Applications of supercritical carbon dioxide technology in soybean processing

by

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To My Parents...

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I. INTRODUCTION

A. Supercritical fluid technology

The trend towards consumership of the processed foods has led to development of the carefully defined and controlled new technologies. The increasing awareness of food safety and increasing market competition have motivated researchers to develop cheaper, safer, and better food products. Separation processes can selectively reduce or remove undesirable compounds or constituents from food product, e.g., cholesterol or calorie reduction and provide a health conscious consumer newer and safer food products. Consequently, separation processes have become a key to unit operations employed in the food processing industry, today. Efforts in recent years have focussed on developing cleaner, safer, and more efficient separation processes. For these reasons, the extension of supercritical fluid extraction (SCFE) into new area of foods and biomaterials research has become an attractive possibility. The stimulus in developing SCF technology is provided by the following factors:

1) Growing consumer concern over harmful residues in foods.
2) Increased government regulation of industrial solvents and stricter pollution control measures.
3) Potential for higher yields and better quality products, i.e., value added products.
4) SCF extraction can be operated under a wide range of conditions to selectively extract tailor-made end products or new products with improved functional and

nutritional characteristics for creating new foods.

A supercritical fluid (SCF) is a compressed gas above its critical pressure and temperature (Figure 1). A supercritical fluid is highly compressible in the vicinity of its critical point. Large density changes (from gas like to liquid like states) can be achieved by relatively small changes in either pressure or temperature, or both. The solvent strength of SCF is approximately linear with respect to density, therefore, extraction and fractionation of a dissolved material is possible by changing the temperature and pressure of a SCF over a continuum (McHugh and Krukonis, 1986; Johnston, 1984).

During the last decade, carbon dioxide has emerged as a preferred supercritical fluid, as it meets the requirements of being a good solvent: inert, nontoxic, nonflammable, inexpensive, and readily available in high purity. Fortunately, the critical temperature and pressure of CO_2 (304 K and 7.3 MPa) are relatively low values in terms of the process equipment requirement to achieve them with ease and without causing substantial product damage (Rizvi et al., 1986).

A variety of promising applications demonstrate the technical and economic viability of SCF technology (Zou et al., 1989). Some are: 1) extraction of flavors, aromas, and colorants; 2) concentration of eicosapentoic acid from fish oil; 3) extraction of cholesterol from butter, lard, tallow, and eggs; 4) decaffeination of coffee and tea; 5) extraction of aroma oils from hops; and 6) removal of nicotine from tobacco.

Although the supercritical fluids exhibit some attractive separation properties, they should not be considered as "magic solvents". Some of the fore-mentioned applications of SCF technology have been commercialized. Kraft General Foods, Inc. is using SC-CO_2

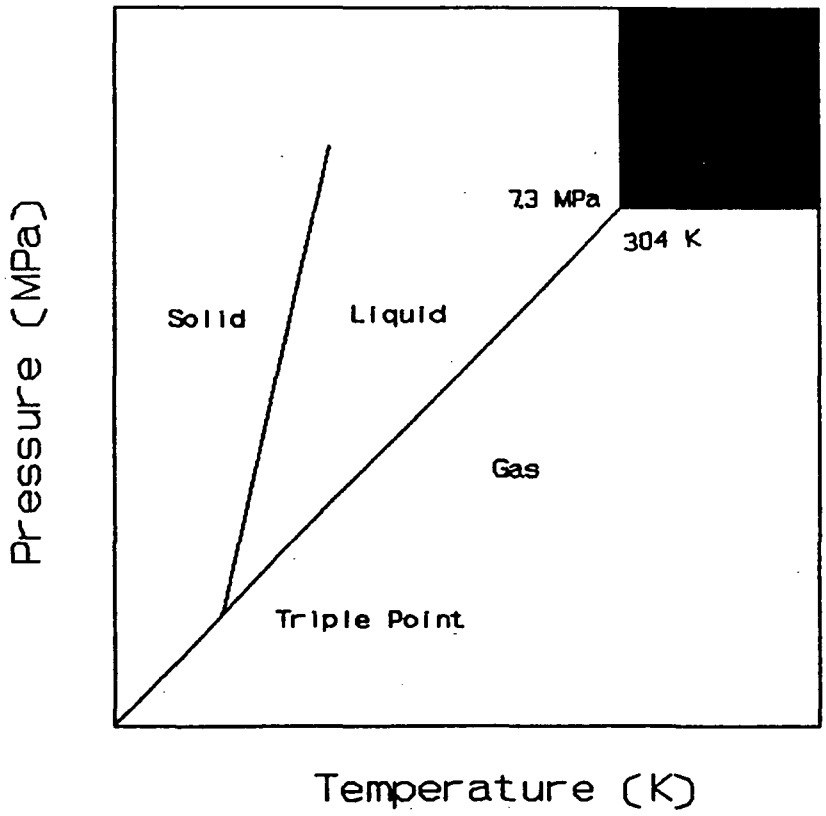

Figure 1. Phase diagram of carbon dioxide. The shaded area represents the supercritical region

for decaffeinating coffee and its parent company, Philip-Morris, Inc. have commissioned a plant for the removal of nicotine from tobacco. In Germany, the SCF technology has been used industrially to obtain oleoresins from spices and aroma oils from hops for beer manufacture. By the end of 1991, John I. Hass, Inc. will complete a plant for extracting hops. There have been number of other successful attempts on lab or pilot plant scale for the extraction or removal of desirable or undesirable food constituents, but the commercialization of those processes have not been practical due to higher operating costs associated with SC-CO_2 technology. The commercial viability of a particular SC fluid application has to be evaluated on a case-by-case basis (Krukonis, 1988).

The major impediments to the broader application of this technology in food processing are high capital investment and lack of equipment capable of continuous feeding and discharging of solids, suspension, and viscous media at high pressure. Although, significant advances have been made in the last couple of years, SC fluid technology is still confined to high value products.

B. Soybean processing

Soybeans dominate U.S. and world vegetable protein and oil markets in spite of competition from other oil seeds. The high qualities of soybean proteins and oil, valuable by-products, reasonable returns to farmers and processors, and plentiful, dependable supply at competitive prices have led to tremendous growth of soybean processing during the last two decades (Pryde, 1980).

Soybean processing involves various steps in order to produce meal and edible oil. Figure 2 shows the various steps of soybean processing. Some of the existing refining stages to make the crude soybean oil edible, e.g., alkali refining, bleaching, and deodorization are costly, inefficient, and constitute losses in yield. Market competitiveness has led researchers to explore the possibility of applying SC-CO_2 technology to refining vegetable oil.

In the conventional process, vegetable oil is deacidified by using alkali refining. The presence of free fatty acids in the fat cause undesirable characteristics, e.g., hydrolytic rancidity and reduced smoke point. Free fatty acids are removed from degummed oil by neutralizing it with ca 0.1% NaOH. After alkali addition and mixing, the crude oil is heated to 75°C to break the emulsion. The soaps generated by alkali saponification are then removed by centrifugation. Since these steps are time consuming and entrain substantial amount of oil constituting significant loss of yield, we studied the feasibility of using SC-CO_2 technology to remove free fatty acids from crude vegetable oil.

Almost all the edible fats and vegetable oils are deodorized before they are consumed to remove native flavors, odors, residual fatty acid, and lower molecular weight impurities. During soybean oil deodorization a valuable byproduct 'deodorizer distillate' is removed. The distillate consists of free fatty acids, neutral oil, mono- and diglycerides and valuable unsaponified compounds, tocopherols (α-tocopherol, i.e., vitamin E) and sterols. The other unsaponifiable materials, hydrocarbons, triterpene alcohols, 4-methylsterols, and squalene are not commercially valuable and are present in very minute amounts. The average composition of deodorizer distillate of soybean oil is as shown in

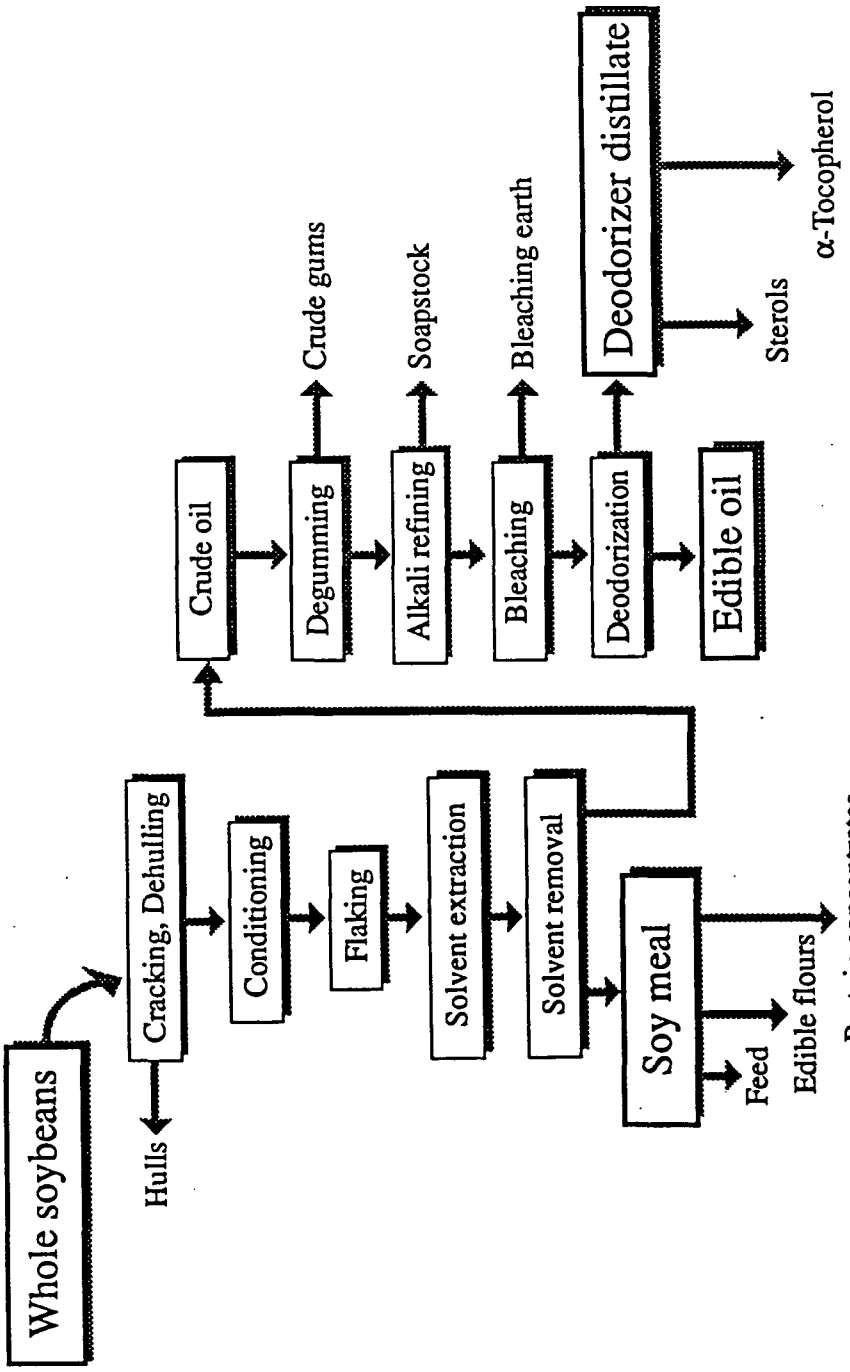

Figure 2. Major products of soybean processing

Table 1. During the 1960s and early 70s, soybean oil deodorizer distillate was a valuable by-product because it was an economical source of α-tocopherol and sterols. More recently, competition from synthetic products has caused the market value of distillate to drop (Herting, 1984; Krukonis, 1984; Gordan and Wolkoss, 1991; Hunsicker, 1991). The world market for synthetic vitamin E is four times larger than the naturally obtained mainly because of the price advantage. The price of natural vitamin E is about $65/kg compared to $32/kg for synthetic. Consumers in Europe, Japan, and Korea prefer natural tocopherols. There is a positive attitude by consumers towards natural products in this country as well, but higher cost continues to be the major disadvantage for natural product acceptance. Therefore, there is a need for lower cost separation technology to recapture the market competitiveness of soybean deodorizer distillate.

Table 1. Average composition of deodorizer distillate of soybean oil (Henkel Corp., LaGrange, IL.)

Fraction	Percentage
Saponifiables	37.6
Sterols, total	18.0
Tocopherols, total	11.1
Other unsaponifiables	33.3

The current annual world production of soy protein ingredients (flours, concentrates and isolates) for food is estimated at about 1 billion pounds with 75% produced alone in the United States. The total production of soybean protein ingredients

is roughly equivalent to about 1.5 billion pounds of 44% protein meal. More than 95% of the soybean meal produced annually in the U.S. (appr. 45 billion pounds) is used to supply protein in animal feeds. Soybean meal constitutes the largest portion of the monetary value of soybeans. In spite of its high protein quality and low cost compared to animal protein, soybean meal has not been accepted in human foods due to off-flavors associated with it. In order to achieve acceptance in human foods, the flavor problem must be solved.

In the 1960s it was recognized that oxidation of the unsaturated fatty acids by the enzyme lipoxygenase in the soybean was the principal cause of the off-flavors. The major off-flavor compounds, pentanal, hexanal, and ethyl-vinyl-ketone are produced during tempering, cracking, and flaking of soybeans. In past, the off-flavor developments were minimized by inactivation or inhibition of lipoxygenase by grinding beans in hot water (100°C), use of dry or moist heat, blanching, grinding at acidic pH, grinding with H_2O_2 and $CaCl_2$, grinding with non-aqueous ethanol, grinding with solvent azeotropes, inhibition by acetylenic compounds, and addition of antioxidants (does not work). The off-flavor compounds were also extracted by the azeotropic mixture of alcohol-hexane (Kinsella and Damodaran, 1980). However, none of these methods were able to provide bland soy protein without sacrificing protein functionality. Therefore, this encouraged us to study the use of SC-CO_2 technology in this area.

C. Background of study

1. Solubilities and fractionation characteristic of fatty acids and triglycerides

Many studies which have reported solubilities of fatty acids and triglycerides in supercritical CO_2 were performed with a major interest in modeling of their behavior in the supercritical phase rather than in evaluating their potential to be fractionated.

Chrastil (1982) measured solubilities of five triglycerides and two fatty acids (stearic and oleic) in SC-CO_2 in the pressure range of 8 to 25 MPa and the temperature range of 40 and 80°C. Under certain processing conditions, fatty acids have much higher solubilities than their corresponding triglycerides. At 15 MPa and 40 and 60°C, the solubility of stearic acid was 10 to 12 times higher compared to tristearin. However, oleic acid was 2 to 7 times more soluble than triolein at 25 MPa and 40 and 60°C.

Supercritical CO_2 with ethanol as an entrainer has been employed to deacidify palm oil (Brunner and Peter, 1981). The amount of free fatty acids in the oil was reduced from 3 to 0.1 wt% by using a multistage counter-current extractor operated at 13.7 MPa and 80°C.

Nilsson et al. (1988) and Rizvi et al. (1988) concentrated the ω3 fatty acids up to 90% from fish oil using a "hot-finger", i.e., temperature gradient technique. The hot-finger process has pressure and temperature induced refluxing capability. These authors have reported that a single pass system was not adequate to enhance the selectivity of the process. Rizvi et al. (1988) reported the use of urea and methanol to remove saturated, mono- and diunsaturated components prior to SCF extraction for better yield and purity of

eicosapentoic acid (EPA). However, Nilsson et al. (1988) recommended the use of a continuous countercurrent process rather than a batch process to eliminate the need of urea crystallization.

Hammam and Sivik (1991) found that mixing 4% ethanol with CO_2 at 15 MPa and 40°C increased the solubility of gluten lipids by at least eight times, and at 20 MPa and 70°C the solubility increased by 20 times when 10% ethanol was used. The authors concluded that with suitable cosolvent the fractionation of gluten lipids was possible.

Bamberger et al. (1988) measured the solubilities of three triglycerides (trilaurin, trimyristin, and tripalmitin) and their corresponding fatty acids at 313 K and at pressures between 8 and 30 MPa. The solubilities of fatty acids or/and their triglyceride homologous series decreased with increasing molecular weight. The solubilities of fatty acids were higher than their corresponding triglycerides. But the solubilities of palmitic acid and trilaurin were similar up to 11 MPa pressure; however pure trilaurin was more soluble in SC-CO_2 compared to pure palmitic acid at higher pressure. This suggests that for deacidification of vegetable oil, knowledge of fatty acid and triglyceride composition is very important, and, the processing conditions must be adjusted accordingly. This group also studied the solubilities of triglyceride mixtures in CO_2 and found that intermolecular interactions in the liquid phase affected the solubilities of individual triglycerides in the supercritical phase. The solubilities of lighter compounds, trimyristin and/or trilaurin were not greatly affected by the presence of the heavier compound, while the solubility of the heavier compound, i.e., tripalmitin was increased two times by the presence of trilaurin and 1.5 times by trimyristin. It has been suggested that lighter

compounds acted as entrainers.

Recently, it was demonstrated that a complex mixture of triglycerides, such as butterfat, can be fractionated into two, four, or even eight fractions depending on the processing temperature and pressure (Biernoth and Merk, 1985; Shishikura et al., 1986; Arul et al., 1987). Biernoth and Merk (1985) used carbon dioxide and propane as supercritical fluids, and fractionated butter oil and palm kernel stearin. At a pressure of 20 MPa and 80°C with SC-CO_2, they reported that extracted material was enriched in C_4-C_{12} fatty acids, whereas, the concentration of C_{18} fatty acids (oleic, linoleic, and stearic) was reduced. Propane at 8.5 MPa and 90°C showed only a 5% decrease in the extractable compounds. Shishikura et al. (1986) modified the dehydrated as well as "as is" butter oil between 13.6 and 31.4 MPa and 40°C. The solubility of dehydrated butter oil increased from 0.41% to 1.88% (w/w). However, the solubility of "as is" oil decreased above 20.9 MPa probably because of co-existing water present in it. The C_{38}-C_{40} triglycerides (TGs) were concentrated in the extract, and higher TGs were found in the residue. Arul et al. (1987) fractionated milk fat with SC-CO_2 at 10-35 MPa and at temperature of 50 and 70°C, respectively. This group also reported the concentration of triglycerides similar to that of Shishikura et al. (1986).

An extensive study of application of the SC-CO_2 in deacidification of olive oil was published by Brunetti et al. (1989). These authors measured solubilities of four fatty acids and two triglycerides at 20 and 30 MPa in the temperature range of 35 to 60°C. The data suggested that, at 60°C and 20 MPa, SC-CO_2 had 12 times and 6 times higher selectivity for stearic and oleic acids, respectively, than for their corresponding triglycerides,

tristearin and triolein. Olive oil was deacidified and it was concluded that the process was feasible especially for oils with relatively high free fatty acid content. Gonsalves et al. (1991) also studied the application of SC-CO_2 extraction to the deacidification of olive oils. This group measured the solubility of glycerol trioleate, husk oil solubility from previously extracted husk oil and also from solid olive husk flakes in SC-CO_2 at 308-313 K temperature and over a pressure range of 9.6-22 MPa. The authors reported that the solubility of free fatty acids were 2 to 3 times higher than triglyceride at 308 and 313 K temperature, respectively. The authors concluded that it may be feasible to deacidify olive oil by SC-CO_2 at relatively mild operating conditions, but they emphasized the need for further work.

