPa/55°C). Although the emulsion stability of yolk extracted at 306 atm/45°C (30.2MPa/45°C) appeared to be poorer than that of the control, the differences were not significant. These results generally indicate that supercritical CO_2 extraction can be utilized to remove cholesterol from spray dried egg yolk without substantially impairing the stability of mayonnaise.

When compared to the control, sponge cake volume was significantly improved at all pressures and temperatures except for the treatment 374atm/55°C (36.9MPa/55°C). The increased protein concentration of egg yolk at the higher extraction temperature and pressure conditions likely played a role in the improved functional properties.

Hunterlab L values significantly increased and a_L and b_L decreased when extraction temperature and pressure were increased. Thus, extraction at higher temperatures and pressures produced a lighter colour dried yolk with less redness and yellows. The removal of some of the xanthophyll pigments from the dried egg yolk would account for the colour change.

From this study, it appears that the best combination of temperature and pressure

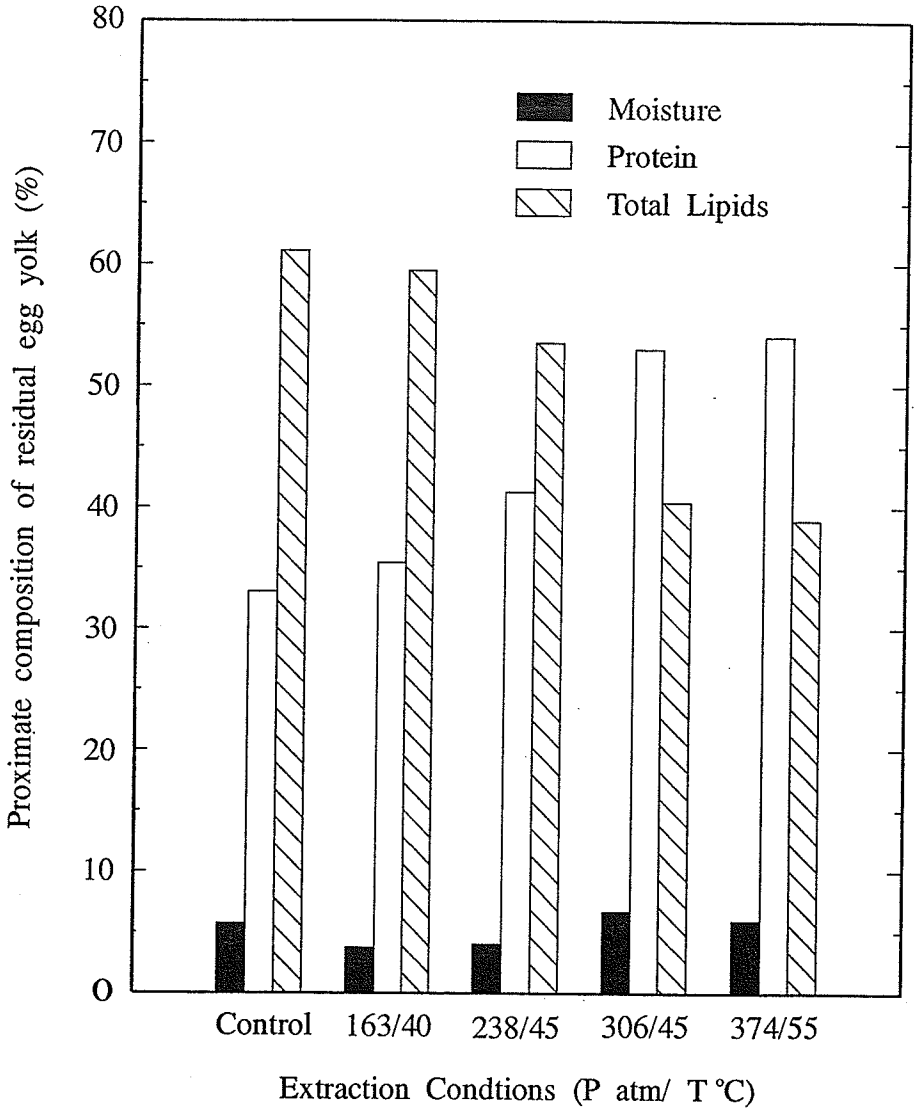

Figure 2.8 Effect of supercritical carbon dioxide extraction of spray-dried egg york at various temperatures and pressures on proximate composition (according to the data from Fronning et al. 1990).

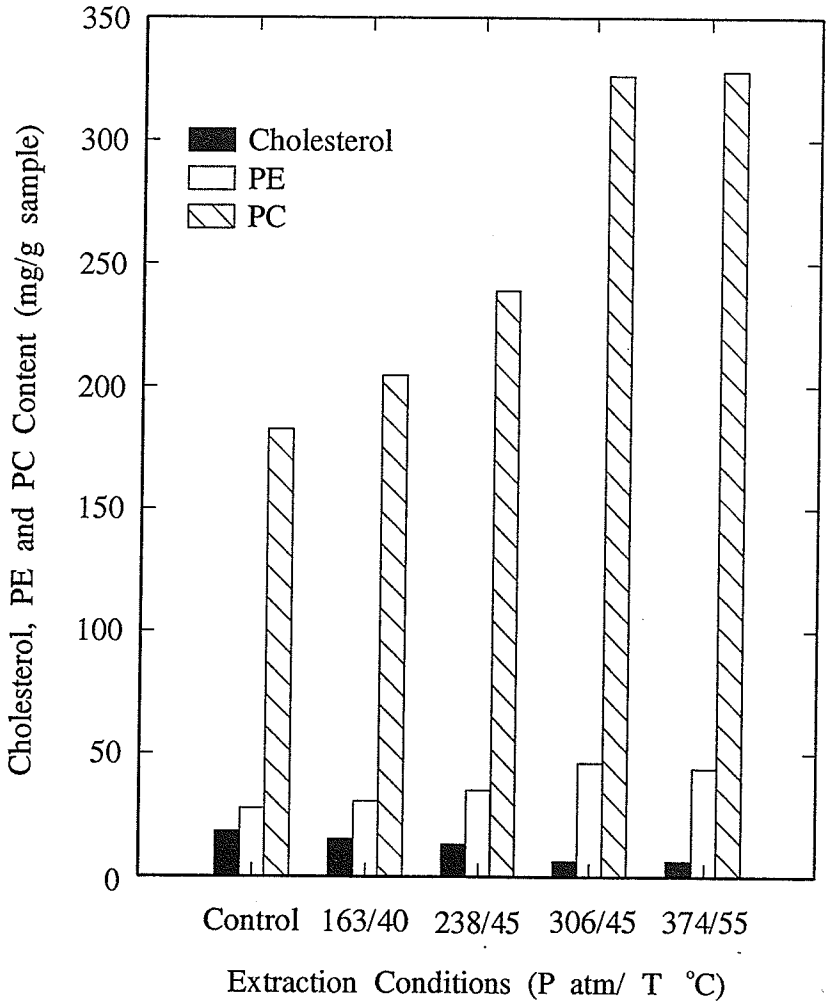

Figure 2.9 Effect of supercritical carbon dioxide extraction of spray-dried egg york at various temperatures and pressures on cholesterol and phospholipid content, PE: phosphatidylethanolamine; PC: phosphatidylcholine (according to the data from Fronning et al. 1990).

for extraction efficiency and product functional quality is 306atm/45°C (30.2MPa/45°C).

Pasin et al. (1991) also reported on the removal of cholesterol from liquid egg yolk with SC CO_2 and entrainer-mixed SC CO_2. Their liquid egg yolk samples were separated from egg white, freeze-dried, and then sieved through a 14-mesh screen, and finally reconstituted to 50% moisture with deionized water. The authors presented equilibrium cholesterol solubility data in pure and entrained CO_2 over the temperature range of 23 - 44 °C and the pressure range of 1500 - 4500 psig (10.3 - 31.0 MPa). The equipment used to measure the solubility is a modified Supercritical Screening Unit, which consists of a 300 ml extractor. Liquid egg yolk sample (about 100 g) was placed in the extractor, and the extractor sealed and purged with carbon dioxide to remove air. The extractor was held at the desired pressure and temperature without flow while the contents were stirred with a magnetic mixer at 20 rpm for two minutes and then allowed to settle for 45 minutes. A multiport valve was used to obtain a 10 microliter sample of the supercritical phase and introduce it directly into a supercritical fluid chromatograph. Cholesterol concentration was then determined with a 25.0 cm * 4.6 mm ID Supelcosil LC-CN column (Supelco) and UV detector, based on an external standard. The supercritical phase was sampled three times for each solubility measurement. The solubility of liquid egg yolk cholesterol in SC CO_2 increased notably at 2500 psig (17.2 MPa), and slightly more at pressures above 3500 psig (24.1 MPa). Cholesterol solubility was higher at 40°C than that at subcritical temperatures. The addition of ethanol (5% w/w) as a cosolvent increased cholesterol solubility by a factor of two. However, the addition of methanol (5% w/w) increased cholesterol solubility by a factor of 10 to 100

over the pressure range and temperature range examined. The batch extraction of liquid egg yolk with SC CO_2 and entrainer-mixed SC CO_2 was also carried out. The samples of feed and residue from batch extractions were analyzed for cholesterol content using an enzymatic method developed by the University of California and by gas chromatography. The extraction with SC CO_2 removed up to 18% of the cholesterol content, however the use of ethanol (1% w/w) as a cosolvent in batch extractions increased cholesterol removal to as high as 46%.

Lim et al. (1991) reported on the continuous SC CO_2 processing of milk fat. They investigated the effect of changes in extraction temperature and pressure, as well as the effect of three processing parameters: recycle, reflux, and temperature gradient in the extraction column on the cholesterol content variation of extract and raffinate. The results indicate that it is impossible to reach the desired 90% cholesterol reduction in either extracted milk fat or raffinate by changing extraction conditions or using either strategy of recycling or refluxing. In order to enhance the cholesterol reduction of the extracted milk fat, the authors added two adsorption columns filled with magnesium silicate in the line, similar to the method employed by Shishikura et al. (1986), and reduced the cholesterol content of raffinate by 88%.

In another paper (Bulley and Labay 1991), the extraction or fractionation of egg yolk using SC CO_2 and alcohol entrainers was reported. They carried out the extractions of freeze-dried egg yolk over the pressure range of 15 to 36 MPa at 40°C and over the temperature range of 40 to 75°C at 36 MPa. The overall solubility of lipids was strongly dependent on extraction pressure. When extraction pressure increased from 15 to 36 MPa

at 40°C, the solubility of lipids also increased from 0.97 mg/g CO_2 to 10.01 mg/g CO_2. The solubility of lipids decreased from 10.01 mg/g to 6.2 mg/g when temperature was increased from 40 to 75°C at 36 MPa. They also performed extractions of freeze-dried egg yolk using methanol or ethanol as entrainer in the CO_2 stream. The inclusion of 3% methanol or ethanol as entrainers increased the solubility of lipids from 10 mg/g to 25 mg/g. The solubility increased again to 44 mg/g when 5% methanol was added. The inclusion of 3% methanol or ethanol as an entrainer also increased total egg lipid recovery from 72.8% to 82 - 86 % of theoretical values. Cholesterol solubility increased (from 0.001 to 0.420 mg/g) with the increase in extraction pressure (from 15 to 36 MPa) and increased (from 0.001 to 0.491 mg/g) with the increase in extraction temperature (from 40 to 55 °C) and then decreased (from 0.491 to 0.390 mg/g) with additional increase in extraction temperature (from 55 to 75 °C) at 36 MPa. The fatty acid composition of the extracts was independent of extraction temperature, pressure and presence of entrainer and remained constant throughout an extraction. SC CO_2 extracted triglycerides and cholesterol preferentially with no measurable phospholipids. The entrained SC CO_2 extracted trace amount of phospholipid during a run. Samples collected during the latter portions of an entrained run after most of the oil had been removed had phospholipid concentrations from 6.8 to 17%.

2.4 Composition of Egg Yolk

Egg yolk consists of about 48.3% moisture (Chung and Stadelman 1965) and 50% solids, and can be physically regarded as a mixture of particulate 'granules' and soluble

plasma (Parkinson 1966). The egg yolk solids are composed of proteins, lipoproteins, carbohydrates, lipids, mineral constituents, and vitamins, which are summarized in Table 2.1. Yolk lipids can be further classified as triglycerides, phospholipids and cholesterol, the portions of which are listed in Figure 2.10. About 84% of cholesterol is present in the free state, and the rest in an esterified form. The cholesterol content of egg yolk is essentially constant although it can be influenced by the strain, age of fowl, laying season,temperature and dietary supplements (Edwards et al. 1960; Beyer and Jensen 1989; Jiang and Sim 1991; Harris and Wilcox 1963; Weiss et al. 1967; Clarenburg et al. 1971; Turk and Barnett 1972; and Nakaue et al. 1980).

Table 2.1 Constituents of egg yolk (Parkinson 1966).

Constituents			Percent of Egg Yolk Solids
Proteins	Livetins		4-10
	Phosphoprotein	Vitellin	4-15
		Vitellenin	8-9
		Phosvitin	5-6
Lipoproteins	Lipovitellin		16-18
	Lipovitellenin		12-13
Lipids	Triglycerides		46
	Phospholipids		20
	Sterols (mainly cholesterol)		3
Carbohydrates			2
Minerals			2
Vitamins			traces

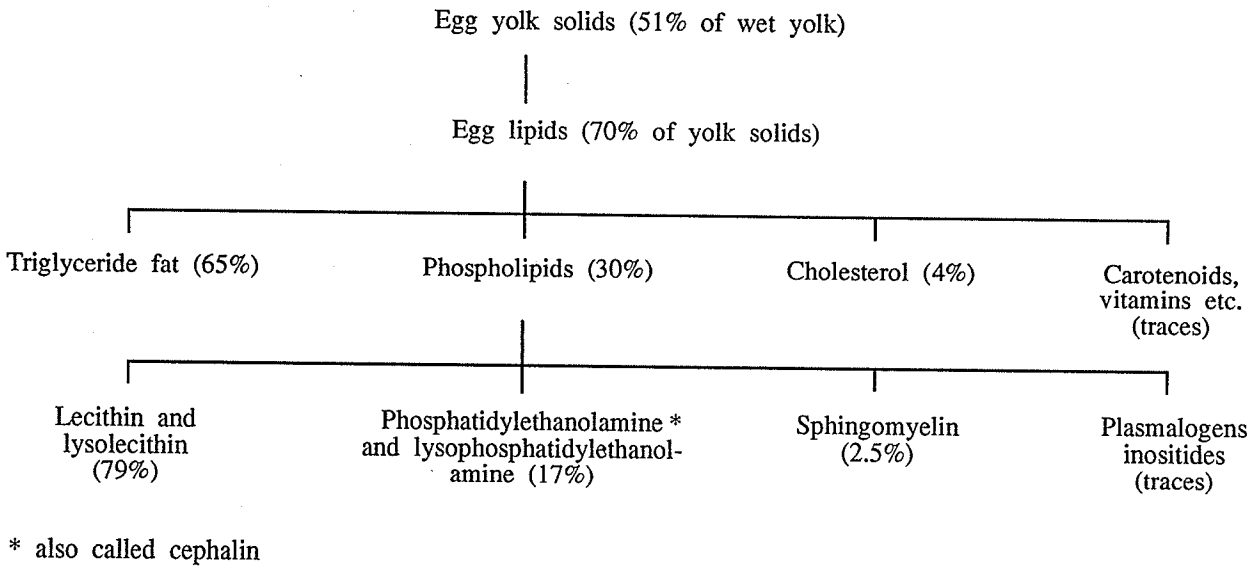

Figure 2.10 Composition of the lipid portion of egg yolk (Parkinson 1966).

3. EXPERIMENTAL METHODS AND MATERIALS

3.1 Experimental Equipment

The extraction experiments were carried out using a modified supercritical fluid extraction (SFE) screening system consisting of three major components: the original SFE screening system, entrainer injection system, and a PC-based control and datalogging system. The details on the modifications to the original SFE screening system have been reported by Crerar (1993).

3.1.1 Modified SFE Screening System

The original SFE screening system was a complete turnkey assembly for extractions up to 69 MPa. The system was equipped with a diaphragm type compressor, a 300 mL extraction vessel with a temperature controller, a feedback pressure regulator, a flow metering valve with a temperature controller, a flow meter, a flow totalizer, and a sampling unit (Figure 3.1). To improve the performance of this SFE screening system, extensive modifications were made by Crerar (1993) including the addition of a temperature controlled chamber (oven), an entrainer injection system, and a PC-based control and datalogging system (Figure 3.2). All of the process temperatures, pressures, and flow rates can be displayed on the monitors of the PC-based control and datalogging system and logged to a disk. The oven temperature and the rate of entrainer addition can be set and controlled by the PC-based control and datalogging system. The temperature inside the extraction vessel, monitored by a T-type thermocouple probe inserted into the

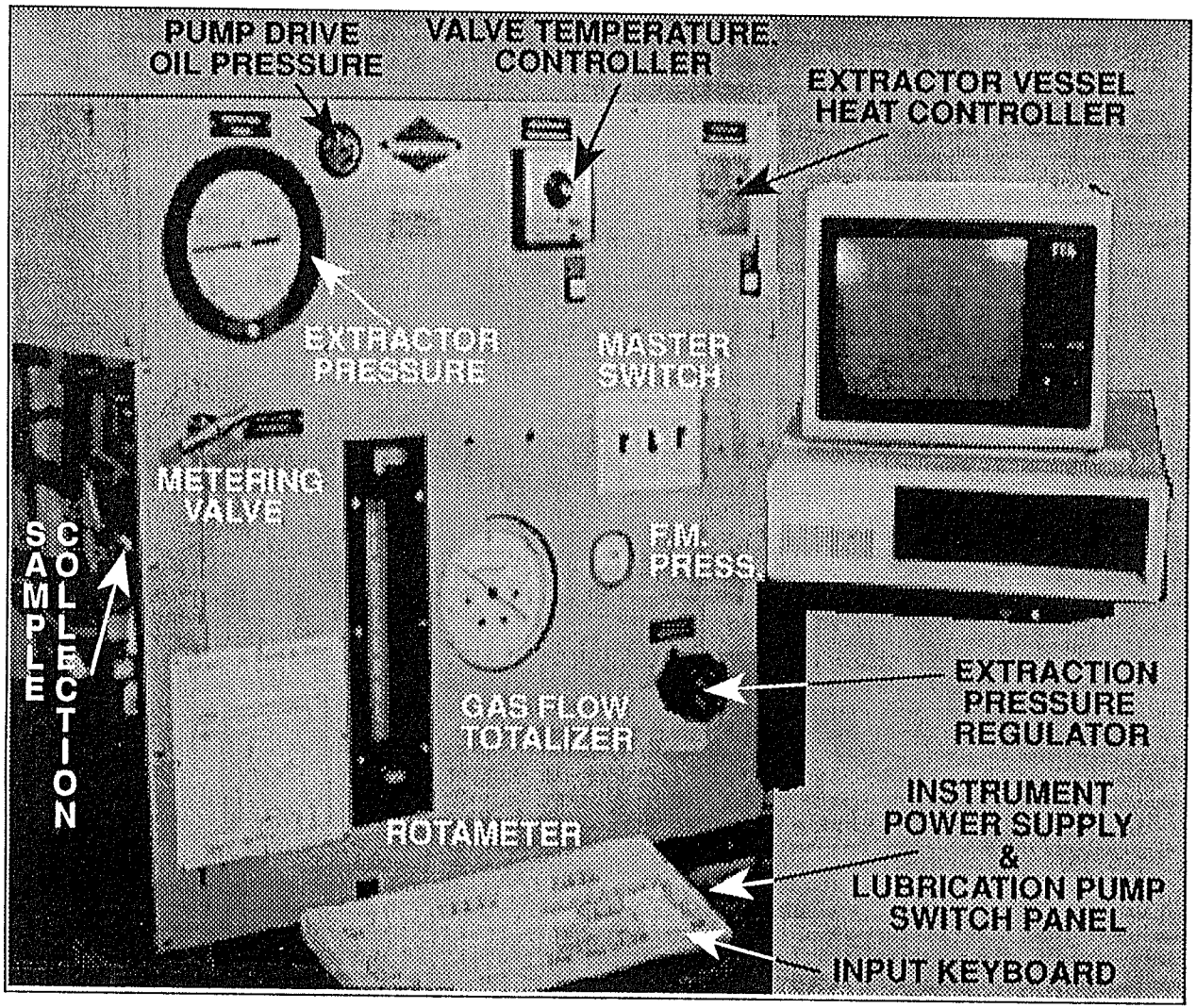

Figure 3.1 Original SFE screening system overview of components (provided by W. J. Crerar).

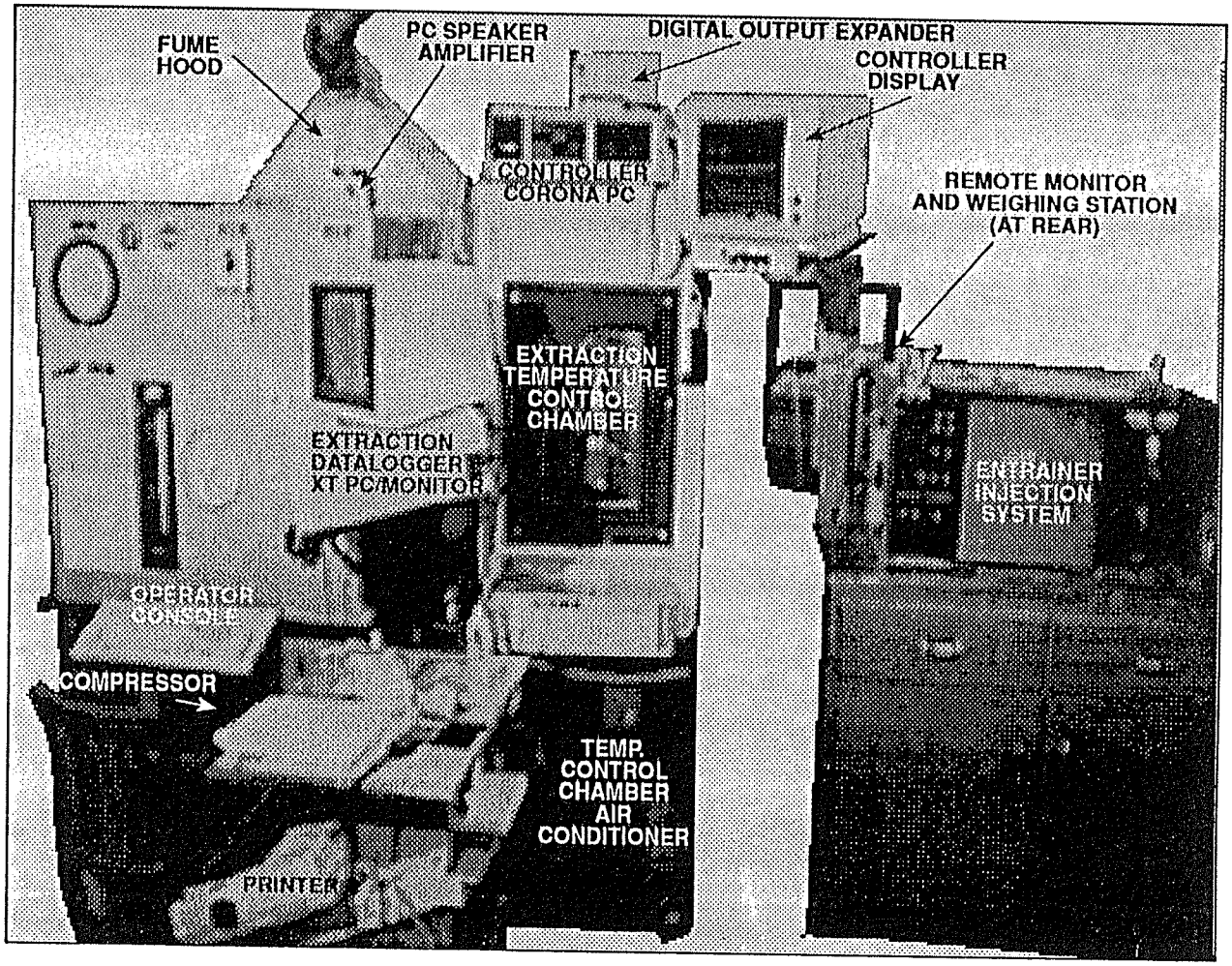

Figure 3.2 Modified SFE screening system overview, showing the original SFE screening system with the added oven, entrainer injection system, and PC-based control and datalogging system (reprinted from Crerar 1993).

vessel, is maintained by heating the extraction vessel with the electrical heater attached to its exterior and controlled by a temperature controller. The metering valve is maintained at the set temperature with an attached heater to promote a constant flow. The heater is controlled by another temperature controller. The pressure in the extraction vessel is controlled by adjusting the feed back pressure regulator and is monitored with a Bourdon-type pressure gauge. The CO_2 pressure in the totalizer is monitored by a pressure sensor. The instantaneous flow rate of CO_2 through the extraction vessel is controlled by the flow metering valve and indicated by the panel mounted flow meter. The total gas flow passed through the extraction vessel is indicated by the flow totalizer readable to 0.1 standard litres. The mass flow of CO_2 is calculated by the computer using the equation of state in terms of the recorded CO_2 volume, temperature (monitored by a thermocouple) and pressure.

3.1.2 Extraction Vessels

The 300 mL extraction vessel supplied with the original SFE screening system was used for the extraction of freeze-dried egg yolk. Another smaller extraction vessel (50 mL) was used for the extraction of pure cholesterol and the mixture of cholesterol and triolein. Both of the vessels are made of 316 stainless steel and can withstand operating pressures up to 63 MPa. The size specifications for the two extraction vessels are reported in Table 3.1.

Figure 3.3 and Figure 3.4 are the assembly drawings of the two vessels, which are composed of several separate components (gland nut, head, collector ring, screw cap,

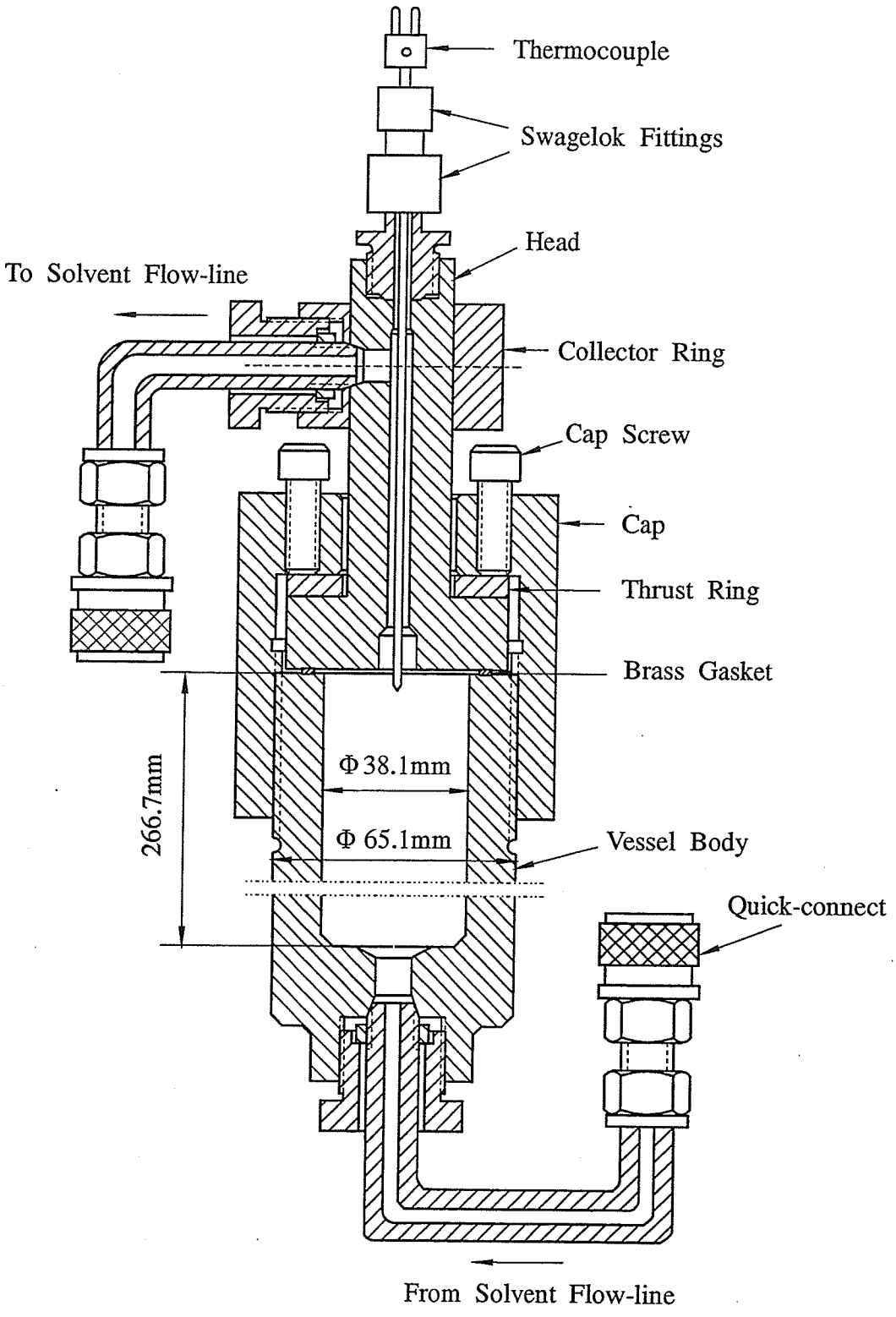

Figure 3.3 Cross sectional view of 300 mL extraction vessel assembly.

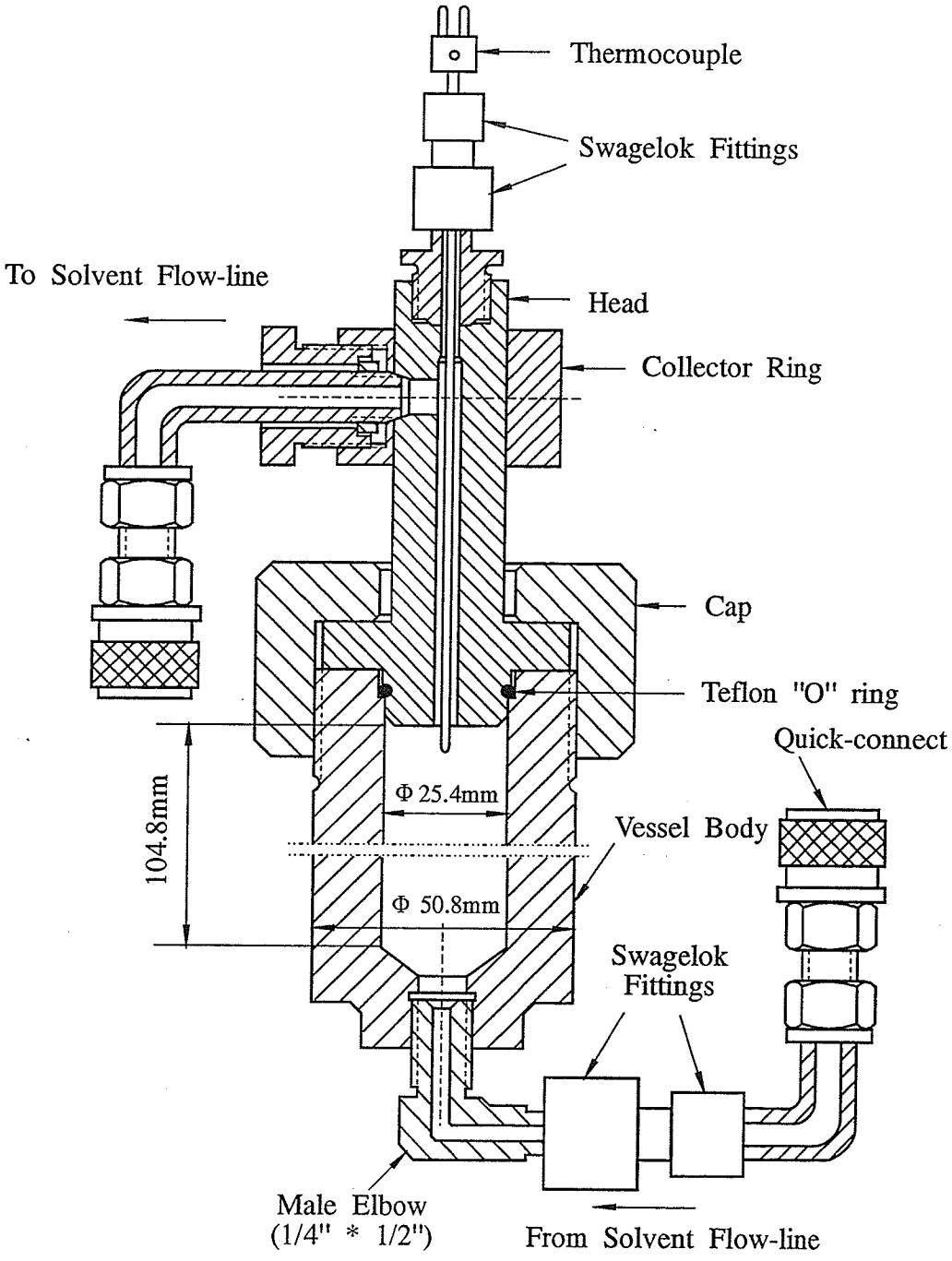

Figure 3.4 Cross sectional view of 55 mL extraction vessel assembly.

thrust ring, gasket, and body). Both of the vessels are mounted on free-standing angle iron bases. The heads can be removed for loading and unloading material. For the larger extraction vessel, the head is tightly secured to the body of the vessel by eight stainless steel bolts and the cap with threads. The contact surfaces of the head and the body were fabricated with a machined circular retaining groove. Before the cap was placed on the body, a piece of brass gasket was placed in the retaining groove. This design was used to eliminate leakage. The bolt size is 10 mm in diameter. Each bolt was tightened with a torque wrench to 45 foot-pounds (61.0 Nm)in the sequence required (shown in Figure 3.5). For the smaller extraction vessel, the head was fabricated with a sealing channel along the circumferential direction and a teflon "O" ring was fitted in the channel to prevent leakage. The head was inserted into the body of the vessel and secured by a cap with threads.