Kramer and Thodos (1989) and White (1990) also measured the solubilities of selected fatty acids at pressures ranging from 14 to 50 MPa and temperatures ranging from 35 to 65°C. Kramer and Thodos (1989) compared solubilities of stearic acid and 1-octadecanol at 45, 55, and 65°C and found that solubilities of both compounds increased with increasing temperature.

White (1990) investigated the solubilities of even-numbered saturated fatty acids, C_{10} through C_{18}. White (1990) measured the solubilities of even-numbered saturated fatty acids at temperature between 35 to 55°C and pressure from 14 to 41.5 MPa . The author reported a greater effect of temperature on the solubility of fatty acids which remain solid under extraction conditions, even with temperature increase. When solute liquifies and reaches or exceeds the uppercritical end point (UCEP), then temperature has a much greater influence on solubility. Lauric and myristic acids have a UCEP of 30 MPa and

45°C, and at this condition, solubility is not measurable using finite measurement techniques. White (1990) observed that the solubility increased with decreasing molecular weight.

Nilsson et al. (1991) reported the solubility isotherms of oleic acid and methyl oleate as well as mono-, di-, and trioleoglycerol in SC-CO_2 at 50 and 60°C and 17.2 to 30.9 MPa. They concluded that SC-CO_2 at moderate temperatures was effective in removing mono- and diacylglycerol by-products from synthetic triglycerides reaction mixtures. The solubility of oleic acid in SC-CO_2 was 6 to 9 times higher than that of triolein at 50°C and at pressures between 17.2 and 20.6 MPa respectively. The solubility of oleic acid was six times more than triolein at 60°C and 19.9 MPa. This group reported lower solubilities of oleic acid, mono-, di or triolein compared to the work of Chrastil (1982) and Brunetti et al. (1989). The authors concluded that these differences were due to lower purity of solutes.

The published data on fatty acid solubilities vary significantly due to differences in experimental conditions and purity of the compounds used. Therefore, to evaluate the feasibility of deacidifying vegetable oils by using SC-CO_2, we measured the solubilities of myristic, stearic, oleic, and linoleic acid and compared them to average solubilities of vegetable oils obtained from del Valle and Aguilera (1988).

2. Thermodynamic modeling of fatty acid and triglyceride solubility

Bamberger et al. (1988) modeled solubility data of fatty acids and triglycerides using the lattice model equation of state. This equation correlated the equilibrium data

from mixture of molecules of disparate sizes to predicted values. The lattice model was found to correlate well experimental and predicted data using binary interaction parameters, δ_{ij} values. However, the model could not predict thermodynamic properties of fatty acids near the critical pressure (9.6 MPa) of the CO_2.

Kramer et al. (1989) predicted the solubility of stearic acid utilizing two approaches. One approach involved only using density as a variable and two adjustable parameters. The other approach utilizes three-dimensional solubility parameter and the Flory-Huggins theory to model all isotherms, using a single adjustable interaction parameter. This approach utilizes the concept of treating the dense SC-CO_2 as an expanded liquid and the solid solute as a subcooled liquid. They showed the calculated values highly dependent on δ, the solubility parameter. Predicted solubility data agreed well with experimental data. However, solubility parameters and other thermodynamic variables make the model more complex.

Chrastil (1982) assumed that molecules of a solute associate with the molecules of a gas with the formation of a solvato complex, which is in equilibrium with the gas. The equilibrium concentration can be calculated by the law of mass action. Because we used Chrastil's approach in modeling fatty acid solubilities, the derivation of his model will be presented here.

In an ideal case if one molecule of a solute A associates with k molecules of a gas B to form one molecule of a solvato complex AB_k in equilibrium with the system, then

$$A + kB \rightleftharpoons AB_k \qquad (1)$$

$$K = [AB_k]/([A][B]) \quad (2)$$

$$\ln K + \ln [A] + k \cdot \ln [B] = \ln [AB_k] \quad (3)$$

where [A] is the molar vapor concentration of a solute, [B] is the molar concentration of a gas. K is the equilibrium constant, which can be expressed as:

$$\ln K = \Delta H_{solv}/RT + q_s, \quad (4)$$

where ΔH_{solv} is the heat of solvation, and q_s is a constant. The vapor phase concentration of a solute can be calculated by Clapeyron-Clausius equation: $\ln [A] = \Delta H_{vap}/RT + q_v$, where ΔH_{vap} is the heat of vaporization of the solute, and q_v is a constant. Usually [A] $\ll [AB_k]$. On combining these expressions into equation 3, we get,

$$\Delta H/RT + q + k \cdot \ln [B] = \ln [AB_k] \quad (5)$$

where $\Delta H = \Delta H_{solv} + \Delta H_{vap}$ is the total heat of reaction, and $q = q_s + q_v$ is constant.

According to Chrastil (1982) it is convenient to express the concentration and density of the gas in g/L, and thus $[AB_k] = c/(M_A + k \cdot M_B)$, $[B] = \rho/M_B$, where c is the concentration of the solute in a gas (g/L), ρ is the density of the gas in (g/L), and M_A and M_B are the molecular weight of the solute and of the gas, correspondingly. From these relationships we get,

$$\Delta H/RT + q + k \cdot \ln\rho - k \cdot \ln M_B = \ln c - \ln (M_A + k \cdot M_B) \quad (6)$$

and thus,

$$c = \rho^k \exp (a/T + b) \quad (7)$$

where k is the association number, $a = \Delta H/R$, and $b = \ln (M_A + k \cdot M_B) + q - k \cdot \ln M_B$.

Using this simple equation Chrastil (1982) predicted the solubility of various solutes in SC-CO_2. The major drawback of this model is its restricted validity for solubilities between 100-200 g/L and 40-70°C temperature.

del Valle and Aguilera (1988) improved Chrastil's equation to relate the solubility of vegetable oil c (g/L) to the density ρ (g/mL) of SC-CO_2 and the absolute temperature T (K). These authors introduced an empirical modification to Chrastil's equation to account for the variation of ΔH_{vap} with temperature to predict the solubility of vegetable oil using literature data using the equation:

$$c = \rho^k \exp (a/T + a'/T^2 + b) \quad (8)$$

Yeh et al. (1991) used Chrastil's equation to describe relationship between solubility of cholesterol (g/L) and density (g/mL) of carbon dioxide. They reported a good agreement among experimental and predicted solubility of cholesterol. However,

this group obtained an association number three times lower compared to Chrastil's value. The authors attributed this deviation to the differences in the experimental conditions.

White (1990) modified Chrastil's model which gave simplified linear equation for predicting the fatty acids solubility with more consistent units, because Chrastil (1982) did not give the values of density or a source that he used in the regression of his data. According to her work, the predicted solubility can be found out by dividing Chrastil equation by density of SC-CO_2:

$$c/\rho = \rho^{(k-1)} \exp (a/T + b) \quad (9)$$

$$y = \rho^{k-1} \exp (a/T + b) \quad (10)$$

where, y is the solubility of fatty acid (g)/CO_2 (g), ρ is the density of SC-CO_2 in g/mL, and T is the temperature in kelvin.

White (1990) used Chrastil's model and reported a good agreement between the measured and predicted solubility data of the saturated fatty acids (lauric, myristic, palmitic, and stearic) determined at a pressure between 14 to 42.5 MPa and temperatures between 35 to 55°C.

3. Off-flavor removal

There have been a number of attempts to find an effective and practical method for controlling and/or removing off-flavors from soy protein. Two processing methods have shown some potential: steam treatment and solvent extraction. Steam treatment is used

commercially (Mustakas et al., 1969; Smith and Circle, 1978) to deactivate lipoxygenase at 100°C but heat reduces the nitrogen solubility index (NSI) of the proteins and thus limits functional properties of concentrates and isolates made from them.

Extraction with alcohols, such as ethanol, methanol, and isopropanol has been used to prepare bland soy concentrates (Eldridge et al., 1971; Rackis et al., 1975, 1979; Baker et al., 1979). Eldridge et al. (1971) used hexane:methanol (75:25), hexane:ethanol (82:18) and hexane:isopropanol (80:20) in a Soxhlet extractor. Hexane-ethanol and hexane-methanol azeotrope were slightly better in removing off-flavors, but the NSI of hexane-methanol treated protein decreased 3 times more compared to the other two azeotropes. The NSI value decreased for all the azeotropes and decreased more readily with increasing extraction time. But overall hexane-ethanol azeotrope was found to be the most effective in removing off-flavor by this group.

Rackis et al. (1975) found that hexane-ethanol azeotrope and steam treatment were effective in deactivating the lipoxygenase and improving flavor scores. NSI values decreased from 51 to 28% when hexane-defatted soy flakes were heated for 30 minutes at 100°C. The authors also cautioned about the loss in nutritive properties of proteins resulting from the destruction of essential amino acids by heat. The hexane-alcohol azeotrope was found to contribute to the deficiency of choline, therefore, choline would have to be supplemented in the diet. Rackis et al. (1979) extracted the off-flavors of soybean and other vegetable proteins with hydrogen bond breaking solvents, such as ethanol and azeotropic mixtures of hexane and alcohols. Ninety nine percent of the lipoxygenase activity was destroyed. The flavor scores were better when azeotropes were

used compared to alcohol washed proteins.

Baker et al. (1979) used ethanol, methanol, and isopropyl alcohol at temperatures ranging from 30 to 75°C. The best flavor scores were obtained when ethanol azeotrope (92.7%) was used at 60°C. Proteins were more extensively denatured when extractions were made with 70% alcohol than with the azeotropes. Methanol caused the most denaturation and isopropyl alcohol the least. Denaturation increased with temperature; however, denaturation was minimal at 60°C, when isopropyl alcohol azeotrope (87.7%) was used.

None of the treatments completely eliminate off-flavors and all denature protein. An additional problem with the solvent extraction approach is the need to eliminate all residual solvent which increases the processing cost and may cause further denaturation of soy proteins.

Christianson et al. (1984) studied the use of SC-CO_2 in place of hexane for corn oil extraction and production of a food-grade germ flour. Full-fat, dry-milled corn germ samples containing 3.5 and 8% moisture were extracted at 50°C and pressures of 34.5 and 66.2 MPa. Moisture levels did not affect oil yield, but the flavor scores of the corn germ samples with 8% moisture were significantly better than those with 3.5% moisture. It should be noted that this group used dry SC-CO_2 and by the end of extraction the final moisture level of the germ was very low (2.0-3.5%). Christianson et al. (1984) also determined the nitrogen solubility index (NSI), and concluded that significant denaturation of proteins in the SC-CO_2-extracted samples had occurred. The NSI value of soy flour decreased from 58 to 30% and even a greater decrease (NSI = 24%) was observed in soy

flour containing 8% moisture after SC-CO_2 treatment. Eldridge et al. (1986) varied the processing conditions temperature (80-100°C), pressure (72.1-84.4 MPa), and moisture content (5-13.5%) for SC-CO_2 extraction of full-fat soybean flakes to produce defatted protein products with improved flavor characteristics and high protein solubilities. The constant moisture of the sample during the extraction was maintained by adding a water-saturated glass-wool plug at the inlet of the extractor. They observed that the presence of moisture in the flakes denatured lipoxygenase and led to improved flavor. However, at higher moisture content (11.4%) and temperatures above 80°C, NSI was reduced. According to these authors, high protein solubility and good flavor scores can be obtained at pressures greater than 83 MPa, temperatures about 80°C, and moisture levels between 10.5 and 11.5%. The usual grassy/beany and bitter flavors of hexane-defatted soybean flours were minimally detectable when extracting under these conditions. The reduced lipoxygenase activity was also responsible in protecting development of undesirable flavors at 37°C during a 2-month storage time.

The most recent work from the same group dealt with the effect of SC-CO_2 extraction on the flavor of corn distillers' grains. Wu et al. (1991) improved the flavor profile of corn distillers' dried grains by treating it with SC-CO_2. They reported that SC-CO_2-extraction with water as an entrainer was more effective in reducing the fermented flavor from the corn distillers' grains than the extraction with 95% ethanol alone or with SC-CO_2-ethanol mixture. As before, the extractions were performed at rather high pressures (64-83 MPa) and temperatures (82-102°C).

The goal of our study was to examine the efficiency of SC-CO_2 in desorbing the

off-flavors from defatted soybean flour at lower temperature and pressure. We also attempted to identify and quantify the off-flavor compounds that were removed.

4. Deodorizer distillate fractionation

Conventional methods for isolation and purification of tocopherol and sterols from deodorizer distillate are chemical treatment, solvent extraction, molecular distillation, ion-exchange, and treatment with hydrogen halide (Smith, 1967; Rubel, 1969; Kim and Rhee, 1982; Herting, 1984). These steps are used together or as alternative steps depending on the proprietary interest of the manufacturer.

Chemical treatments include saponification of triglycerides and esterification of fatty acids to ease their removal from deodorizer distillate. Smith (1967) initially saponified deodorizer sludge by adding caustic soda in order to free the sludge of fatty acid esters. The saponified deodorizer sludge is then acidulated with mineral acid such as sulfuric acid and result in a mixture of free fatty acids, free tocopherols, free sterols, water, excess mineral acid and alkali salts. The glycerol and water with the excess acid and salts in solution separate from the oily mixture and are usually removed therefrom.

Smith (1967) also described the importance of the esterification process by treating deodorizer distillate with alcohol to separate sterol and tocopherols. This process is performed by mixing the deodorizer distillate with a lower aliphatic alcohol such as methanol, preferably in stoichiometric excess relative to the fatty acid content of the distillate, and a mineral acid such as sulfuric acid, under reflux. A reaction mixture is obtained which consists of free tocopherols, free sterols, excess lower, monohydric,

aliphatic alcohol, water, glycerol, and mineral acid. The sterols are then removed by adding 5-60% water of the sludge contents. The water crystallized sterols are separated from the mixture by centrifugation or filtration.

Rubel (1969) and Kim and Rhee (1982) explained the solvent extraction process for separating tocopherol, sterols from other component of the deodorizer distillate based on the polarity of each component. However, the disadvantage of this process is that it requires very large amounts of solvent, and the tocopherols and sterols may migrate to the raffinate fraction of the polar and nonpolar liquid solvent pairs.

Molecular distillation was found to be the most efficient and important step in terms of yield and purity of tocopherol and sterols compared to other methods (Herting, 1984; Smith, 1967). The alkali-refined soybean oil containing 0.19% of a mixture of α-, γ-, and δ-tocopherols is distilled in a centrifugal-type molecular still; the tocopherols fraction, which distills below 240°C under 0.53 Pa (0.004 mm Hg) pressure, is collected. These conditions minimize loss of heat-sensitive materials. The sterols and other substances in the fraction are removed by crystallization from acetone at -10°C and the glycerides are removed by saponification. After this the tocopherols in the unsaponifiable matter are further concentrated by a second molecular distillation and a fraction containing at least 60% mixed tocopherols is obtained.

Rubel (1969) described that when fatty acid free deodorizer sludge is subjected to anionic exchange resins at temperatures between 10 to 40°C, the tocopherols are preferentially adsorbed on the resin and other impurities such as sterols, hydrocarbons, glycerides, higher alcohols, and pigments are separated from free tocopherols which are

adsorbed. The tocopherols are then eluted using any substance which can produce anions. Molecular distillation followed by resin treatment produces tocopherols of high purity.

Tocopherols are also purified from a mixture of sterols and tocopherols. The mixture is treated with gaseous or aqueous HCl (the most common hydrogen halide). Sterols form insoluble halides and are separated from the mixture by filtration or centrifugation. Tocopherols can be purified by this method not only from deodorizer distillate, but from any mixture containing sterols and tocopherols.

The chemical methods require very complex process, large amounts of organic solvent, result in loss of biological activity, and high energy cost (Herting and Drury, 1967; Shishikura et al., 1988; Lee et al., 1991). Herting (1984) reported that tocopherols are sensitive to oxidation, heat and alkaline conditions. Since the tedious process of separating or purifying of tocopherols and their possible thermal degradation products during conventional process are the negative factors, SCFE offers the possibilities of overcoming these drawbacks.

Fractionation of tocopherols and sterols from deodorizer distillate using SC fluid technology has not been extensively studied. Shishikura et al. (1988) and Lee et al. (1991) were the only investigators who have examined the fractionation of tocopherols and sterols from the deodorizer distillate of soybean oil. Shishikura et al. (1988) reported that at 50°C and pressures between 9 to 35 MPa, separation was neither feasible by SC-CO_2 nor by SC-N_2O alone. About 2.2 wt% distillate was extracted by SC-N_2O at 15 MPa which was about three times more than when SC-CO_2 was used. SC-N_2O was more effective in the extraction of substances with low volatility like sterols.

The esterification of free fatty acids with ethanol increased extraction efficiency of fatty acid removal by seven times because of decreased fatty acid polarity and viscosity. A total of 79.3% tocopherols in the SC-CO_2 extracted sludge could be recovered in the final fraction. However, this fraction contained 62.7% of sterols which increased viscosity and made further concentration of sterols difficult. Therefore, the authors recommended the simple pretreatment of sludge to remove sterols by crystallization before supercritical processing to enhance processing efficiency.

Shishikura et al. (1988) used silica gel as an adsorbent. They reported that the separation efficiency increased by silicic acid column. The fatty acid ester content of the extract decreased steeply from 98 to 3%. A maximum of 64.3% tocopherols could be obtained in the final fraction before the separation of sterol ester and wax. This correspond to 11 times the concentration in the feed sludge. The recovery of tocopherol was more than 90% of the tocopherol content of feed sludge. The authors recommended the need to remove sterol esters before esterification of the sludge. The free sterols were largely adsorbed by the silicic acid column. The column was regenerated by the SCF extraction with an entrainer such as ethanol or methanol. Therefore, the use of a silicic acid column further aided the concentration of tocopherols.

Lee et al. (1991) also studied the feasibility of tocopherol concentration from sterol-free soybean sludge and esterified soybean sludge with SC-CO_2 at temperature and pressure ranging from 35 to 70°C and 20 to 40 MPa. The esterified sludge had 4 to 6 times higher solubility in SC-CO_2 than the sterol-free soybean sludge. The batch-type method used by this group was able to concentrate the tocopherols up to 40 wt% from the

esterified soybean sludge. The authors emphasized the need of a countercurrent multistage reactor vessel to obtain higher tocopherol concentrations.

Although, Shishikura et al. (1988) emphasized the need of multiple steps in order to fractionate and purify tocopherols by supercritical technology, we decided to generate the fractionation profile of deodorizer distillate of soybean oil by SC-CO_2 using 40°C temperature and a different pressure increment.

II. MATERIALS AND METHODS

A. Apparatus

A custom assembled supercritical fluid extraction system was used. A schematic diagram of the flow-through apparatus is shown in Figure 3. Carbon dioxide (Matheson Gas Products) gas (99.9% pure) was delivered at a pressure of 5 MPa (725 psi) by the regulator to the air driven single-stage gas booster (Haskel Inc., Burbank, CA). The carbon dioxide (CO_2) was compressed in a stainless steel surge tank, which was maintained at a pressure around 48 MPa (7,000 psi), higher than the operating pressure. The operating pressure of SC-CO_2 was controlled by a Alphagaz Model 2612 regulator (Cooks Inc., Algona, IA) at the downstream of surge tank. The carbon dioxide was heated to the process temperature by flowing through a stainless-steel (S.S.) coil immersed in a constant temperature water bath before contacting the samples in the extraction vessels (E1 or E2). The temperature in the water bath was controlled to ± 0.3°C by a Fisher immersion circulator and temperature was measured with a mercury thermometer to ± 0.1°C.

The extraction vessel (E1) was a 300 mL Magnedrive assembly (Autoclave Engineering, Erie, PA). This vessel served as water saturator in the experiment with soybean flour. In the experiments with fatty acids and deodorizer distillate, a 20 cm long and 1 cm internal diameter (i.d.) 316 S.S. column (E2) of a 55 MPa (8,000 psi) pressure rating was obtained from Alltech (Deerfield, IL).

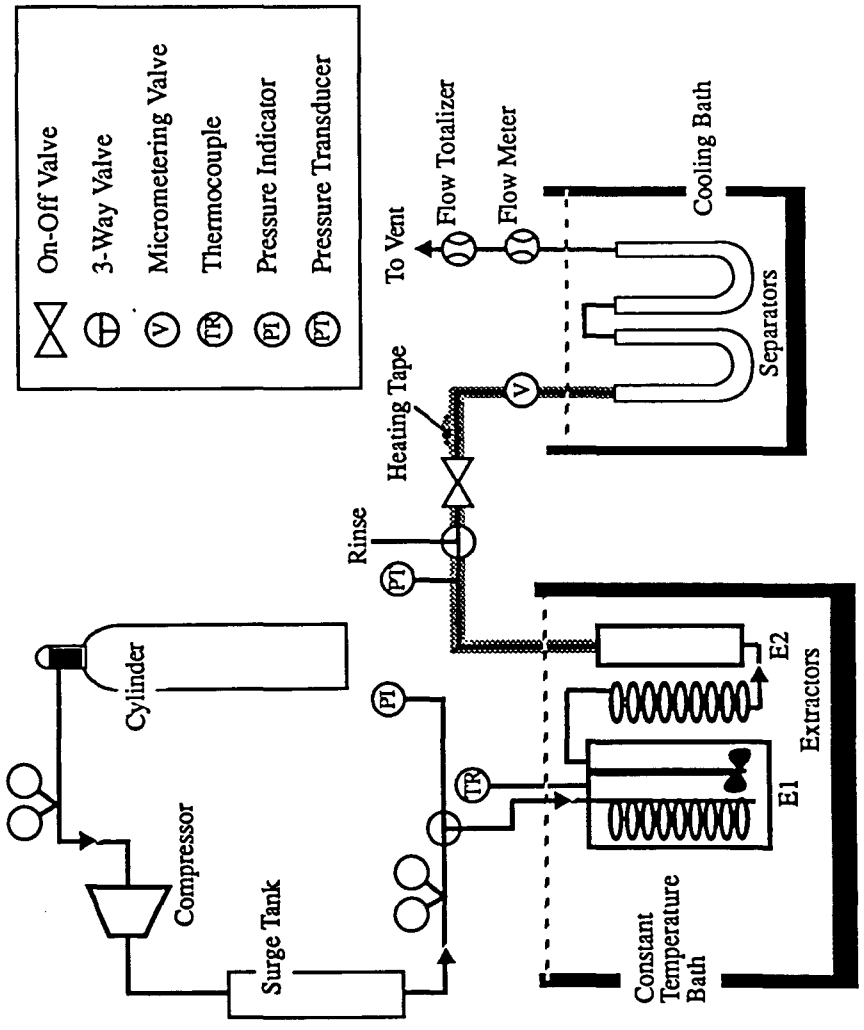

Figure 3. Schematic process diagram of the supercritical fluid extraction system

In the off-flavor removal experiment, a 20 cm long and 2.2 cm i.d. column was used for soy flour extraction, and a 10 cm long and 0.7 cm i.d. packed with an adsorbent. The SC-CO_2 was allowed to equilibrate before depressurizing for one hour.

The SC-CO_2 was expanded across a micrometer valve (Model 10VRMM-2812, Autoclave Eng., Erie, PA). An on/off valve (Model 10V-2075, Autoclave Eng., Erie, PA) upstream from the micrometering valve was also installed for complete gas shut-off. The gas flow rate was monitored by rotameter (Model FL-3840C, Omega Eng., Stamford, CT). The precipitated solute was collected in glass-tube separators which were immersed in a zero or sub-zero temperature ethylene glycol bath. All the lines and valves were heated with heating tapes to 20-30°C above the process temperature to avoid cooling during expansion and precipitation of the extracted material in lines and valves. The amount of CO_2 consumed during the process was measured by a mass flow controller (Model FMA767-I) and a totalizer (Model DP-350, Omega Eng., Stamford, CT) calibrated for carbon dioxide.

B. Measurement of fatty acid solubility

1. Extraction procedure

Solubilities of four fatty acids (FAs) at three different temperatures and pressures were studied. These were: myristic acid (MW 228.4, m.p. 54.5°C) 99-100% purity; stearic acid (MW 284.5, m.p. 69-70°C) 99% purity; oleic acid (MW 282.5, m.p. 4°C) 99% purity; and linoleic acid (MW 280.4, m.p. -12°C) 99% purity. All FAs were purchased from

Sigma Chemical Company (St. Louis, MO).

Between 5 and 10 g of solid FA samples (myristic and stearic) were packed in the extractor (E2) in three alternating layers of glass wool and solid fatty acid sample to minimize channelling of SC-CO_2. In the case of liquid FA samples (oleic and linoleic), glass wool was soaked with 4 to 5 g sample and the plugs were packed in the column in the same way as the solid samples. The system was then pressurized and left to equilibrate for at least one hour before opening the micrometer valve to start SC-CO_2 flow.

The equilibrium solubility measurements of myristic acid were carried out at the pressures of 15.2, 20.7 and 27.6 MPa. At each pressure, three different temperatures 40, 50 and 60°C were used for solubility determination. Since myristic acid has a melting point of 54.5°C, we performed the solubility study in two separate experiments with different amounts of myristic acid packed in the column. Eight and half g of myristic acid were packed for the extraction at 40 and 50°C, and 4.7 g of myristic acid was used at 60°C. For the lower temperature extraction, the column was packed evenly in three beds, but at higher temperature, more myristic acid was packed in the top bed. At 60°C, it was expected that melted myristic acid would migrate into the lower part of the reactor.

The SC-CO_2 flow rate was kept between 0.5 and 0.1 SL (standard liter) per min. (1-2 on the rotameter) as measured at standard pressure and temperature of 101.3 kPa and 273 K, respectively. Extraction was performed with 2.5 to 10 SL of CO_2, depending on the solubility of myristic acid at the particular extraction conditions. Solubility measurements were performed in triplicate for the same batch of myristic acid. Whenever

the pressure or temperature was changed, the first run of the extraction was discarded.

Stearic acid solubility measurements were carried out at pressures of 13.8, 20.7, and 27.6 MPa, and temperatures of 40 and 50°C. Stearic (4.8 g) acid was packed in the stainless steel column and was extracted in the same manner as before. The valves and the lines were heated between 100 and 125°C temperature to prevent precipitation of stearic acid in the lines and the valves. The CO_2 flow rate was maintained between 0.5 and 0.1 SL/min and the extraction was performed with 15 SL of CO_2. The other process conditions were the same as for myristic acid extraction. The solubility experiments were performed in duplicate for two batches of stearic acid.

In the case of liquid fatty acids, oleic and linoleic, about 4 to 5 g of fatty acids were packed in the extraction vessel. Three different pressures 13.8, 20.7 and 27.6 MPa, and temperatures of 40, 50 and 60°C were used. The solubilities were measured from two separate batches for each fatty acid because the material inside the column was exhausted as the extraction progressed. The extraction at 27.6 MPa pressure and three temperatures was performed with a fresh charge of sample. The flow rate of CO_2 was maintained between 0.15 and 0.2 SL/min (5-6 on the rotameter). The amount of carbon dioxide used to solubilize fatty acid ranged from 5 to 40 SL. Solubility measurements were performed in triplicates. The other process conditions were kept as before.

2. Quantitative analysis

The extracted material after depressurization of SC-CO_2 was collected in a 15 cm long glass tube with a side arm. The amount of extract collected was weighed to ±

0.0001g. The residual fatty acid that precipitated in the valves and the lines were solubilized and rinsed with 15-20 mL hexane. Hexane was then evaporated in water bath at 50°C. The final weight after evaporation was used for solubility determination. Solubilities of various samples were expressed as weight percent and as gram weight of fatty acid per gram of CO_2. The total mass of CO_2 used in the extraction was calculated from standard liters of CO_2 measured by the flow totalizer.

C. Fatty acid modeling

The fatty acid solubilities were fit to Chrastil's model (Equation 7) using the multilineal regression analysis by SAS (r) proprietary software release 6.06.01 (SAS institute Inc., Cary, NC). This software was used to get the unknown constants, *k*, a, and, b of the linear equation.

The experimental values of the various researchers were used to obtain a general equation to predict the solubility of each fatty acids. The experimental data that were used to generate the model are summarized in Tables 8, 9, 10 and 11 in Appendix.

D. Off-flavor removal from soybean flour

1. Extraction procedure from Vinton 81 soybeans

Cold defatted soybean flour was used for this study. The flour (25-30 g) was packed in three beds separated by glass wool in a 20 cm long and 2.2 cm i.d. 316 S.S.

column. Downstream of this column, a second 10 cm long and 0.7 cm i.d. 316 S.S. column was connected. The smaller column was packed with 0.75 g Tenax-GC, 20/35 mesh polymer (Alltech Ass., Deerfield, IL). Tenax is a porous polymer based on 2,6-diphenyl-p-phenyl-p-phenyllene oxide which is used as an adsorbent for trapping volatiles such as aldehydes, ketones and alcohols. Dry as well as water-saturated SC-CO_2 was used at a pressure of 27.6 MPa and a temperature of 40°C to remove off-flavor compounds. Water-saturated CO_2 was prepared by bubbling the SC-CO_2 through a reactor vessel (Autoclave Eng., Erie, PA) containing about 150 mL deionized water at 40°C. The flow rate of CO_2 was maintained between 0.15 and 0.2 SL/min and the extraction was performed using 500-600 SL of CO_2. At this pressure and temperature, SC-CO_2 contained 0.002 g H_2O/g CO_2. The depressurized SC-CO_2 was passed through a glass U-tube containing 10 g Tenax-GC to entrap solutes from the gas stream. Both columns and the U-tube were weighed before and after the extraction. Upon completing the experiment, the lines were flushed with double distilled diethyl ether to remove any residual volatiles in the lines. A control run using gaseous CO_2 was performed at 4.1 MPa and 40°C for 43 hr. The CO_2 flow rate was maintained between 0.2 and 0.25 SL/min and a total volume of 600 SL CO_2 was used in the latter experiment.

2. Soybean flour and extract analysis

The beds of SC-CO_2 treated, defatted soybean flour were stored in the refrigerator until analyzed. The extracted volatiles that were adsorbed on the Tenax in the second column and in the U-tube were eluted using about 50 mL double distilled ether. The

ether was evaporated using a Vigreux distillation column (Pyrex) and the residue left in the round bottle flask was immediately freezed to avoid loss of volatiles.

3. Gas chromatography

The headspace gas chromatographic (GC) analysis was performed on the control as well as SC-CO_2 treated soy flour. The volatiles extracted from the flour were analyzed by using a Varian 3400 (Sunnyvale, CA) gas chromatograph.

For headspace analysis, 1 g of flour was weighed in a 50 mL glass vial. The SC-CO_2 treated flour was analyzed separately for the volatiles present in the top, middle, and the bottom bed of the flour. A slurry of 1 g flour was also prepared by adding water. After sample preparation, the glass vials were equilibrated at least one hour in an oven at 37°C.

A 30 m long DB-WAX capillary column (J & W Scientific Inc., Rancho Cordova, CA) was used for GC analysis. The column temperature was maintained at 40°C for 8 min. Then the temperature was raised to 200°C and held at that temperature for 22 min. The column injector temperature was set at 210°C, and temperature at the flame ionization detector (FID) was 250°C. Hydrogen at a rate of 4 mL/min was used as a carrier gas. Nitrogen was used as an inert diluent at a rate of 26 mL/min. The air and hydrogen flow rates in the FID were set at 30 mL/min. The sensitivity of measurement ranged between 10^{11}-10^{12}, and the attenuation was set between 4-8.

Five milliliters of headspace over the dry as well as over a soy flour slurry were injected in the GC. The injection time was two min and the column was cryofocussed

during that time using liquid nitrogen. The analysis was performed in triplicate in each case. Three standards, butanal, pentanal, and hexanal were used to determine the retention times of these compounds. Butanal and pentanal (98+% pure) were purchased from Polyscience Corp. (Niles, IL), and hexanal (99% pure) were obtained from Aldrich (Milwaukee, WI).

The adsorbed volatiles that were eluted from the Tenax column and the U-tubes were also analyzed by using capillary GC. About 0.5 µL sample of extract was injected on the DB-WAX column under the same conditions as above. The analysis was performed in triplicate.

4. Total protein and protein functionality determination

The total protein and NSI of defatted soy flour were expressed on dry weight basis. The moisture content was determined by drying the defatted soy flour in vacuum oven at 80°C for 5 hr. Control as well as SC-CO_2 treated soy flour were analyzed in duplicate for total protein content by using Kjeldahl AOCS Method Ac 4-41 (1964).

a. Nitrogen solubility index (NSI) The official AACC Method 46-23 was modified to determine NSI values from smaller amounts of sample. All the three portions of SC-CO_2 treated soy flour were mixed for NSI determination. The analysis was performed in triplicate for control as well as SC-CO_2 treated soy flour.

About 2.5 g of sample was weighed and transferred into 180 mL bottle. Then, 80 mL of water at 30°C was added and the bottle was shaken. The mixture was shaken in a water bath at 120 rpm and 30°C for 120 min and then it was transferred into a 100-mL

volumetric flask. A couple of drops of antifoam (silicone oil) were added and the mixture was diluted with water to 100 mL. The suspension was shaken thoroughly and let to stand for a few minutes. After that the suspension was transferred into a 50 mL centrifuge tube and centrifuged for 10 min at 1,500 rpm (309 g force). The supernatant was filtered through a funnel plugged with glass wool. The clear supernatant (10 mL) was pipetted into a Kjeldahl flask and protein content determination was performed according to the AOCS Method Ac 4-41. The analysis was performed in triplicate, both the for control as well as SC-CO_2 treated soy flour. NSI was calculated as:

$$\%NSI = \frac{\%water\text{-}soluble\ nitrogen}{\%total\ nitrogen} \times 100 \qquad (11)$$

5. Sensory evaluation

A Triangle test (Larmond, 1977) was performed to determine the difference between the control and the SC-CO_2 treated soy flour. Fifteen panelists familiar with the soybean flavor were chosen at random. The panelists received three coded samples in 20-mL glass vials containing 2 g of soybean flour. The panelists were told that two (control) samples were the same and one (SC-CO_2-treated) was different. They were asked to identify the odd sample based on its odor, and to comment on the differences between samples. The level of significance for correct responses was determined using statistical chart (Larmond, 1977).

E. Deodorizer distillate

1. Extraction procedure

The deodorizer distillate of soybean oil was obtained from Archer Daniels Midland Company (Des Moines, IA). The sample was stored at 4-5°C. Between 4 to 5 g of deodorizer distillate were soaked in the glass wool and packed in three beds separated by glass wool in a 20 cm long and 1 cm i.d., 316 S.S. column. The fractionation was started at a pressure of 10.3 MPa and temperature of 40°C for 45 hr. The carbon dioxide flow rate was maintained between 0.15 and 0.2 SL/min. The extracted fractions were collected in the glass tubes after every 50 to 100 SL of CO_2. The glass tubes were wrapped with aluminum foil and kept in an ice bath at 2-3°C during the extraction to avoid degradation of tocopherols. The lines and valves were flushed with 10 mL ether at the end of each run to dissolve any residual material not collected in the tubes. The ether was evaporated by blowing nitrogen gas in the tubes. The solubility was expressed in weight percent as before.

After 45 hr of extraction at 10.3 MPa, the pressure was raised to 20.7 MPa, and the extraction was performed for another 26 hr at 40°C. The pressure was further increased to 27.6 MPa, and the fractions were collected for the next 18 hr. The temperature of the lines and valves was increased to 70°C because at pressures of 20.7 and 27.6 MPa, the extracted material blocked the lines.

2. Analytical methods

After the extraction, the various fractions obtained at different pressures were analyzed by using thin layer chromatography (TLC). The TLC was performed on the 20 cm x 20 cm Adsorbosil Plus 1 glass plates with 25 μm adsorbent thickness (Alltech Ass., Deerfield, IL). The standards oleic acid 99% purity, stigmasterol, 90% purity, and α-tocopherol of 95% purity, were obtained from Sigma Chemical Company (St. Louis, MO).

In order to select the optimum mobile phase to identify various compounds in the deodorizer distillate, and in the extracted fractions, different solvent systems were examined. Solvent systems investigated were: 1) petroleum ether:ether:acetic acid at a volume ratios of 80:20:1, 70:30:2:, and 65:35:2; 2) cyclohexane:ether at 80:20; 3) benzene:chloroform at 70:30; 4) chloroform:benzene at 70:30; and 5) chloroform:methanol at 65:35 ratio. Twenty five μg of oleic acid, α-tocopherol, and stigmasterol, and 100 μg of deodorizer distillate were spotted on the TLC plate. Each solvent system was allowed to equilibrate in TLC chamber for half an hour. The plate was then placed in the chamber. The solvent front took about 45 min to travel 2.5 cm below the top of the TLC plate. The plate was then taken out of the TLC chamber and was dried. The migration of different compounds was visualized by spraying 0.2% solution of 2',7'-dicholorofluroscein. The locations of various compounds were observed under UV light. The α, γ, and δ-tocopherols were observed as purple spots, and other compounds as bright yellow spots (Christie, 1973). The relative migrations of various compounds were also observed as black spots when 50% H_2SO_4 was sprayed on the TLC plate, and the lipid material was charred at 110°C for 15-20 min (Christie, 1973).