Table 3.1 Dimensions of the extraction vessels.

Extraction Vessel Number	Inside Diameter (mm)	Inside Length (mm)	Vessel Volume (mm^3)	Vessel Wall Thickness (mm)
1	38.1	266.7	$304x10^3$	13.5
2	25.4	104.8	$53x10^3$	12.6

The bottom of the smaller vessel was fitted with a male elbow (1/4" x 1/2"), by which the vessel was connected to the shutoff valve through quarter inch tubing. The bottom of the larger vessel was fitted with a Swagelok fitting and was also connected to

the shutoff valve through quarter inch tubing. The thermocouple probe monitoring the temperature inside the vessel was inserted into the head from the top and fastened to the head by Swagelok fittings. The solvent flow line connections to and from the vessels were made via quick-connect couplings. The vessel could be taken out of the oven upon loading and unloading material by disconnecting the quick-connect couplings.

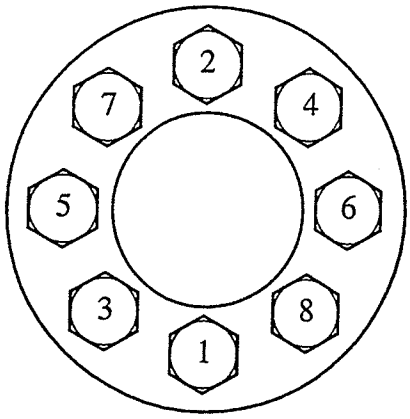

Figure 3.5 Cap screw tightening sequence recommended by Supercritical Fluid Extraction System Operation Manual (1985) for 300 mL extraction vessel locking cap.

3.1.3 Entrainer Injection System

The HPLC 1081B, a microprocessor controlled instrument incorporating a reciprocating diaphragm pump and a solvent flow system, was modified and used as an entrainer injector (Crerar 1993). An isolating valve and a 3-way selector valve were added in the solvent line. The isolating valve is used to prevent the gas solvent from entering

the entrainer line when shutting off the HPLC pump. The 3-way selector valve is used to switch the entrainer flow from "bypass" line, which is equipped with a back pressure regulator and used to adjust the back pressure, to "inject" position. When the HPLC 1081B is controlled by the internal microprocessor, the flow rate can be selected between 0 to 9.90 mL/min in increments of 0.01 mL/min; the pressure can be selected from 0 to 40.0 MPa with 0.1 MPa increments. To adapt the HPLC flow/pressure electronics for better control at lower flow rates, an alternate range was added to the flow amplifier, enabling the gain of the amplifier to be increased 10 times when pumping small volumes. Both flow ranges were recalibrated to indicate solvent flow rate in g/min rather than mL/min. When the PC-based control and datalogging system is activated and controlling the HPLC 1081B, the desired solvent flow rate and the desired entrainer concentration can be entered through the keyboard of the controller (i.e. PC Corona). The desired entrainer flow rate is calculated in terms of these two values. The actual entrainer flow rate is adjusted to the calculated flow rate by an appropriate stepper motor movement.

The principle of entrainer flow control is shown in Figure 3.6. When program "cntrolr1" entrainer mode (installed in the controller) is activated and <F1> pressed, PRE RUN on the DIK board (Display and Keyboard) of HPLC 1081B is turned on and the information sent to the CPU (Central Processing Unit). If an error condition is present, the pump does not turn on. The error can be accessed in the check mode of HPLC 1081B. If no errors are present, the PON (Pump On) line goes to low and relay K1 on the PRI board (Primary board) turns on gating power to the pump drive motor. When the flywheel tab passes through the photo switch, the PIF (pump interface) generates a pump pulse

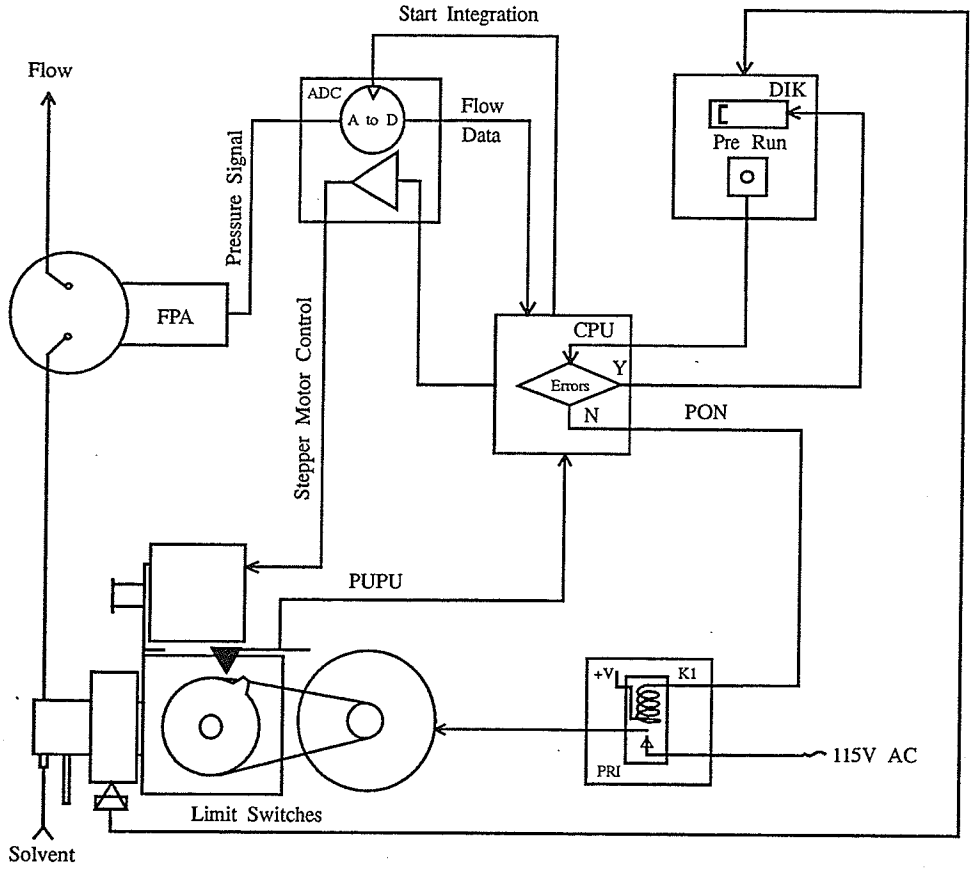

Figure 3.6 Schematic diagram of entrainer flow control of HPLC 1081B (from Liquid Chromatograph 1081B Service Manual 1980).

(PUPU). Coincident with the PUPU is a flow pulse which is discharged from the pumphead into the flow transducer. This flow pulse is translated into a voltage by the FPA (Flow Transducer Preamp board) and sent to the ADC (Analog to Digital Converter). The CPU instructs the ADC to integrate this voltage spike when it receives the PUPU and then compares the digital flow information from ADC with the setpoint stored in memory. If the measured flow does not agree with the setpoint, the CPU instructs the pump stepper motor to take appropriate action. This stepper motor command is decoded and amplified on the ADC and sent to the pump. When the pumphead strikes the limit switch, a signal is sent through the DIK signal multiplexing circuitry to the CPU which ceases pumphead movement.

3.1.4 Sampling Units

Two sampling units were used to collect samples in the experiments and are shown in Figure 3.7 and Figure 3.8. The sampling unit in Figure 3.7 was used for collecting egg yolk lipids and mixture extracts. It consists of a brass cap, a 16 x 150 mm (O.D. x L) vial, and a 44 mm long section of 2 mm O.D. brass tubing connected to the metering valve via a Swagelok adaptor. A 115 mm long section of 3 mm O.D. stainless steel tubing is connected to the other end of the brass tubing and inserted into the vial for 100 mm in depth to assist in the precipitation of egg yolk lipids and mixture extracts. Another piece of brass tubing having one end fitted with a pipe connector, is connected to the brass cap and used to convey lipid-free gas to the flow meter. A teflon O-ring is used for sealing the vial-brass cap fitting. The gas and the dissolved lipids flow through

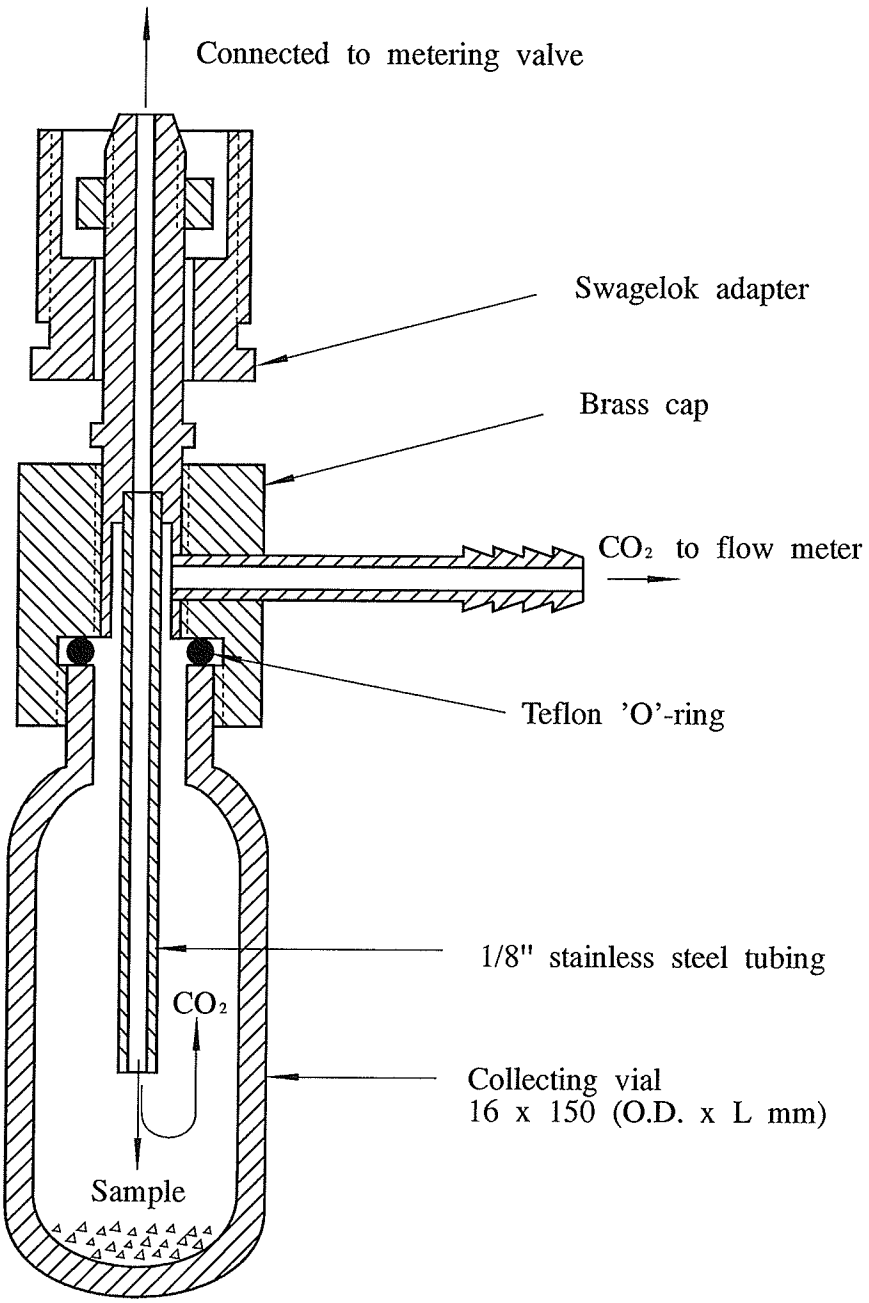

Figure 3.7 Cross sectional view of sampling unit for extracts from egg yolk and mixtures.

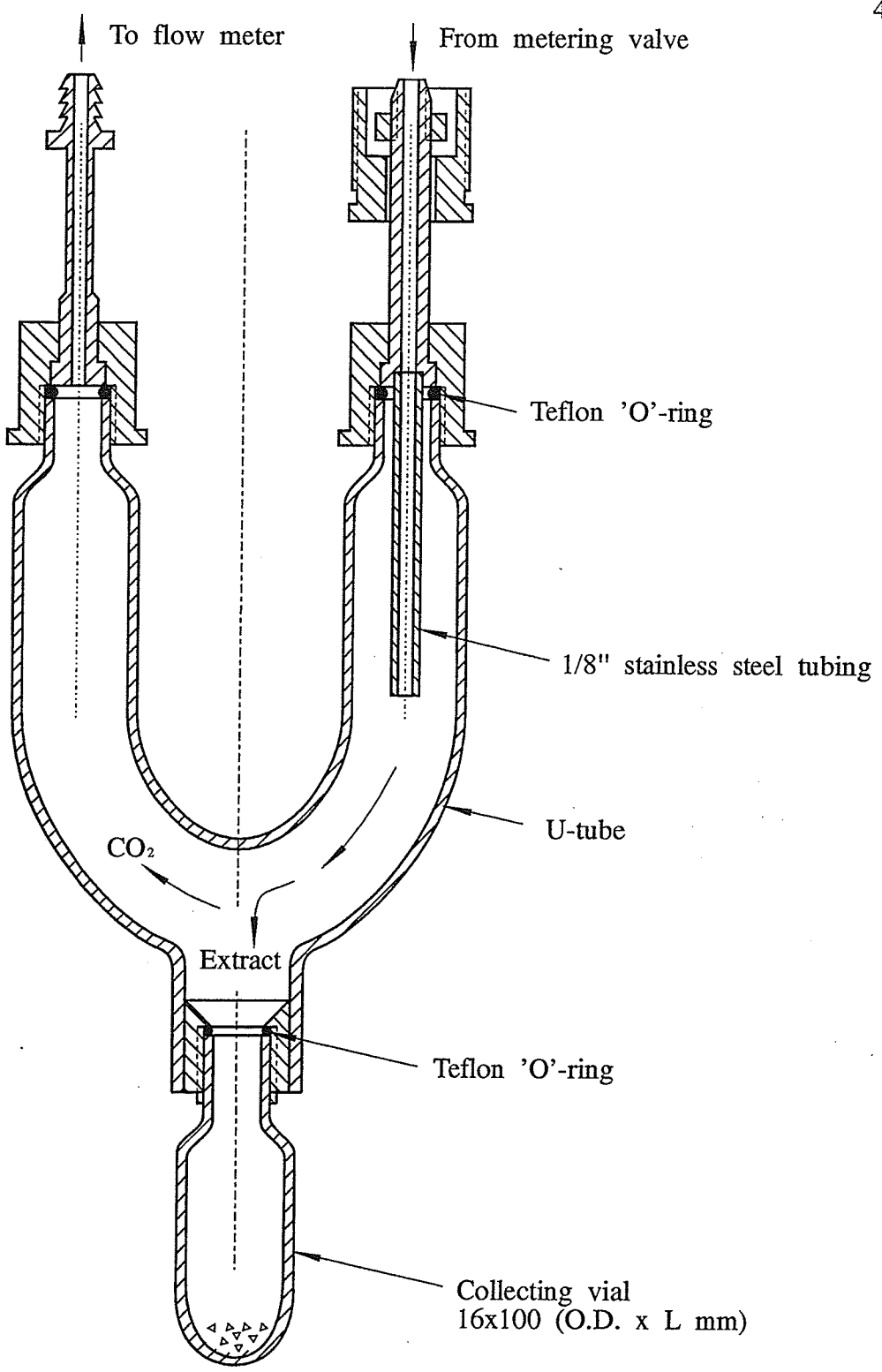

Figure 3.8 Cross sectional view of the sampling unit for pure cholesterol.

the stainless steel tubing. When they exit from the tubing, the lipids deposit on the bottom of the vial and the gas flows through the exit brass tubing to the flow meter. The sampling unit is very convenient for changing vials. However, it is not suitable for collecting cholesterol. Powdered cholesterol deposits very slowly and is easily resuspended by the flowing gas and carried into the flow meter. To reduce the losses of the cholesterol during precipitation, the sampling unit in Figure 3.8 was specially designed. It consists of a U-tube (made in the University of Manitoba glass shop), a 16 x 100 mm (O.D. x L) vial, and two brass connectors. One arm of the U-tube is connected to the metering valve via one brass connector and a Swagelok adapter. The other arm of the U-tube is connected to the plastic pipe directing gas to the flow meter. Teflon O-rings are used for connector-U-tube fitting seal and U-tube-vial fitting seal. A handful of glass wool is placed in each arm of the U-tube and serves to filter the gas and prevent the extract from entering the flow meter. The unit works effectively, though it makes changing vials awkward.

3.1.5 Extract Drying Unit

If an entrainer is added to the CO_2 during the course of an extraction, it normally precipitates with the dissolved materials in the collecting vials. Before the mass of the dissolved materials can be determined, the entrainer in the vials must be evaporated. The drying unit used in this work is shown in Figure 3.9. The water bath is used to maintain a constant water bath temperature. A continuous nitrogen flow serves to protect the extracts from oxidation and speed up the drying process.

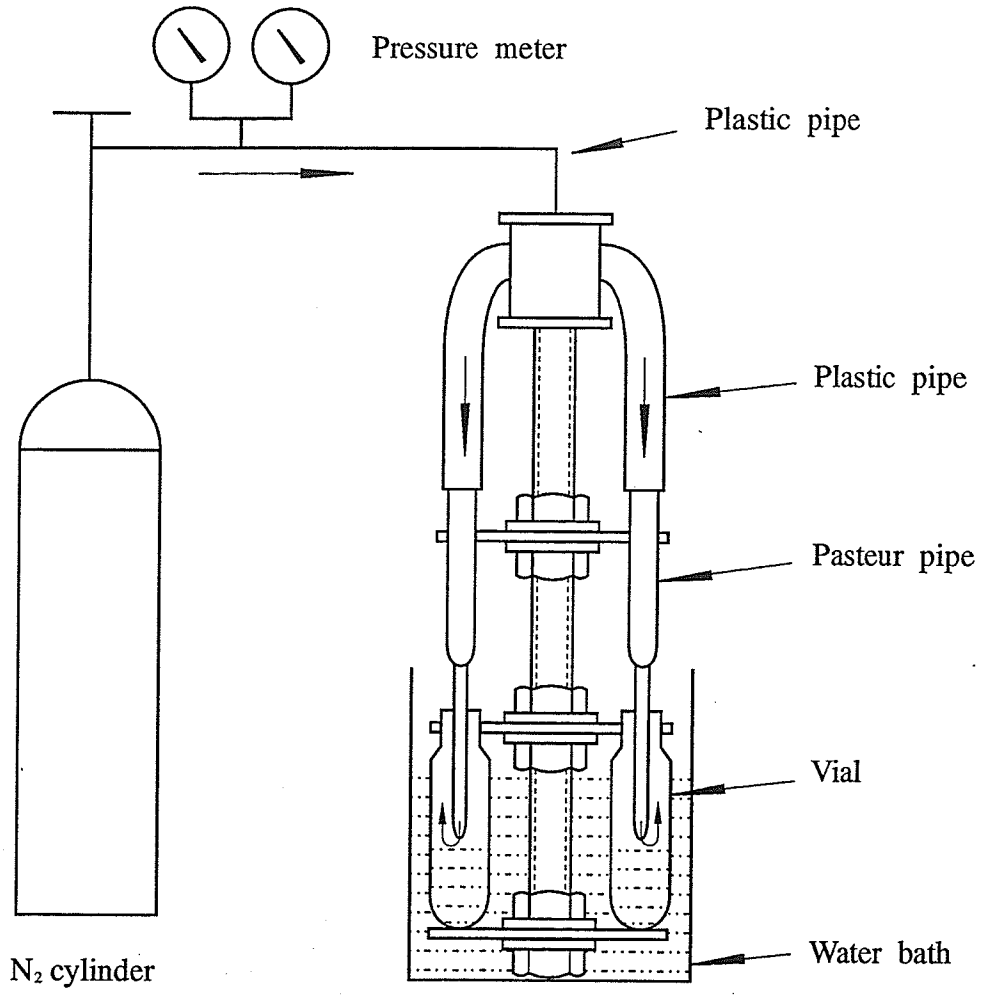

Figure 3.9 Extract drying unit.

3.1.6 Solvent Flow Path

The flow path of CO_2 and entrainer is shown in Figure 3.10. Liquid carbon dioxide from a commercial cylinder passes through a Nupro 7 μm sintered filter and enters the single-ended diaphragm compressor. The excess compressor capacity is sent back into the compressor's suction end. This "feed back" function is controlled by a back pressure regulator which also controls the extraction vessel pressure. After compression, CO_2 enters the preheating coil in the oven. If no entrainer is used, the preheated CO_2 then flows through a shutoff valve and into the extraction vessel. If entrainer is used, the preheated CO_2 and entrainer enter a small vessel filled with 0.5 mm glass beads to increase mixing, flow through a shutoff valve, and enter the extraction vessel. In the extraction vessel, the temperature of the CO_2 or the mixture is brought to the desired value by heating the extraction vessel with the electric heater attached to its exterior. It is here that the CO_2 or entrained CO_2 dissolves the components of interest. The solute-laden CO_2 with or without entrainer exits from the extraction vessel and flows through 1/8" O.D. stainless steel tubing to the metering valve. The CO_2 and the dissolved solute separate into two phases due to the pressure reduction across the metering valve. The solute or the solute/entrainer mixture precipitate on the bottom of the collecting vial. The gaseous CO_2 flows through the plastic tubing, the flow meter, the totalizer and is finally vented to the atmosphere.

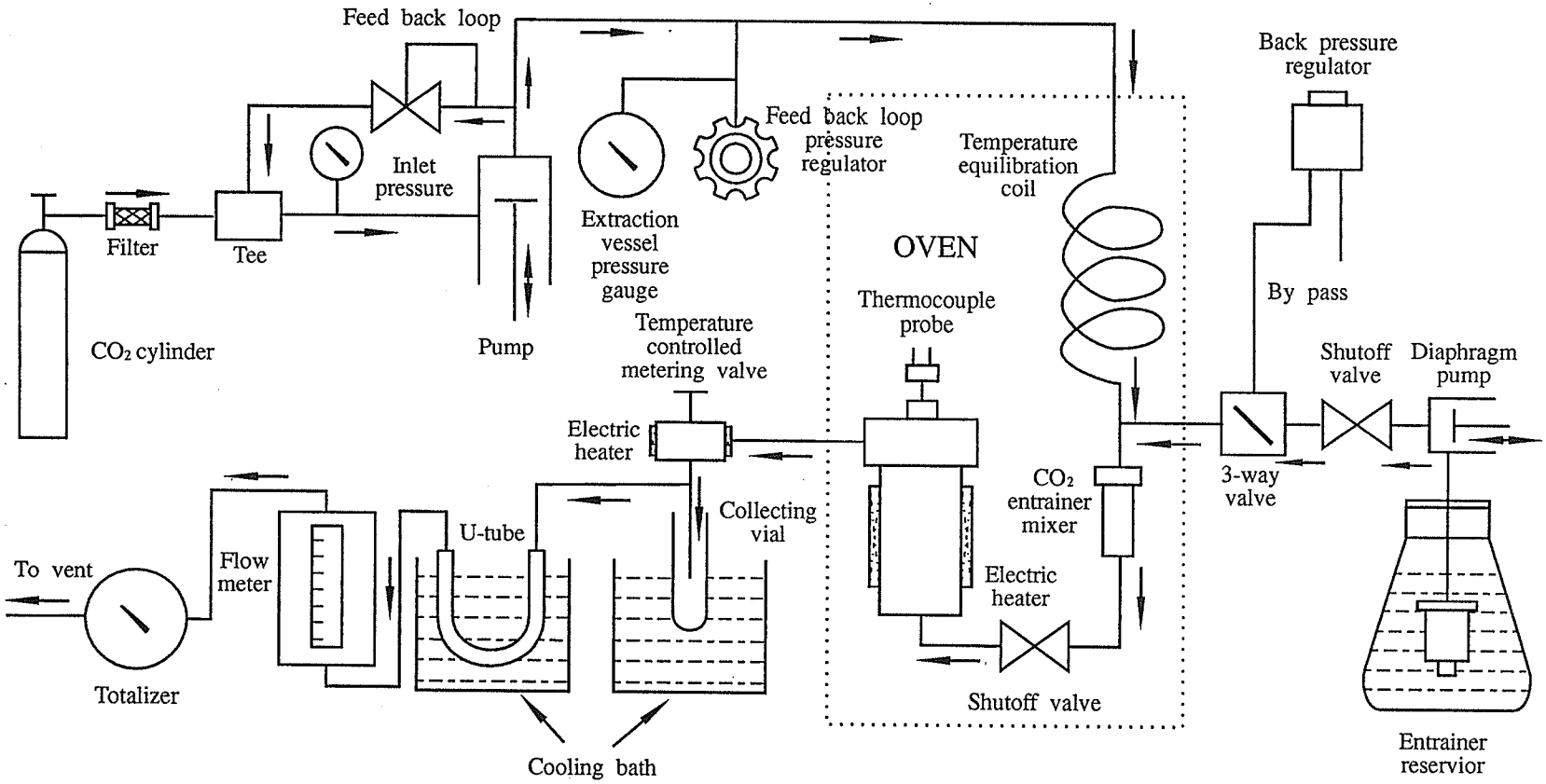

Figure 3.10 Flow path of CO_2 and entrainer.

3.2 Experimental Materials

3.2.1 Carbon Dioxide and Nitrogen

In this research, siphon CO_2 to be used as the solvent was obtained from Linde Union Carbide (Winnipeg, Canada). The siphon CO_2 is standard grade, which is compatible with the commercial grade supplied by Canadian Liquid Air Ltd. (Winnipeg, Canada). The specifications of the commercial grade siphon CO_2 are listed in Table 3.2.

Nitrogen used to protect extract samples and freeze-dried egg yolk from oxidation was also obtained from Linde Union Carbide (Winnipeg, Canada). Its purity is 99.95% with a moisture content of less than 25 µL/L.

Table 3.2 Specifications of commercial grade siphon carbon dioxide.

Purity	99.5%
Cylinder pressure at 15°C	5.7 MPa (830 psig)
Typical impurities	
	CO < 300 µL/L
	H_2 < 100 µL/L
	Air < 0.5%
	CH_4 < 30 µL/L
	H_2O < 200 µL/L

3.2.2 Methanol and Ethanol

Methanol and ethanol were used separately as entrainers in this research. HPLC grade methanol was supplied by Mallinckrodt Specialty Chemicals Co. (Mississauga, Canada). It meets American Chemist Society specifications. The ethanol obtained from Consolidated Alcohols Ltd. (Toronto, Canada) was guaranteed to meet the specifications

of the British and U.S. pharmacopoeia. The physical properties of the two alcohols with some specifications are listed in Table 3.3.

The methanol was also used as a coolant in the sample chilling bath. The mixture of methanol and chloroform with the portion of 1:1 v/v was used for extraction system flushing.

3.2.3 Chloroform and Acetone

HPLC grade chloroform and reagent grade acetone were purchased from Mallinckrodt Specialty Chemicals Co. The chloroform was mixed with methanol and used for extraction system flushing. The acetone was used for cleaning up the extraction vessel sealing surfaces.

3.2.4 Cholesterol and Triolein

Cholesterol (99+% purity) and triolein (approximate 95% purity) were obtained from Sigma Chemical Company (St. Louis, MO). Their specifications are given in Table 3.4. When not in use, cholesterol was stored at less than 0°C and triolein at 0 - 5 °C.

3.2.5 Freeze-dried Egg Yolk

Freeze-dried egg yolk used in this study was supplied by Export Packers Ltd. (Winnipeg). It was produced from fresh egg yolk by drying overnight in a freeze dryer at a condensator temperature of around -40°C and a vacuum pressure of 0.25 - 0.5 ATM (0.025 - 0.05 MPa). The composition of the freeze-dried egg yolk is listed in Table 3.5.

Table 3.3 Physical properties and some specifications of methanol and ethanol (Timmermans 1950).

Physical properties	Methanol	Ethanol
boiling point (760 mm Hg)	64.7°C	78°C
dielectric constant (20 °C)	33.6	25.0
refractive index (n 20°/D)	1.33	1.36
viscosity (20 °C)	0.60 cP	1.2 cP
density (20 °C)	0.792 (g/mL)	0.789 (g/mL)
Purity	99.9%	100%
Residue after evaporation	<0.0001%	<0.006%
Water	0.01%	0

Table 3.4 Specifications of cholesterol and triolein.

Name	Molecular Formula	Molecular Weight	Melting Point (°C)	Purity
Cholesterol	$C_{27}H_{26}O$	386.7	148	99+%
Triolein	$C_{57}H_{104}O_6$	885.4	4.9	95%

Table 3.5 Composition of freeze-dried egg yolk.

Composition	Percentage in Yolk (%)
Triglycerides	41.99
Cholesterol	3.79
Phosphatidylcholine (PC)	16.59
Phosphatidylethanolamine (PE)	7.77

3.2.6. Lipid Standards

Cholesterol, triolein, and egg yolk phosphatidylcholine standards for HPLC analysis were purchased from Sigma Chemical Co. Egg yolk triglyceride standards were

obtained by isolating the lipids which had been extracted from the original freeze-dried egg yolk on thin layer chromatography using a modified Folch method (Folch et al. 1956). All standards were stored below -40°C until used.

3.3. Experimental Procedures

3.3.1 Operating Conditions

3.3.1.1 Extraction Pressure

The four pressure levels selected (20, 25, 30, and 36 MPa) were between the critical pressure of CO_2 (7.3 MPa) and the maximum operating pressure of the modified SFE screening system. The maximum pressure of the original SFE screening system of 40 MPa has been reduced to 36 MPa by the solvent line Swagelok connection. During the course of the experiments, the extraction pressure was regulated to the setpoint ± 0.5 MPa.