Based on the above experiment, the petroleum ether:ether:acetic acid (80:20:1) solvent system was found to be the best for qualitative analysis of the compounds present in the deodorizer distillate, extracted fractions, and in the residue left after extraction. Hence, this solvent system was chosen as the mobile phase.

III. RESULTS AND DISCUSSION

A. Deacidification of vegetable oil: Solubility of fatty acids in SC-CO_2

The effects of pressure and temperature on the solubilities of the four fatty acids are plotted in Figure 4. The solubility isotherms for individual fatty acids showed an increase of solubility with increasing pressure at all temperatures. Myristic acid had the highest solubility at the pressures and temperatures studied, followed by linoleic, oleic and stearic acid. The solubility isotherms of stearic acid at 40 and 50°C are only reported in Figures 4a and 4b because reliable solubility measurements at 60°C were difficult to obtain due to precipitation of stearic acid in the lines and the micrometering valve. The solubility of stearic acid was much lower than the other three fatty acids probably due to the difference in physical state. At the process temperatures below 50°C, stearic acid was probably in a solid state whereas the other three fatty acids, including myristic acid, were liquid.

Bamberger et al. (1988) recently determined experimentally that myristic acid (mp 54.5°C) is a liquid at 15 MPa and 40°C in the presence of SC-CO_2. Our solubility data of stearic acid are 1/2 to 1/5 of those reported by Chrastil (1982), White (1990), and Kramer and Thodos (1989) and an order of magnitude lower than those of Brunetti et al. (1989) under the same experimental conditions. Our review of the literature data reveals that stearic acid solubilities had the largest reported discrepancies in solubility. The solubility of myristic acid was in the range of reported values in the literature (Bamberger et al.,

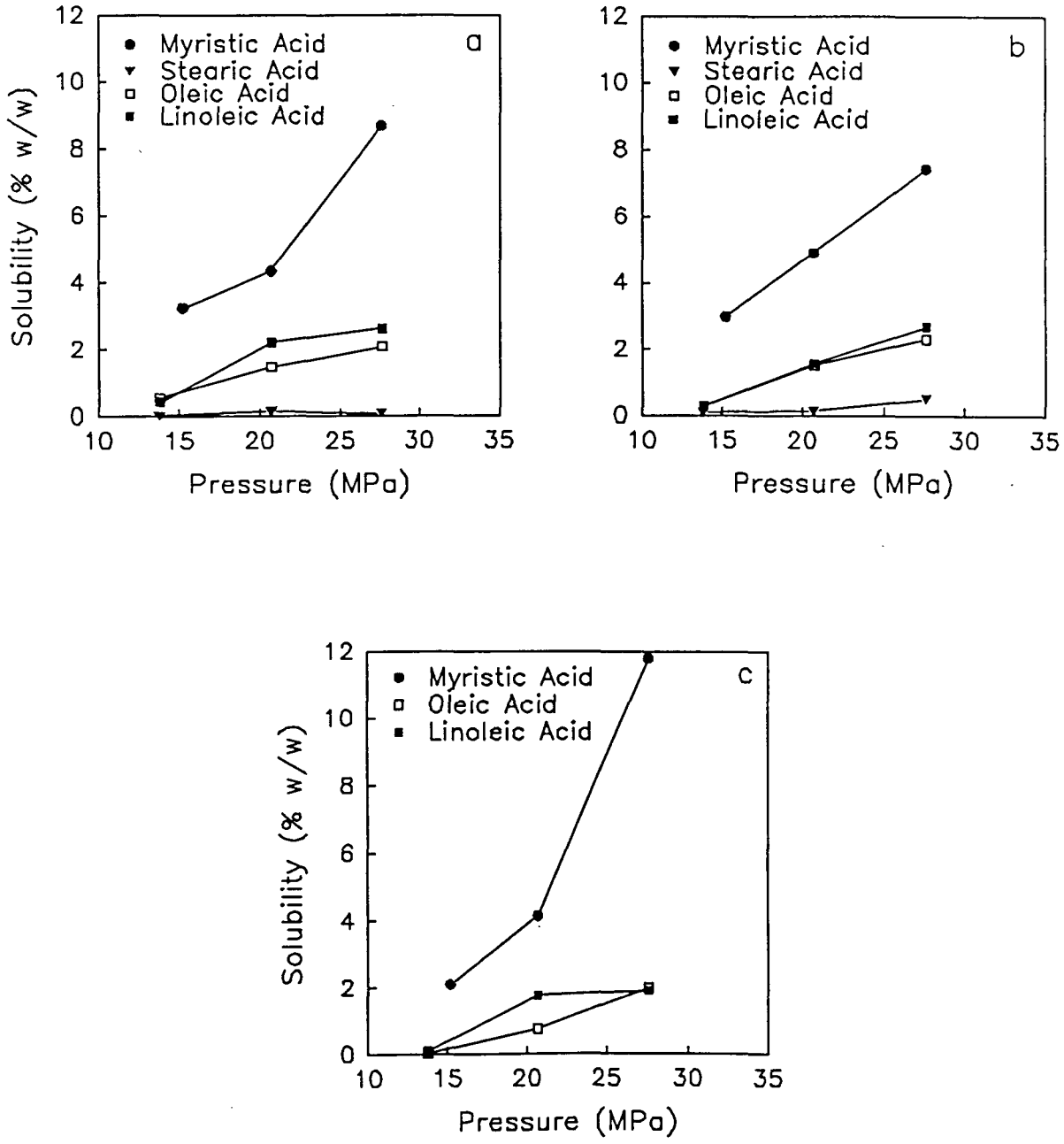

Figure 4. Solubility isotherms of fatty acids in SC-CO_2 as a function of pressure: (a) 40°C; (b) 50°C; and (c) 60°C

1988; Brunetti et al, 1989; White, 1990). Oleic acid solubilities shown in Figure 4 agree with the values of Nilsson et al. (1991) and with the solubilities measured by Chrastil (1982) at pressures below 20 MPa. Both Chrastil (1982) and Brunetti et al. (1989) found oleic acid solubilities at 25 and 30 MPa, respectively, and at 60°C they were almost four times higher than our values. This discrepancy could be, in part, explained by the lower purity of their starting material as pointed out by Nilsson et al., (1991). Linoleic acid had a very similar solubility to oleic acid as would be expected on the basis of similar molecular weight and structural characteristics. Myristic acid (C_{14}:0) has a higher solubility in SC-CO_2 than any of C_{18} fatty acid because of its significantly higher vapor pressure compared to the other three fatty acids (Singleton, 1960).

At lower pressures, near and below 15 MPa, the solubilities of the liquid fatty acids (myristic, oleic, and linoleic) were found to decrease with increasing temperature (Figure 5). The solubility decrease was more for oleic and linoleic acids at lower pressure and higher temperature. Myristic acid solubility was affected to a lesser extent with temperature increase, but the stearic acid solubility increased with increasing temperature. Figure 5 shows that a cross-over point exists in the experimentally measured solubility at pressures close to 20 MPa. At pressures near the critical point, a moderate temperature increase can cause a large decrease in fluid density resulting in a decrease in solute solubility. Such behavior is called retrograde behavior (Marentis, 1988) and it has been explained in terms of temperature effect on the vapor pressure of the solute and on the density of SC-CO_2 (Peter and Brunner, 1978). Vapor pressure of the fatty acids increases exponentially with temperature whereas the density of SC-CO_2 decreases almost linearly

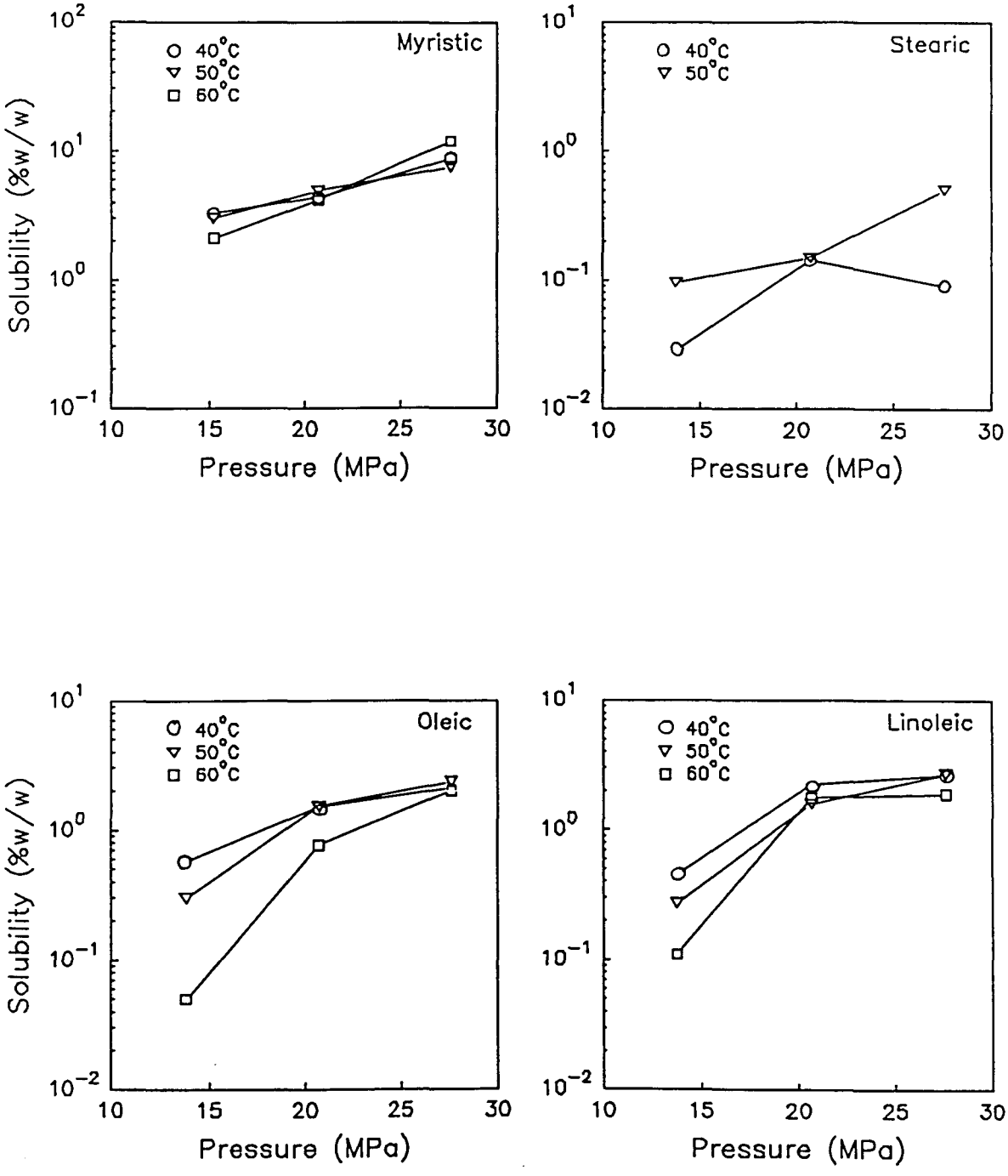

Figure 5. Retrograde behavior of fatty acid solubility of this work in SC-CO_2 as a function of pressure and temperature

under operating conditions used in this work (Singleton, 1960; Angus et al., 1976). Therefore, at lower pressure, where the fluid is highly compressible, the density of SC-CO_2 decreases significantly with small increases in temperature. At the higher pressures, the fluid becomes less compressible and temperature affects density slightly and the solubility increases with temperature as does the vapor pressure (Wong and Johnston, 1986). Retrograde behavior was not observed for stearic acid as it exhibited an increase in solubility with increase of temperature from 40 to 50°C. A temperature of 50°C is probably close to the depressed melting point of stearic acid which offsets the negative temperature effect on the CO_2 density. Similar observations were made by Brunetti et al. (1989) about another solid fatty acid, palmitic acid, whose solubility also increased with as temperature increased from 35 to 50°C. Brunetti et al. (1989) suggested that compounds which are solids under experimental conditions tend to show an increase of solubility with an increasing temperature.

To eliminate the effect of pressure on the density, solubility isotherms in Figure 6 were plotted versus density instead of pressure. The difference of solubilities between C_{18}-fatty acids in Figure 6 at a particular density is mainly due to vapor pressure effects and, therefore, by increasing the temperature at constant density the solubility should be expected to increase. The solubility of each fatty acid increased proportionally to the density of the supercritical fluid. The small decrease of solubility observed for stearic acid at the SC-CO_2 density of 0.9×10^{-3} (kg/m^3) (Figure 6a) was probably due an experimental error. The standard deviation (n=3) of the average solubility of stearic acid at this particular density was unusually high, almost 45%. An order of magnitude higher

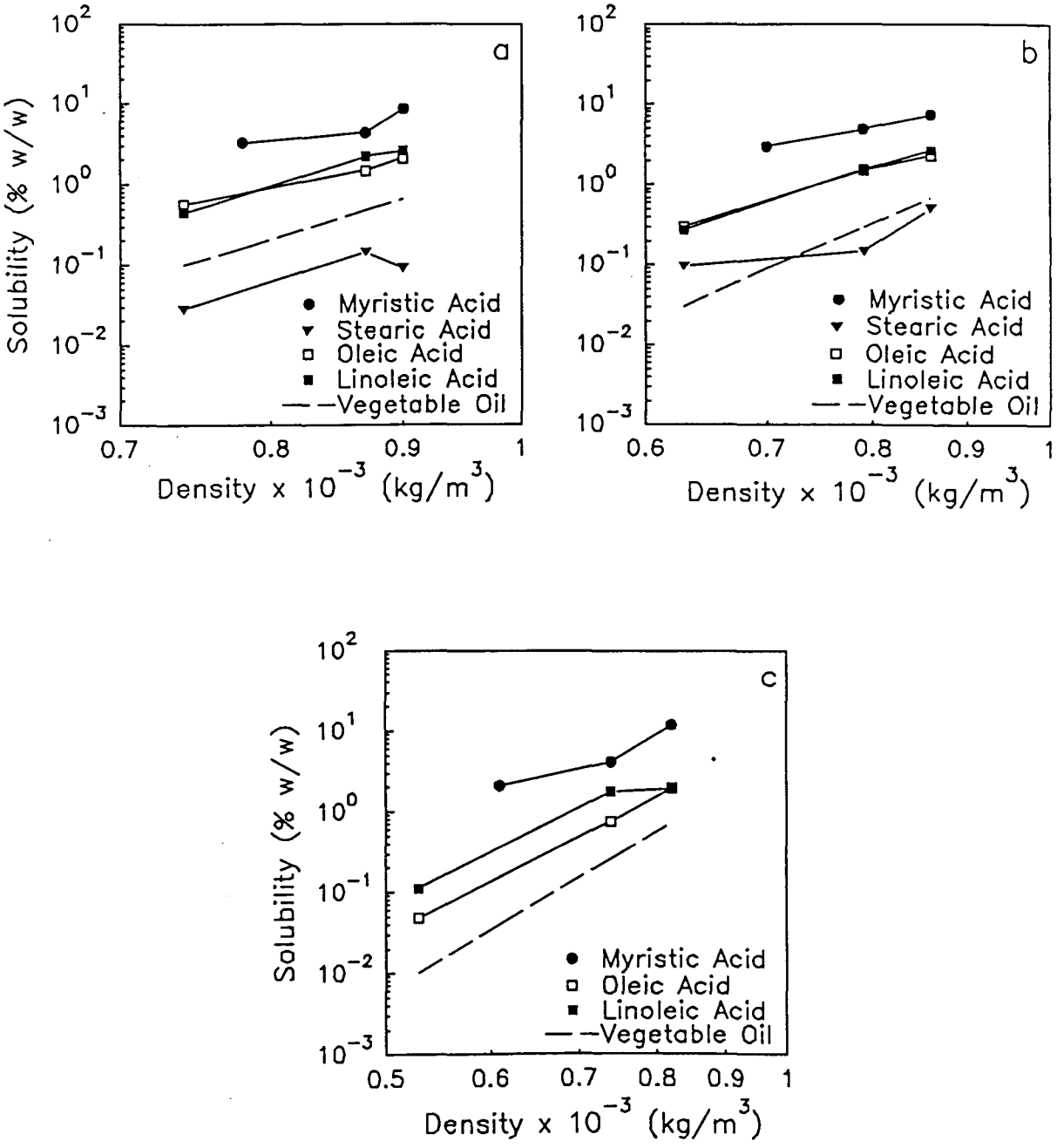

Figure 6. Solubility isotherms of vegetable oil and fatty acids in SC-CO_2 as a function of density: (a) 40°C; (b) 50°C; and (c) 60°C

Table 2. Experimental solubility data of fatty acids in SC-CO_2

y (g/g)	$\rho x10^{-3}$ (kg/m^3)	T (K)	Fatty Acid
0.0325	0.78	313	Myristic
0.0435	0.87	313	Myristic
0.0869	0.90	313	Myristic
0.0298	0.70	323	Myristic
0.0490	0.79	323	Myristic
0.0744	0.86	323	Myristic
0.0210	0.61	333	Myristic
0.0414	0.74	333	Myristic
0.1181	0.82	333	Myristic
0.00028	0.74	313	Stearic
0.00145	0.87	313	Stearic
0.00090	0.90	313	Stearic
0.00096	0.63	323	Stearic
0.00150	0.79	323	Stearic
0.00510	0.86	323	Stearic
0.005700	0.74	313	Oleic
0.014900	0.87	313	Oleic
0.020900	0.90	313	Oleic
0.003000	0.63	323	Oleic
0.015200	0.79	323	Oleic
0.023100	0.86	323	Oleic
0.000480	0.53	333	Oleic
0.007600	0.74	333	Oleic
0.019800	0.82	333	Oleic
0.0045	0.74	313	Linoleic
0.0222	0.87	313	Linoleic
0.0263	0.90	313	Linoleic
0.0027	0.63	323	Linoleic
0.0158	0.79	323	Linoleic
0.0269	0.86	323	Linoleic
0.0011	0.53	333	Linoleic
0.0176	0.74	333	Linoleic
0.0189	0.82	333	Linoleic

vapor pressure of myristic acid than vapor pressures of other three acids (Singleton, 1960) is reflected in its higher solubility as shown in Figures 4 and 6. The stronger effect of temperature on the solubility of stearic acid (solid) than on the solubilities of the other (liquid) acids, as discussed above, is more apparent from the solubility isotherms in Figures 6a and 6b.

The solubilities of myristic acid were significantly higher than stearic acid (α=0.05%). Tables 2, 3, 4, and 5 in Appendix shows the standard deviation for the solubility measurements of each fatty acid studied. However, there was no significant difference (α=0.05) in the solubilities of two liquid fatty acids, oleic and linoleic acid.