3.3.1.2 Extraction Temperature

Four temperature levels were selected at 32, 40, 50, and 60°C. All of the temperatures were above the critical temperature of CO_2 (31.2°C) to ensure that the CO_2 was in the supercritical state. At high temperatures, the fluid density may be reduced to a point where the solvation properties are no longer favourable. In addition, the material to be extracted may be adversely affected by high temperatures. These two considerations set the upper boundary on the temperatures selected. During the course of the experiments, the extraction temperature was regulated to the setpoint ± 2°C.

3.3.1.3 Solvent Flow Rate

It has been shown that the equilibrium solubility of a solute in SC CO_2 is independent of the CO_2 flow rate passing through the extraction bed providing that there is sufficient contact time for the solvent/solute to reach equilibrium (Fattori 1986; Stahl et al. 1980). The CO_2 flow rate in these experiments was controlled to 10 ± 1 g/min for the extraction of egg yolk lipids, 5 ± 0.5 g/min for the extraction of pure cholesterol, and 2 ± 0.2 g/min for the extraction of the mixture of cholesterol and triolein.

3.3.1.4 Entrainer type and concentration

Either methanol or ethanol was used as entrainer. Entrainer content levels of 3, 6, and 9% by weight were chosen to test the effect of entrainer concentration on the solubility of solutes in SC CO_2 and the selectivity of SC CO_2 for solutes. All of the experiments using an entrainer were conducted at the operating conditions of 40°C and 36MPa.

All treatments were performed at least in duplicate.

3.3.2 Operating Procedures

3.3.2.1 Sample Preparation

Freeze-dried egg yolk that had been stored in sealed plastic bags under nitrogen was pulverized using an onion chopper and sieved using standard sieve series No. 10 (2.00mm) and No. 20 (0.85mm). The egg yolk particles that passed through sieve No. 20 were discarded. The egg yolk particles that remained on sieve No. 10 were crushed again

and passed over the sieve. The egg yolk particles caught between the two sieves were collected in a beaker and used for the different extraction experiments. The egg yolk samples to be used in a run were weighted just before loading the extraction vessel to protect the egg yolk from autoxidation. Normally, 60 to 70 grams of egg yolk were used for each extraction run.

Cholesterol and triolein from Sigma were used as received. The mixtures of cholesterol and triolein were prepared in the following way: the required amount of triolein was weighed into a plastic tray, the required amount of cholesterol was weighed into another plastic tray and then transferred to the tray containing the triolein. The triolein and cholesterol were mixed thoroughly. Normally about 10 grams of mixture were used for each run. The mixtures were then added to 30 - 35 mL of 0.5 mm glass beads and completely mixed before being loaded into the extraction vessel. The addition of glass beads into the mixtures was used to increase the surface area available for mass transfer during extraction.

3.3.2.2 Entrainer Degassing

Prior to injection, entrainer solvents were degassed under vacuum. The side arm of a 500 mL filter flask, placed on a magnetic stirrer, was connected to a water-powered aspirator. A Gooch-type porcelain funnel with a tapered rubber seal was inserted into the flask and a piece of No.29 filter was put on the flat, perforated bottom of the funnel. The water tap providing the power for the aspirator was turned on, entrainer poured into the funnel and filtered into the flask. After the flask was filled up to 500 mL level, the funnel

was removed, the flask sealed with a rubber stopper, and the entrainer stirred under vacuum until no further gas bubble formed. The stirrer was then turned off, the vacuum line and the rubber stopper removed, and the entrainer transferred to a 1,000 mL flask, which served as the entrainer reservoir of the injector pump.

3.3.2.3 Extraction Vessel Loading

A container filled with glass wool was weighed. A small plug of glass wool from the container was first placed on the bottom of the vessel to prevent egg yolk particles from entering the CO_2 flow line. The preweighed, pulverized freeze-dried egg yolk (approximate 60 to 70 g) was placed in the vessel on top of the glass wool. Another plug of glass wool also from the container was inserted into the vessel on top of the egg yolk for the same purpose mentioned above. The glass wool protruding out of the vessel was tamped down and carefully trimmed. The sealing surfaces of the body and the head and brass gasket were cleaned with a acetone-wetted tissue. Then the brass gasket was placed in the retaining groove on the sealing surface. After it was closed, the vessel was placed in the oven and connected to the CO_2 flow line. During the course of an extraction, carbon dioxide flowed through the vessel from bottom to top. The surplus egg yolk with its container and the surplus glass wool with its container were weighed. The masses of egg yolk particles and glass wool added into the extraction vessel were determined using a difference method.

The loading procedures for cholesterol/triolein mixtures were the same as those for freeze-dried egg yolk. The loading procedures for cholesterol were slightly different.

The extraction vessel was packed with alternating layers of cholesterol and glass wool. The glass wool was added to reduce the chance that the cholesterol would compact during a run.

3.3.2.4 Equipment Startup

Following the loading procedure, the cooling bath was turned on, the CO_2 cylinder and the shutoff valve on the CO_2 flow line were turned on while the metering valve was closed. Liquid leakage detector was used to check the leakage at the Swagelok connections of solvent flow line. If there was no leakage, the controlling program "cntrolr1" installed in PC Corona was booted, and "No Entrainer" mode was run. The desired extraction temperature, pressure and CO_2 flow rate were entered. The oven heater was turned on. When the extraction temperature was within 5 C° of the set point, the electric heater attached to the exterior of the vessel was turned on to bring the extraction temperature to the set point. The system was then left to stabilize for about 45 minutes. In the meantime, the heater on the metering valve was turned on. Usually the metering valve temperature was set 20 - 25 C° above the extraction temperature. Another PC XT, in which the program "logr" was installed, was also turned on and "logr" was started. The extraction conditions (pressure, temperature, CO_2 flow rate, and entrainer addition), the masses of egg yolk and glass wool added into the vessel, and the description of the extraction matrix bed were typed in through the keyboard of PC XT. The masses of collecting vials were weighed in via the balance and the remote terminal. All of the masses were logged to a disk. Then, the option "Acquiring Extraction Data" was selected.

The oiler pump was activated to lubricate the compressor pump drive and the compressor was started. If no entrainer was used, the extraction pressure was adjusted to the set point by rotating the pressure regulator knob, the metering valve was opened slightly and adjusted to produce the desired flow rate after the system reached the set pressure value. If entrainer was used, the entrainer injector power supply was turned on one hour before the extraction started. The "cntrolr1" program was stopped and re-booted, "Entrainer" mode selected, and the desired entrainer concentration and CO_2 flow rate entered. When the extraction vessel pressure reached two-thirds of the set point, the entrainer precharging was started by pressing <F3> on the PC Corona keyboard. The metering valve was opened to allow a flow rate of 1 - 1.5 g/min CO_2 to enhance mixing of the CO_2 and entrainer. Once the precharging was done and the entrainer pumphead had moved back to the initial position, the CO_2 flow rate was adjusted to the desired value. The extraction was started by simultaneously pressing <F1> on the keyboards of PC Corona and PC XT.

3.3.2.5 Extract Sampling

The extract sampling began immediately after the extraction started. A preweighed vial was fitted to the sampling head. It was removed and replaced by another preweighed vial after a desired period of time. This action was repeated for as many times as needed. In simple SC CO_2 extractions, the extraction sample vials were weighed in via the balance and remote terminal during the course of an extraction. The entrainer-extracted samples were dried under nitrogen and then weighed in. The masses of the samples collected could be determined from the differences between the "before" and "after" masses of

vials. The volume of gaseous CO_2 passing through each collecting vial during the collecting period was measured by the gas totalizer. The gas temperature and pressure were measured by a thermocouple and a pressure sensor, respectively, and all three variables were recorded in the computer. The corresponding mass of CO_2 was calculated by the computer from the ideal gas law using the three variables. Entrainer flow rate was dependent on the desired entrainer concentration and the flow rate of CO_2. The average entrainer flow rate during a run was calculated after the completion of the run.

3.3.2.6 Equipment Shutdown

After the extraction run had been completed and if no entrainer was used in the run, the shutoff valve on the CO_2 flow line was turned off first to prevent CO_2 backflow and the CO_2 cylinder was shut off. The compressor was turned off. All heaters were switched off and the system was allowed to depressurize. When the extraction vessel pressure dropped to the CO_2 cylinder pressure, the shutoff valve was switched on to let the remaining CO_2 pass through the system downstream. "Acquiring Extraction Data" was stopped. After the system pressure dropped to 0 MPa, the extraction vessel was disconnected and removed from the CO_2 flow line. If entrainer was used in the run, the entrainer injection system was shut off first. The shutoff valve on the entrainer flow line was turned off to prevent CO_2 from entering the entrainer flow line. The entrainer pump was also turned off by setting the injection system back to the standby mode. The 3-way valve was turned back to "bypass" position from "inject" position. Then the extraction system was shut off following the procedures described previously.

3.3.2.7 Post-extraction Treatment and System Cleaning

After the completion of a run, the extraction vessel was removed from the oven and opened. The contents were removed and placed on a piece of preweighed paper towel. The sealing surfaces of the extractor body and head were wiped clean with two to three preweighed tissues. If no entrainer was used in the run, the tissues, the paper towel and the contents removed from the vessel were weighed. If entrainer was used in the run, the contents as well as the tissues and the paper towel were weighed, then left to dry for 4 - 6 hours in the ambient temperature. The dry contents, the tissues and the paper towel were weighed again. The egg yolk residue was then separated from the glass wool and transferred into a plastic bag with a zipper. Nitrogen was added to the bag and the bag was stored in a freezer. The mass of the egg yolk residue recovered from the extraction vessel was determined by subtracting the masses of the tissues and the paper towel from the total mass of dry contents, the tissues and the paper towel.

The empty extraction vessel was rinsed with a solvent of 1:1 (v/v) chloroform/methanol. The rinsed material and the solvent were collected in a preweighed beaker. The solvent was evaporated and the mass of the rinsed material from the extraction vessel determined.

The system downstream of the extraction vessel (including the metering valve and sampling unit) was flushed with 1:1 (v/v) chloroform/ methanol to clean up any material left behind from the previous run.

The cleaning conditions were the same as the extraction conditions under which the previous run was carried out. The extraction vessel was filled with approximately 1:1

chloroform/methanol solution and connected to the solvent flow line. The CO_2 cylinder and the shutoff valve were opened. The metering valve was closed. The heating system was turned on and the temperature inside the extraction vessel brought to the desired value. The compressor was then started. When the set points of pressure and temperature were reached, the metering valve was opened slowly and set to allow the chloroform/methanol solution and the CO_2 to flow through the downstream line freely. The solution was collected in a preweighed beaker. When approximately half of the solution emerged from the sampling head, the system was shut down and depressurized. When the extraction vessel pressure meter reading was 0 MPa, the shutoff valve was closed, the quick-connect couplings were disconnected and reconnected so that the direction of CO_2 flow was reversed. The extractor was reheated, repressurized, and the system downstream was again flushed. The material being flushed from the lines was collected in the same beaker. When the solution in the extraction vessel was completely discharged, the CO_2 was still allowed to continue flushing the system for twenty minutes to ensure that trace amounts of the solution did not remain in the system. Finally, the CO_2 cylinder was turned off, the system was depressurized and the empty vessel was removed. The washing solvent in the beaker was evaporated and the mass of the flushed material from the system downstream was determined.

If no entrainer was used in the run, the sample vials were added with nitrogen to reduce oxidation, covered with caps, and stored in a freezer.

If entrainer was used in the run, 500 µL BHT (butylated hydroxytoluene (1.0024 mg/mL)) was added to each sample vial and then all the vials were placed in the sample

drying unit. Continuous nitrogen flow was supplied to each vial. When the entrainer in each vial was evaporated, the vials were taken out of the drying unit, weighed, and the data entered into the extraction data file. The BHT was used to prevent the samples from oxidation when being dried at 45°C. The continuous nitrogen flow protected the samples from oxidation and increased the rate of drying.

After the completion of the above work, the extraction log file was updated by entering, through the keyboard, the mass of the residue recovered from the extraction vessel, the mass of the rinsed material from the extraction vessel, and the mass of the flushed material from the system lines. The extraction log file was saved and then converted to ASCII format, which could be imported to QuattroPRO as a .PRN file for further analysis.

3.4 Methods

3.4.1 Extract Analysis

For each extraction run, four to eight samples were picked from the extracts collected at the beginning, the middle and the end of each run (Figure 3.11) for cholesterol and triglycerides or triolein analysis. Phospholipid analysis was carried out only for the freeze-dried egg yolk entrainer runs.

The lipid components of the extracts of supercritical extraction process were isolated using a modified Folch Method (Folch et al. 1956). The isolated lipids from the supercritical CO_2 extracts were then analyzed for triglyceride and cholesterol content. Triglyceride and cholesterol were analyzed using the method of Hamilton and Comai

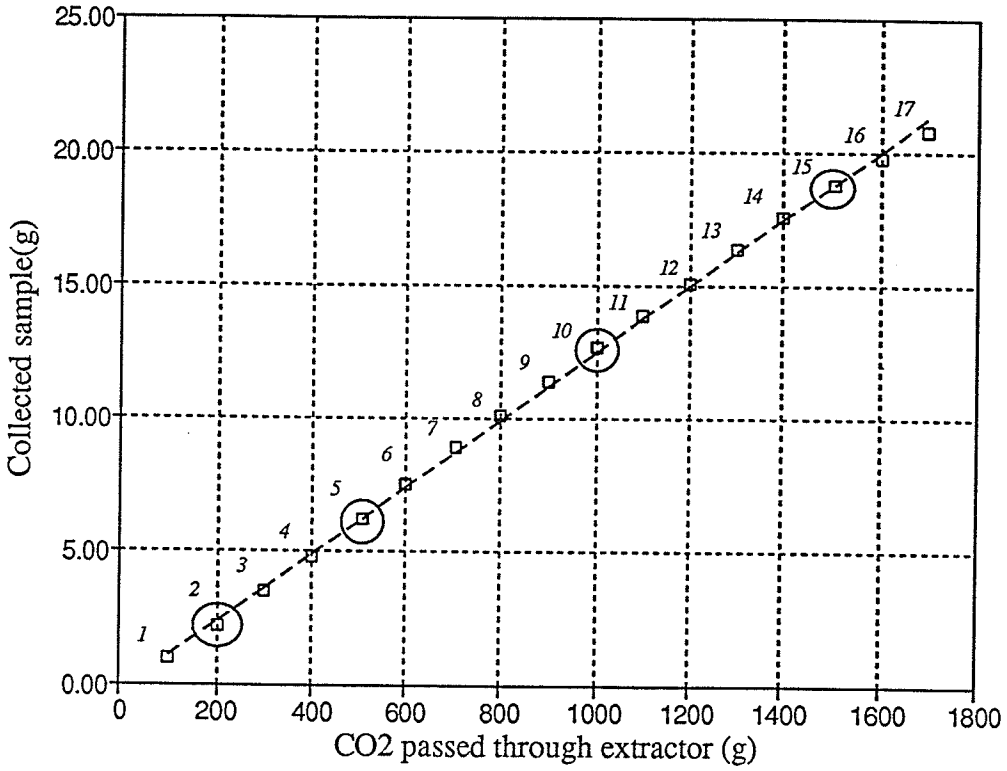

Figure 3.11 Typical extraction curve, showing samples being selected for analysis.

(1988a) with pure egg yolk triglycerides isolated on thin layer chromatography and cholesterol purchased from Sigma Chemical Co. as references. A Waters HPLC with a M45 pump connected to a 30 cm x 3.9 mm μPorasil column was used for the separation. A Shimadzu 8PD-6A UV detector was used at 206 nm.

The isolated lipids from the supercritical alcohol entrained CO_2 extracts were further separated into polar lipids (phospholipids) and neutral lipids (cholesterol and triglycerides) following the method of Hamilton and Comai (1988b) prior to separation by HPLC. Triglycerides and cholesterol were analyzed as previously described. Phospholipids were separated on a HPLC using an isocratic elution with acetonitrile-methanol-water (50:45:6.5 v:v:v) at a flow rate of 0.4 ml/min on a μPorasil column and detected by UV absorption at 206 nm. Egg yolk phosphatidylcholine and phosphatidylethanolamine, purchased from Sigma, were used as standards. Only phosphatidylcholine and phosphatidylethanolamine data were quantified.

All the samples were analyzed at least in duplicate for component content. The average concentration of each component in each sample was recorded.

3.4.2 Extraction Data Handling

The ASCII format extraction data file was imported into a macro-driven spreadsheet/graphics data analysis program for supercritical extraction (developed by Crerar 1993), which was run under QuattroPRO. The data was then formatted. The regression analysis was performed for the data from the initial linear portion of the accumulated sample mass vs. accumulated CO_2 mass curve, accumulated CO_2 mass vs.

accumulated time curve, entrainer mass added vs. accumulated time curve, and percent recovery vs. specific CO_2 curve, and the corresponding experimental data and fitting curves were graphed. An example of a typical output obtained from the analysis program is shown in Table 3.6 (extraction with entrained SC CO_2), Figures 3.12, 3.13, 3.14, and 3.15. In Table 3.6 at the top of the spreadsheet, the date, the starting time, and the extraction conditions were recorded, as well as the mass of the material added in the extraction vessel, the total mass of the samples collected in the vials, the mass of the residue recovered from the extraction vessel, and the masses of the materials washed from the vessel and the solvent flow line. These masses were used to estimate the overall mass balance of a run. The difference between the mass of the input and the total mass of the materials recovered is expressed as a "Loss". For the run shown in Table 3.6, a "Loss" of - 4.29% indicates that the total mass of the materials recovered is 4.29% more than the input material, which is attributed to the inability to remove the trace entrainer in the extract samples and the residue.

In the middle of the spreadsheet, columns 1 - 12 contain the original data recorded in the extraction data file for each collected sample, column 1 shows the number of collecting vials, column 2 the mass of CO_2 that passed through each vial during the sampling interval, column 3 the accumulated mass of CO_2 that passed through the sampling vials since the beginning of the run, column 4 records the vial changing time, column 5 the time elapsed for each sample collection, column 6 records the accumulated time elapsed since the run started. The masses of the initial and final vials are recorded in columns 7 and 8. The mass of entrainer reservoir, and the entrainer mass used since

Table 3.6 Typical spreadsheet obtained from the macro-driven spreadsheet/graphics data analysis program for supercritical extraction with entrainer.

Date 03-10-1992
Time 11:06:10
SC-CO2 Extraction with 3% EtOH
Sample Freeze-dried egg yolk with particle size of 0.85 - 2 mm
Bed Matrix coarsely granulated (0.85-1 mmØ)

Extractor capacity	300	ml	Captured in vials	29.109	g
Mass of sample added	72.912	g	Trapped in U-tube	0.695	g
			Recovered from vessel	46.138	g
Extraction temperature	40.0	C	Washed from vessel	0.038	g
Extraction pressure	36.0	MPa	Flushed from lines	0.057	g
Valve temperature	75.0	C	Total Recovered	76.037	g
CO2 flowrate	10.0	g CO2/min			
Atmospheric pressure	100.417	kPa	=>1.055	-4.20	%
Entrainer type	3%	EtOH	Starting Reservoir Mass	1023.2	g

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Sample Vial #	CO2 Flo Interval (g)	Accum'd Flow (g)	Start Time (HH:MM	Elapsed Time (s)	Accum'd Time (min)	Mass of Collector (g) Initial	Final	Reservoir Mass (g)	Entr'r Added (g)	Collecte Sample (g)	Accum'd Sample (g)	Fit Curve Sample (g)	Fit Curve Entr'r (g)	Fit Curve CO2 (g)	Specific CO2 (g/g smpl	% Recovery	% Fit
1	200.9	200.9	00:00	1341	22.3	17.021	19.220	1016.521	6.707	2.199	2.199	1.806	7.155	188	2.76	3.02	2.477
2	100.1	301.0	00:22	664	33.4	16.938	18.677	1013.140	10.088	1.739	3.938	4.534	10.362	294	4.13	5.40	6.218
3	99.6	400.5	00:33	656	44.4	16.974	20.229	1009.754	13.474	3.255	7.193	7.249	13.554	398	5.49	9.87	9.942
4	100.2	500.8	00:44	665	55.4	16.861	19.789	1006.355	16.873	2.928	10.121	9.982	16.767	503	6.87	13.88	13.690
5	99.8	600.5	00:55	651	66.3	16.979	19.865	1003.086	20.142	2.886	13.007	12.701	19.964	606	8.24	17.84	17.420
6	100.6	701.1	01:06	644	77.0	17.089	19.340	999.771	23.457	2.251	15.258	15.444	23.189	709	9.62	20.93	21.182
7	100.2	801.3	01:17	630	87.5	17.000	19.127	996.578	26.650	2.127	17.385	18.175	26.400	809	10.99	23.84	24.927
8	99.5	900.8	01:27	630	98.0	16.918	18.826	993.381	29.847	1.908	19.293	20.887	29.588	909	12.35	26.46	28.647
9	101.0	1001.8	01:38	626	108.5	17.000	18.662	990.219	33.009	1.662	20.955	23.641	32.826	1008	13.74	28.74	32.423
10	99.2	1101.0	01:48	609	118.6	17.051	18.545	987.120	36.108	1.494	22.449	26.346	36.007	1104	15.10	30.79	36.134
11	100.3	1201.4	01:58	623	129.0	16.932	18.407	983.927	39.301	1.475	23.924	29.082	39.223	1203	16.48	32.81	39.886
12	100.5	1301.9	02:09	626	139.4	17.044	18.359	980.763	42.465	1.315	25.239	31.823	42.446	1303	17.86	34.62	43.646
13	99.2	1401.1	02:19	608	149.6	16.976	18.170	977.660	45.568	1.194	26.433	34.528	45.626	1399	19.22	36.25	47.355
14	100.1	1501.2	02:29	601	159.6	16.880	17.949	974.618	48.610	1.069	27.502	37.257	48.834	1494	20.59	37.72	51.098
15	99.7	1600.9	02:39	601	169.6	17.041	18.031	971.558	51.670	0.990	28.492	39.974	52.029	1590	21.96	39.08	54.825
Blowdow	76.1	1676.9	02:49			16.991	17.608	971.282	51.946	0.617	29.109				23.00	39.92	57.669

Sample Regression Output:

Constant	-3.6709
Std Err of Y Est	0.40635
R Squared	0.99495
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	0.027263
Std Err of Coef.	0.000971
Sample Solubility	27.26 mg/g CO2

CO2 Regression Output:

Constant	-24.54834
Std Err of Y Est	7.1178688
R Squared	0.999765
No. of Observations	15
Degrees of Freedom	13
X Coefficient(s)	9.517889
Std Err of Coef.	0.040474
CO2 Flowrate	9.52 g CO2/min

Entrainer Regression Output:

Constant	0.715918
Std Err of Y Est	0.241589
R Squared	0.999737
No. of Observations	15
Degrees of Freedom	13
X Coefficient(s)	0.032053
Std Err of Coef.	0.000144
Entrainer content	3.11 %

Specific Recovery Regression Output:

Constant	-5.03
Std Err of Y Est	0.557
R Squared	0.995
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	2.7263
Std Err of Coef.	0.09713
Spec. Recovery	2.7263

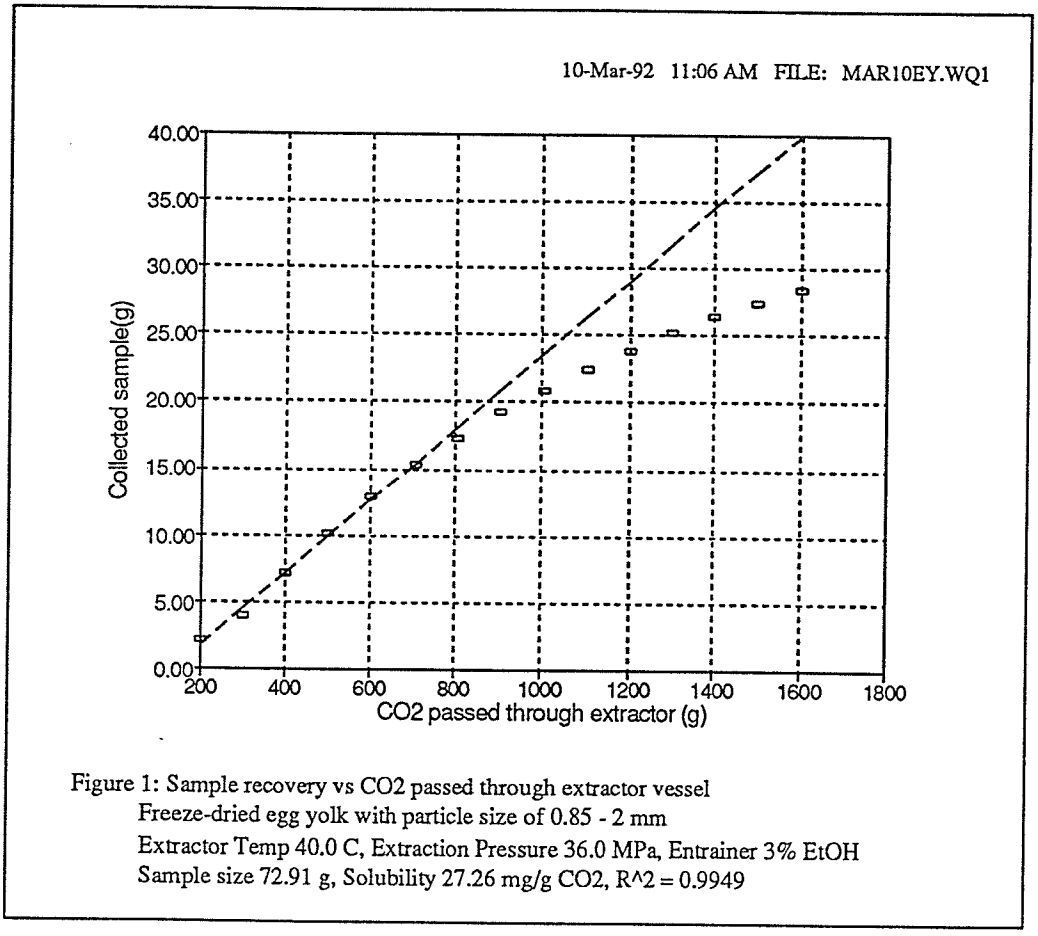

Figure 3.12 Typical graphic output from macro-driven spreadsheet/graphics data analysis program for supercritical extraction: sample recovery vs. CO_2 passed through the extractor (sample solubility).

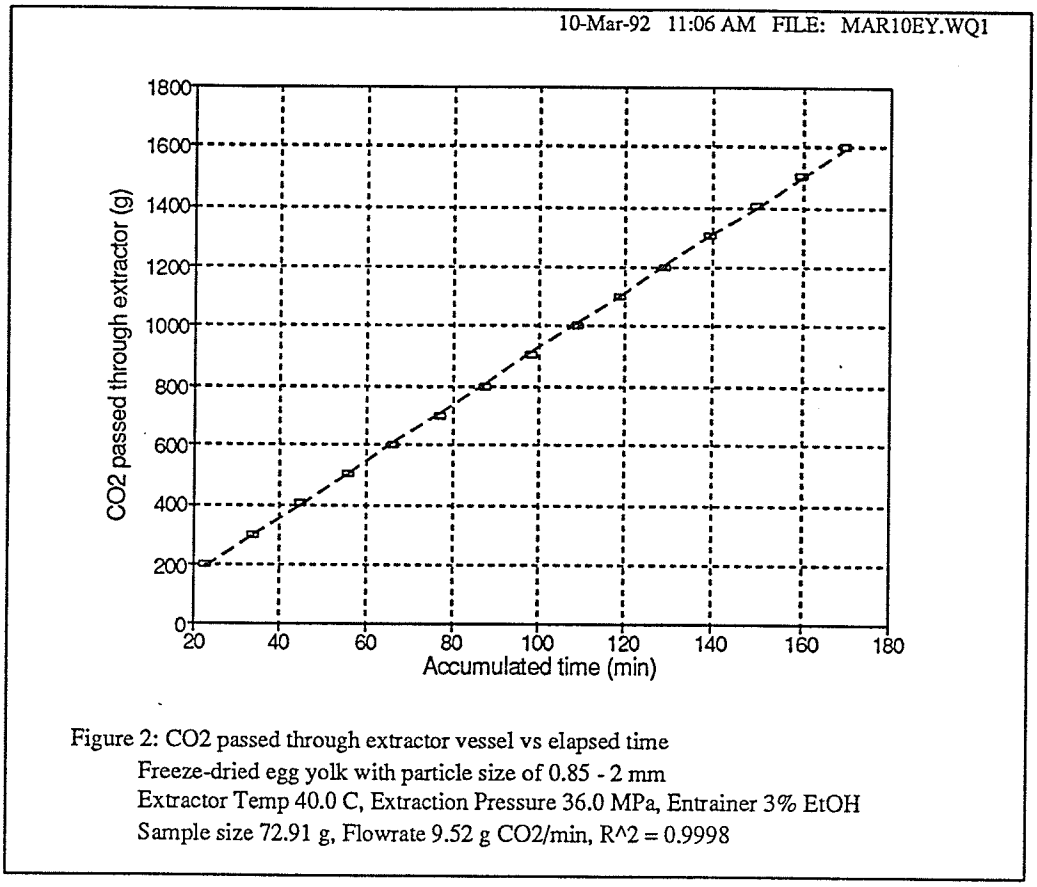

Figure 2: CO2 passed through extractor vessel vs elapsed time
Freeze-dried egg yolk with particle size of 0.85 - 2 mm
Extractor Temp 40.0 C, Extraction Pressure 36.0 MPa, Entrainer 3% EtOH
Sample size 72.91 g, Flowrate 9.52 g CO2/min, R^2 = 0.9998

Figure 3.13 Typical graphic output from macro-driven spreadsheet/graphics data analysis program for supercritical extraction: CO_2 passed through the extractor vs. elapsed time (CO_2 flow rate).

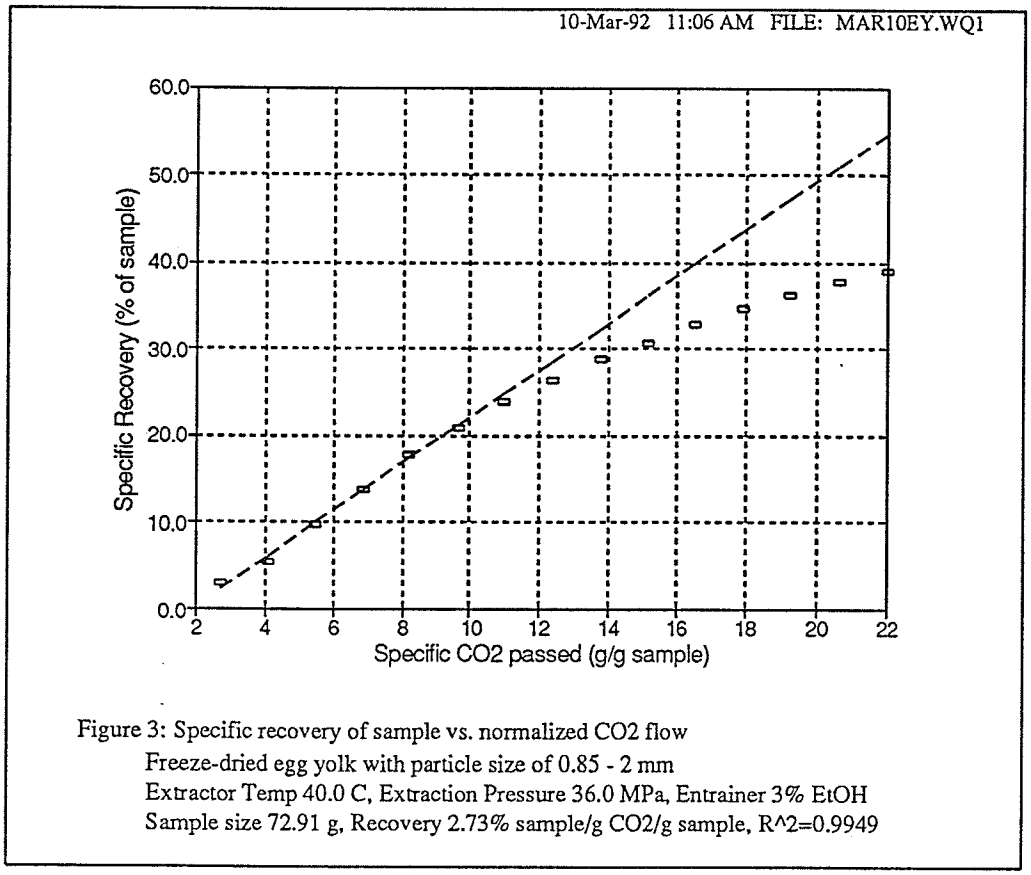

Figure 3: Specific recovery of sample vs. normalized CO2 flow
Freeze-dried egg yolk with particle size of 0.85 - 2 mm
Extractor Temp 40.0 C, Extraction Pressure 36.0 MPa, Entrainer 3% EtOH
Sample size 72.91 g, Recovery 2.73% sample/g CO2/g sample, R^2=0.9949

Figure 3.14 Typical graphic output from macro-driven spreadsheet/graphics data analysis program for supercritical extraction: percent sample recovery vs. specific CO_2 usage (specific recovery).

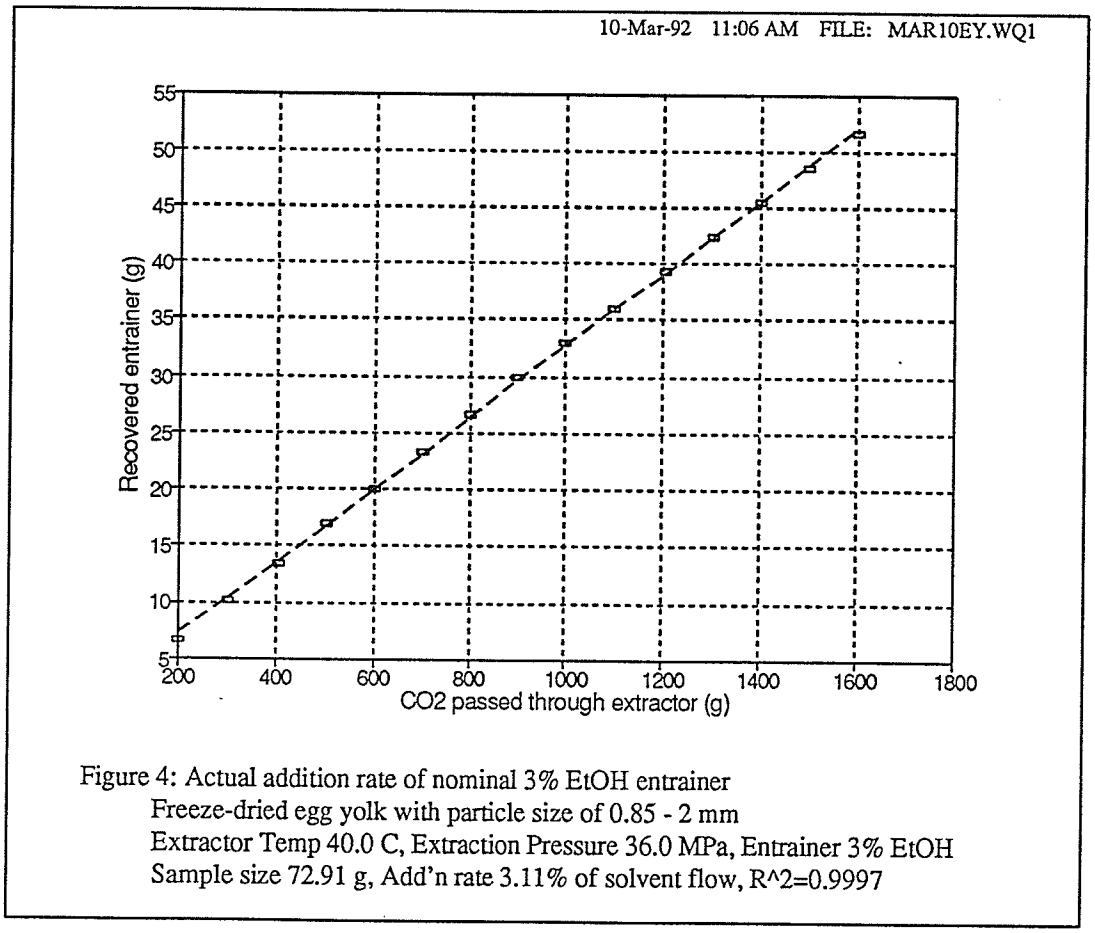

Figure 4: Actual addition rate of nominal 3% EtOH entrainer
Freeze-dried egg yolk with particle size of 0.85 - 2 mm
Extractor Temp 40.0 C, Extraction Pressure 36.0 MPa, Entrainer 3% EtOH
Sample size 72.91 g, Add'n rate 3.11% of solvent flow, R^2=0.9997

Figure 3.15 Typical graphic output from macro-driven spreadsheet/graphics data analysis program for supercritical extraction: entrainer addition vs. CO_2 usage (entrainer addition rate).

the beginning of the run are recorded in columns 9 and 10. The mass of the sample collected in each vial is obtained by subtracting column 7 from column 8 and the result recorded in column 11. The accumulated masses of the collected samples are recorded in column 12. "Specific CO_2" (the mass of CO_2 passing through per gram of sample) and "Percent Recovery" are shown in column 16 and column 17 of the spreadsheet. Columns 13, 14, 15, and 18 are the fitting values for accumulated sample mass, accumulated entrainer mass, accumulated CO_2 mass, and percent recovery. The regression results are also shown on the bottom of the spreadsheet. The actual CO_2 flow rate, rate of entrainer addition, and specific recovery rate during the run can be obtained from these regression results.

3.4.3 Solubility Determination of Extract

The solubility of an extract in SC CO_2 is determined as the slope of the initial linear portion of its extraction curve, i.e. the accumulative mass of the extract vs. the accumulative CO_2 mass curve. Figure 3.12 is a typical extraction curve of egg yolk lipids. Therefore, the X coefficient shown in Sample Regression Output in Table 3.6 represents the solubility of egg yolk lipids in CO_2. Labay (1991) provided an explanation for the use of the linear portion of the curve of extract mass vs. CO_2 mass to determine the solubility of the extract in CO_2. Other researchers (Fattori 1986; Ikushima et al. 1986; and Taniguchi et al. 1985) have also experimentally supported this method as applied to lipid solubility in SC CO_2.

3.4.4 Solubility Determination of Individual Components in Mixture and Egg Yolk Extracts

3.4.4.1 Solubility Determination of Individual Components in the Extracts from Mixtures

The solubility of each component in the extracts from the mixtures of cholesterol and triolein was determined in a method similar to that used for total solubility determination; i. e., establishing extraction curve - accumulated mass of component vs. accumulated mass of CO_2 for each component, and the slope of initial linear portion of the extraction curve represents the solubility of the component in SC CO_2. The procedures for establishing extract curves include: determining the concentration of each component in each extract sample collected during a run, calculating the mass of each component in each sample by multiplying its concentration by sample mass, calculating the accumulated mass of each component by adding its mass in each sample in collecting order since the start of the run, and drawing the curve of accumulated mass of each component vs. accumulated mass of CO_2 passed through the extractor.

3.4.4.2 Solubility Determination of Individual Components in the Extracts from Egg Yolk

Only selected extract samples from discrete intervals were analyzed for the concentrations of the components in egg yolk extracts. The following procedures were used to determine the solubility of components in egg yolk extracts. The concentration of each component in each analyzed extract sample was determined and recorded. The

product of the concentration and the extract sample mass yields the mass of the component. Dividing the mass of the component by the mass of CO_2 passed through the extractor during sample collection gives the concentration of the component in SC CO_2 (mg/g CO_2). The value reported is the arithmetic mean of four extract samples on the initial portion of total extraction curve. The value represents the solubility of the component in SC CO_2. An example of a solubility calculation is summarized in Table 3.7. Columns 1 and 2 give the extraction pressure and temperature. Column 3 is the number of extract samples on the extraction curve. Column 4 records the mass of CO_2 passed through vial during the sampling interval, column 5 the mass of extract sample collected. Column 6 records triglyceride concentration determined by HPLC analysis, Column 7 cholesterol concentration determined by HPLC analysis. Column 8 records the calculated triglyceride concentration in SC CO_2 (column 5 x column 6 / column 4) during each sampling interval, and Column 9 cholesterol content in SC CO_2 (column 5 x column 7 / column 4). The average values for triglyceride and cholesterol concentrations in CO_2 are recorded in the last row.

Table 3.7 Solubility calculation of components in yolk lipid extracts.

Extraction Condition Pressure (MPa)	Temperature (°C)	Extract Number	CO_2 Interval (g)	Extract Mass (g)	TG Conc. (%)	CHO Conc. (%)	TG Content in CO_2 (mg/g)	CHO Content in CO_2 (mg/g)
36	32	2	100.1	1.037	65.6	10.7	6.8	1.1
		5	99.9	1.159	67.3	10.2	7.8	1.2
		12	100.1	1.093	70.5	10.7	7.7	1.2
		15	99.2	1.044	65.0	9.55	6.8	1.0
						Mean	7.27	1.12

4. RESULTS AND DISCUSSION

The results and discussion section is divided into four parts. In the first part, the information about the effects of extraction conditions (extraction temperature, pressure and entrainer concentration) on the solubility of pure cholesterol in SC CO_2 is presented. In the second part, the information about the effects of one component in a binary mixture and its content on the solubility of the other component is presented. The relationship between the total solubility of the mixture and the solubilities of individual components is discussed. The third part deals with the effects of extraction temperature and pressure on the total solubility of egg yolk lipids, the solubilities of egg yolk cholesterol and triglycerides, as well as on the selectivity of SC CO_2 for cholesterol over triglycerides. The effects of the presence of triglycerides in egg yolk on the solubility of cholesterol and the optimum operating conditions for the SC CO_2 extraction of egg yolk lipids are discussed. The fourth part deals with the effects of entrainer type and concentration on the total solubility of egg yolk lipids, the solubilities of egg yolk cholesterol and triglycerides, as well as on the selectivity of SC CO_2 for cholesterol over other lipids present in egg yolk.

4.1 Extraction of Pure Cholesterol

The solubilities of pure cholesterol in either SC CO_2 or SC CO_2-ethanol mixtures were determined from the corresponding extraction curves using the procedures described in section 3.4.3.

4.1.1 Cholesterol Solubility as a Function of Temperature and Pressure

The equilibrium solubility of cholesterol in SC CO_2 is plotted in Figure 4.1 as a function of temperature. The data points shown on the graph are the arithmetic means of the experimental solubilities. The error bar shown on each curve in the graph represents the maximum standard error for the set of solubility means. At constant pressure, cholesterol solubility increases with increasing temperature over the range studied. At 20 MPa, the solubility increases fairly slowly with increasing temperature. An increase in the temperature from 32°C (solubility = 0.49 mg/g CO_2) to 60°C (solubility = 0.56 mg/g CO_2) leads to an increase in the solubility of only 0.07 mg/g CO_2. At the other three pressures examined, the solubility increases faster with increasing temperature.

The same solubility data for cholesterol are also plotted in Figure 4.2 as a function of pressure. Figure 4.2 shows that at constant temperature cholesterol solubility increases almost linearly with increasing pressure. At 60°C, the solubility increases faster with increasing pressure. The highest solubility for cholesterol in SC CO_2 (1.27 mg/g CO_2) was observed at 60°C and 36 MPa. The effects of changes in extraction temperature and pressure on cholesterol solubility in SC CO_2 are best understood by referring to the principle on which the SFE process is based (section 1.1). For any substance, its solubility in a supercritical solvent is strongly dependent on the temperature and pressure at which the SFE process is carried out. At constant temperature, the density of CO_2 increases with increasing pressure which tends to enhance the solvation capacity of the solvent. At constant pressure, temperature may affect the solvation power of a supercritical solvent through its effects on the solvent density and on the vapour pressure of the solute. As the

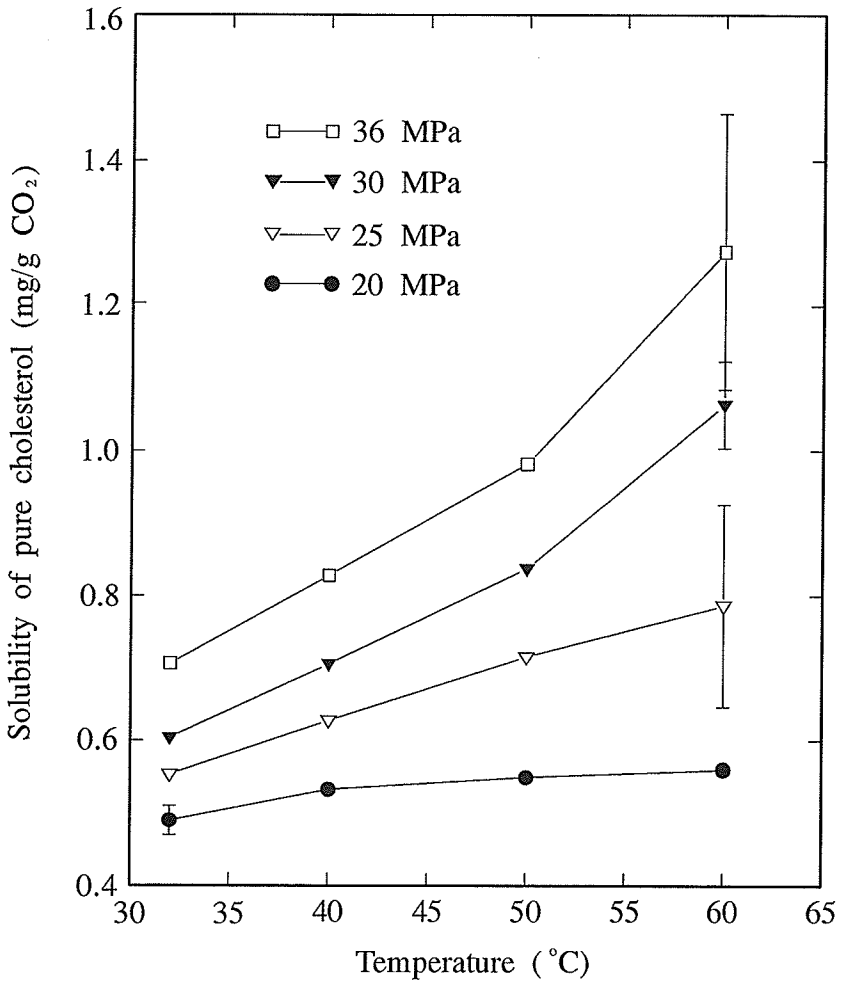

Figure 4.1 Solubility of pure cholesterol as a function of temperature at four pressures.

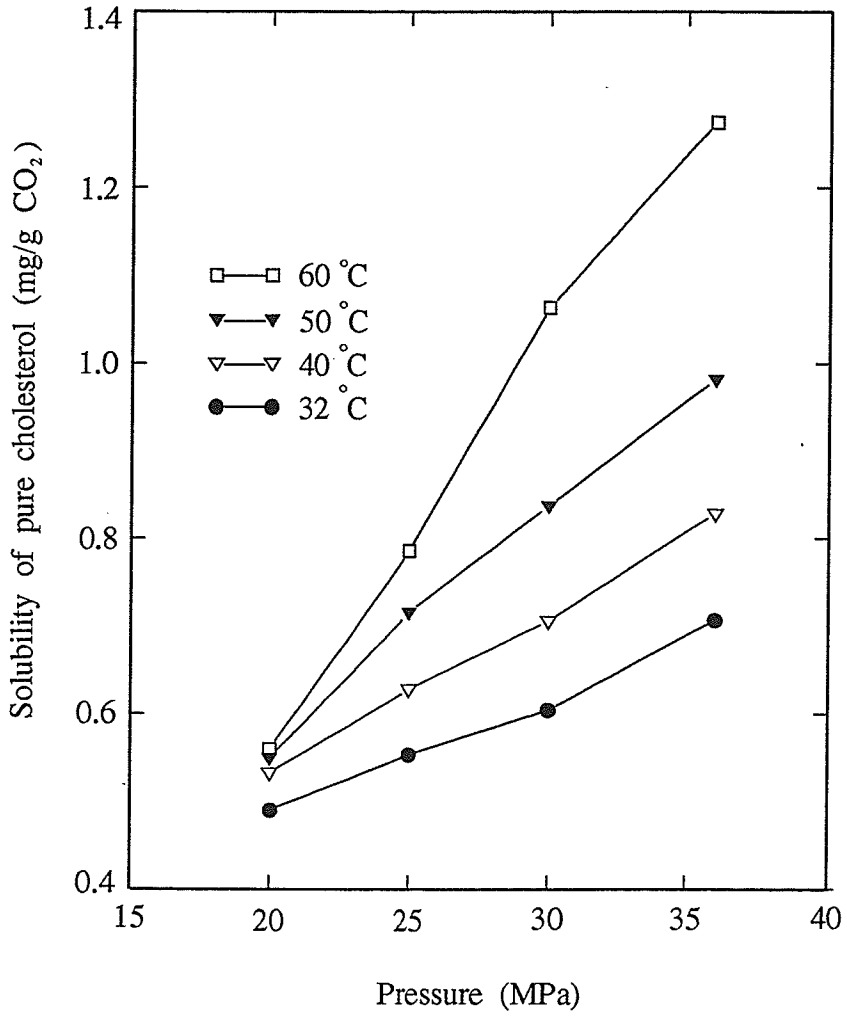

Figure 4.2 Solubility of pure cholesterol as a function of pressure at four temperatures.

extraction temperature increases, the density of the supercritical solvent decreases resulting in a decrease in the solvation power of the solvent. On the other hand, an increase in the temperature also leads to an exponential increase in the vapour pressure of the solute and results in a potential increase in the concentration of the solute in the supercritical phase (Peter and Brunner 1978; Nilsson et al. 1988). However, the effect of temperature on the density of the solvent and as a result on its solvation capacity changes with pressure (Figure 4.3). At the low pressures (for example, in the vicinity of the critical pressure) where the solvent is highly compressible, the density decreases significantly with small increases in temperature. At low pressures, the density effect might be dominant, resulting in a solubility decrease with an increase in temperature (referred to as retrograde behaviour in the literature). At higher pressures, the density is affected only slightly by a change in temperature and the vapour pressure effect might be dominant, resulting in a solubility increase with temperature due to the increase in vapour pressure (Wong and Johnston 1986). At a moderate pressure, the density effect balances the vapour pressure effect and the solubility remains constant with the changes in temperature. In the literature, this moderate pressure is referred to as crossover pressure.

In this study, cholesterol solubility was observed to increase with increasing temperature and pressure. This was expected since Yun et al. (1991) have reported that the crossover pressure for cholesterol/SC CO_2 system is located at approximate 16 MPa and all the cholesterol extraction experiments in this study were conducted at pressures above 16 MPa.

In Figure 4.4, the solubility of cholesterol is plotted as a function of CO_2 density

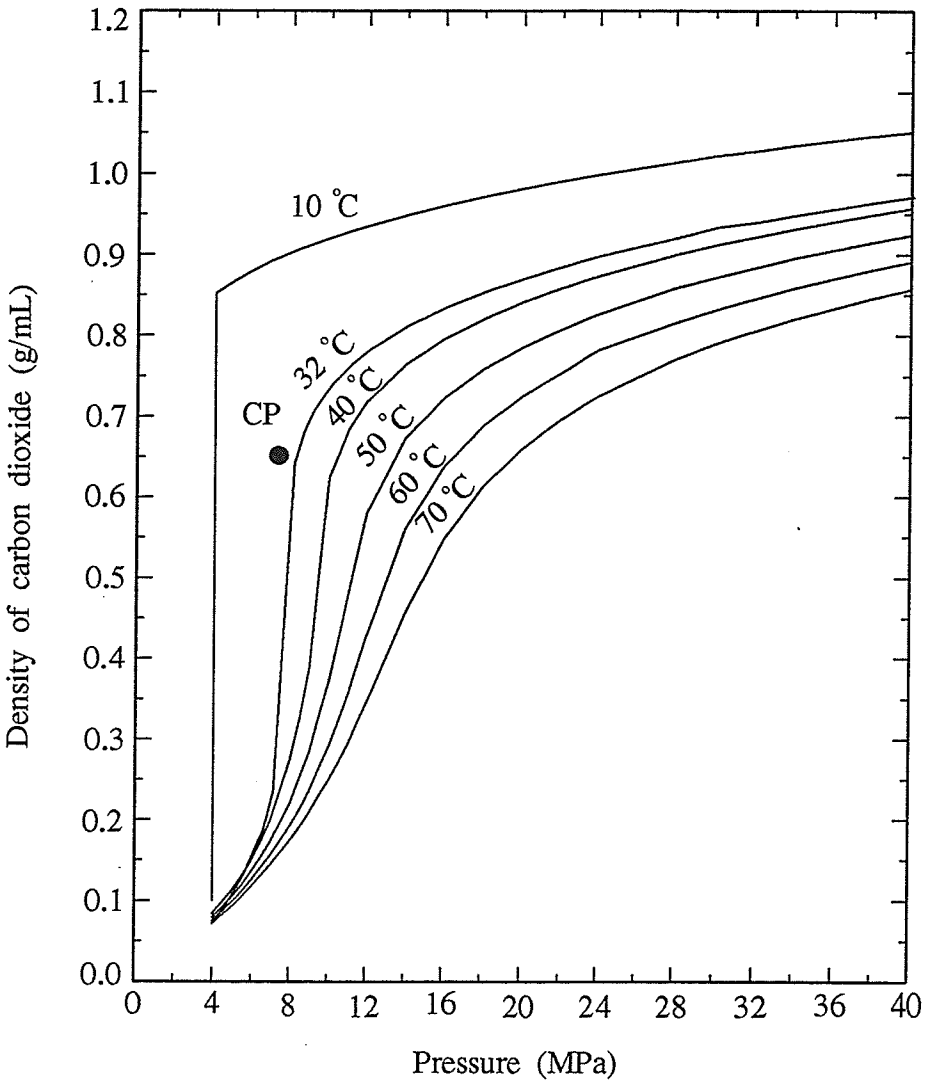

Figure 4.3 Density of carbon dioxide as a function of pressure at different temperatures. The critical point is designated as CP (drawn according to the data from Angus et al. 1976).

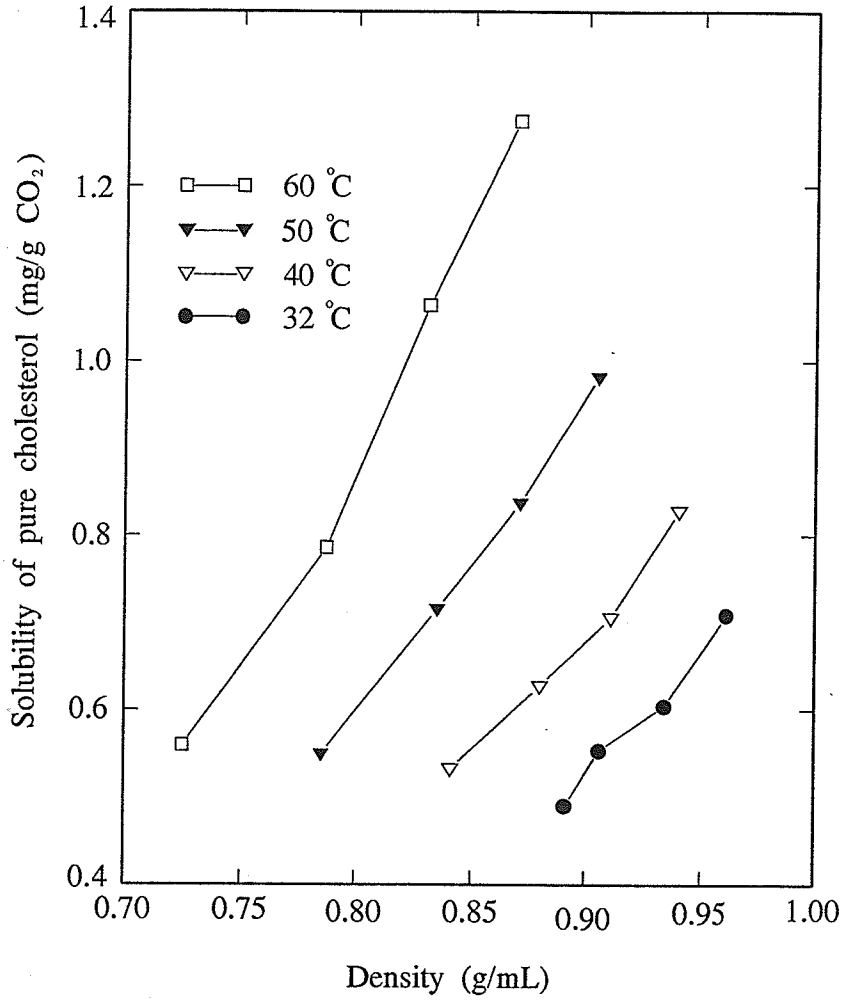

Figure 4.4 Solubility of pure cholesterol as a function of CO_2 density at four temperatures.

at four temperatures. It is apparent from this figure that for any density, the solubility increases with increasing temperature; at any temperature the solubility increases with increasing solvent density. These results support the general rules relating solvent power, temperature and solvent density proposed by Brogle (1982). The separation of these solubility isotherms reflects the vapour pressure effect.

From Figure 4.1 and Figure 4.2 it can be seen that the extraction of cholesterol should be carried out at a pressure greater than 20 MPa since the solubilities of cholesterol at 20 MPa are low, ranging from 0.49 to 0.56 mg/g CO_2.

So far the solubility of cholesterol in supercritical CO_2 has been measured at 40, 60 and 80 °C by Chrastil (1982), at 35, 40, and 60 °C by Wong and Johnston (1986), at 40, 50, and 60 °C by Yun et al. (1991), at 55 and 60°C by Lee et al. (1991), and at 40 and 55 °C by Yeh et al. (1991). However, there are significant differences among the solubilities reported by these researchers. Part of the differences may be attributed to the different methods used. Chrastil used an equilibrium method while other groups used a continuous flow method. The sampling methods and the determination methods of solubility used by these researchers are also different, but it is difficult to rationalize the differences in the data. The solubilities of cholesterol obtained in this study are consistently lower than those obtained by Yun et al. (1991), although a continuous flow method was used in both studies. Figure 4.5 provides a comparison of the two sets of data. The tendency of the variation in the solubility with pressure reported in these two studies is similar. The crossover pressure for cholesterol/SC CO_2 was observed by Yun et al. (1991) at 17 MPa. If my results (Figure 4.5) were extrapolated to 16 MPa, it

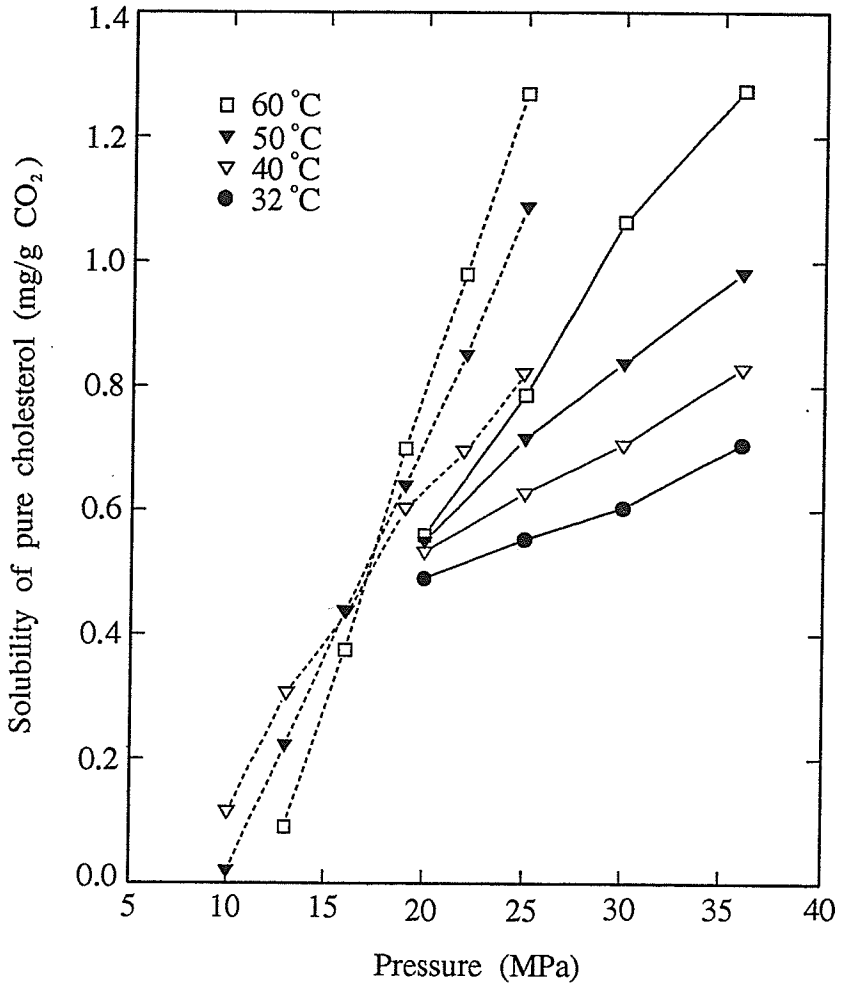

Figure 4.5 Comparison of cholesterol solubilities obtained by Yun et al. (1991) (dashed lines) with those obtained in this study (solid lines).

appears that they would produce a crossover point at about the same pressure. The solubilities of cholesterol in SC CO_2 at 40 °C and 60 °C determined in this study are significantly lower than those obtained by Wong and Johnston (1986) (Figure 4.6). I am unable to provide a reason for the differences among these two sets of data.

4.1.2 Cholesterol Solubility as a Function of Entrainer Concentration

The solubilities of pure cholesterol in SC CO_2 and ethanol mixtures at 40°C and 36 MPa were plotted as a function of ethanol concentration (Figure 4.7). The data points shown on the graph are single solubility values. The error bar represents the maximum standard error obtained for the slopes of the lines which could be drawn through the initial portion of the extraction curves used to calculate the solubilities. The figure indicates that as the ethanol concentration increases, the cholesterol solubility also increases. As the ethanol concentration increases from 0 to 10 %, the cholesterol solubility increases from 0.83 mg/g CO_2 to 4.0 mg/g CO_2, a 4.8-fold increase. Based on the mass balances carried out for each run, the solubility values may be inflated 4 to 6 % by weight due to trace amount of ethanol in the samples.