In Figure 6, solubility isotherms of vegetable oils were also plotted for a comparison with fatty acid isotherms. The solubility values of the oil for operating conditions in this work were calculated from a model developed by del Valle and Aguilera (1988). Their model-equation is based on experimental solubilities of three vegetable oils, including soybean oil, and estimates triglyceride solubilities as a functions of temperature and SC-CO_2 density. By comparing the solubility isotherms of liquid fatty acids and vegetable oil shown in Figure 6, it can be concluded that deacidification of oils would be possible. Higher temperatures and lower densities (lower pressure) would favor selectivity of CO_2 for fatty acids. The proper extraction conditions should be chosen to achieve good yield of fatty acids and satisfactory selectivity. Based on the relative solubilities of vegetable oil and liquid fatty acids, it appears that pressure between 15 and 20 MPa and temperatures at or above 50°C would result in higher selectivity for fatty acids than the oil. For example, at 50°C and 15 MPa the solubility of pure myristic acid

relative to that of vegetable oil is 30 times larger. For both oleic acid and linoleic acid, solubilities are about 7 times larger. At higher pressure (20 MPa), the relative solubilities are somewhat lower -- 13, 4.5, and 4.5 for myristic, linoleic, and oleic acid, respectively. But, at 20 MPa and 50°C the extraction yield (g FA/g CO_2) of these three fatty acids is almost 50% higher than that at 15 MPa.

At low densities of SC fluid, the short chain fatty acids are more soluble in the supercritical fluid phase. As the pressure (density) of the SC fluid is increased at a constant temperature, the solubility increases. Variation in molecular weight and unsaturation leads to the difference in the volatility of triglycerides (Perry et al., 1949). Similar observation are expected for fatty acids as well. According to regular solution theory (Hildebrand and Scott, 1950), the solute-solvent mixing is best achieved when the nature and the cohesive energy densities of the solute and solvent molecules are matched. When the heat of mixing or interchange energy density, $(\delta_{solute} - \delta_{solvent})^2$ is zero, then the solubility should be maximal. The regular solution Scatchard-Hildeband model (Prausnitz, 1969) also indicates that with increasing molar volume (size) of the solute molecules of similar cohesive energy densities, the solubility would decrease, i.e., a larger number of solvent molecules would be required to cluster around or saturate a larger molecule than for a smaller one to effect the dissolution process. This suggests that molecular size has a primary influence in the molecular separation. However, other principles which may or may not depend on the molecular size, are also important in the separation of fatty acids (Arul et al., 1987).

Therefore, operating conditions need to be optimized depending on the main

objective of the extraction, i.e., fractionation verses oil deacidification. If fractionation of fatty acids is the goal, then lower pressure and higher temperatures are favored. For deacidification of vegetable oils, higher pressures, as suggested above, would be preferred because of better yields. Stearic acid cannot be discussed in this context because its interaction with vegetable oil (triglycerides) would probably change its physical state and that would probably result in a different solubility than estimated in this study.

B. Fatty acid modeling

Out of four fatty acids studied in this work, solubilities of all but linoleic acid have been investigated by number of researchers in the past. The three dimensional Figures 7, 8, 9, and 10 summarize the solubility of myristic, stearic, oleic and linoleic acid as functions of temperature and density. In general, solubility increases with density and with temperature at the constant density. However, there are considerable differences among the experimental solubility values of literature. The experimental solubility values of myristic acid agrees well for most of researchers as shown in Figure 7 except the data of White (1990) at 0.94 g/mL SC-CO_2 density and 318 K. White (1990) has attributed this exceptionally high solubility (62.3 wt%) to the existence of the uppercritical end point (UCEP) at that condition. The stearic acid solubility data are fairly comparable for most of the investigators up to 318 K as shown in Figure 8, but a lot of discrepancies are observed at higher temperature. We believe that this difference was mainly due to experimental error in measuring stearic acid. The high melting point (69.5°C) of the

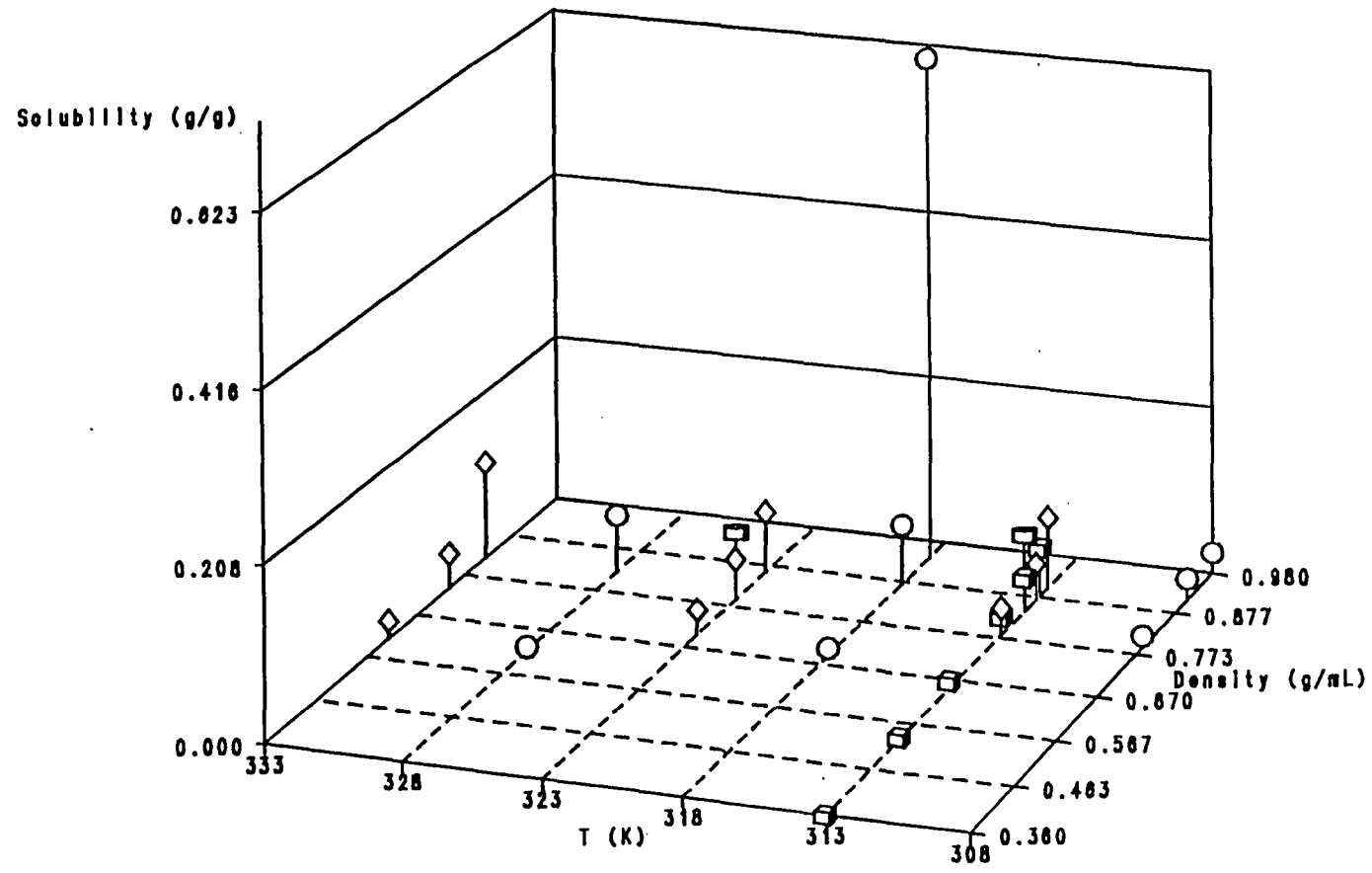

Figure 7. Experimental solubility (g/g) of myristic acid as a function of density (g/mL) and temperature (K), using literature data. Symbols: (⊟) Bamberger et al. (1988); (▭) Brunetti et al. (1989); (○) White (1990); and (◇) This work (1991)

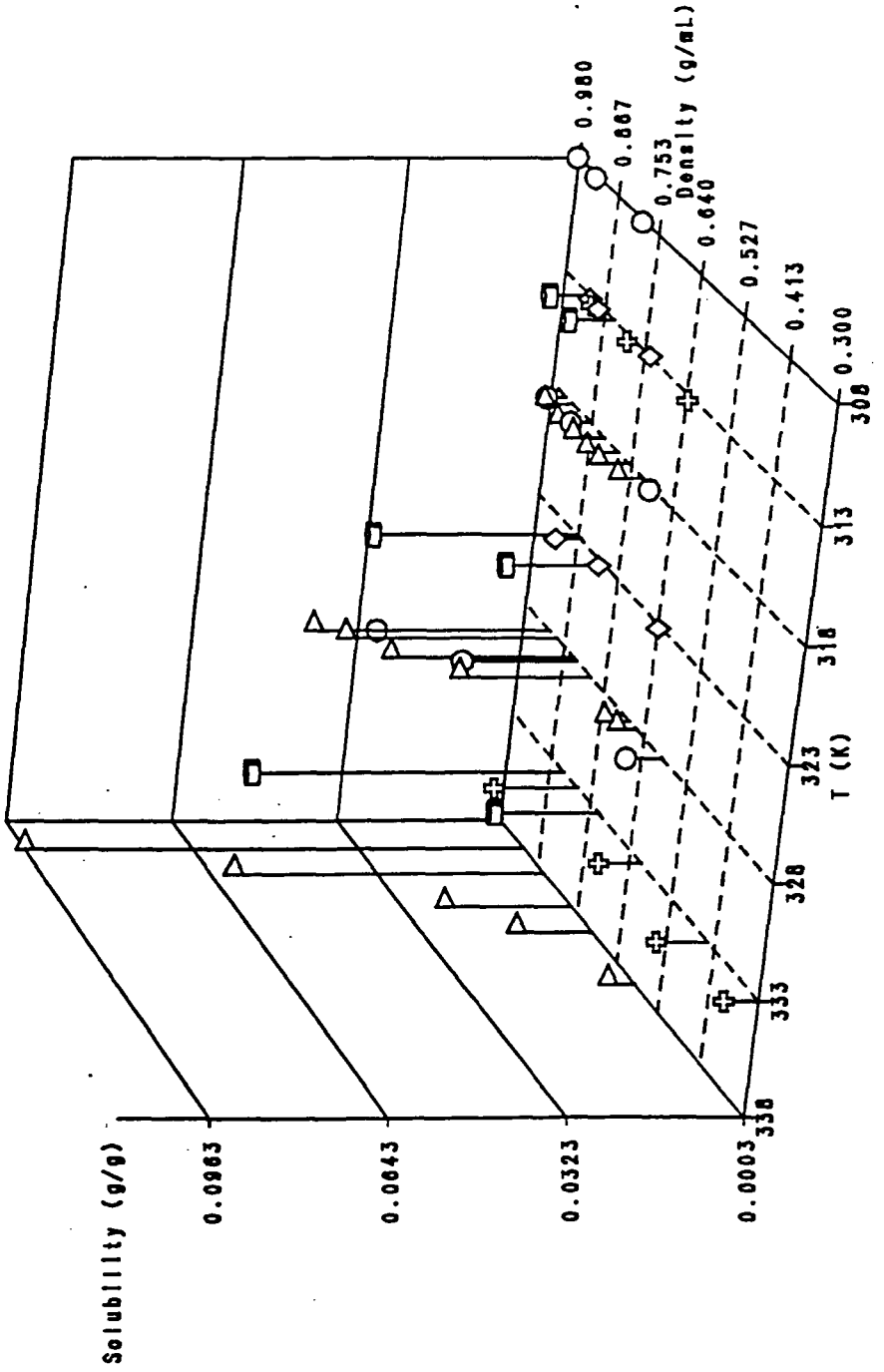

Figure 8. Experimental solubility (g/g) of stearic acid as a function of density (g/mL) and temperature (K), using literature data. Symbols: (□) Brunetti et al. (1988); (✙) Chrastil (1982); (▷) Kramer and Thodos (1989); (○) White (1990); and (◇) This work (1991)

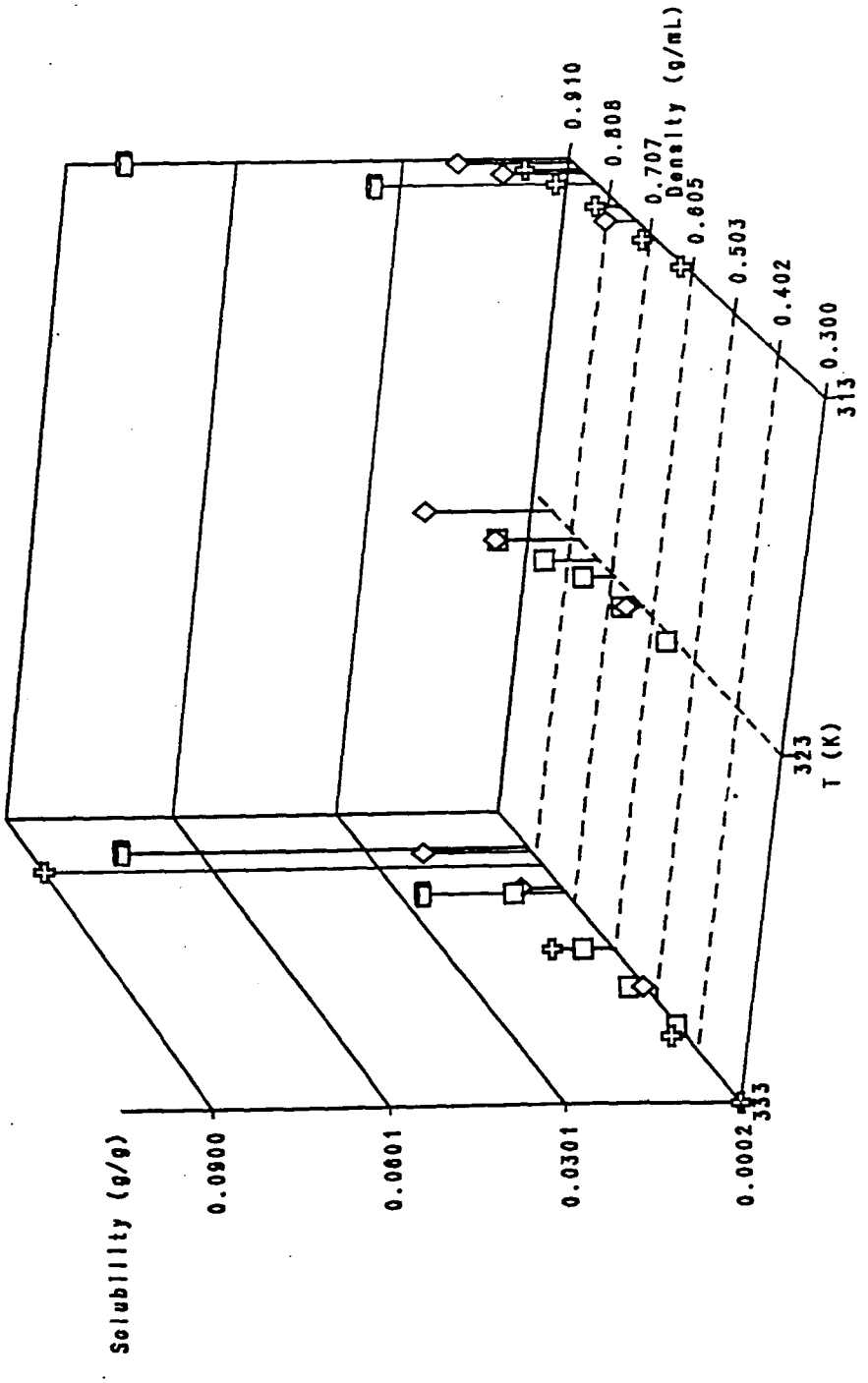

Figure 9. Experimental solubility (g/g) of oleic acid as a function of density (g/mL) and temperature (K), using literature data. Symbols: (▣) Brunetti et al. (1988); (✙) Chrastil (1982); (□) Nilsson et al. (1991); and (◇) This work (1991)

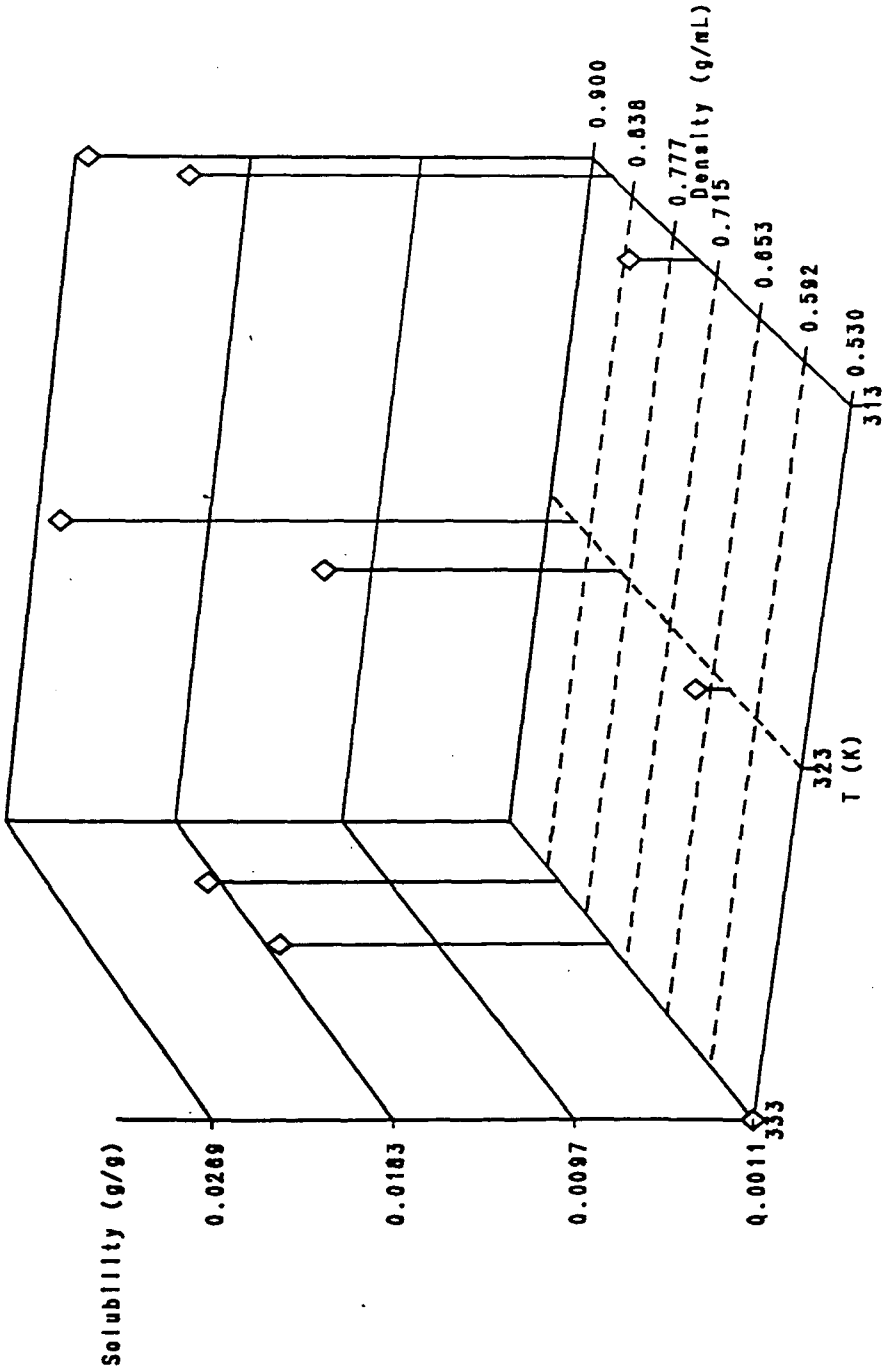

Figure 10. Experimental solubility (g/g) of linoleic acid as a function of density (g/mL) and temperature (K). Symbols: (◇) This work (1991)

stearic acid can cause its precipitation in process lines and valves before the depressurization as the tubing diameter is substantially smaller compared to the extractor and a thin layer of solubilized material may start depositing at the tubing walls. Figure 9 shows that solubility data of oleic acid in this work and Nilsson et al. (1991) were low compared to data of Chrastil (1982) and Brunetti et al. (1989). Nilsson et al. (1991) have attributed the higher solubility data of Chrastil (1982) and Brunetti et al. (1989) to the lower purity of the oleic acid used by these investigators. Yeh et al. (1991) reported that the lower solubility could also be due the result of the dynamic measurement technique if equilibrium has not been reached.