The increase in solvent power of CO_2 resulting from the addition of small amount of entrainer has been noted by Dobbs et al. (1987), Dobbs and Johnston (1987), Brunner and Peter (1982), Wong and Johnston (1986), VanAlsten et al. (1984), and Schmitt and Reid (1986). Dobbs et al. (1987) and VanAlsten et al. (1984) suggest that the solubility enhancement induced by the addition of entrainers is due to the increase in the density of the solvent mixture and the specific chemical interactions between the solutes and the

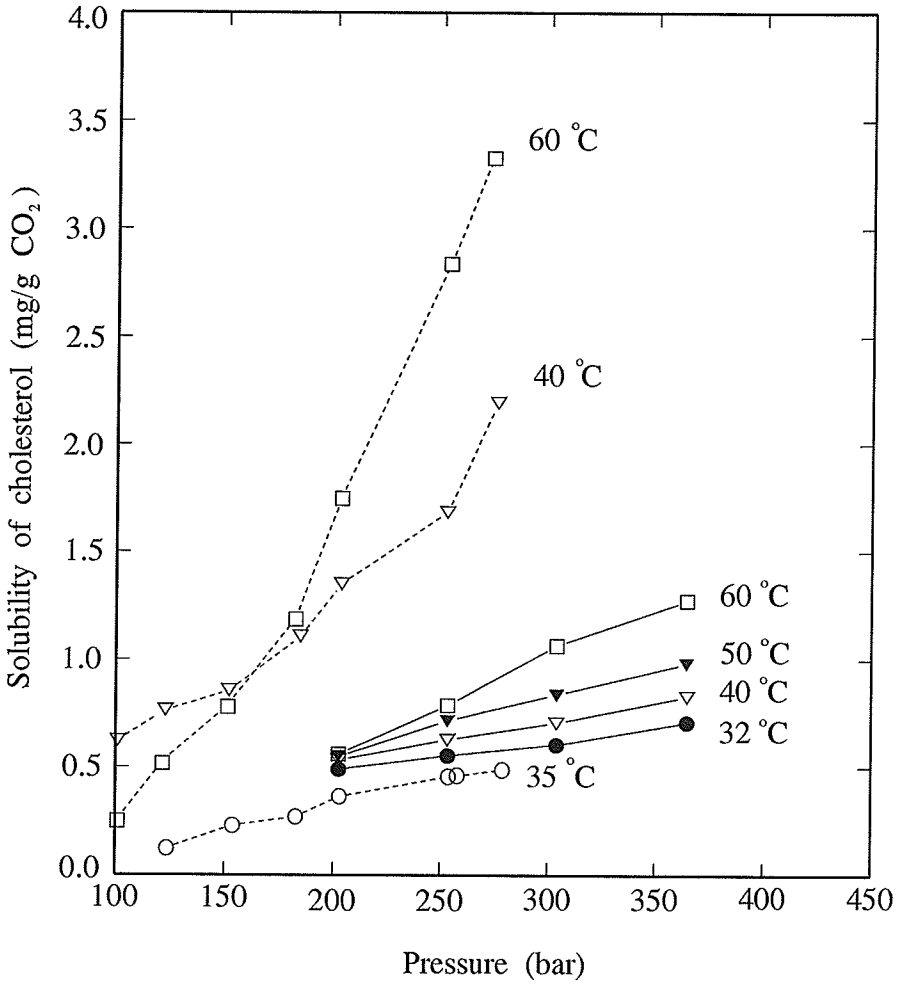

Figure 4.6 Comparison of cholesterol solubility data obtained by Wong and Johnston (1986) (dashed lines) with those obtained in this study (solid lines).

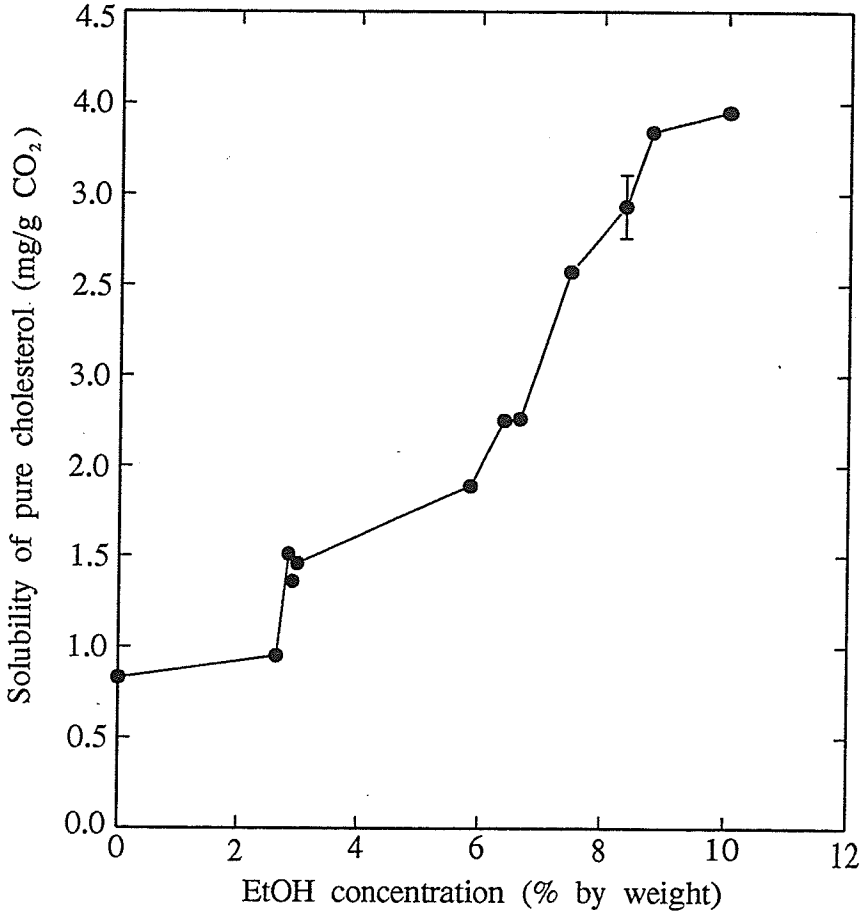

Figure 4.7 Solubility of pure cholesterol as a function of ethanol concentration at 40 ℃ and 36 MPa.

entrainer, such as hydrogen bonding, Lewis acid-base interaction. However, in the dense supercritical region (ρ_r, which is the ratio of CO_2 density at the extraction pressure and temperature to that at the critical point, is greater than 1.3), the modification in the density of the solvent due to the addition of an entrainer contributes only slightly to the solubility enhancement. In our study, the extractions of cholesterol were carried out in the dense supercritical region ($\rho_r > 2$), the solubility enhancement of cholesterol caused by the addition of ethanol could be attributed to the specific interactions between the solute cholesterol and the entrainer ethanol. Cholesterol has a hydroxyl group, which can form hydrogen bonds (one of Lewis acid-base interactions) with ethanol (Wong and Jonston 1986); and ethanol has a much greater tendency to donate a hydrogen bond than does carbon dioxide, therefore the solubility enhancement for cholesterol is expected when an ethanol-CO_2 mixture is used as solvent. This result is also in agreement with Wong and Johnston's (1986).

In addition to the specific chemical interactions, the supercritical solvent itself and the concentration of entrainer are also of importance (Walsh et al. 1987). The data of VanAlsten et al. (1984) and Schmitt and Reid (1986) show that the acid-base interactions are a secondary entrainer effect superimposed on a primary effect determined by cosolvent concentration. The results of Dobbs et al. (1987) also demonstrate that when the solute-cosolvent interaction constant exceeds the solute-solvent interaction constant by approximately a factor of 3, the solubility is a strong function of the cosolvent concentration. Our result, that the solubility of cholesterol increases with the ethanol concentration, agrees with the above observations.

4.2 Extraction of the Mixture of Cholesterol and Triolein

The equilibrium solubilities of cholesterol and triolein mixtures with the approximate weight ratios of 75:25, 50:50, and 25:75 were measured at 40°C and 36 MPa. The equilibrium solubility of pure triolein was also measured at the same temperature and pressure. About 10 grams of the mixtures were used for each run. Examples of the extraction curves are shown in Figure 4.8. The mass of the collected mixture increased linearly with the mass of CO_2 passed through the extractor during the course of the extraction. Thus, it is likely that the saturation had been achieved within the extractor. The total solubilities of the mixtures were calculated from the extraction curves using the method described in section 3.4.3. The mean values from two runs for each of the three mixtures are 7.51, 7.84, and 6.96 mg/g, respectively. To evaluate the effect of the mixing of the two components on the solubility of each individual component, the sequentially collected extracts were subsequently analyzed on HPLC to determine the mass fraction of each component in the extracts. The corresponding extraction curves for cholesterol and triolein were plotted in Figure 4.9, Figure 4.10, and Figure 4.11. Cholesterol solubilities obtained from these curves are 2.49, 3.03, and 2.21 mg/g. Triolein solubilities are 5.08, 4.62, and 5.23 mg/g. It can be seen from Figure 4.12 that the total solubilities of the mixtures, cholesterol solubility, and triolein solubility do not vary significantly with the composition of the mixture over the composition range studied. The addition of triolein into cholesterol increased the solubility of cholesterol in SC CO_2 by a factor of 1.6 - 2.6 fold compared with its solubility in SC CO_2 without triolein present. However, the solubility of triolein itself was reduced by a factor of 1.8 - 2.2 fold compared with the

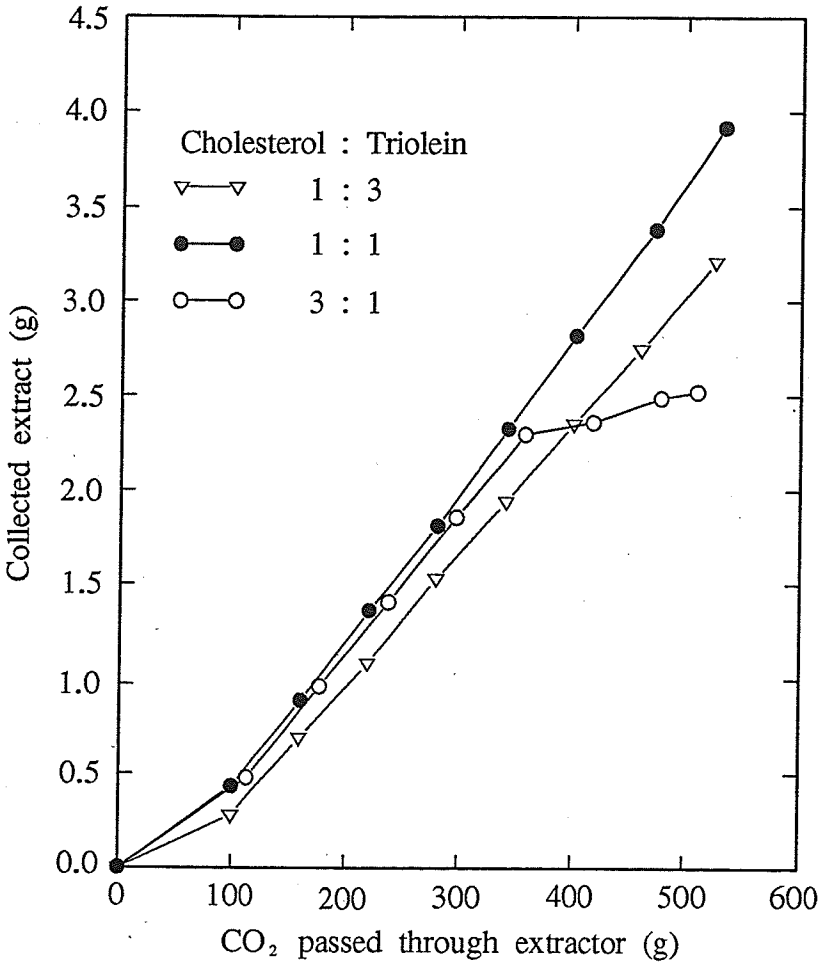

Figure 4.8 Extraction curves for cholesterol and triolein mixtures with approximate weight ratios of 75:25, 50:50, and 25:75. The extractions were performed from a glass bead matrix. Extraction conditions: temperature 40 °C, pressure 36 MPa, flow rate 2.0 g/min, extractor 55 mL.

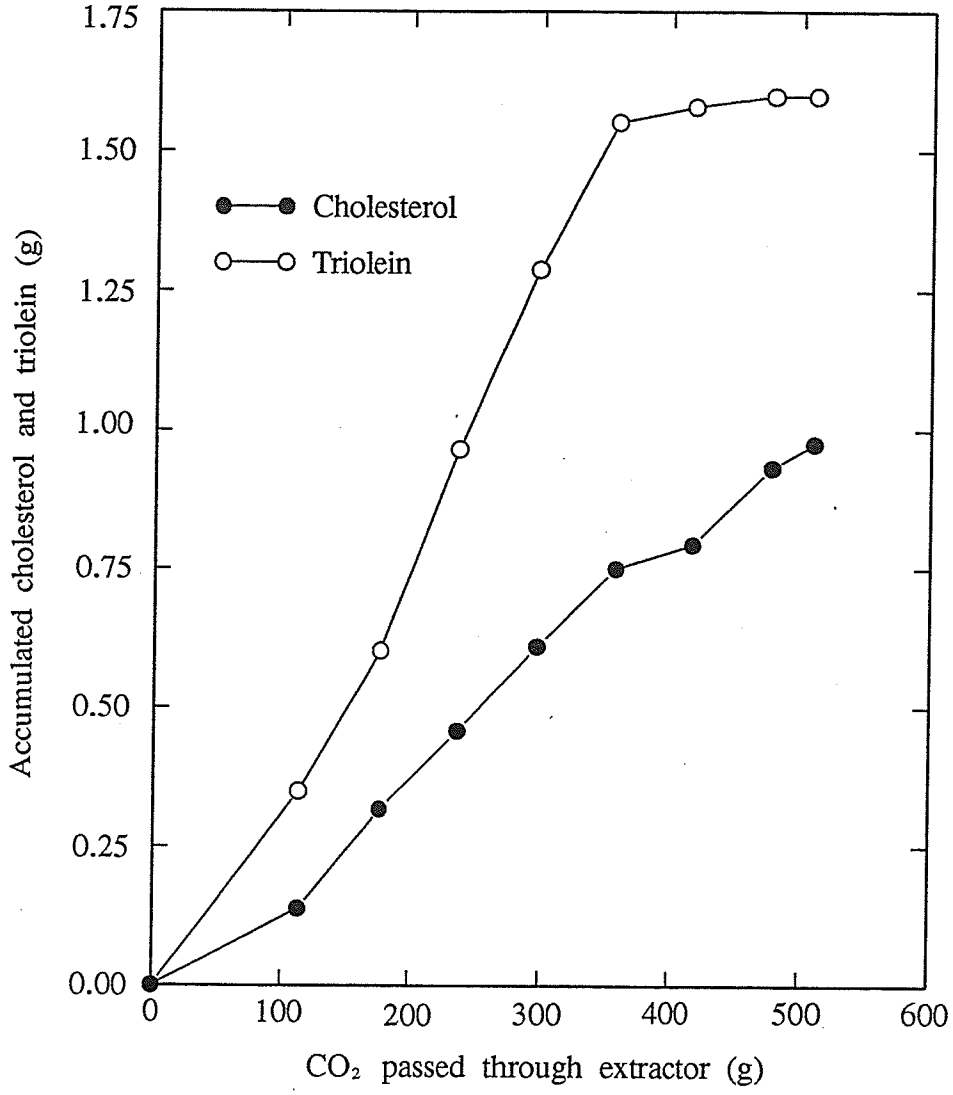

Figure 4.9 Extraction curves for cholesterol and triolein when extraction was performed on their mixture with weight ratio of 75:25. Extraction conditions: temperature 40 °C, pressure 36 MPa, flow rate 2.0 g/min, extractor 55 mL.

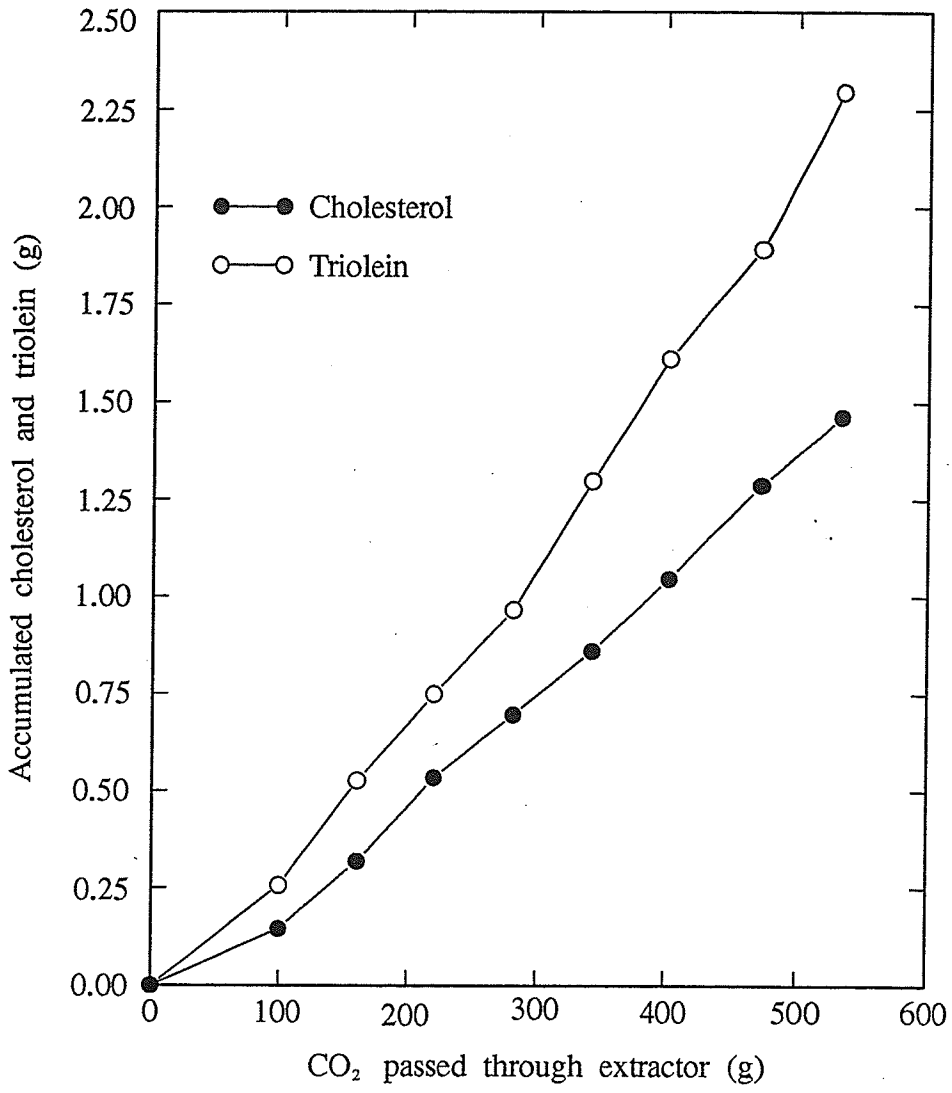

Figure 4.10 Extraction curves for cholesterol and triolein when extraction was performed on their mixture with weight ratio of 50:50. Extraction conditions: temperature 40 °C, pressure 36 MPa, flow rate 2.0 g/min, extractor 55 mL.

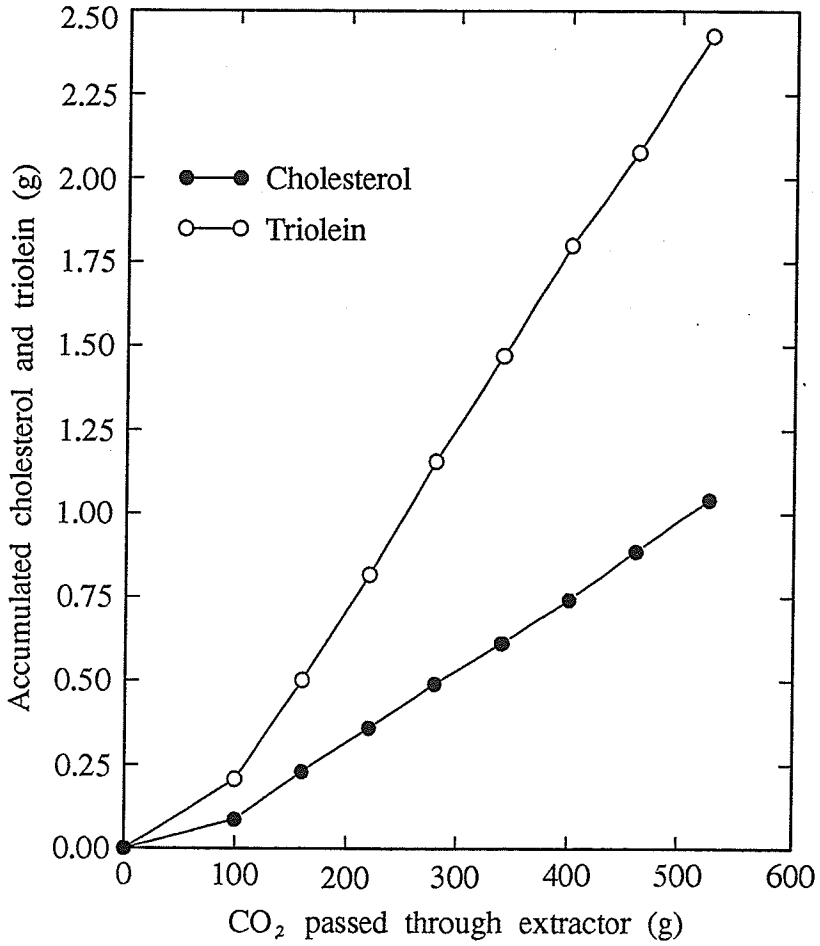

Figure 4.11 Extraction curves for cholesterol and triolein when extraction was performed on their mixture with weight ratio of 25:75. Extraction conditions: temperature 40 °C, pressure 36 MPa, flow rate 2.0 g/min, extractor 55 mL.

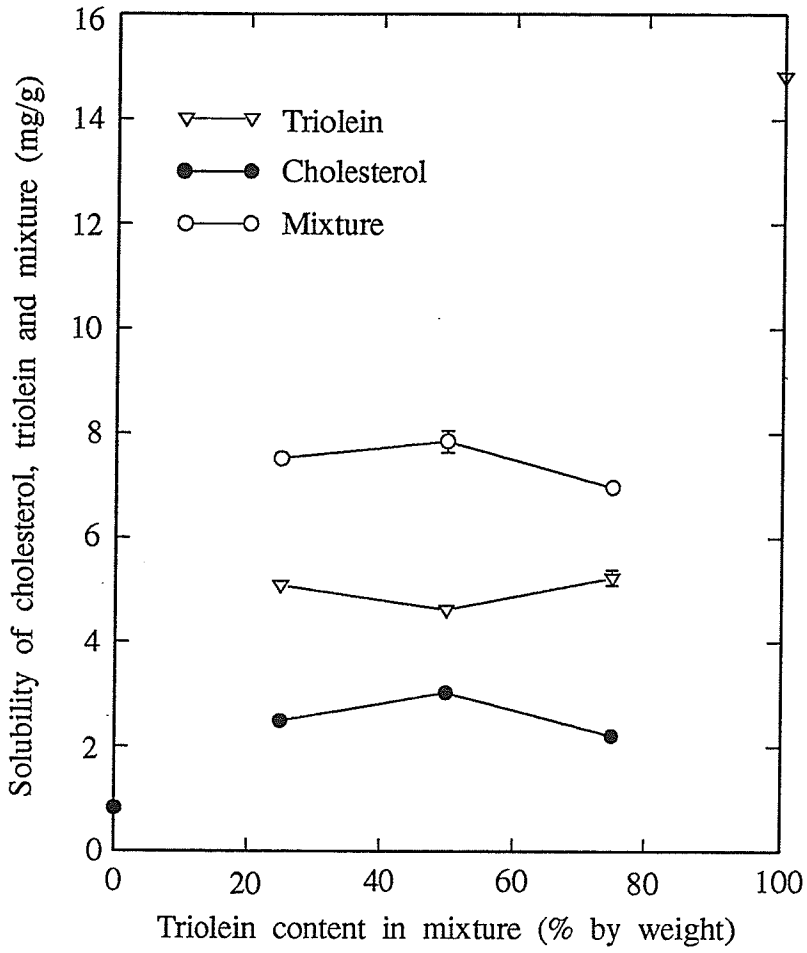

Figure 4.12 Solubility of cholesterol and triolein mixture, cholesterol, and triolein present in mixture as a function of triolein content in mixture. Extraction conditions: temperature 40 °C, pressure 36 MPa, flow rate 2.0 g/min, extractor 55 mL.

solubility of pure triolein in SC CO_2. From these results it can be concluded that more soluble compounds increase the solubilities of less soluble compounds in SC CO_2 when mixed with less soluble compounds. It has been reported that the presence of more soluble compounds in the supercritical fluid phase helps to solubilize the less soluble compounds (Kosal et al. 1992).

Fattori (1986) has reported that the solubility of mixtures of homologous monounsaturated triglycerides can be predicted using a modified version of Raoult's equation:

$$S_t = \Sigma X_i * S_i$$

where S_t is the total solubility of the mixture in SC CO_2 (g/g); S_i is the solubility of the pure substance in SC CO_2 (g/g); and X_i is molar fraction of the pure substance in the mixture.

To examine whether the total solubility of the mixture of triolein and cholesterol can also be described by the above equation, the calculated and measured solubility values of each component and the mixture were compared (Table 4.1). The calculated solubility value of each component was obtained by multiplying the solubility of each pure component in SC CO_2 by its molar fraction in the mixture. From the information displayed in Table 4.1, it is evident that the calculated values are not close to the measured ones. This result indicates that the mixture of cholesterol and triolein did not behave in an ideal manner as Fattori described. This phenomena is not surprising. Because cholesterol is a solid, triolein is a liquid and they are miscible and structurally different, the molecular interaction between them could be significant. For cholesterol-

triolein-CO_2 system, the total solubility of mixture can not be simply predicted by the composition of the mixture and the solubility of each pure component in SC CO_2.

Table 4.1 Comparison of calculated and measured solubility values for the mixture of cholesterol and triolein and individual component present in the mixture. Extraction conditions: temperature 40°C; pressure 36 MPa; flow rate 2.0 g/min.

Name of Solute	Molar Fraction in Mixture	Solubility of Pure Component (mg/g)	Calculated Solubility (mg/g)	Measured Solubility (mg/g)
Cholesterol	0.837	0.83	0.72	2.49
Triolein	0.127	14.8	1.88	5.08
Mixture			2.60	7.51
Cholesterol	0.696	0.83	0.58	3.03
Triolein	0.304	14.8	4.50	4.62
Mixture			5.08	7.84
Cholesterol	0.433	0.83	0.36	2.21
Triolein	0.567	14.8	8.40	5.23
Mixture			8.76	6.96

4.3 Extraction of Freeze-dried Egg Yolk Using SC CO_2

To investigate the optimum operating pressure and temperature for removing cholesterol from freeze-dried egg yolk by SC CO_2 extraction, freeze-dried egg yolk was extracted using SC CO_2 in the temperature range of 32 - 60 °C and the pressure range of 20 - 36 MPa. The total solubilities of egg yolk lipids were determined from the extraction curves using the method described in section 3.4.3. The solubilities of egg yolk triglycerides and cholesterol were determined using the method described in section 3.4.4.2.

4.3.1 Effects of Temperature and Pressure on Total Solubility of Egg Yolk Lipids in SC CO_2

The total solubility of lipids as a function of temperature at different pressures is shown in Figure 4.13. The data points shown on the graph are arithmetic means of the experimental values. The error bar shown on each curve is the maximum standard error for the set of solubility means. It is evident that the effect of changes in temperature on the solubility of the lipids in CO_2 changes with pressure. At 30 MPa, the total solubility of lipids with temperature changes from 32°C to 60°C is almost constant. At pressures below 30 MPa (20, 25 MPa), the total solubility of lipids decreases with increasing temperature, whereas at 36 MPa the solubility increases with increasing temperature. This temperature effect is not unusual and has been reported for soybean oil dissolved in SC CO_2 (Friedrich et al. 1982), naphthalene dissolved in supercritical ethylene (Williams 1981) and in CO_2 (de Fillippi 1982), and cholesterol dissolved in supercritical CO_2 (Yun et al. 1991). A rationale for the observation has been given in section 4.1.1.

In Figure 4.14 the total solubility of lipids in SC CO_2 is plotted as a function of pressure at four temperatures. The figure indicates that for a fixed temperature, as the pressure of CO_2 increases, the total solubility of lipids also increases. The solubility isotherms intersect at approximate 30 MPa. It can be seen that excellent separation of lipids and CO_2 solvent could be achieved by a simple pressure reduction. At 32°C, when the pressure is reduced from 36 MPa (solubility = 11.0 mg/g CO_2) to 20 MPa (solubility = 4.38 mg/g CO_2), 60% of the dissolved lipids are separated from the CO_2. The same pressure drop at 60°C will yield 86% of the dissolved lipids. Labay (1991) has reported

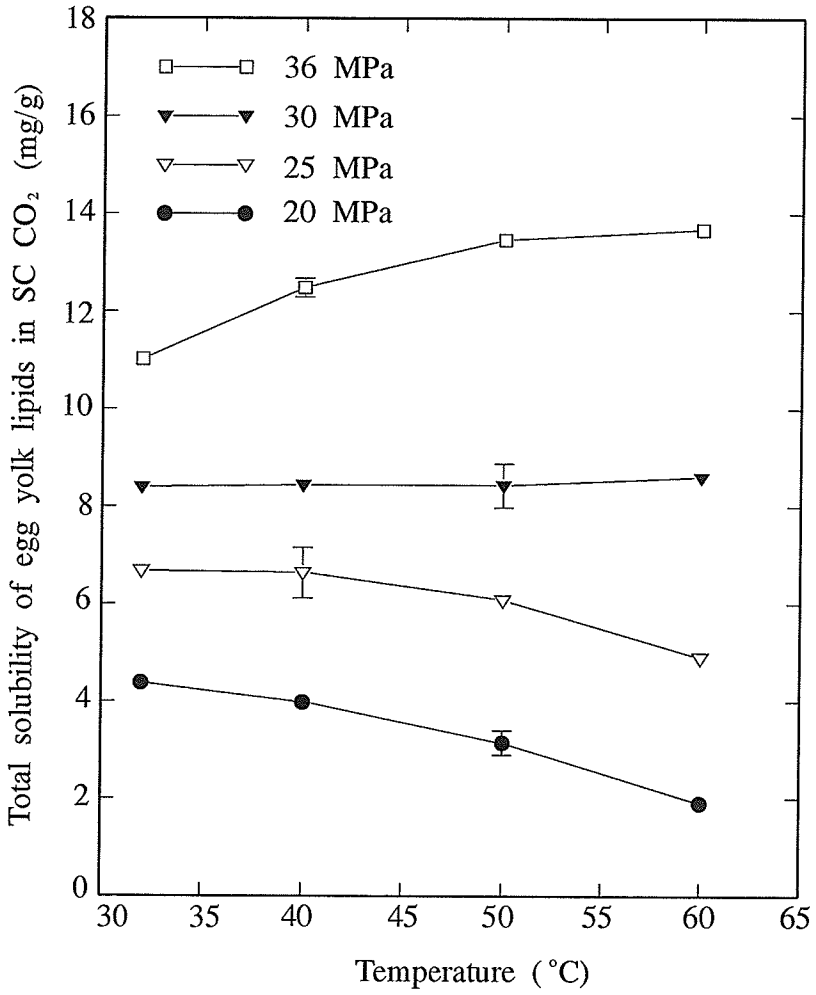

Figure 4.13 Total solubility of egg yolk lipids in SC CO_2 as a function of temperatue at four pressures.

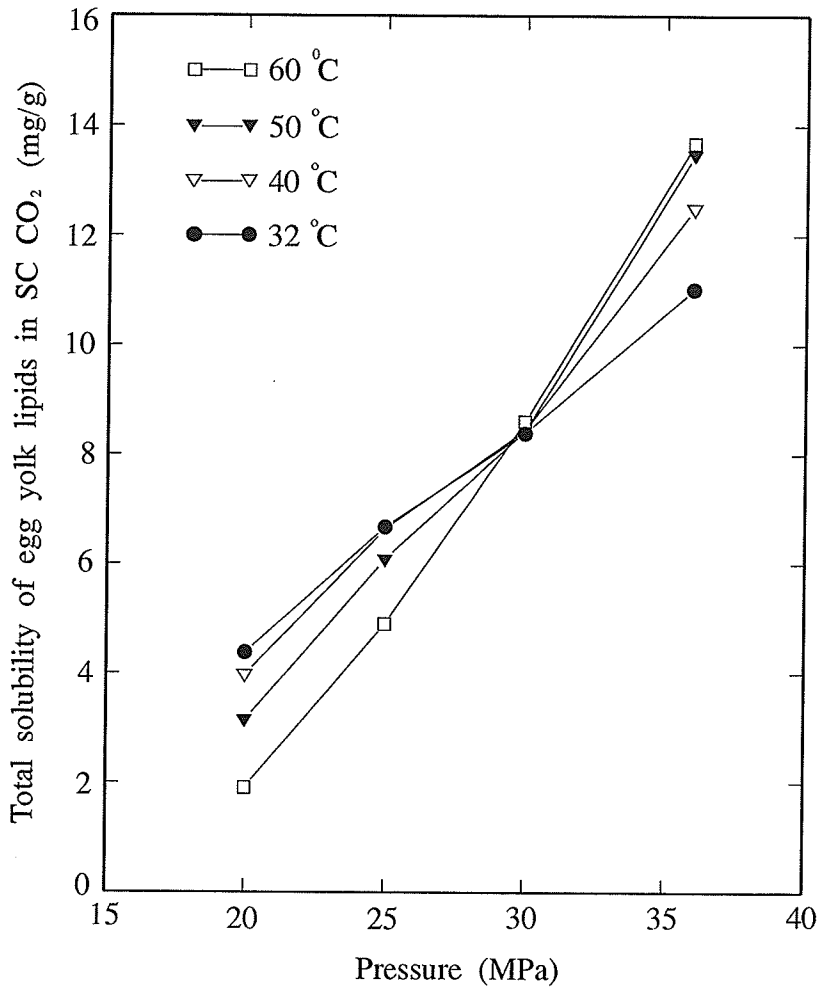

Figure 4.14 Total solubility of egg yolk lipids in SC CO_2 as a function of pressure at four temperatures.

that the total solubility of lipids in SC CO_2 at 40°C and 15 MPa is 0.67 mg/g CO_2 and that at pressures below 10 MPa, yolk lipid solubility is extremely low (< 0.01 mg/g CO_2) (Labay, 1991). Thus, a pressure reduction to about 15 MPa will recover most of the lipids and a reduction to atmospheric pressure is not necessary to achieve an excellent separation of lipids and CO_2. In a re-circulating extraction system, the energy costs can be greatly reduced if the CO_2 requires recompression from only 15 MPa to 36 MPa instead of from atmospheric to 36 MPa. The moderate changes in solubility for changes in temperature of up to 28 C° as shown in Figure 4.13 indicate why lipids can not be effectively separated from CO_2 solely by a temperature change in this system.