The pooled literature data of the four fatty acids solubilities in SC-CO_2 were modeled using modified Chrastil's equation developed by White (1990). The coefficients *k*, a and b are different for each fatty acids as shown in Table 3 and 6 and 7 in the Appendix. The Figure 11 represents the predicted (literature) and our experimental solubility of all acids studied. Our experimental values for myristic acid solubility at all temperatures agree with the predicted solubility values (Figure 11).

Stearic acid data showed the largest deviation from the predicted literature values. Our solubility data were about 1/2 to 1/5 of predicted values. At higher temperatures and pressures, the solubility of the stearic acid increases, but precipitation of solute before depressurization step could have resulted in erroneous solubility measurement. Figure 11 shows that there was not good agreement between our experimental values and predicted values till 50°C. As the temperature increased, the discrepancy between predicted and experimental values increases. We experienced extensive blockage of the process lines

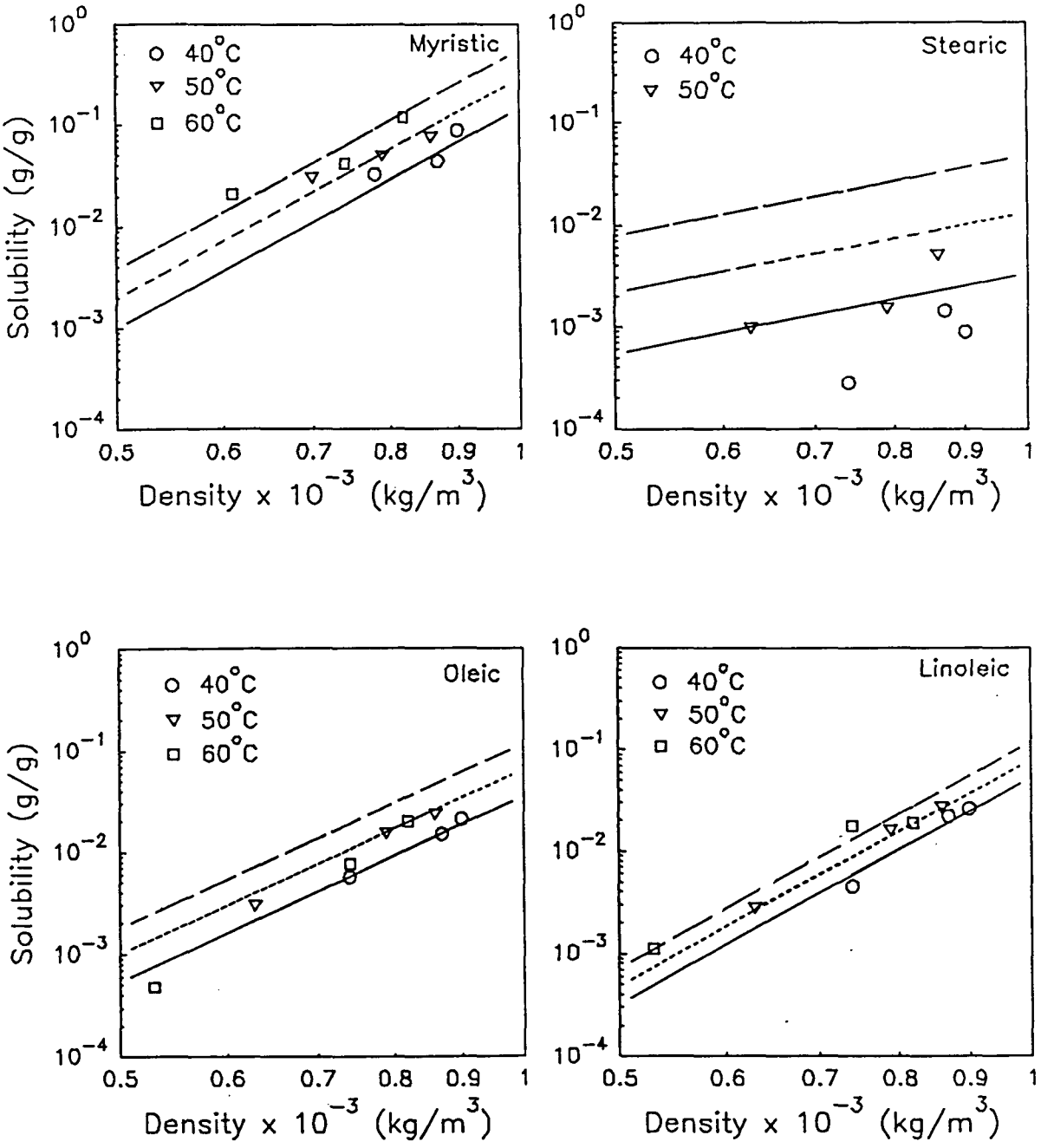

Figure 11. Solubility of fatty acids as a function of density. Lines represent predicted isotherms (literature): (——) 40°C; (·····) 50°C; and (– –) 60°C

and valves, and could not achieve reproducible results. The equilibrium solubility measurement as performed by Chrastil (1982) could give more reliable results compared to a dynamic system for these kind of solutes.

Our experimental solubility data for oleic acid is comparable with predicted values from literature at 40 and 50°C (Figure 11). But, at 60°C we measured 1/2 to 1/5 lower solubility than predicted. This difference in the solubility is mainly due to the higher solubilities measured by Chrastil (1982) and Brunetti et al. (1989) as shown in Figure 9. Chrastil (1982) measured about 4 times higher solubility of oleic acid at 60°C, whereas Brunetti et al. (1989) reported 3 to 5 times higher solubility at 40 and 60°C, compared to ours and the Nilsson et al. (1991) work at the same temperature as shown in Figure 9. We believe that lower purity of the solute was responsible for high solubility data of these authors at the fore-mentioned process conditions. Brunetti et al. (1989) also used only 68% pure oleic acid, but in this work all the solutes were at least 99% pure. According to Yeh et al. (1991), the high experimental solubility data of Chrastil (1982) could also have been due to the equilibrium method of measurement. To verify the modified Chrastil's method for oleic acid, we used only the data of this work and Nilsson et al. (1991) as shown in Figure 12. Our experimental data agrees well with the predicted isotherms of 40 and 50°C. There is lesser agreement at the lower density of SC-CO_2, this could be due to the limitation of this model to predict solubility at these conditions or due to the "hot-finger" effect of 20-30°C higher temperature of the lines coming out of extractor.

The predicted solubility data of this work agree well with the experimental work for oleic acid, and similar observations were made for linoleic acid as well. However,

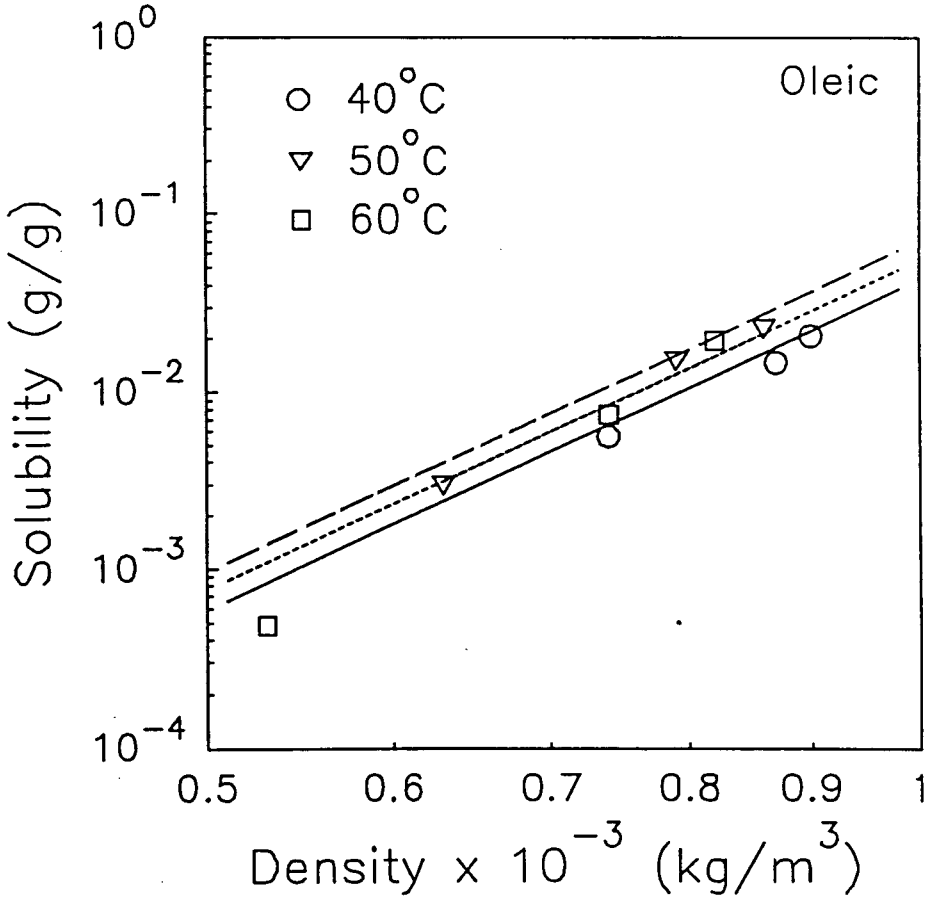

Figure 12. Solubility of oleic acid as a function of density. Lines represent predicted isotherms (This work and Nilsson et al., 1991): (——) 40°C; (·····) 50°C; and (– –) 60°C

Figure 11 shows that the linoleic acid solubility was influenced more by temperature compared to oleic acid. The reason for this difference could be the fact that linoleic acid solubility data were averages of two runs, but oleic acid data was obtained after averaging four different runs.

In general, our solubility data for fatty acids in SC-CO_2 and their average predicted values using literature data compared well for myristic and oleic acid. However, the largest discrepancy in solubility was observed for stearic acid. This work suggests that unless there is an agreement in the experimental data, a prediction model cannot be effective for stearic acid. We eliminated some of the experimental values which were significantly different than the averaged predicted values to observe the effect that would have on the model fit. We did not eliminate any data points for stearic acid because it was very difficult to judge the most reliable observation at a particular process condition. We eliminated the White (1990) data (Figure 7) of myristic acid at UCEP, because more than 62 wt% solubility at that condition was reported. For oleic acid, the high solubility (9 wt%) of Chrastil, 1982 (0.79 g/mL; 60°C) and Brunetti et al., 1989 (0.84,40°C; 0.91 g/mL,40°C) and (0.83 g/mL SC-CO_2, 60°C) were eliminated and resulting model fits are shown in Figure 13.

It is clear from Figure 13 that the elimination of selected data points from myristic and oleic acid, the predicted literature values can be correlated better with our experimental data. When comparing Figures 11 and 13 for myristic acid, although the removal of White (1990) data at UCEP improved the correlation between predicted data of literature and this work. For oleic acid, the correlation was better at 60°C after bad

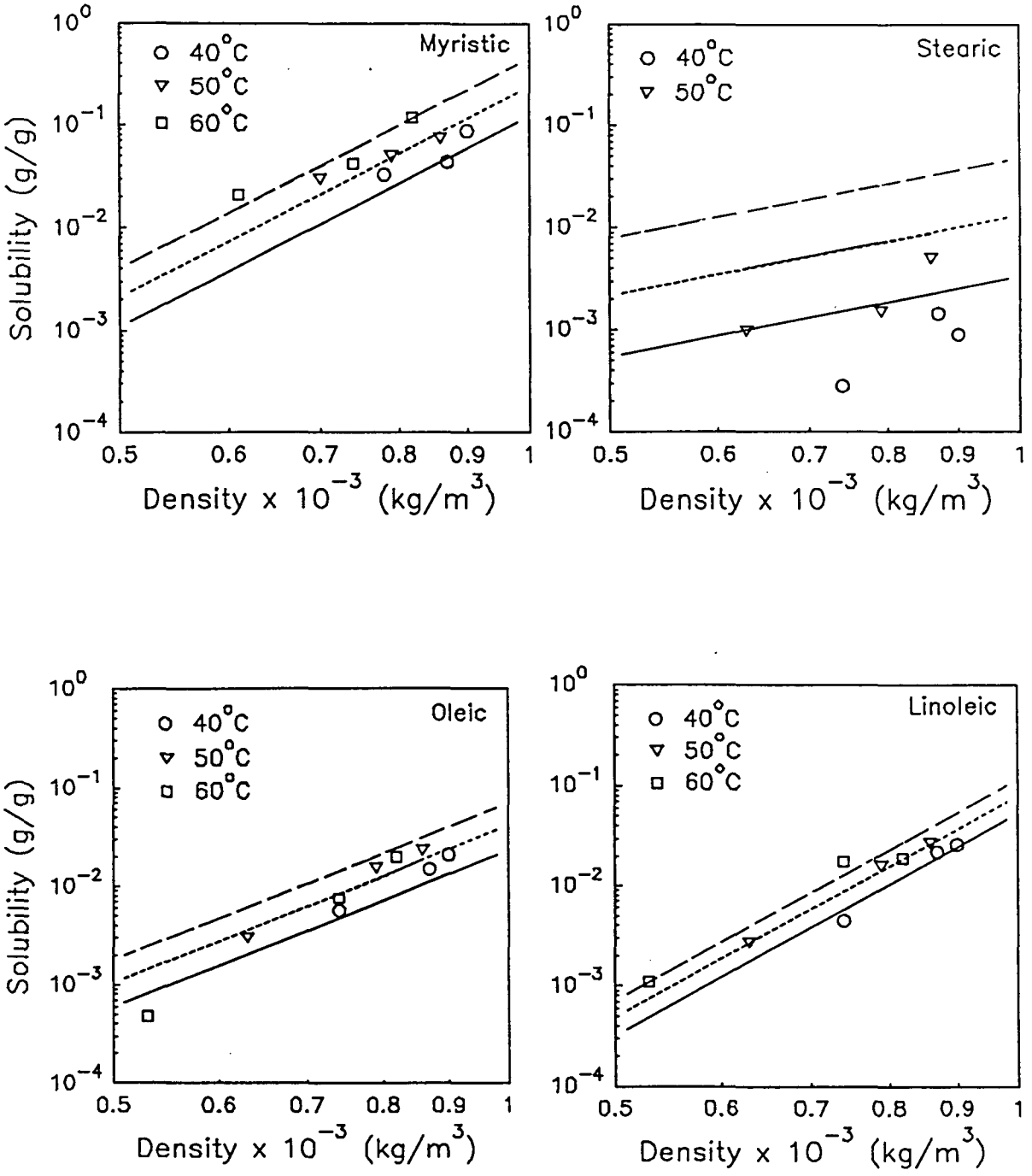

Figure 13. Solubility of fatty acids as a function of density. Lines represent predicted isotherms (literature, after bad point removal): (——) 40°C; (·····) 50°C; and (– –) 60°C

point removal, but deviated more at 40 and 50°C, and at higher density. Nilsson et al. (1991) experimental data also shows the same trend. The experimental and predicted values of linoleic acid shows the similar results.

Closer agreement between predicted and experimental values was found at lower densities compared to higher densities. After analyzing the literature data, it appears that most discrepancies in solubility occur either at the lower or at the density extremes. Therefore, this model is reliable only between intermediate values (0.5-0.9 x 10^{-3} kg/m_3) of densities. del Valle and Aguilera (1988) concluded that improved Chrastil's equation adequately represented the solubilities of several vegetable oils in SC-CO_2 between 0.45 to 0.98 g/mL density of solvent. In addition, changes in the physical states of the solute under processing conditions should always be taken into consideration. This linear model cannot predict changes in the solubility if there is a physical transformation of solute.

Table 3 shows the association number *k* for different fatty acids. Ideally the association number of a fatty acid should be the same, but various factors can contribute to the different numbers as reported by various authors. Yeh et al. (1991) have suggested that differences in solubility measurement techniques can result in different association numbers. If the solute undergoes any physical transformation, the number of molecules of supercritical solvent adhering to the solute might change; hence a different association number. White (1990) suggests that reduction of the surface area of the solute on liquefaction can cause erratic results and consequently a different association constant. A larger association constant (*k*) indicates greater influence of density on solubility; whereas the constant 'a' shows the importance of temperature on solubility. For instance, the

Table 3. Model constants of literature data

Fatty Acid	*k*-1	a	b	R^2
Myristic	7.1 ± 0.6	-6955 ± 1558	20.3 ± 4.9	0.88
Stearic	2.6 ± 0.6	-13934 ± 1522	38.8 ± 4.8	0.67
Oleic	6.0 ± 0.6	-6253 ± 1822	16.6 ± 5.7	0.79
Linoleic	7.4 ± 0.6	-4145 ± 1165	10.3 ± 3.7	0.97

greater influence of temperature on the solubility of stearic acid is shown by the larger a-value; whereas density (lower *k* value) has a lesser effect on solubility. The effects of *k* and a-values can be seen in Figures 11 and 13. The solubilities of the other three fatty acids are largely controlled by density and temperature has smaller effect. The effect of constant 'b' is dependent on the molecular weight of solute and the association number. It is difficult to determine how a constant 'b' influences solubility. The value of constant 'b' appears to be influenced by the molecular weights and the physical states of the fatty acids. Fatty acids with higher melting points have larger 'b' values compared to the lower melting fatty acids. Among the three fatty acids of similar molecular weight, it seems (Table 1 in Appendix and Table 3) that the value of 'b' decreases as melting point decreased. These physical constants help determine the optimum condition for the fatty acid solubility studies.

Figure 14 compares predicted solubilities for fatty acids and triglycerides. This Figure shows that the predicted solubilities of fatty acids (literature) are two to ten times higher than the predicted solubility of vegetable oil reported by del Valle and Aguilera (1988). Therefore, it is possible to deacidify vegetable oil using SC-CO_2.

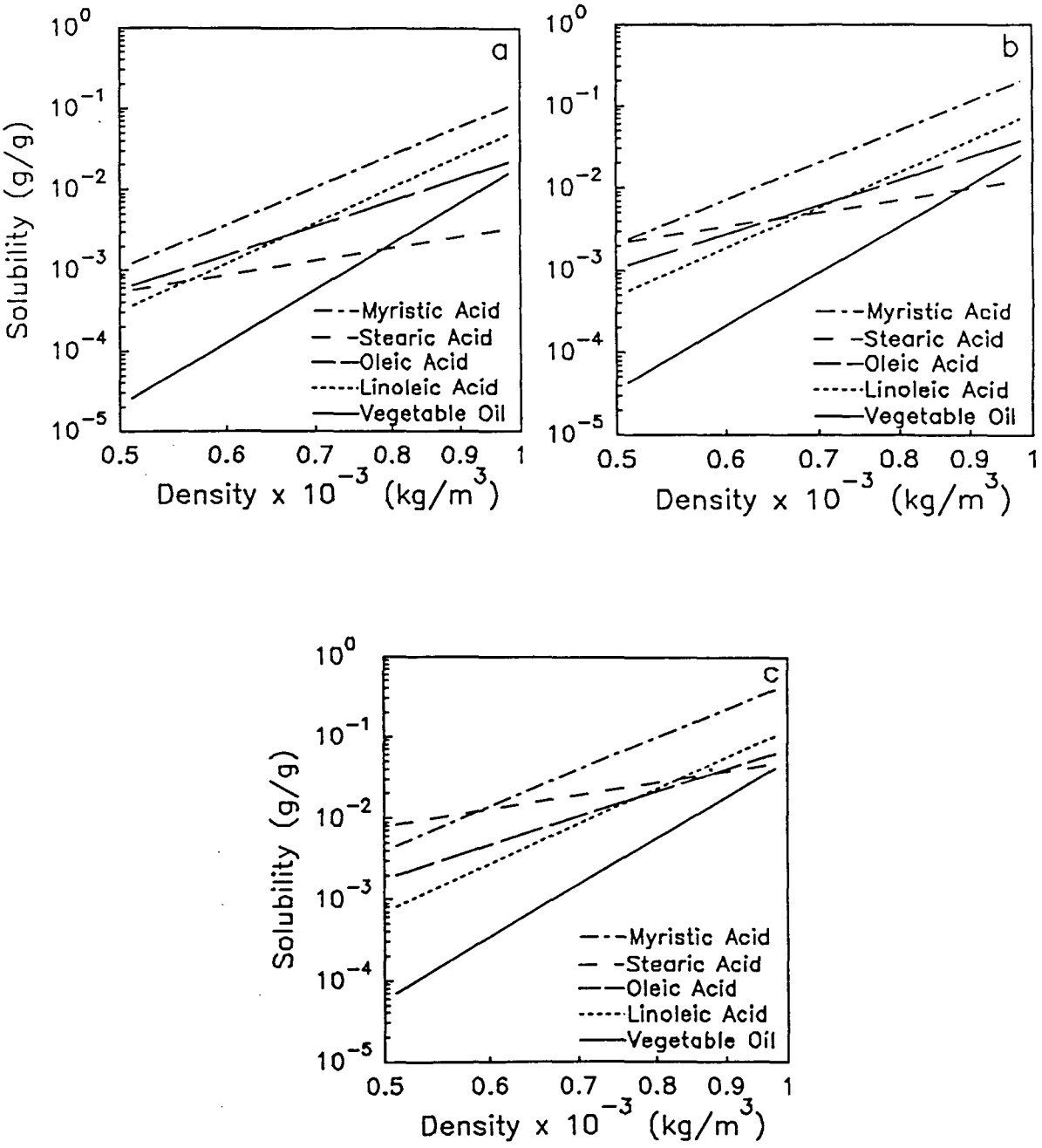

Figure 14. Predicted solubility isotherms of vegetable oil and fatty acids in SC-CO_2 as a function of density: (a) 40°C; (b) 50°C; and (c) 60°C

C. Off-flavor removal from soybean flour

Our preliminary work in which we compared the extraction of defatted soybean flour with dry and water-saturated SC-CO_2 indicated that more efficient removal of off-flavors was achieved by using water-saturated SC-CO_2. This observation is in agreement with the results of Christianson et al. (1984) and Eldridge et al. (1986) who also found that flavor improvement of SC-CO_2-extracted protein was directly related to the moisture content of the corn and soy protein samples. Therefore, we further studied the effects of the water-saturated SC-CO_2 on the desorption of soybean off-flavor compounds.

Under our experimental conditions (27.6 MPa, 40°C) the solubility of water in the supercritical CO_2 was about 0.2 wt% (Chrastil, 1982). The results of total volatiles present in the soy flour before and after the SC-CO_2 are shown in Figure 15. Headspace analysis revealed about an elevenfold reduction in volatiles for the samples treated with the SC-CO_2. When soy flour was flushed with gaseous CO_2 at 4 MPa and 40°C, no change in volatiles' content was measured. This suggests that the solvent power of SC-CO_2 is responsible for desorption. Butanal, pentanal, and hexanal were identified as the off-flavor compounds removed during the extraction (Figure 16). The amounts of all three compounds were significantly reduced after SC-CO_2 extraction. The SC-CO_2 was more effective in desorbing butanal and pentanal than hexanal from the protein. This effect could be explained by the expected higher solubility of butanal and pentanal compared to hexanal in supercritical CO_2. The higher solubility was expected due to the lower molecular weight of butanal and pentanal compared to hexanal. Butanal and

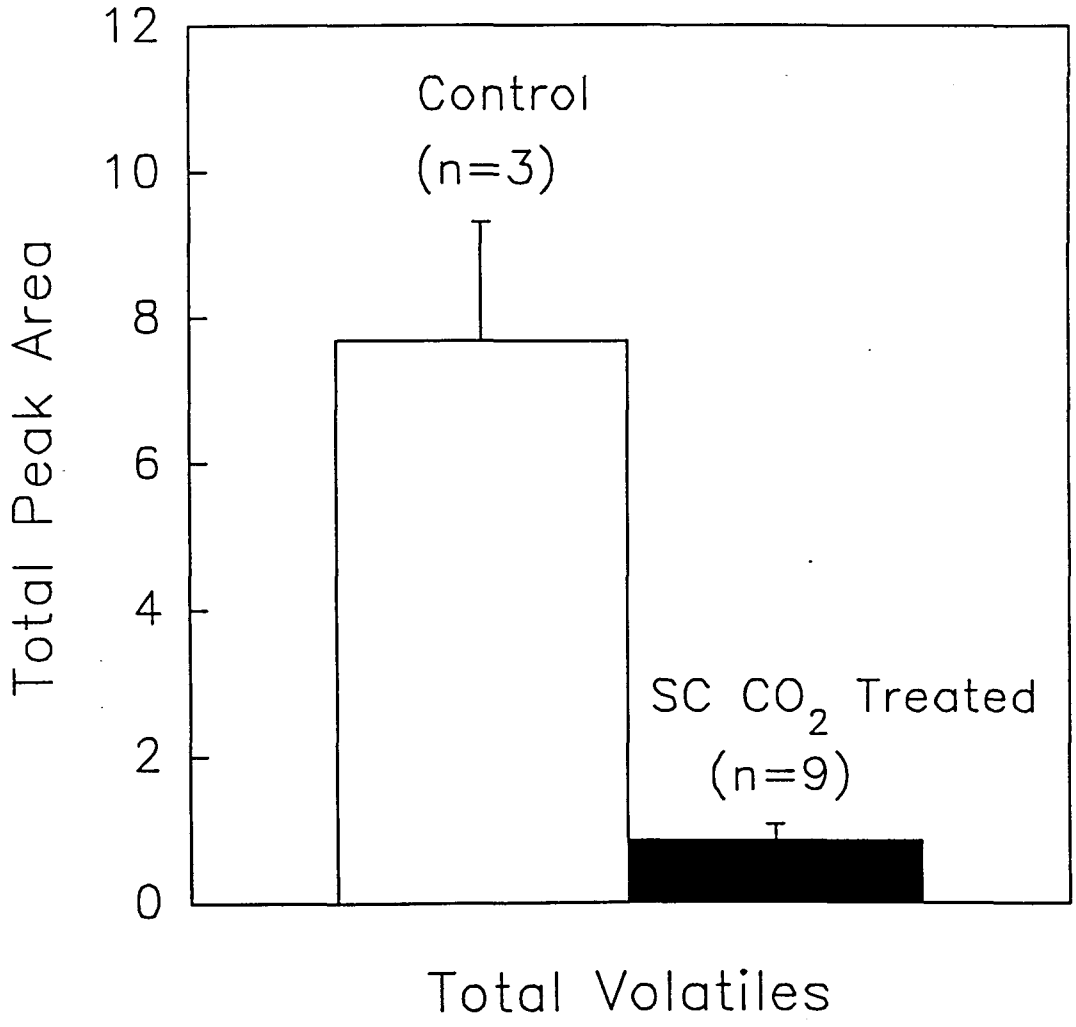

Figure 15. Headspace analysis of the control and SC-CO_2 treated defatted soybean flour for the total volatiles

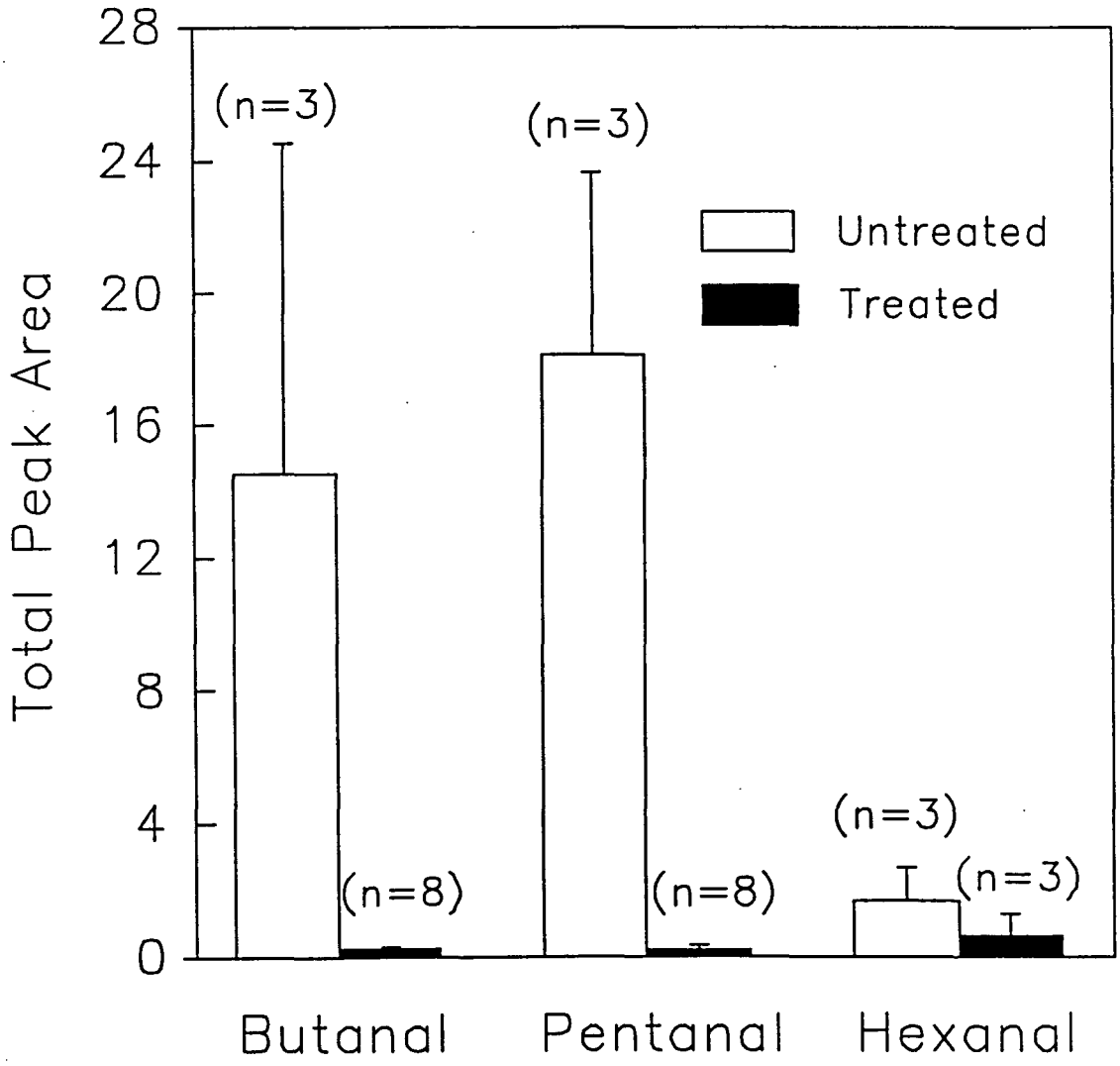

Figure 16. Off-flavor compounds identified in the untreated and SC-CO_2- treated soybean flour. Bars represent standard deviation

pentanal concentrations were reduced 55 and 75 times, respectively, whereas hexanal concentration was reduced only threefold. The effect of moisture on the extraction efficiency is probably related to the higher polarity of the moist CO_2 due to entrained water as well as to enhanced mass transfer due to "swelling" of the flour (Peter, 1984). The swelling of flour by water probably results in the higher available surface area for CO_2 to interact with off-flavor molecules. Also, water can help release aldehydes from soy flour by favoring a shift in the predicted Schiff's base equilibrium, thus, SC-CO_2 can extract these compounds with relative ease (O'Keefe et al., 1991). The difficulty in removing hexanal compared to its lower MW counterparts suggests that higher pressures or density of SC-CO_2 may be needed to desorb hexanal from soy proteins. Since we have a minimal understanding of soy flavor and protein interaction, it is difficult to draw a conclusion about the role of SC-CO_2 at the molecular level. Kinsella and Damodaran (1980) also emphasized the need of more information concerning the nature of flavor-protein binding.

Analyses of the extract from the U-tube and the smaller column containing Tenax showed that butanal and pentanal were deposited on the Tenax. Hexanal could not be detected in the extract, which further confirmed that the process conditions for SC-CO_2 need further optimization for the effective removal of hexanal. We believe that the presence of entrainer, such as ethanol, may help remove larger quantities of off-flavor compounds. Effects of the entrainer on the protein functionality will have to be examined.

The results of gas chromatographic analysis were confirmed by the triangle test.

Thirteen out of fifteen panelists found that treated soy flour with moist SC-CO_2 was different (α=0.001) than the untreated flour. The panelists commented on the lower, less strong or absence of beany flavor in the treated flour. However, some of the panelists detected fishy odors in the SC-CO_2-extracted soy flour.

The NSI values of untreated and SC-CO_2-treated soy flour samples are given in Table 4. The NSI value of the dry SC-CO_2-treated soy flour was found to be significantly (α=0.05) lower than the untreated flour. There was no significant (α=0.05) difference between the NSI values of the treated and untreated flour with moist SC-CO_2. The total protein values of the untreated and the SC-CO_2-treated soy flour were also not significantly different. These results agree with the previous report by Christianson et al. (1984) that the NSI value of corn germ flour extracted with dry SC-CO_2 was significantly lower than that of the hexane extracted material. The 8% initial moisture in the corn germ helped the extraction of off-flavors, but the concomitant removal of water dried the sample and apparently affected protein solubility. This conclusion is supported by subsequent work of this group where moist SC-CO_2 was used throughout the

Table 4. Effect of dry and moist SC-CO_2 extraction on nitrogen solubility index

Analysis	Untreated Flour	Treated Flour
Total protein (%)	$43.7^{a} \pm 1.7^{f}$	$43.9^{b} \pm 5.3$
NSI, moist SC-CO_2 (%)	$67.0^{c} \pm 6.4$	$63.1^{d} \pm 7.6$
NSI, dry SC-CO_2 (%)	$67.0^{c} \pm 6.4$	$50.4^{e} \pm 0.3$

[a]n=4; [b]n=7; [c]n=4; [d]n=7; [e]n=2
[f] standard error

extraction of soybean flakes Eldridge et al. (1986). These authors found that the higher moisture content of soy flakes caused denaturation of lipoxygenase and a decrease in NSI. But, Weder (1984) treated commercial lysozyme with moist SC-CO_2 and N_2 at 30 MPa and 80°C. He reported that, although there was some unfolding of the protein, SC-CO_2 did not adversely influence protein functionality. Since both of the fluids produced similar results, he concluded that the heating of proteins in presence of water at 80°C was responsible for unfolding of proteins, not SC-CO_2. We also believe that the change in protein solubility was caused primarily by the higher temperatures of extraction (above 80°C) used in that study (Weder, 1984). Our work indicates that extraction of defatted flour at lower temperatures and pressures is effective in off-flavor removal without causing denaturation of the soy protein.