The total solubility of lipids can be expressed as a function of the density of CO_2. In Figure 4.15, the interactive effects of temperature and pressure changes become much clearer and illustrate the very close connection between lipid solubility and CO_2 density. This pattern has been reported previously for cholesterol in section 4.1.1. In this study, the highest total solubility of egg yolk lipids in SC CO_2 was observed at 60°C/36MPa. However, a burning smell was observed during the extraction process and the raffinate was a dark brown color. These phenomena show that some chemical changes were taking place during the process and the extraction of egg yolk lipids should not be carried out at temperatures as high as 60°C.

4.3.2 Composition Analysis of Extracts from SC CO_2 Extraction

For each set of extraction temperatures and pressures, four extraction samples from the early, intermediate, and late stages of the run were selected and analyzed for

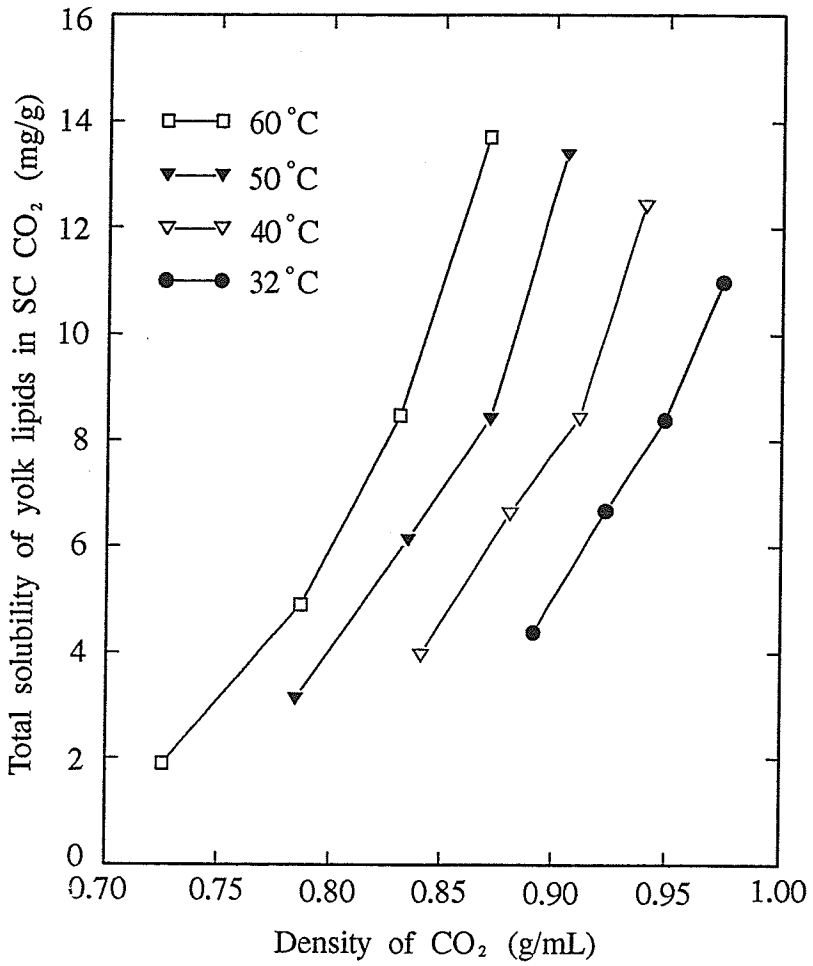

Figure 4.15 Total solubility of egg yolk lipids in SC CO_2 as a function of CO_2 density at four temperatures.

triglyceride content and cholesterol content.

The composition of each analyzed extract is shown in Table 4.2. From the information displayed in this table, it is noticed that the triglycerides and cholesterol account for 85 to 95% of the extracts collected at 20, 25, and 30 MPa. Changes in temperature at these pressures do not change the overall content of the samples collected. It is possible that the unidentified materials such as diglycerides, sterol esters, pigments, and vitamin alcohols could account for the unidentified 5 to 15% of the extracts. It is also possible that the experimental error as a result of the UV detector not measuring the triglycerides with saturated fatty acids accounts for a part of the unidentified percentage. For the runs at 36 MPa, the overall lipid content in the extracts collected varies over a much greater range (77.4 - 98.4%). There is no readily explainable cause for these variations at this time.

The partition coefficient for triglycerides, i.e., the ratio of triglyceride concentration in the extract to its concentration in the original egg yolk sample, ranges between 1.55 and 2.24; the partition coefficient for cholesterol from 1.22 and 4.24. These data show that triglycerides and cholesterol can be concentrated from egg yolk substrate by SC CO_2 extraction.

4.3.3 Effect of Temperature and Pressure on Solubilities of Egg Yolk Triglycerides and Cholesterol in SC CO_2

The solubility of egg yolk triglycerides is plotted as a function of temperature and pressure in Figure 4.16 and Figure 4.17, respectively. The error bar shown on each curve

Table 4.2 Composition of the CO_2 extracts of egg yolk and the partition coefficients of their components.

Extraction Conditions		Extract Number	Specific CO_2 (g/g sample)	Triglycerides Concentration (%)	Cholesterol Concentration (%)	Partition Coefficient for TG*	Partition Coefficient for CHO*
Pressure (MPa)	Temperature (°C)						
36	32	2	3.50	65.6	10.7	1.56	2.83
		5	8.70	67.3	10.2	1.60	2.70
		12	20.9	70.5	10.7	1.68	2.83
		15	26.1	65.0	9.55	1.55	2.52
36	40	2	2.88	68.2	8.99	1.62	2.37
		5	7.24	78.2	8.77	1.86	2.31
		12	17.2	83.5	11.6	1.99	3.06
		15	21.5	68.9	10.9	1.64	2.88
36	50	2	2.92	63.2	11.7	1.50	3.09
		5	7.27	81.4	13.3	1.94	3.51
		12	17.4	70.5	13.0	1.68	3.42
36	60	2	3.26	80.4	16.1	1.91	4.24
		5	8.07	73.1	15.4	1.74	4.07
		12	19.4	93.6	11.1	2.23	2.93
		15	24.2	92.5	11.5	2.20	3.02
30	32	2	5.03	84.6	5.61	2.02	1.48
		3	7.54	85.7	5.56	2.04	1.47
		11	27.6	87.7	5.99	2.09	1.58
		12	30.1	90.3	5.62	2.15	1.48
30	40	2	5.17	81.7	7.79	1.95	2.06
		3	7.74	73.6	6.48	1.75	1.71
		12	30.9	78.0	7.14	1.86	1.88
		13	33.5	90.8	6.75	2.16	1.78
30	50	2	4.74	93.5	8.61	2.23	2.27
		3	7.12	79.3	10.3	1.89	2.73
		13	30.8	84.2	5.96	2.01	1.57
		14	33.2	87.5	6.02	2.08	1.59
30	60	2	4.42	71.2	9.21	1.69	2.43
		3	6.62	77.8	8.30	1.85	2.19
		11	24.3	85.0	6.93	2.02	1.83
		12	26.5	86.4	7.34	2.06	1.94

Table 4.2 Composition of the CO_2 extracts of egg yolk and the partition coefficients of their components (cont'd).

Extraction Conditions Pressure (MPa)	Extraction Conditions Temperature (°C)	Extract Number	Specific CO_2 (g/g sample)	Triglycerides Concentration (%)	Cholesterol Concentration (%)	Partition Coefficient for TG*	Partition Coefficient for CHO*
25	32	2	6.01	91.2	5.07	2.17	1.34
		3	8.58	83.6	4.62	1.99	1.22
		13	34.3	91.6	5.99	2.18	1.58
		14	36.9	92.7	5.61	2.21	1.48
25	40	2	5.49	76.9	7.59	1.83	2.00
		3	7.83	76.1	5.73	1.81	1.51
		13	31.3	74.4	5.77	1.77	1.52
		14	33.7	87.4	6.62	2.08	1.75
25	50	2	5.27	89.8	8.30	2.14	2.19
		3	7.54	78.5	7.18	1.87	1.89
		11	25.6	73.7	7.49	1.76	1.98
		12	27.9	72.0	8.03	1.72	2.12
25	60	2	6.16	70.2	9.11	1.67	2.40
		3	9.23	79.9	11.0	1.90	2.89
		12	35.4	91.0	6.22	2.17	1.64
		13	38.2	94.1	5.91	2.24	1.56
20	32	2	8.38	73.5	6.89	1.75	1.82
		3	13.4	90.3	7.21	2.15	1.90
		9	43.6	88.5	6.75	2.11	1.78
		10	48.6	79.4	5.83	1.89	1.54
20	40	2	7.78	80.0	11.1	1.90	2.92
		3	12.4	78.4	9.08	1.87	2.40
		9	40.4	81.3	7.89	1.93	2.08
		10	47.1	84.8	13.1	2.02	3.47
20	50	2	7.86	76.0	13.7	1.81	3.63
		3	12.6	83.8	11.8	2.00	3.11
		9	40.9	74.8	8.39	1.78	2.21
		10	45.6	81.7	11.2	1.95	2.96
20	60	2	7.63	82.7	13.0	1.97	3.42
		3	12.2	73.4	11.7	1.75	3.09
		9	39.6	77.8	12.3	1.85	3.25
		10	44.2	86.8	11.7	2.07	3.10

* CHO: cholesterol; TG: triglycerides.

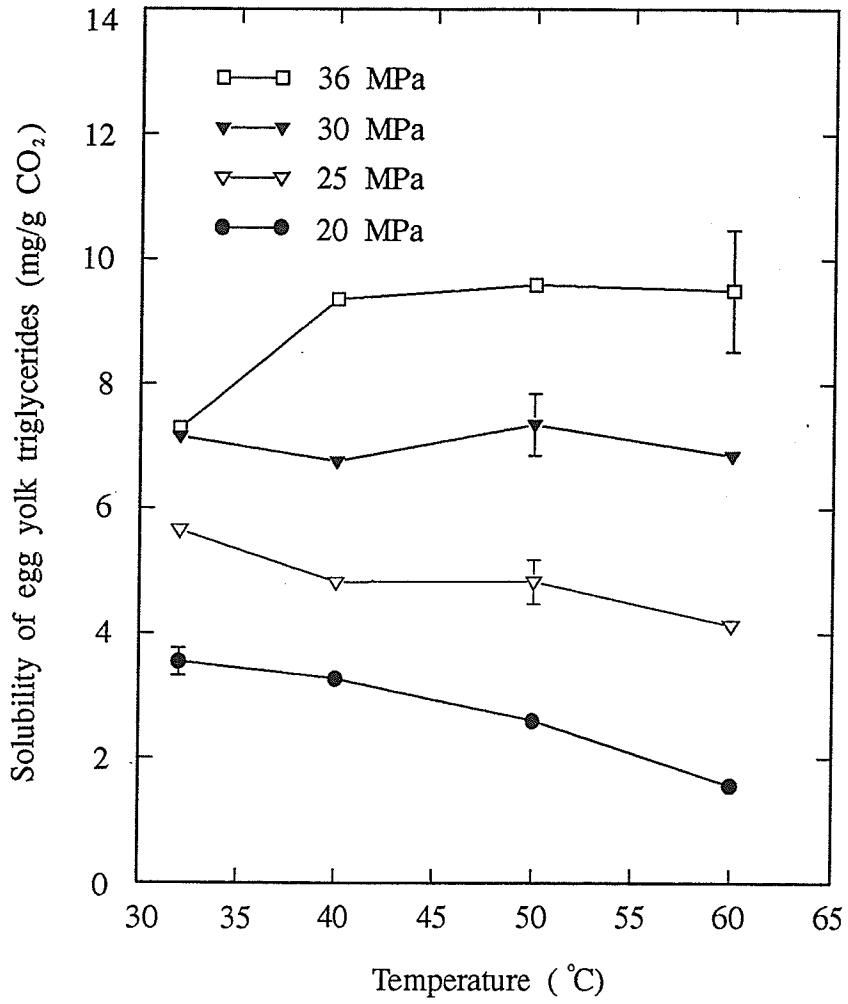

Figure 4.16 Solubility of egg yolk triglycerides in SC CO_2 as a function of temperature at four pressures.

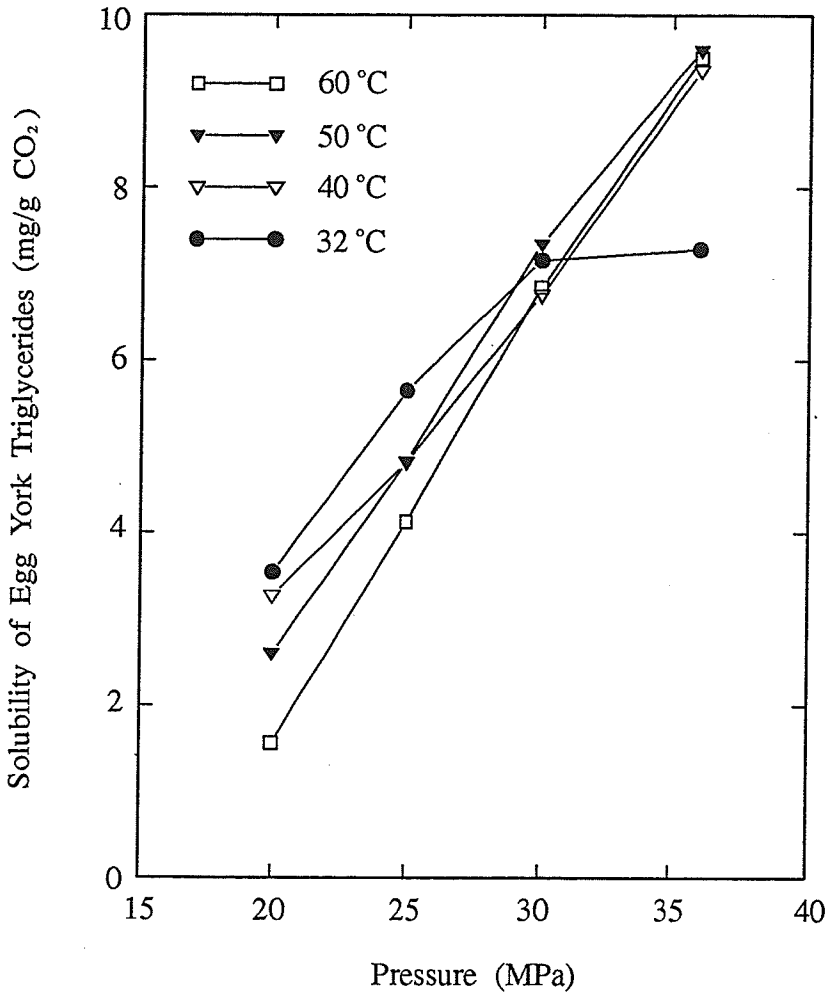

Figure 4.17 Solubility of egg yolk triglycerides in SC CO_2 as a function of pressure at four temperatures.

in the Figure 4.16 represents the maximum standard error for the set of solubilities. These two figures indicate that the effects of pressure and temperature on the solubility of egg yolk triglycerides follow similar patterns reported previously for the total solubility of lipids. This is not unexpected since the triglycerides make up about 63 % of egg yolk lipids. The solubility isotherms of triglycerides do not intersect at a fixed location as previously shown for the total lipids. This could be attributed to the interactions between the components present in egg yolk or that triglycerides are a mixture. The solubility of egg yolk cholesterol is plotted as a function of temperature and pressure in Figure 4.18 and Figure 4.19, respectively. There are small variations in the solubility of cholesterol with the temperature at the extraction pressures equal to or below 30 MPa. At 36 MPa, the solubility of cholesterol increases with increasing temperature. Figure 4.19 shows that the solubility of egg yolk cholesterol in SC CO_2 increases fairly slowly with increasing pressure when the pressure of CO_2 is below 30 MPa. As the pressure of CO_2 is increased to 36 MPa, the solubility of cholesterol increases dramatically, from 0.47 - 0.69 mg/g CO_2 at 30 MPa to 1.12 - 1.69 mg/g CO_2 at 36 MPa.

4.3.4 Effect of Other Components Present in Egg Yolk Lipids on the Solubility of Cholesterol

For the sake of discussing other components present in egg yolk lipids on the solubility of cholesterol, the solubilities of pure cholesterol and egg yolk cholesterol are plotted against temperature at four pressure levels in Figure 4.20. At pressures of 20, 25, and 30 MPa, the solubility of pure cholesterol in SC CO_2 is 20 to 133% higher than that

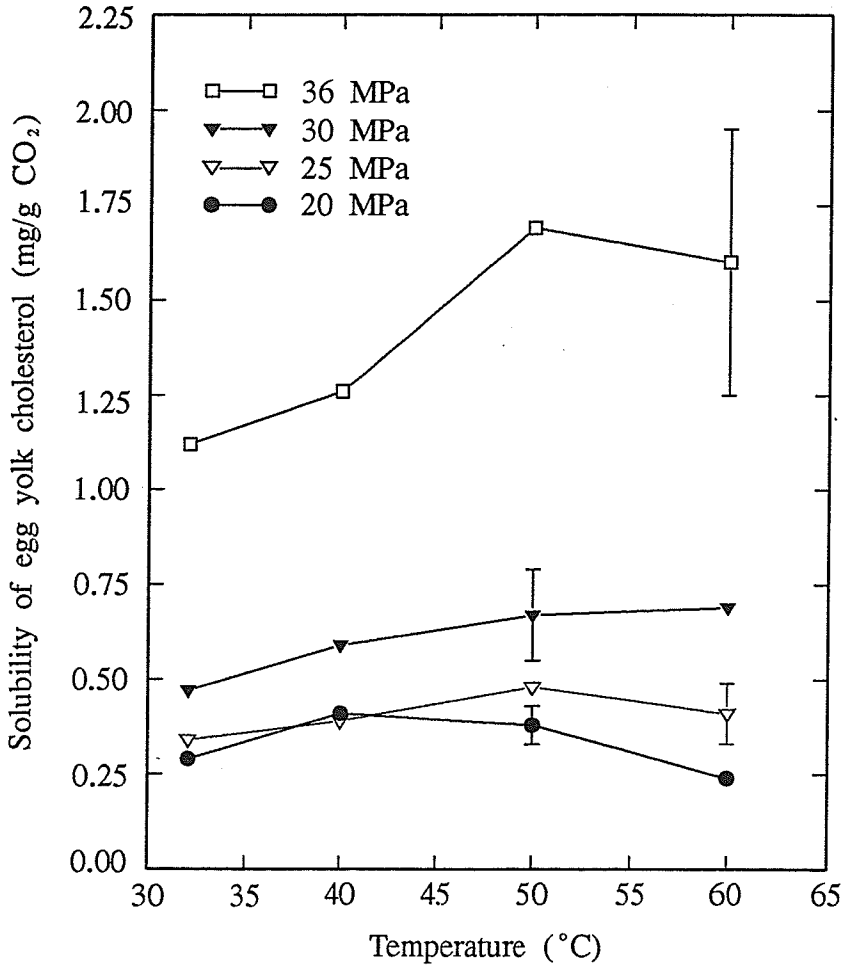

Figure 4.18 Solubility of egg yolk cholesterol in SC CO_2 as a function of temperature at four pressures.

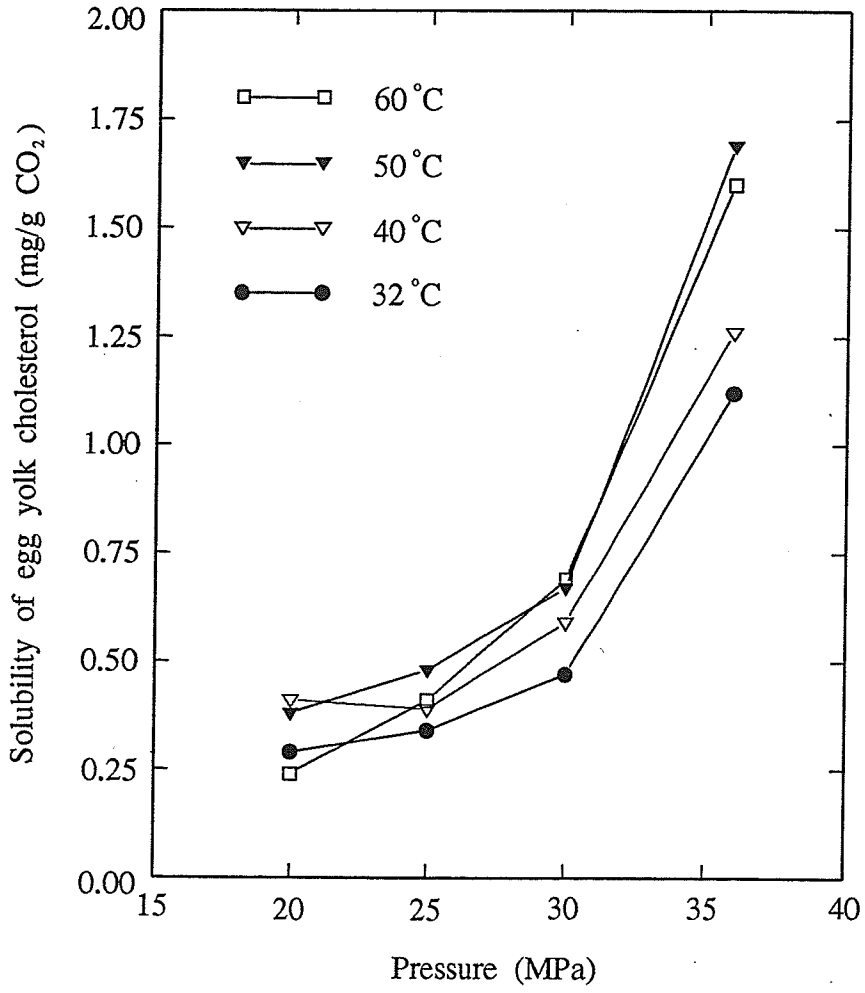

Figure 4.19 Solubility of egg yolk cholesterol in SC CO_2 as a function of pressure at four temperatures.

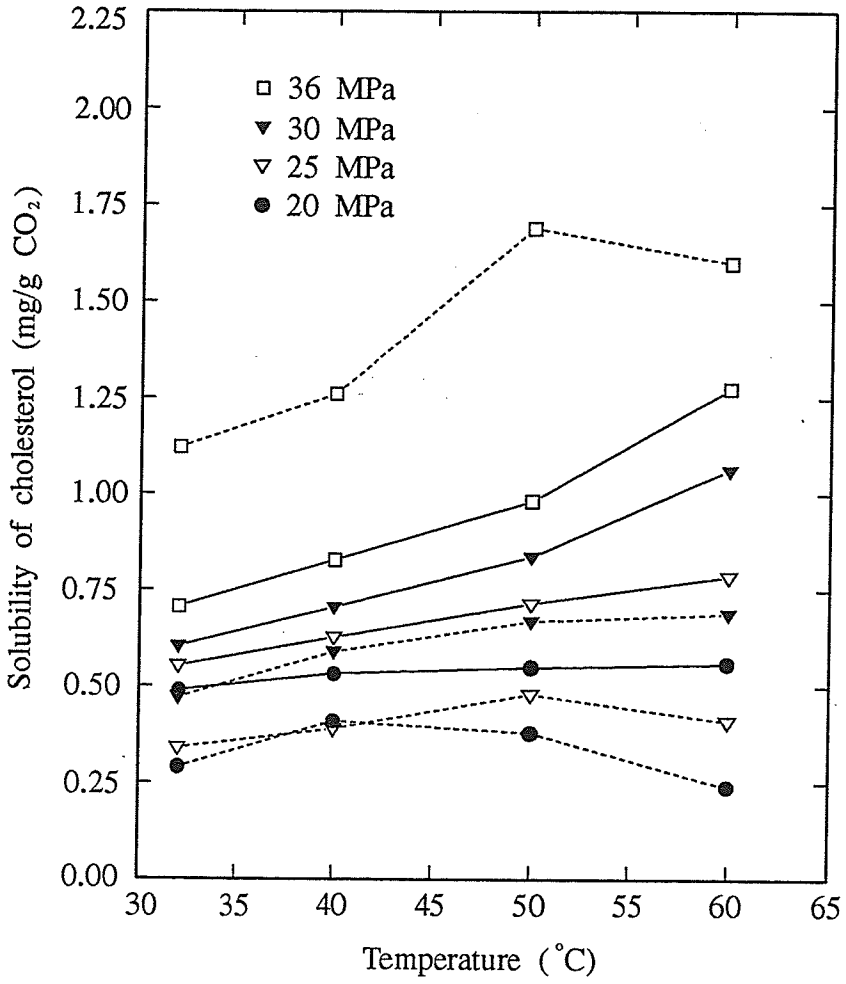

Figure 4.20 Comparison of solubilities of pure cholesterol (solid lines) and egg yolk cholesterol (dashed lines) in SC CO_2 at various temperatures and pressures.

of egg yolk cholesterol, which is in association with other egg yolk lipids. At 36 MPa, the solubility of pure cholesterol in SC CO_2 is 25 to 72% lower than that of egg yolk cholesterol, which agrees with the result reported in Section 4.2 that the solubility of cholesterol is enhanced due to the presence of triglycerides in the substrate. The observed results could be explained if cholesterol and triglycerides have a strong repulsive interaction in SC CO_2. The low extraction pressures are unable to overcome the strong repulsive interaction between cholesterol and triglycerides and lead to the decrease in cholesterol solubility. The high extraction pressures are able to overcome the repulsive interaction and result in the increase in cholesterol solubility. Schaeffer et al. (1988) postulated a similar hypothesis to explain their results for monocrotaline. They reported that the solubility of monocrotaline in the presence of other plant material was smaller by 50% to 98% when compared with the solubility of pure monocrotaline in SC CO_2. They thought that the depression of the monocrotaline solubility in the presence of the plant material indicated that the lipids and monocrotaline have a strong repulsive interaction in the fluid phase resulting in a decrease in the solubility of the less volatile component - monocrotaline.

4.3.5 Effect of Temperature and Pressure on Selectivity of SC CO_2 for Cholesterol over Triglycerides

Since one of the main purposes of this work is to investigate the optimum extraction temperature and pressure for selectively removing cholesterol from egg yolk, the selectivity of SC CO_2 for cholesterol over other components in egg yolk is the key

criteria and even more important than the solubilities of the components in SC CO_2. In this study, the selectivity of SC CO_2 for one component over another component is defined as the ratio of their partition coefficients. The selectivity of SC CO_2 for cholesterol over triglycerides was calculated in terms of this definition and listed in Table 4.3. It can be seen from Table 4.3 that the selectivity of SC CO_2 for cholesterol over triglycerides is almost constant during each individual run with the exception of the two runs at 60°C/36 MPa and 60°C/25 MPa. The variations in the selectivity during these two runs could be due to the chemical changes of egg yolk components at high temperature.

The average selectivity of SC CO_2 for cholesterol over triglycerides during each run is plotted against temperature in Figure 4.21 and against pressure in Figure 4.22. Figure 4.21 shows that the selectivity generally increases with the increases in temperature at all pressures examined except for 36 MPa. Figure 4.22 shows that at a fixed temperature, the selectivity decreases as the pressure increases from 20 to 25 MPa. The selectivity insignificantly changes with increasing pressure from 25 MPa to 30 MPa and then increases as the pressure increases from 30 to 36 MPa. These variations in the selectivity with temperature and pressure are best understood based on the effects of temperature and pressure on the solubilities of the solutes. The solubilities of different solutes change with temperature and pressure in different rates, resulting in the changes in the selectivity of SC CO_2 with temperature and pressure. Similar results have been reported by Schaeffer et al. (1988) and Temelli et al. (1988). Schaeffer et al. observed that the selectivity of CO_2 for monocrotaline increased with increasing temperature and with decreasing pressure. Temelli et al. (1988) observed that the selectivity of CO_2 for

Table 4.3 Selectivity of SC CO_2 for cholesterol over triglycerides at various temperatures and pressures.

Extraction Conditions		Extract	Specific	Selectivity of CO_2
Pressure (MPa)	Temperature (°C)	Number	CO_2 (g/g sample)	for CHO over TG*
36	32	2	3.50	1.81
		5	8.70	1.69
		12	20.9	1.69
		15	26.1	1.63
36	40	2	2.88	1.46
		5	7.24	1.24
		12	17.2	1.54
		15	21.5	1.76
36	50	2	2.92	2.06
		5	7.27	1.81
		12	17.4	2.04
36	60	2	3.26	2.22
		5	8.07	2.34
		12	19.4	1.32
		15	24.2	1.37
30	32	2	5.03	0.73
		3	7.54	0.72
		11	27.6	0.76
		12	30.1	0.69
30	40	2	5.17	1.06
		3	7.74	0.97
		12	30.9	1.01
		13	33.5	0.82
30	50	2	4.74	1.02
		3	7.12	1.44
		13	30.8	0.78
		14	33.2	0.76
30	60	2	4.42	1.43
		3	6.62	1.18
		11	24.3	0.90
		12	26.5	0.94

Table 4.3 Selectivity of SC CO_2 for cholesterol over triglycerides at various temperatures and pressures (cont'd).

Extraction Conditions		Extract	Specific	Selectivity of CO_2
Pressure (MPa)	Temperature (°C)	Number	CO_2 (g/g sample)	for CHO over TG*
25	32	2	6.01	0.62
		3	8.58	0.61
		13	34.3	0.72
		14	36.9	0.67
25	40	2	5.49	1.09
		3	7.83	0.83
		13	31.3	0.86
		14	33.7	0.84
25	50	2	5.27	1.02
		3	7.54	1.01
		11	25.6	1.13
		12	27.9	1.24
25	60	2	6.16	1.44
		3	9.23	1.52
		12	35.4	0.76
		13	38.2	0.70
20	32	2	8.38	1.04
		3	13.4	0.89
		9	43.6	0.84
		10	48.6	0.81
20	40	2	7.78	1.53
		3	12.4	1.28
		9	40.4	1.08
		10	47.1	1.72
20	50	2	7.86	2.00
		3	12.6	1.56
		9	40.9	1.24
		10	45.6	1.52
20	60	2	7.63	1.74
		3	12.2	1.77
		9	39.6	1.75
		10	44.2	1.50

* CHO: cholesterol; TG: triglycerides.

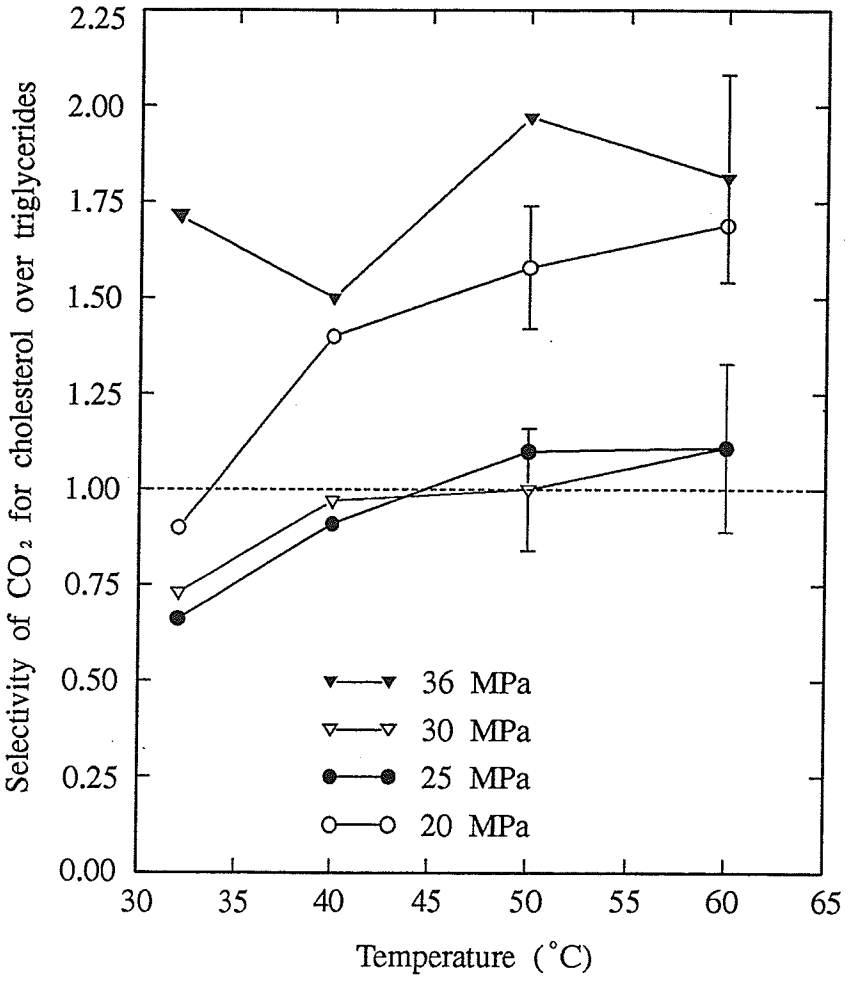

Figure 4.21 Selectivity of SC CO_2 for cholesterol over triglycerides as a function of temperature at four pressures.

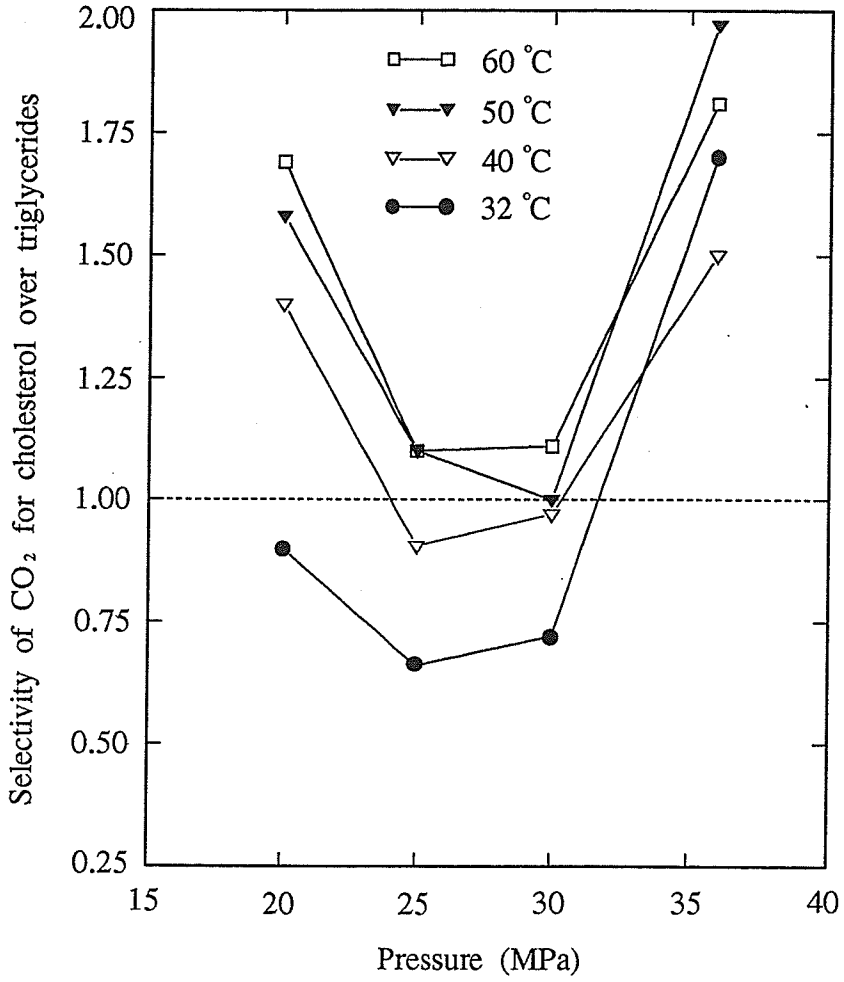

Figure 4.22 Selectivity of SC CO_2 for cholesterol over triglycerides as a function of pressure at four temperatures.

oxygenated compounds over the terpene hydrocarbons changed with temperature and pressure in the SFE process of citrus oil.

On the other hand, Dobbs and Johnston (1987) reported that for 2-aminobenzoic acid and anthracene system, the selectivity was insensitive to pressure with or without cosolvent present. Kosal et al. (1992) also reported that in their physical mixture systems of progesterone + testosterone, testosterone + cholesterol, and progesterone + cholesterol, the selectivities did not change with the pressure at high temperatures. These results are not contradictory to ours. The selectivity of SC CO_2 for a component in a mixture of organic solids is related primarily to the solute vapour pressures and only secondarily to intermolecular forces in the SC phase (Dobbs and Johnston 1987). The vapour pressures of the components are the key properties affecting the selectivity in the system in which solute-solute interactions are negligible. If the ratio of the vapour pressures of the components is insensitive to pressure, the selectivity should not be sensitive to pressure. In the systems of Dobbs and Johnston (1987) and Kosal et al. (1992), the solubilities of the components are sufficiently low so that the effects of solute-solute interactions are negligible. Thus, the selectivities in their systems should not be sensitive to pressure if the ratio of the vapour pressures of the components in their system is insensitive to pressure. In this study, however, it is evident from the previous results that the interactions between the components of egg yolk could not be negligible. In addition to the vapour pressure of each component, the intermolecular forces would play a role in changing selectivity. Our results indicate that the selectivity of SC CO_2 is related not only to the vapour pressures of the solutes but also to the intermolecular forces in the

supercritical phase and agree with Dobbs and Johnston's statement.

It is evident from Figure 4.22 that at 36 MPa and all temperatures as well as at 20 MPa and 40°C, 50°C and 60°C, fractionation occurred between triglycerides and cholesterol and cholesterol was preferentially extracted. At other temperature and pressure combinations examined, the fractionation of cholesterol from other egg yolk lipids is not significantly favoured. The highest selectivity of SC CO_2 for cholesterol over triglycerides occurs at 36 MPa and 50°C and is 1.97. From the pointviews of both solubility and selectivity, the optimum extraction pressure and temperature for removing cholesterol from egg yolk using SC CO_2 are 36 MPa and 50°C.

4.4 Extraction of Freeze-dried Egg Yolk Using SC CO_2 and Alcohol Mixtures

To investigate the effects of entrainer on the solubility of egg yolk lipids in SC CO_2 and the selectivity of SC CO_2 for cholesterol over other lipids present in egg yolk, extractions were also carried out on freeze-dried egg yolk using SC CO_2 and different concentrations of either methanol or ethanol at 40°C and 36 MPa. At least two runs were performed at each entrainer concentration. The total solubility of lipids in SC CO_2 was determined from the corresponding extraction curves using the procedures described in section 3.4.3. The solubilities of the individual components in SC CO_2 were calculated using the method discribed in section 3.4.4.2.

4.4.1 Effect of Entrainer Type and Concentration on the Total Solubility of Lipids in SC CO_2

The effects of entrainer type and concentration on the total solubility of lipids in SC CO_2 are shown in Figure 4.23. The error bar shown on each curve represents the maximum standard error for the slopes of the lines which could be drawn through the initial portion of the extraction curves used to calculate the solubilities. The figure indicates that: (1) the total solubility of egg yolk lipids increases from 12.5 mg/g CO_2 to 68.6 mg/g CO_2 when the ethanol concentration in the SC CO_2 is increased from 0 to 10% by weight; (2) the total solubility of egg yolk lipids in SC CO_2 increases from 12.5 mg/g CO_2 to 66.7 mg/g CO_2 when the methanol concentration in SC CO_2 is increased from 0 to 9% by weight; (3) the increase in solubility with entrainer concentration is similar for both methanol and ethanol; (4) as the concentration approaches 9%, this difference in enhancement effect becomes less pronounced; (5) for the same entrainer concentration when expressed on weight basis, ethanol induced a larger solubility enhancement than methanol; (6) on a molar concentration basis, the increase in enhancement effect of ethanol when compared to methanol would be even greater.

The mass balance calculations carried out for each entrainer run showed that the alcohol entrainer in the lipid extracts could not be removed completely. The residual entrainer in the extracted egg yolk lipids could result in an apparent 4 to 6% increase in solubility over the true solubility. Thus, the interpretation of the data must be carried out with care.

The solubility enhancement of yolk lipids induced by the addition of either

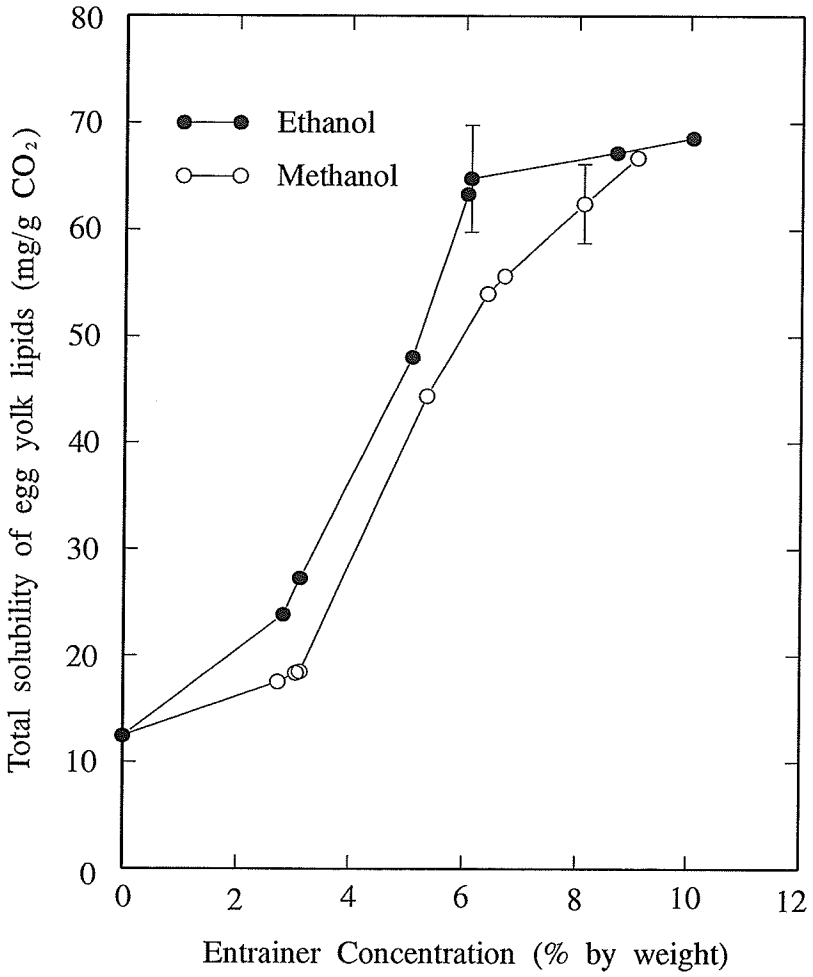

Figure 4.23 Total solubility of egg yolk lipids in SC CO_2 as a function of entrainer type and concentration. Extraction conditions: temperature 40 °C, pressure 36 MPa, flow rate 10.0 g/min.

methanol or ethanol follows the similar pattern reported for cholesterol in Section 4.1.2 and could be attributed to the specific interactions between the lipid components and the entrainer. The details will be discussed in Section 4.4.3.

The effect of entrainer type on the solubility of a compound is dependent on the specific interactions between the entrainer and the solutes and the relative concentration of the entrainer and the solvent. Only the addition of an appropriate entrainer can enhance the solubility of solutes. Schmitt and Reid (1986) used benzene, cyclohexane, acetone, and methylene chloride as entrainers when measuring the solubilities of phenanthrene and benzoic acid in either carbon dioxide or ethane. They found a significant entrainer effect only in benzoic acid/ethane/acetone system, not in any of the other systems studied.

Methanol and ethanol were examined as different types of entrainers in this study. Ethanol has physical properties similar to those of methanol except for its lower toxicity. Their various solubility parameters are listed in Table 4.4.

Table 4.4 Solubility parameters of methanol and ethanol (Barton 1983).

	δ $(MPa)^{1/2}$					
Name	δ_t	δ_d	δ_o	δ_i	δ_a	δ_b
Methanol	29.7	12.7	10.0	1.6	17.0	17.0
Ethanol	26.0	13.9	7.0	1.0	14.1	14.1

δ_t: total cohesion parameter; δ_d: dispersion cohesion parameter; δ_o: orientation cohesion parameter; δ_i: induction cohesion parameter; δ_a: Lewis acid cohesion parameter; δ_b: Lewis base cohesion parameter.

The solubility of egg yolk lipids in CO_2 was enhanced a little more by ethanol than by methanol. The comparison of solubility parameters of methanol and ethanol

suggests that the solubility enhancement for egg yolk lipids be affected more by dispersion than other interaction forces.

4.4.2 Composition Analysis of Extracts from SC CO_2 and Entrainer Extraction

Four samples, collected in the early, intermediate, and late stage of each run, were analyzed for lipid composition. The results were tabulated in Table 4.5. The triglyceride concentration and cholesterol concentration in the samples collected from the same run were not significantly different. The triglyceride concentration in the samples decreased as the methanol concentration increased from 3.05% to 6.7% by weight, and then did not change significantly as the methanol concentration was increased to 9.06%. The triglyceride concentration in the samples decreased with the increase in ethanol concentration. The cholesterol concentration in the samples decreased with increasing methanol concentration but did not change with increasing ethanol concentration. The phosphatidylcholine (PC) and phosphatidylehtanolamine (PE) concentrations in the samples did not change with the extraction time during the duration of each entrainer run and generally increased with increasing entrainer concentration. PC was absent in the extracts with 3% ethanol. PE was absent in both of the 3% ethanol and methanol extracts. These results demonstrate that the selectivity of solvent mixtures changes with the entrainer type and concentration, which agrees with the work of Temelli (1992) and Schaeffer et al. (1988). About 12% - 36% of the materials in the extract samples remain unidentified. Except for the compounds mentioned in Section 3.4.2, the unremoved entrainer could be a part (<6%) of the unknown materials. Further experiments are needed

Table 4.5 Composition of the CO_2-alcohol extracts of egg yolk and the partition coefficients of their components.

Entrainer Type	Entrainer Conc. (%)	Extract Number	Specific CO2 (g CO2/ g sample)	Triglyceride Conc. (%)	Cholesterol Conc. (%)	PC Conc. (%)	PE Conc. (%)	Partition Coefficient of TG	Partition Coefficient of CHO	Partition Coefficient of PC	Partition Coefficient of PE
Methanol	3.1	2	4.35	71.0	5.05	--	0.00	1.69	1.33	0.00	0.00
		6	10.1	71.1	5.50	0.81	0.00	1.69	1.45	0.05	0.00
		10	15.9	70.2	5.30	0.15	0.00	1.67	1.40	0.01	0.00
		14	21.6	70.7	5.42	0.07	0.00	1.68	1.43	0.00	0.00
	6.7	2	4.01	55.5	4.67	5.00	0.31	1.32	1.23	0.30	0.04
		6	6.97	56.0	5.07	5.78	0.35	1.33	1.34	0.35	0.05
		10	9.93	57.2	4.72	4.73	0.04	1.36	1.25	0.29	0.01
		14	15.9	53.9	5.00	4.11	0.40	1.28	1.32	0.25	0.05
	9.1	2	3.20	58.9	3.94	12.6	6.60	1.40	1.04	0.76	0.85
		6	5.52	54.1	4.31	17.8	5.35	1.29	1.14	1.08	0.69
		10	8.27	56.4	4.36	20.1	5.64	1.34	1.15	1.21	0.73
		14	13.6	51.3	4.48	19.3	5.47	1.22	1.18	1.17	0.70
Ethanol	2.8	2	4.45	70.7	5.63	0.00	0.00	1.68	1.49	0.00	0.00
		6	10.4	74.2	4.54	0.00	0.00	1.77	1.20	0.00	0.00
		10	16.4	77.5	5.13	0.00	0.00	1.85	1.35	0.00	0.00
		13	20.7	73.0	5.22	0.00	0.00	1.74	1.38	0.00	0.00
	5.1	2	4.33	74.6	5.11	1.45	0.27	1.78	1.35	0.09	0.03
		5	7.71	74.8	5.70	1.48	0.29	1.78	1.50	0.09	0.04
		8	12.3	67.2	4.69	2.15	0.55	1.60	1.24	0.13	0.07
		11	16.9	69.8	4.94	1.00	0.06	1.66	1.30	0.06	0.01
	6.1	3	4.37	76.5	6.14	1.90	0.04	1.82	1.62	0.11	0.01
		7	7.27	65.6	4.96	1.84	0.20	1.56	1.31	0.11	0.03
		10	11.6	67.5	4.95	2.78	0.66	1.61	1.31	0.17	0.08
		14	16.0	67.3	4.90	4.14	2.65	1.60	1.29	0.25	0.34
	8.7	2	2.96	62.4	4.85	13.7	2.24	1.49	1.28	0.83	0.29
		6	5.32	67.6	4.60	13.1	1.84	1.61	1.21	0.79	0.24
		10	10.3	67.3	4.94	12.7	1.69	1.60	1.30	0.76	0.22
		14	16.3	62.0	4.68	15.9	4.83	1.48	1.23	0.96	0.62

to identify the unknown materials.

The comparison of partition coefficients with and without entrainer suggests that the addition of an entrainer into CO_2 decreases the partition coefficients for cholesterol and increases the partition coefficients for PC and PE at high entrainer concentrations (6.7% and 9.1% methanol, 6.1% and 8.7% ethanol). The partition coefficient for triglycerides was decreased at 6.7% and 9.1% methanol levels and did not changed at 3% methanol level and all the ethanol levels examined. It is evident from these partition coefficients that triglycerides and cholesterol can also be concentrated from egg yolk substrate by SC CO_2 and entrainer extraction, and PC and PE can not be concentrated directly from egg yolk by SC CO_2 and entrainer extraction.

4.4.3 Effect of Entrainer Type and Concentration on Solubilities of Individual Components in Extracts

The solubilities of triglycerides, cholesterol, PC, and PE are plotted against entrainer concentration in Figures 4.24, 4.25, 4.26, and 4.27. From these figures it can be seen that the addition of entrainer into SC CO_2 increases the solubilities of all components at alcohol concentrations greater than 3%. Addition of ethanol resulted in a greater increase in the solubilities of cholesterol and triglycerides than methanol. The solubilities of both PC and PE increased from trace amounts at alcohol concentration less than 5% to significant amounts (PE solubility: 1.64 to 3.20 mg/g; PC solubility: 8.25 to 10.0 mg/g) at 9% (Figure 4.26 and Figure 4.27). These results support the previous reports of the enhancing effects of alcohol on lipid solubility in CO_2 (Temelli 1992, Labay 1991). The

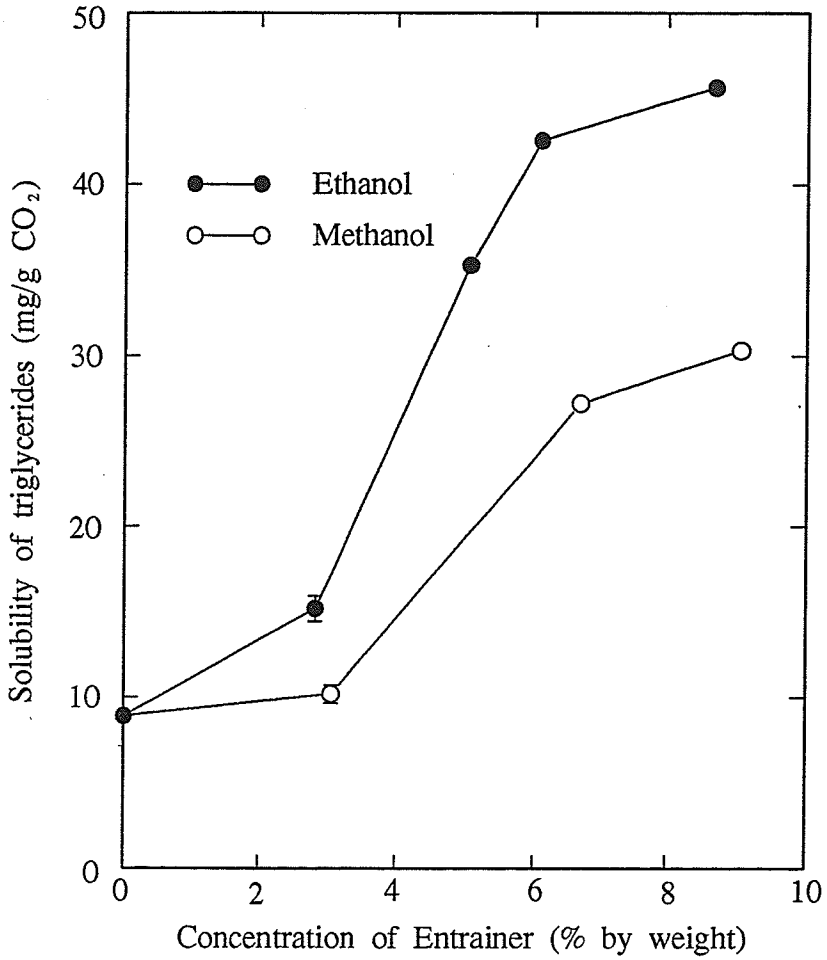

Figure 4.24 Solubility of egg yolk triglycerides in SC CO_2 as a function of entrainer type and concentration. Extraction conditions: temperature 40 °C, pressure 36 MPa, flow rate 10.0 g/min.

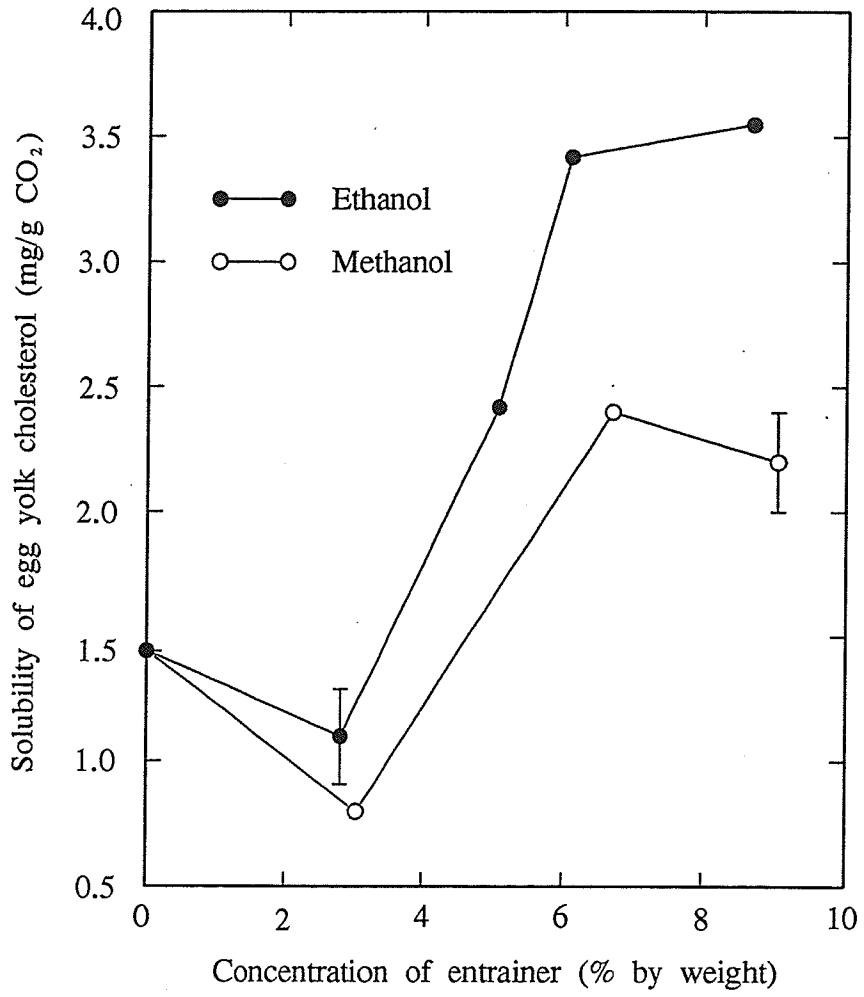

Figure 4.25 Solubility of egg yolk cholesterol in SC CO_2 as a function of entrainer type and concentration. Extraction conditions: temperature 40 °C, pressure 36 MPa, flow rate 10.0 g/min.

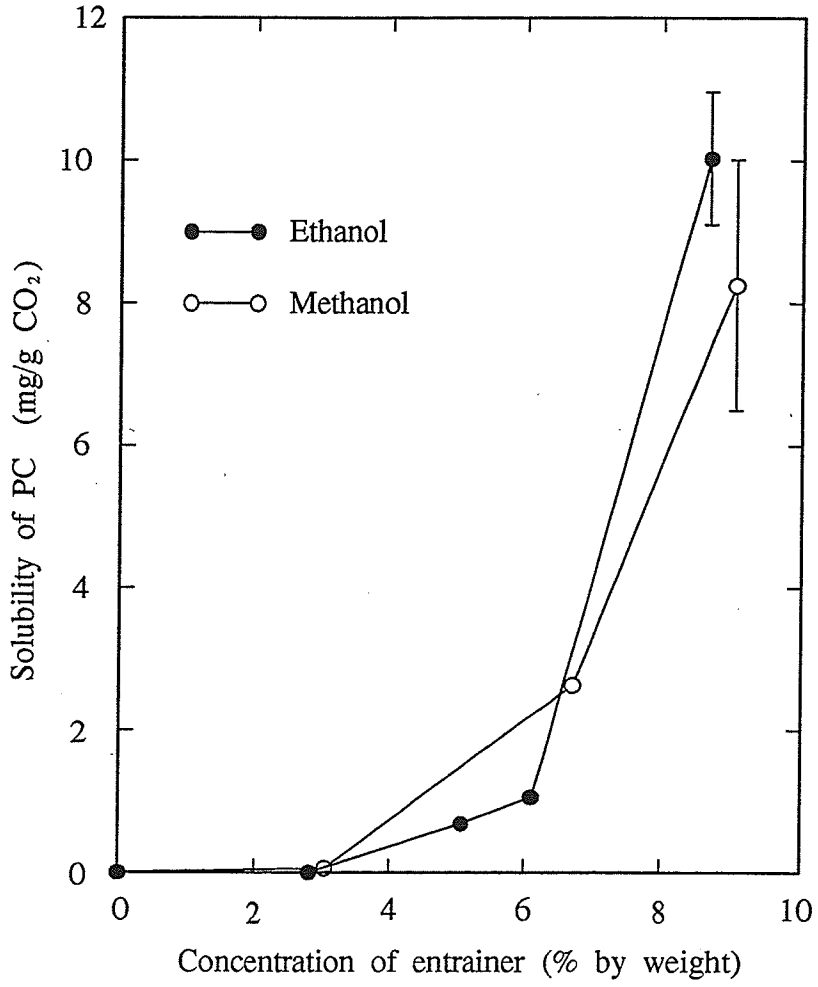

Figure 4.26 Solubility of egg yolk PC in SC CO_2 as a function of entrainer type and concentration. Extraction conditions: temperature 40 °C, pressure 36 MPa, flow rate 10.0 g/min.

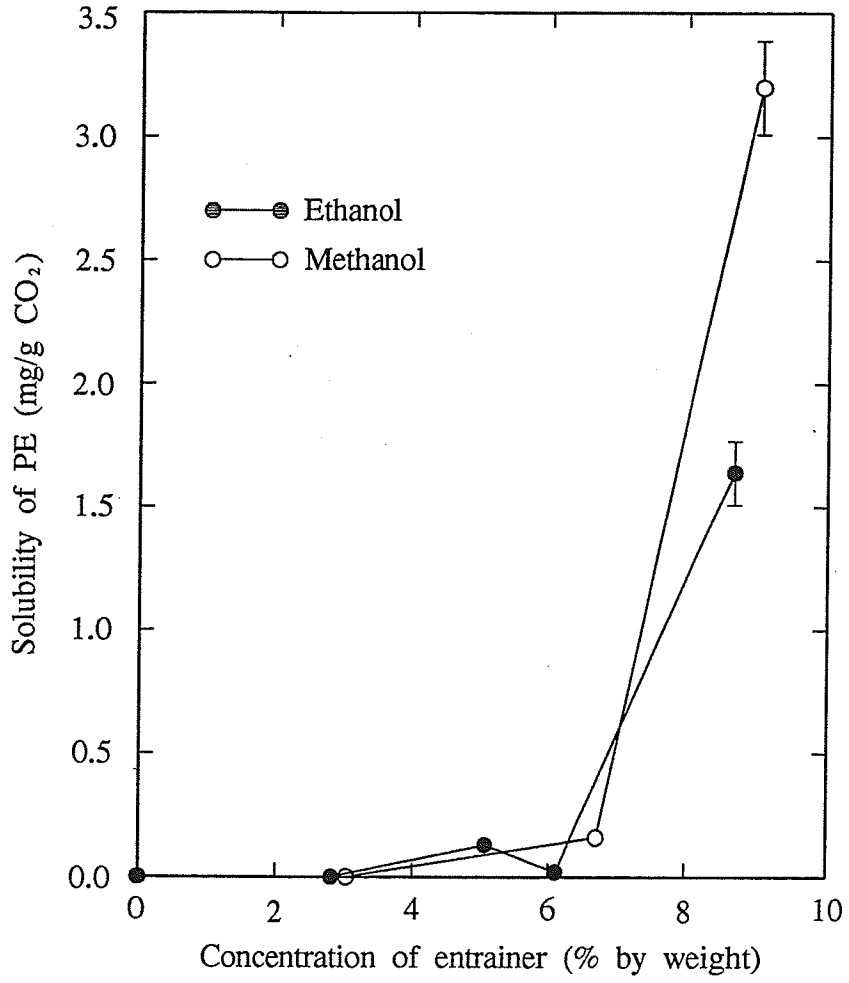

Figure 4.27 Solubility of egg yolk PE in SC CO_2 as a function of entrainer type and concentration. Extraction conditions: temperature 40 °C, pressure 36 MPa, flow rate 10.0 g/min.

effects have been attributed to: (1) the addition of methanol or ethanol into CO_2 increases the dipole moment of the solvent mixture; (2) some specific interactions occur between lipids and entrainer; for example, cholesterol has a hydroxyl group and can form hydrogen bonding with methanol or ethanol (Wong and Johnston 1986); (3) PC and PE are strongly polar and hence soluble in polar solvent mixtures (Labay 1991); (4) the addition of methanol or ethanol into SC CO_2 may have disrupted the lipoprotein complexes to which cholesterol and its esters are intimately bound and the noncovalent bonds between the phospholipids and the proteins. Disruption of the complexes may release cholesterol, its esters and phospholipids and make them available for extraction (Hardardottir and Kinsella 1986). These enhancements in the solubilities of individual components lead to the enhancement of the total solubility of egg yolk lipids in CO_2-alcohol mixtures.

The above results also support McNally and Wheeler's result (1988) that the polarity of the solvent mixture must be optimized to match the polarity of the solute in order to maximize the solvation capacity of the solvent mixture.

From Figures 4.26 and 4.27 it can be seen that PC and PE are not soluble in pure CO_2 and CO_2-3% alcohol mixtures but are soluble in CO_2 modified with high concentration (>6% by weight) alcohols. This result shows that it may be possible to concentrate phospholipids from egg yolk using SC CO_2 and high concentration (>6% by weight) alcohol mixtures after the neutral lipids have been removed by SC CO_2.

4.4.4 Effect of Entrainer Type and Concentration on Selectivity of SC CO_2 for Cholesterol over Other Lipids Present in the Extracts

The selectivities of SC CO_2 for cholesterol over triglycerides and phospholipids during the extraction processes are tabulated in Table 4.6. All the average selectivity data except for one at 9% methanol level are greater than 1, indicating that cholesterol was preferentially extracted by CO_2 and alcohol mixtures. The average selectivity of SC CO_2 and alcohol mixtures for cholesterol over triglycerides and phospholipids (TG, PC and PE) is plotted as a function of entrainer concentration in Figure 4.28. The results indicate that the selectivity of SC CO_2 and alcohol mixtures for cholesterol decreases with an increase in entrainer concentration over the ranges of 0 - 3% and 3 - 9%. In the range of 3 - 6%, the selectivity increases slightly with increasing entrainer concentration. This result supports Temelli's result that the selectivity depends on entrainer concentration. The comparison of the selectivities of CO_2 for cholesterol with and without alcohol added into the CO_2 suggests that the addition of alcohol reduced the selectivity of SC CO_2 for cholesterol. The selectivity was reduced more by ethanol than by methanol. These results indicate that the selectivity is not only related to the entrainer concentration but also the entrainer type and agree with earlier reports (Nilsson et al. 1992, and Liang and Yeh 1991). Nilsson et al. (1992) observed in their study that the addition of ethanol reduced the selectivity of supercritical fluid. Liang and Yeh (1991) concluded that high solubilities were associated with low separation efficiency for model mixture.

Nilsson et al. (1992) defined 'entrainer effect' as an increase in both solvent power and selectivity of a supercritical fluid upon addition of a small amount of a certain

Table 4.6 Selectivity of SC CO_2 - alcohol mixtures for cholesterol over triglycerides and phospholipids at different concentrations.

Entrainer Type	Entrainer Concentration (%)	Extract Number	Specific CO_2 (g CO_2/g sample)	Selectivity of CO_2 for CHO over TG, PC and PE*
Methanol	3.1	2	4.35	1.24
		6	10.1	1.34
		10	15.9	1.32
		14	21.6	1.34
	6.7	2	4.01	1.34
		6	6.97	1.43
		10	9.93	1.34
		14	15.9	1.50
	9.1	2	3.20	0.88
		6	5.52	0.98
		10	8.27	0.93
		14	13.6	1.03
Ethanol	2.8	2	4.45	1.40
		6	10.4	1.07
		10	16.4	1.16
		13	20.7	1.25
	5.1	2	4.33	1.17
		5	7.71	1.30
		8	12.3	1.18
		11	16.9	1.22
	6.1	3	4.37	1.37
		7	7.27	1.28
		10	11.6	1.22
		14	16.0	1.16
	8.7	2	2.96	1.08
		6	5.32	0.97
		10	10.3	1.06
		14	16.3	0.99

* CHO: cholesterol; PC: phosphatidylcholine; PE: phosphatidylethanolamine.

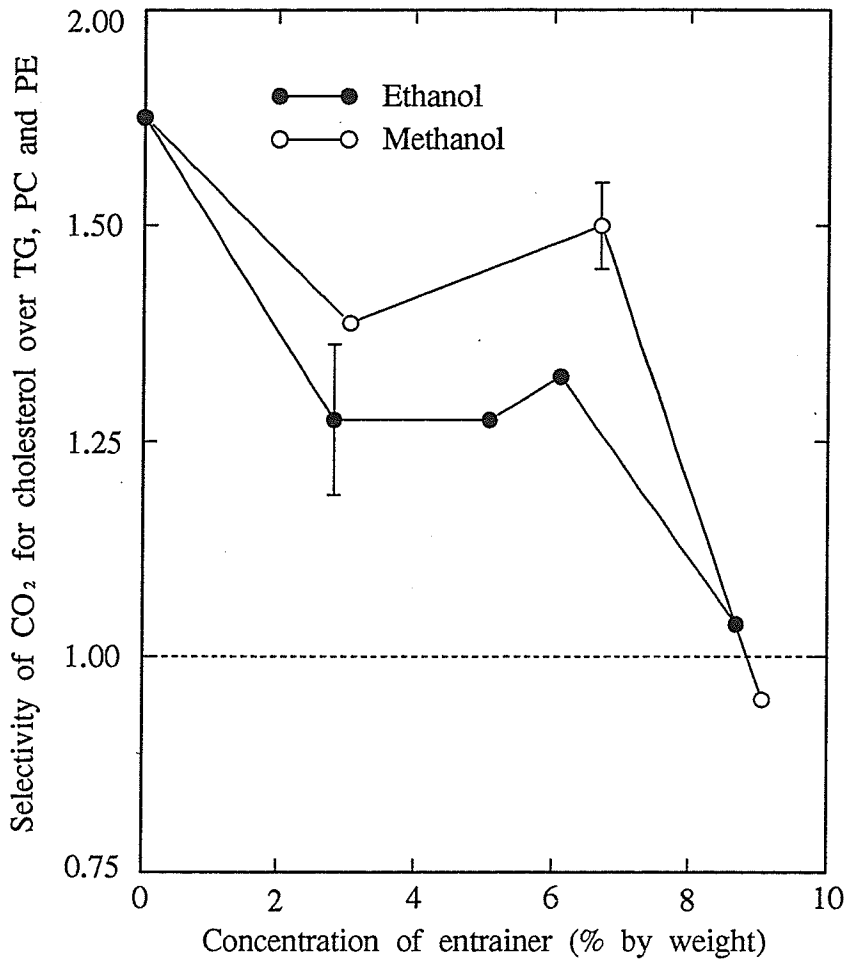

Figure 4.28 Selectivity of SC CO_2 for cholesterol over triglycerides and phospholipids as a function of entrainer type and concentration. Extraction conditions: temperature 40 ℃, pressure 36 MPa, flow rate 10.0 g/min.

cosolvent. Methanol and ethanol do enhance the solubilities but do not increase the selectivity of CO_2 in the removal of cholesterol from egg yolk lipids by SC CO_2.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The findings of this study are summarized as follows:

1. The solubility of pure cholesterol in SC CO_2 is a function of extraction temperature, pressure, and entrainer concentration. These results support but do not confirm the previous study that the crossover pressure for pure cholesterol is about 16 MPa. The solubility increases with increasing pressure, temperature, and entrainer concentration over the ranges examined.

2. The solubility of a less soluble component, cholesterol, is enhanced due to the presence of a more soluble component, triolein, in the mixture. The solubility of triolein itself is reduced.

3. The solubility of cholesterol does not significantly change with the triolein content in the mixtures.

4. The total solubility of the mixture can not be predicted based on the solubility of each pure component in supercritical CO_2 and the mixture composition.

5. The overall solubility of egg yolk lipids and the solubilities of individual components in SC CO_2 are functions of extraction temperature, pressure, and entrainer type and concentration. They increase with increasing extraction pressure and temperature when the extraction pressure is above 30 MPa. These solubilities change insignificantly or decrease with increasing temperature when the extraction pressure is equal to or lower than 30 MPa.

6. The influence of the other components present in egg yolk on the solubility of cholesterol depends on pressure. At the pressures equal to or less than 30 MPa, the presence of the other components reduces the solubility of cholesterol in SC CO_2. At 36 MPa, the solubility of cholesterol is enhanced.

7. The selectivity of SC CO_2 for cholesterol over triglycerides is a function of extraction pressure, temperature. The results confirm the previous statement that the selectivity of SC CO_2 for a component in a mixture of organic solids is related primarily to the solute vapour pressures and only secondarily to intermolecular forces in the supercritical phase.

8. The addition of an entrainer into CO_2 enhances the total solubility of lipids, and the solubilities of individual components present in egg yolk. The overall solubility of lipids and the solubilities of triglycerides and phospholipids increase with increasing entrainer concentration. The solubility of cholesterol fluctuates with entrainer concentration. Different entrainers induce different levels of enhancement in solubility. Ethanol increases the solubilities more than methanol.

9. The extraction selectivity of SC CO_2 and alcohol mixtures for cholesterol is a function of entrainer concentration and type. The addition of alcohol into CO_2 decreases the selectivity of SC CO_2 for cholesterol.

10. The optimum extraction conditions for selectively removing cholesterol from egg yolk by SC CO_2 are 36 MPa and 50 °C and with no alcohol as an entrainer.

5.2 Recommendations

The following recommendations are suggested for future research:

1. Other substances such as acetone, ethyl acetate, or ethyl ether as an entrainer in the SC CO_2 extraction of cholesterol from egg yolk lipids should be investigated to find a suitable entrainer for the extraction process.

2. An investigation on the supercritical CO_2 extraction of the mixtures of cholesterol and triglycerides should be carried out at different extraction pressures to obtain the information about the interaction between cholesterol and triglycerides in a supercritical phase and the effect of extraction pressure on their interaction.

3. More experiments are needed to investigate the effect of the mixture composition on the total solubility of the mixture in supercritical CO_2 in other composition ranges.

REFERENCES

Angus, S., Armstrong, B. and deReuck, K. M., 1976. 'IUPAC: Carbon Dioxide International Thermodynamic Tables of Fluid State-3', IUPAC Project Center, Imperial College, London, Pergamon Press, 1-382.

Artz, W. E. and Sauer, R. M., Jr., 1992. 'An improved Micro-extraction Cell for Supercritical Fluid Extraction and Chromatography of Fatty Acids', JAOCS, 69:309-313.

Balaban, M., 1989. 'Supercritical Extraction: Recovering Process Materials', Agricul. Eng., March/April, 24-25.

Barton, A. F. M., 1983. 'Handbook of Solubility Parameters and Other Cohension Parameters', CRC Press Inc., Boca Raton, FL., 43.

Basta, N., 1984. 'Supercritical Fluids', High Technology, June, 385-390.

Basta, N. and McQueen, S., 1985. 'Supercritical Fluids: Still Seeking Acceptance', Chem. Eng., 92:14-17.

Beyer, R. S. and Jensen, L. S., 1989. 'Research Note: Cholesterol Content of Commercially Produced Egg in Georgia', Poultry Sci., 68:1703-1706.

Brogle, H., 1982. 'CO_2 as a Solvent: Its Properties and Applications', Chemistry and Industry, June, 385-390.

Brunner, G. and Peter, S., 1982. 'On the Solubility of Glycerides and Fatty Acids in Compressed Gases in the Presence of an Entrainer', Separation Sci. Technol., 17:199-214.

Buchner, E. H., 1906. 'Liquid Carbon Dioxide as a Solvent', Zeitschr. Phys. Chem., 54:665.

Bulley, N. R., Fattori, M. J., Meisen, A. and Moyls, L., 1984. 'Supercritical Fluid Extraction of Vegetable Oil Seeds', JAOCS, 61:1362-1365.

Bulley, N. R. and Labay, L., 1991. 'Extraction/Fraction of Egg Yolk Using Supercritical CO_2 and Alcohol Entrainers', the 2nd International Symposium on Supercritical Fluids, Boston, Massachusettes, May, 10-12.

Chao, R. R., Mulvaney, S. J., Bailey, M. E. and Fernando, L. N., 1991a. 'Supercritical CO_2 Conditions Affecting Extraction of Lipid and Cholesterol from Ground Beef',

J. Food Sci., 56:183-187.

Chao, R. R., Mulvaney, S. J., Sanson, D. R., Hsieh, F. and Tempesta, M. S., 1991b. 'Supercritical CO_2 Extraction of Annatto (Bixa Orellana) Pigments and Some Characteristics of the Color Extracts', J. Food Sci., 56:80-83.

Chimowitz, E. H. and Pennisi, K. J., 1986. 'Process Synthesis Concepts for Supercritical Gas Extraction in the Crossover Region', AIChE J., 32:1661-1676.

Chrastil, J., 1982. 'Solubility of Solids and Liquids in Supercritical Gases', J. Phys. Chem., 66:3016-3021.

Christianson, D. D., Friedrich, J. P., List, G. R., Warner, K., Bagley, E. B., Stringfellow, A. C. and Inglett, G. E., 1984. 'Supercritical Fluid Extraction of Dry-Milled Corn Germ with Carbon Dioxide', J. Food Sci., 49:229-232.

Chung, R. A. and Stadelman, W. J., 1965. 'A Study of Variations in the Structure of the Hen's Egg', Brit. Poultry Sci., 6:277-282.

Clarenburg, R. K., Chung, I. A. and Wakefield, L. M., 1971. 'Reducing the Egg Cholesterol Level by Including Emulsified Sitosterol in Standard Chicken Diet', J. Nitr., 101:289-297.

Crerar, W. J., 1993. 'Modification and Instrumentation of a Screening Unit for Extraction of Egg Yolk Lipids with Supercritical Carbon Dioxide and Entrainers', Master's Thesis, University of Manitoba.

Cygnarowicz-Provost, M. C., O'Brien, D. J., Maxwell, R. J. and Hampson, J. W., 1991. 'Supercritical Fluid Extraction of Fungal Lipids Using Mixed Solvents', the 2nd International Symposium on Supercritical Fluids, Boston, Massachusetts, May, 17-20.

deFillipi, R. P., 1982. 'CO_2 as a Solvent: Application to Fats, Oils and Other Materials', Chemistry and Industry, June, 390-394.

Degnan, A. J., von Elbe, J. H. and Hartel, R. W., 1991. 'Extraction of Annatto Seed Pigment by Supercritical Carbon Dioxide', J. Food Sci., 56:1655-1659.

Dobbs, J. M., Wong, J. M., Lahiere, R. J. and Johnston, K. P., 1987. 'Modification of Supercritical Fluid Phase Behavior Using Polar Cosolvents', Ind. Eng. Chem. Res., 26:56-65.

Dobbs, J. M. and Johnston, K. P., 1987. 'Selectivities in Pure and Mixed Supercritical Fluid Solvents', Ind. Eng. Chem. Res., 26:1476-1482.

Dobbs, J. M., Wong, J. M., and Johnston, K. P., 1986. 'Nonpolar Cosolvents for Solubility Enhancement in Supercritical Fluid Carbon Dioxide', J. Chem. Eng. Data, 30:303-308.

Edwards, H. M., Jr., Driggers, J. E., Dean, R. and Carmon, J. L., 1960. 'Studies on the Cholesterol Content of Eggs from Various Breeds and/or Strains of Chickens', Poultry Sci., 39:487-489.

Eggers, R., 1978. 'Large-Scale Industrial Plant for Extraction with Supercritical Gases', In: Extraction with Supercritical Gases, Editors: G. M. Scheider, E. Stahl and G. Wilke, Verlag Chemie, 155-175.

Eggers, R., Sievers, U. and Stein, W., 1985. 'High Pressure Extraction of Oil Seed', JAOCS, 62:1222-1229.

Eggers, R. and Sievers, U., 1989. 'Processing Oilseed with Supercritical Carbon Dioxide', J. Chem. Eng. Japan, 22:641.

Fattori, J. M., 1986. 'Supercritical Fluid Extraction of Canola Seed', PhD. Thesis, University of British Columbia.

Fattori, M., Bulley, N. R. and Meisen, A., 1988. 'Carbon Dioxide Extraction of Canola Seed: Oil Solubility and Effect of Seed Treatment', JAOCS, 65:968.

Favati, F., King, J. W., Friedrich, J. P. and Eskins, K., 1988. 'Supercritical CO_2 Extraction of Carotene and Lutein from Leaf Protein Concentrates', J. Food Sci., 53:1532-1536.

Folch, J., Lees, M. and Sloane-Stanley, G. H. 1956. 'A Smiple Method for the Isolation and Puritication of Total Lipids from Animal Tissues', J. Biol. Chem., 226:497-509.

Friedrich, J. P., 1984. 'Supercritical CO_2 Extraction of Lipids from Lipid Containing Materials', U. S. Patent, 4,466,923, August 21.

Friedrich, J. P., List, G. R. and Heakin, A. J., 1982. 'Petroleum-Free Extraction of Oil from Soybeans with Supercritcal CO_2', JAOCS, 59:288-292.

Friedrich, J. P. and Pryde, E. H., 1984. 'Supercritical CO_2 Extraction of Lipid - Bearing Materials and Characterization of the Products', JAOCS, 61:223-228.

Froning, G. W., Wehling, R. L., Cuppett, S. L., Pierce, M. M., Niemann, L., and Siekman, D. K., 1990. 'Extraction of Cholesterol and Other Lipids from Dried Egg Yolk Using Supercritical Carbon Dioxide', J. Food Sci., 55:95-98.

Goodrum, J. W. and Kilgo, M. B., 1989. 'Rapeseed Oil Recovery by CO_2 Solvent: Recovery Kinetics and Extraction Models', Trans. ASAE, 32:727-731.

Haeffner, E. A. and Coenen, H. F., 1986. 'Color Extraction from Paprika Using Supercritical Gases', Paper No. 105, Presented at 191st ACS, National Meeting, New York, NY, April, 13-18.

Hamilton, J. G. and Comai, K., 1988a. 'Classes by Normal Phase HPLC', Lipids, 23:1150-1153.

Hamilton, J. G. and Comai, K., 1988b. 'Rapid Separation of Neutral Lipids, Free Fatty Acids and Polar Lipids Using Prepacked Silica Sep-Pak Columns', Lipids, 23:1146-1149.

Hardardottir, I. and Kinsella, J. E., 1988. 'Extraction of Lipid and Cholesterol from Fish Muscle with Supercritical Fluids', J. Food Sci., 53:1656-1658.

Harris, P. C. and Wilcox, F. H., 1963. 'Studies on Egg Yolk Cholesterol: 1. Genetic Variation and Some Phenotypic Correlations in a Randombred Population; 2. Influence of Season; 3. Effect of Dietary Cholesterol', Poultry Sci., 42:178-189.

Hubert, P. and Vitzthum, O. G., 1978. 'Fluid Extraction of Hops, Spices, and Tobacco with Supercritical Gases', Angew. Chem. Int. Ed. Engl., 17:710-715.

Ikushima, Y., Saito, N., Hatakeda, K., Ito, S., Asano, T. and Goto, T. 1985. 'Effects of entrainers on the extraction of Triglycerides with Supercritical Carbon Dioxide', Chemistry Letter, 1789-1792.

Ikushima, Y., Saito, N., Hatakeda, K., Ito, S., Asano, T. and Goto, T. 1986. ¾A Supercritical Carbon Dioxide Extraction from Mackerel (Scomber Japonicus) Power: Experiment and Modeling', Bull. Chem. Soc. Jpn., 59:3709-3713.

Ikushima, Y., Hatakeda, K., Ito, S., Saito, N., Asano, T., and Goto, T., 1988. 'A Supercritical Carbon Dioxide Extraction from Mixtures of Triglycerides and Higher Fatty Acid Methyl Esters Using a Gas-Effusion-Type System', Ind. Eng. Chem. Res. 27:818-823.

Ikushima, Y., Saito, N., and Goto, T., 1989. 'Selective Extraction of Oleic, Linoleic, and Linolenic Acid Methyl Esters from Their Mixture with Supercritical Carbon Dioxide - Entrainer Systems and A Correlation of the Extraction Efficiency with a Solubility Parameter', Ind. Eng. Chem. Res., 28:1364-1369.

Irani, C. A. and Funk, E. W., 1977. 'Separation Using Supercritical Gases, in CRC Handbook: Recent Developments in Separation Science', Vol. III, Part A, CRC

Press, Boca Raton, Florida, 171-193.

Jiang, Z, and Sim, J. S., 1991. 'Egg Cholesterol Values in Relation to the Age of Laying Hens and to Egg and Yolk Weights', Poultry Sci., 70:1838-1841.

Johnston, K. P., and Eckert, C. A., 1981. 'An Analytical Carnahan-starling Van der Waals Model for Solubility of Hydrocarbon Solids in Supercritical Fluids', AIChE J., 27:773-779.

Johnston, K. P., Peck, D. G., and Kim, S., 1989. 'Modeling Supercritical Mixtures - How Predictive is it?', Ind. Eng. Chem. Res., 28:1115-1123.

Kim, S., Wong, J. M., and Johnston, K. P., 1985. 'Theory of the Pressure Effect in Dense Gas Extraction', In Supercritical Fluid Technology; Penninger, J. M. L., Radosz, M., McHugh, M. A., Krukonis, V. J. , Eds.; Elvesier: New York, 45-66.

Kosal, E., Lee, C. H. and Holder, G. D., 1992. 'Solubility of Progesterone, Testosterone, and Cholesterol in Supercritical Fluids', J. Supercrit. Fluids, 5:169-179.

Labay, L. T., 1991. 'Extraction/Fractionation of Egg Yolk Using Supercritical CO_2 and Alcohol Entrainers', Master's Thesis, Department of Food Science, University of Manitoba.

Larson, K. A. and King, M. L., 1986. 'Evaluation of Supercritical Fluid Extraction in the Pharmaceutical Industry', Biotechnol. Prog., 2:73-81.

Lee, C.-H., Kosal, E., and Holder, G. D., 1991. 'Solubility of Biological Compounds in Supercritical Fluids', the 2nd International Symposium on Supercritical Fluids, Boston, Massachusetts, May, 308-311.

Li, S. and Hartland, S., 1991. 'Influence of Co-solvent on Solubility and Selectivity in Extraction of Xanthine Stimulants and Cocoa Butter from Cocoa Beans with Supercritical CO_2', the 2nd International Symposium on Supercritical Fluids, Boston, Massachusetts, May, 13-16.

Liang, J. H. and Yeh, A., 1991. 'Process Conditions for Separating Fatty Acid Esters by Supercriticla CO_2', JAOCS, 69:305-308.

Lim, S., Lim, G. B. and Rizvi, S. S. H., 1991. 'Continuous Supercritical CO_2 Processing of Milk Fat', the 2nd International Symposium on Supercritical Fluids, Boston, Massachusettes, May, 292-296.

List, G. R. and Friedrich, J. P.,1985. 'Processing Characteristics and Oxidative Stability of Soybean Oil Extracted with Supercritical CO_2 at 50°C and 8,000 psi', JAOCS,

62:82-84.

Manabe, A., Tokumori, T., Sumida, Y., Yoshida, T., Hatano, T., Yazaki, K. and Okuda, T., 1987. 'Application of Supercritical Fluid Extraction to Components of Crude Drugs and Plants, III. Extraction of Pigments from Lithospermum Root and Licorice Root', Yakugaku Zasshi, 107:506.

Mangold, H. K., 1983. 'Liquefied Gases and Supercritical Fluids in Oil Seed Extraction', JAOCS, 60:178A-180A.

McNally, M. E. and Wheeler, J. R., 1988. 'Increasing Extraction Efficiency in Supercritical Fluid Extraction From Complex Matrices, Predicting Extraction Efficiency of Diuron and Linuron in Supercritical Fluid Extraction Using Supercritical Fluid Chromatographic Retention', J. Chromatogr., 447:53-63.

Nakaue, H. S., Lowry, R. R., Cheeke, P. R. and Arscott, G. H., 1980. 'The Effect of Dietary Alfalfa of Varying Saponin Content on Yolk Cholesterol Level and Layer Performance', Poultry Sci., 59:2744-2748.

Nilsson, W. B., Gauglitz, E. J., Hudson, J. K., Stout, V. F. and Spinelli, J., 1988. 'Fractionation of Menhaden Oil Ethyl Esters Using Supercritical Fluid CO_2', JAOCS, 65:109-117.

Nilsson, W. B., Seaborn, G. T., and Hudson, J. K., 1992. 'Partition Coefficients for FAtty Acid Esters in Supercritical Fluid CO_2 with and without Ethanol', JAOCS, 69:305-308.

Ortmanis, A., 1986. 'Supercritical Extraction (SCE) Technology', Feature Article, March, 1-8.

Parkinson, T. L., 1966. 'The Chemical Composition of Eggs', J. Sci. Food Agr., 17:101-111.

Parkinson, G., and Johnson, E., 1989. 'Supercritical Processes Win CPI Acceptance', Chem. Eng., 96:35-39.

Pasin, G., Novak, R. A., Reightler, W. J., King, A. J. and Zeidler, G., 1991. 'Cholesterol Removal from Liquid Egg Yolk by Supercritical Extraction', the 2nd Symposium on Supercritical Fluids, Boston, Massachusetts, May, 312-314.

Paul, P. F. M. and Wise, W. S., 1971. 'The Principles of Gas Extraction', Mills and Boon Limited, London.

Peter, S. and Brunner, G., 1978. 'The Separation of Nonvolatile Substances by Means

of Compressed Gases in Countercurrent Processes', Angew. Chem. Int. Ed. Engl., 17:746-750.

Rizvi, S. S. H., Benado, A. L., Zollweg, J. A. and Daniels, J. A., 1986. 'Supercritical Fluid Extraction: Fundamental Principles and Modeling Methods', Food Tech., June, 55-65.

Robey, R. J., and Sunder, S., 1984. 'Application of Supercritical Processing to the Concentration of Citrus Oil Fractions. Presented at the AIChE meeting, San, Francisco, Calif. Nov. 25-30.

Roselius, R., Vitzthum, O. and Hubert, P., 1972a. 'Nicotine Removal from Tobacco', German Patent 2,043,537, May 31.

Roselius, R., Vitzthum, O. and Hubert, P., 1972b. 'Recovery of Coffee Oil Containing Aroma Ingredients from Roasted Coffee', German Patent 2,106,133, August 31.

Roselius, R., Vitzthum, O. and Hubert, P., 1973a. 'Selective Extraction of Nicotine from Tobacco', German Patent 2,142,205, May 15.

Roselius, R., Vitzthum, O. and Hubert, P., 1973b. 'Extracting Cocoa Butter', U. S. Patent 3,923,847, May 23.

Sakaki, K., Yokochi, T., Suzuki, O. and Hakuta, T., 1990. 'Supercritical Fluid Extraction of Fungal Oil Using CO_2, N_2O, CHF_3, and SF_6', JAOCS, 67:553-557.

Schaeffer, S. T., Zalkow, L. H., Teja, A. S., 1988. 'Extraction of Monocrotaline from Crotalaria Spectabilis Using Supercritical Carbon Dioxide and Carbon Dioxide-Ethanol Mixture', Biotechnol. Bioeng. in press.

Schmitt, W. J., and Reid, R. C., 1986. 'The Use of Entrainers in Modifying the Solubility of Phenanthrene and Benzoic Acid in Supercritical Carbon Dioxide and Ethane, accepted for publication in Fluid Phase Equil..

Schneider, G. M., 1978. 'Physicochemical Principles of Extraction with Supercritical Gases', Angew. Chem. Int. Ed. Engl., 17:716-727.

Shishikura, A., Fujimoto, K., Kaneda, T., Arai, K. and Saito, S., 1986. 'Modification of Butter Oil by Extraction with Supercritical Carbon Dioxide', Agric. Biol. Chem., 50:1209-1215.

Stahl, E., Schutz, E. and Mangold, H. K., 1980. 'Extraction of Seed Oils with Liquid and Supercritical CO_2', J. Agric. Food Chem., 28:1153-1157.

Stahl, E. and Quirin, K. W., 1983. 'Dense Gas Extraction on a Laboratory Scale: A Survey of Some Recent Results', Fluid Phase Equilibra, 10:269.

Stahl, E., Schilz, W., Schutz, E. and Willing, E., 1978. 'A Quick Method for the Microanalytical Evaluation of the Dissolving Power of Supercritical Gases', Angew. Chem. Int. Ed. Engl., 17:731-738.

Taniguchi, M., Tsuji, T., Shibata, M. and Kobayashi, T., 1985. 'Extraction of Oils from Wheat Germ with Supercritical Carbon Dioxide', Agric, Biol. Chem., 49:2367-2372.

Temelli, F., Chen, C. S., and Braddock, R. J., 1988. 'Supercritical Fluid Extraction in Citrus Oil Processing', Food Tech., June, 145-150.

Temelli, F., 1992. 'Extraction of Triglycerides and Phospholipids from Canola with Supercritical Carbon Dioxide and Ethanol', J. Food Sci., 57:440-442,457.

Timmermans, J., 1950. ¾Physico-chemical Constants of Pure Organic Compounds', Elsevier Publishing Company, 302-312.

Turk, D. E. and Barnett, B. D. 1972. 'Diet and Egg Cholesterol Content', Poultry Sci., 51:1881.

VanAlsten, J. G., Hansen, P. C., and Eckert, C. A., 1984. 'Supercritical enhancement factors for nonpolar and polar systems. Paper 84a presented at AIChE 1984 annual meeting, San Francisco.

Vidal, J. P., Fort, J. J., Gaultier, P. and Richard, H., 1989. 'Vanilla Aroma Extraction by Dense Carbon Dioxide', Sci. Aliments, 9:89-100.

Vitzthum, O. and Hubert, P. 1972. 'Recovering Fats and Oils from Plant Seeds', German Patent 2,127,596, December 21.

Vitzthum, O. and Hubert, P. 1973. 'Extraction of Caffeine from Coffee', German Patent 2,212,281, September 27.

Vitzthum, O. and Hubert, P. 1976. 'Spice Extracts', Canadian Patent 989,662, May 25.

Vitzthum, O., Hubert, P., and Sirtl, W., 1975. 'Decaffeination of Crude Coffee', German Patent 2,357,590, May 28.

Vitzthum, O., Hubert, P., and Sirtl, W., 1976. 'Hop Extracts', Canadian Patent 987,250, April 13.

Walsh, J. M., Ikonomou, G. D. and Donohue, M. D., 1987. 'Supercritical Phase Behavior: the Entrainer Effect', Fluid Phase Equilibria, 33:295-314.

Weiss, J. F., Johnson, R. M. and Neber, E. C., 1967. 'Effect of Some Factors and Drugs on Cholesterol Concentration in the Egg and Plasma of the Hen', J. Nutr., 91:119-128.

Williams, D. F., 1981. 'Extraction with Supercritical Gases', Chem. Eng. Sci., 36:1769-1788.

Willson, R. C., 1985. 'Supercritical Fluid Extraction', In Comprehensive Biotechnology. The Principles, Applications, Regulations of Biotechnology in Industry, Agriculture and Medicine, C. L. Cooney and A. E. Humphrey (Eds.). Pergamon Press, New York, NY., 567-574.

Wong, J. M. and Johnston, K. P., 1986. 'Solubilization of Biomolecules in Carbon Dioxide Based Supercritical Fluids', Biotechnol. Prog., 2:29-38.

Yamaguchi, K., Murakami, M., Nakano, H., Konosu, S., Kokura, T., Yamamoto, H., Kosaka, M. and Hata, K., 1986. 'Supercritical Carbon Dioxide Extraction of Oils from Antarctic Krill', J. Agric. Food Chem., 34:904-907.

Yeh, A., Liang, J. H., and Hwang, L. S., 1991. ¾Separation of Fatty Acid Esters from Cholesterol in Esterified Natural and Synthetic Mixtures by Supercritical Carbon Dioxide', JAOCS, 68:224-229.

Yun, S. L. J., Liong, K. K., Gurdial, G. S. and Foster, N. R., 1991. 'Solubility of Cholesterol in Supercritical Carbon Dioxide', Ind. Eng. Chem. Res., 30:2476-2482.

Zosel, K., 1971. 'Decaffeinizing Coffee', French Patent 2,079,261, December 17.

Zosel, K., 1972. 'Caffeine from Crude Coffee', German Patent 2,221,560, November 30.

Zosel, K., 1974. 'Deodorization of Plant Oils', German Patent 2,332,038, January 24.

Zosel, K., 1975. 'Simultaneous Hydrogenation and Deodorization of Fats and/or Oils', German Patent 2,441,152, March 6.

Zosel, K., 1978. 'Separation with Supercrtical Gases: Pratical Applications', Angew. Chem. Int. Ed. Engl., 17:702-709.