D. Deodorizer distillate fractionation

Deodorizer distillate was fractionated at 40°C and 10.3, 20.7 and 27.6 MPa by SC-CO_2. A typical thin layer chromatography profile for identifying the various constituents is shown in Figure 17. The analysis of the fractions collected at 10.3 MPa pressure for 45 hr showed the presence of sterols, α-, γ, and δ-tocopherols. α-tocopherol was identified using a standard whereas the γ and δ-tocopherol were identified using elution profile of literature data (Guzman and Murphy, 1986; Taniguchi et al., 1985). The TLC analysis showed that at this pressure fatty acid (oleic acid standard), sterols, and tocopherols were extracted. Two other compounds were also found to be present in the fractions, but they

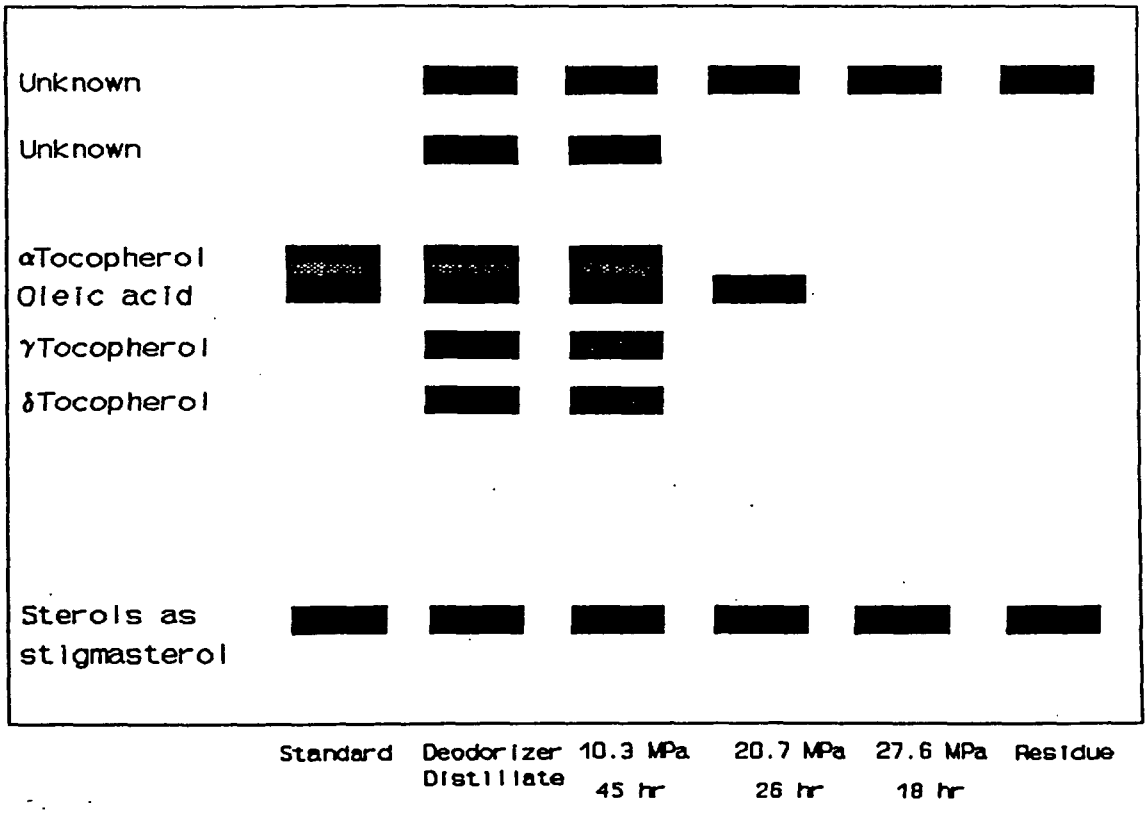

Figure 17. Thin layer chromatography of deodorizer distillate fractionation in SC-CO_2 at 40°C

were not identified. The analysis showed that SC-CO_2 did not selectively separate α-tocopherol and sterol at this pressure. The analysis also showed that α-tocopherol and fatty acids had similar polarities, but γ- and δ-tocopherols were more polar compared to α-tocopherol and fatty acids. Sterols were found to be the most polar compounds among all the identified compounds as they travelled the least. The two unknowns, which could be mono- or diglycerides, sterol esters, wax esters or, hydrocarbons (Stahl and Quirin, 1983), were the most hydrophobic compounds based on TLC profile. Similar compounds were identified in the original deodorizer distillate as shown in Figure 17.

The TLC profile of the fractions collected at 20.7 MPa showed the absence of tocopherols. Fatty acids and sterols were found to be present as in 10.3 MPa fractions. The absence of tocopherols at this fraction suggests that they were either completely extracted at the lower pressure, or degraded due to the long time of extraction at the previous stage of extraction. The analyses of the fractions collected at 27.6 MPa and the deodorizer distillate residue left after extraction showed the same TLC profile. Only sterols and other unidentified compounds were found in the fractions and the residue. This indicated that fatty acids were completely exhausted at 20.7 MPa and sterols were continuously extracted at all pressure. In general, the solubility of sterols increased with increasing pressure.

Our results show that the fractionation of α-tocopherol and sterols from deodorizer distillate of soybean oil is not feasible by SC-CO_2 alone. This preliminary study confirms the results reported by Shishikura et al. (1988). The polar nature of sterols result in very low solubility and pose a problem in their removal by SC-CO_2. In addition, due to their

high melting point, the sterols precipitated in the lines and interfered with the normal flow of SC-CO_2. Therefore, it is important to remove sterols by crystallization with organic solvent (Herting, 1984; Shishikura et al., 1988) before deodorizer distillate is subjected to SC extraction to separate α-tocopherol from fatty acids.

Although the molecular weight of α-tocopherol (MW ~ 425) is higher than that of fatty acids (MW ~ 280), the polarities of these two compounds are very close. Shishikura et al. (1988) esterified fatty acids with ethanol to make them more hydrophobic and to enhance their extraction. Also, this group recommended the use of an ion-exchange column to increase the yield of tocopherol.

IV. CONCLUSIONS

A. Vegetable oil deacidification

The solubility studies of pure compounds provided useful information about the effects of pressure and temperature (extraction conditions) on their solubilities in SC-CO_2. The solubilities of fatty acids are higher than triglycerides at most of the process conditions; however, at 20.7 MPa and 50°C the selectivity and yield of fatty acid was found to be better. Hence, under optimal conditions, it would be possible to deacidify vegetable oil using SC-CO_2.

B. Thermodynamic modeling of fatty acid solubilities

The predicted solubility data obtained by using the modified Chrastil's equation is fairly reliable in the pressure range from 15 to 25 MPa and at temperatures from 40 to 60°C that were used by most of the investigators. More experimental data is required to obtain better predicted solubility values. The model is not effective in explaining possible phase changes of the solutes that may be occurring at different supercritical conditions. The model is limited to the solute-solvent interactions in the SC-phase.

C. Off-flavor removal

The SC-CO_2-extraction technology was found to be effective in removing the off-flavor compounds from soy proteins. The water-saturated SC-CO_2 at 27.6 MPa and 40°C was found to lower the levels of off-flavor compounds, butanal and pentanal. Hexanal was not removed in the same proportion as butanal and pentanal. The supercritical conditions were not optimum for hexanal removal, but volatiles were significantly lower in SC-CO_2 treated soy flour. Protein solubility did not change significantly due to SC-CO_2 treatment.

D. Deodorizer distillate fractionation

The experimental work on the fractionation of α-tocopherol and sterols from deodorizer distillate of soybean oil suggests that the SC-CO_2 alone is not effective in purifying and separating these compounds.

V. SUGGESTIONS

A. Vegetable oil deacidification

The effects of intermolecular interaction in the liquid phase between the components of the system, including SC-CO_2 should be taken into account before a final conclusion is made about feasibility of SC-CO_2 deacidification. It is also important to know the fatty acid composition of particular vegetable oil in order to select optimum conditions for deacidification. For example, soybean oil is rich in the fatty acids (oleic, C_{18}:1; linoleic, C_{18}:2; and linolenic, C_{18}:3) which are liquid at the supercritical extraction conditions used in this work. But, it may not be suitable for other vegetable oil, such as palm oil which has high as well as low melting fatty acids (palmitic acid, C_{16}:0 and oleic, C_{18}:1) or coconut oil which has short chain saturated fatty acids (caprylic, C_8:0; capric, C_{10}:0; lauric, C_{12}:0; and myristic, c_{14}:0). However, del Valle and Aguilera (1988) have reported that similar solubility behavior of vegetable oil such as soybean and safflower can be expected because of their similar physical properties. More work is needed to understand the feasibility of deacidification of vegetable oil with different lipid compositions.

B. Thermodynamic modeling of solubility of fatty acids

Although the modified Chrastil's model predicts the solubilities of pure fatty acids

and triglycerides in SC-CO_2, but its effectiveness in determining the optimum conditions for deacidification of vegetable oil cannot be confirmed unless a mixture of triglycerides and fatty acid is studied. Chrastil (1982) developed an equation for calculating the density of SC-CO_2 for selectively separating one solute from another in a mixture. However, the major drawback of this equation is the assumption made by Chrastil (1982) that molecules of solutes in solution do not affect one another. Bamberger et al. (1988) discussed entrainer effects of one solute on another which was suspected to be the major cause of erroneous solubility measurements of Chrastil (1982) and Brunetti (1989). Therefore, we believe that this model should not be used in determining the selectivity of removal for one particular compound from another. The knowledge of binary interaction parameters between solute-solute and solute-solvent will be crucial. This emphasizes the need for a better thermodynamic model to account for these factors as well as the possible phase change during the process.

C. Off-flavor removal

Although SC-CO_2-extraction technology was found to be effective in reducing off-flavor compounds from soy proteins, its commercialization will depend on the processing concept. If soy oil is extracted with supercritical CO_2 instead of hexane, the processing conditions at the end of the extraction can be adjusted for optimum off-flavor removal in the same reactor without significantly increasing the cost of the meal. On the other hand, upgrading the soy meal ($0.10/lb) or food-grade flours ($0.15-0.35/lb) in a separate

process would not be very attractive since the added cost due to SC-CO_2 processing would be between $0.20 and $0.30/lb (Moses and Cody, 1984). Therefore, unless soybean extraction is performed with supercritical solvents, only the higher value products such as protein isolates ($1.00-1.25/lb) would probably be able to bear the increased cost of the off-flavor removal. The operating cost of extraction can be reduced by carefully optimizing the process condition and use of an entrainer such as ethanol. Future work should concentrate on high value soy products. There is also a need for a storage study to monitor any development of off-flavors and effect on protein functionality. The SC-CO_2 treated soy protein should also be in incorporated in a food system, such as summer sausage to evaluate its organoleptic properties.

D. Deodorizer distillate fractionation

Multiple steps such as alcohol crystallization of sterols prior to SC-CO_2-extraction, esterification of fatty acids, use of a cosolvent such as ethanol, and use of an adsorbent material, such as silica, at upstream and/or downstream separation stages will be required to make this technology feasible. Suitable adsorbent should be chosen which can selectively adsorb and desorb the α-tocopherol from a mixture of extracted material. Since each additional step results in a more complex process and increase the operating cost, unless there is greater demand for naturally obtained α-tocopherol and sterol, the commercial feasibility of this technology in this area is not promising. Since the price of synthetic vitamin E is about $70/lb, which is less than half the price of natural vitamin E

from the deodorizer distillate, efforts should be concentrated decreasing the processing cost of natural tocopherol. There are several chemical treatments involved in the enrichment of α-tocopherol from deodorizer distillate which increase the process cost and complexity of the process. Therefore, research efforts should focus on minimizing the number of steps without sacrificing the economics of the process. Recently, the Food and Drug Administration (FDA) has become stricter in defining what is "natural" on the food label. α-Tocopherol enriched from deodorizer distillate undergoes several chemical treatments (saponification, esterification, solvent extraction and methylation), therefore, it will be important to carefully monitor FDA guidelines for a product to be called natural or derived from natural source which could possibly affect the price and market share of synthetic and natural vitamin E.

Finally, as a rule of thumb, industry will resist the acceptance of any new technology unless the market for the product is large and profitable. A new supercritical plant of a $15-30 million cost is not likely to be commissioned unless there is convincing evidence that this new technology is significantly better than conventional processes.

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APPENDIX

Table 1. Physical Properties of Fatty Acids

Fatty Acid	MW	Melting Pt. (°C)	Vapor Pressure[a] x 10^3 (Pa)		
			40°C	50°C	60°C
Lauric, C12:0	200.3	44	105	302	807
Myristic, C14:0	228.4	58.5	18	55	159
Palmitic, C16:0	256.3	63.5	4.2	14	42
Stearic, C18:0	284.5	69.5	0.8	2.7	9.0
Oleic, C18:1	282.5	4	1.3	4.5	14.0
Linoleic, C18:2	280.4	-12	*	*	*

[a]Singleton (1960).
*Data not available.

Table 2. Solubility of myristic acid in SC-CO_2[a]

Temperature (°C)	Pressure (MPa)		
	15.2	20.7	27.6
	wt%	wt%	wt%
40	3.25 ± 0.25[b]	4.35 ± 0.35	8.69 ± 0.84
50	2.98 ± 1.73	4.90 ± 0.49	7.44 ± 1.16
60	2.1 ± 0.89	4.14 ± 0.96	11.81[a] ± 0.50

[a]n = 3
[b] standard error

Table 3. Solubility of stearic acid in SC-CO_2[a]

Temperature (°C)	Pressure (MPa)		
	13.8	20.7	27.6
	wt%	wt%	wt%
40	0.028 ± 0.002[b]	0.145 ± 0.015	0.09 ± 0.04
50	0.096 ± 0.04	0.15 ± 0.03	0.51 ± 0.23

[a] n = 2
[b] standard error

Table 4. Solubility of oleic acid in SC-CO_2[a]

Temperature (°C)	Pressure (MPa)		
	13.8	20.7	27.6
	wt%	wt%	wt%
40	0.57 ± 0.2[b]	1.49 ± 0.15	2.09 ± 0.77
50	0.3 ± 0.08	1.52 ± 0.1	2.31 ± 0.16
60	0.048 ± 0.01	0.76 ± 0.1	1.98 ± 0.28

[a] n = 4
[b] standard error

Table 5. Solubility of linoleic acid in $SC\text{-}CO_2$[a]

Temperature (°C)	Pressure (MPa)		
	13.8	20.7	27.6
	wt%	wt%	wt%
40	0.45 ± 0.04[b]	2.22 ± 0.36	2.63 ± 0.52
50	0.27 ± 0.04	1.58 ± 0.28	2.69 ± 0.22
60	0.11 ± 0.01	1.76 ± 0.08	1.89 ± 0.86

[a] n = 2
[b] standard error

Table 6. Model constants of literature data after bad point removal

Fatty Acid	*k*-1	a	b	R^2
Myristic	6.8 ± 0.5	-6837 ± 1275	19.7 ± 4.0	0.91
Stearic	2.6 ± 0.6	-13934 ± 1522	38.8 ± 4.8	0.67
Oleic	5.3 ± 0.5	-5739 ± 1644	14.6 ± 5.2	0.81
Linoleic	7.4 ± 0.6	-4145 ± 1165	10.3 ± 3.7	0.97

Table 7. Model constants for this work only

Fatty Acid	*k*-1	a	b	R^2
Myristic	5.3 ± 0.8	-4272 ± 1146	11.5 ± 3.7	0.88
Stearic	5.4 ± 1.8	-15670 ± 4514	43.8 ± 14.4	0.83
Oleic	7.7 ± 0.7	-2346 ± 1347	4.6 ± 4.3	0.96
Linoleic	7.4 ± 0.6	-4145 ± 1165	10.3 ± 3.7	0.97

Table 8. Experimental and predicted data (literature) of myristic acid solubility in SC-CO_2

y (g/g)		$\rho x10^{-3}$ (kg/m^3)	T (K)	Author
exp.	pred.			
0.0138	0.0169	0.79	308	White
0.0260	0.0442	0.91	308	White
0.0253	0.0732	0.98	308	White
0.0001	0.0001	0.36	313	Bamburger
0.0025	0.0016	0.53	313	Bamburger
0.0088	0.0064	0.65	313	Bamburger
0.0219	0.0221	0.78	313	Bamburger
0.0408	0.0366	0.84	313	Bamburger
0.0592	0.0502	0.88	313	Bamburger
0.0940	0.0366	0.84	313	Brunetti
0.0325	0.0221	0.78	313	This work
0.0435	0.0464	0.87	313	This work
0.0869	0.0585	0.90	313	This work
0.0087	0.0135	0.69	318	White
0.0733	0.0655	0.87	318	White
0.0830	0.0474	0.79	323	Brunetti
0.0298	0.0208	0.70	323	This work
0.0490	0.0474	0.79	323	This work
0.0744	0.0844	0.86	323	This work
0.0050	0.0126	0.62	328	White
0.0705	0.0843	0.82	328	White
0.0210	0.0154	0.61	333	This work
0.0414	0.0573	0.74	333	This work
0.1181	0.1152	0.82	333	This work

Table 9. Experimental and predicted (literature) data of stearic acid solubility in SC-CO_2

y (g/g)		$\rho x10^{-3}$ (kg/m^3)	T (K)	Author
exp.	pred.			
0.0009	0.0009	0.79	308	White
0.0011	0.0013	0.92	308	White
0.0006	0.0016	0.98	308	White
0.0090	0.0021	0.84	313	Brunneti
0.0080	0.0026	0.91	313	Brunneti
0.0016	0.0010	0.62	313	Chrastil
0.0020	0.0018	0.78	313	Chrastil
0.0022	0.0024	0.88	313	Chrastil
0.0003	0.0015	0.74	313	This work
0.0015	0.0023	0.87	313	This work
0.0009	0.0026	0.90	313	This work
0.0012	0.0026	0.69	318	White
0.0041	0.0047	0.87	318	White
0.0040	0.0058	0.94	318	White
0.0051	0.0029	0.72	318	Kramer
0.0060	0.0033	0.76	318	Kramer
0.0063	0.0037	0.79	318	Kramer
0.0062	0.0042	0.83	318	Kramer
0.0065	0.0047	0.87	318	Kramer
0.0056	0.0055	0.92	318	Kramer
0.0190	0.0072	0.79	323	Brunneti
0.0390	0.0093	0.87	323	Brunneti
0.0010	0.0040	0.63	323	This work
0.0015	0.0072	0.79	323	This work
0.0051	0.0090	0.86	323	This work
0.0071	0.0064	0.59	328	White
0.0219	0.0158	0.83	328	White
0.0334	0.0202	0.91	328	White
0.0040	0.0087	0.66	328	Kramer
0.0050	0.0094	0.68	328	Kramer
0.0249	0.0139	0.79	328	Kramer
0.0348	0.0164	0.84	328	Kramer
0.0406	0.0190	0.89	328	Kramer
0.0456	0.0202	0.91	328	Kramer
0.0200	0.0213	0.73	333	Brunneti

0.0600	0.0300	0.83	333	Brunneti
0.0065	0.0020	0.30	333	Chrastil
0.0097	0.0053	0.43	333	Chrastil
0.0084	0.0133	0.61	333	Chrastil
0.0162	0.0263	0.79	333	Chrastil
0.0053	0.0226	0.59	338	Kramer
0.0146	0.0369	0.71	338	Kramer
0.0241	0.0457	0.77	338	Kramer
0.0599	0.0593	0.85	338	Kramer
0.0963	0.0710	0.91	338	Kramer

Table 10. Experimental and predicted data (literature) of oleic acid solubility in SC-CO_2

y (g/g)		$\rho x10^{-3}$ (kg/m^3)	T (K)	Author
exp.	pred.			
0.0011	0.0018	0.62	313	Chrastil
0.0028	0.0033	0.69	313	Chrastil
0.0047	0.0062	0.78	313	Chrastil
0.0075	0.0092	0.84	313	Chrastil
0.0103	0.0118	0.88	313	Chrastil
0.0057	0.0047	0.74	313	This work
0.0149	0.0111	0.87	313	This work
0.0209	0.0133	0.90	313	This work
0.0017	0.0017	0.55	323	Nilsson
0.0039	0.0036	0.63	323	Nilsson
0.0058	0.0062	0.70	323	Nilsson
0.0098	0.0083	0.74	323	Nilsson
0.0149	0.0118	0.79	323	Nilsson
0.0030	0.0036	0.63	323	This work
0.0152	0.0118	0.79	323	This work
0.0231	0.0185	0.86	323	This work
0.0002	0.0001	0.30	333	Chrastil
0.0028	0.0008	0.43	333	Chrastil
0.0109	0.0051	0.61	333	Chrastil
0.0260	0.0132	0.73	333	Brunetti
0.0005	0.0024	0.53	333	This work
0.0076	0.0152	0.74	333	This work
0.0198	0.0245	0.82	333	This work
0.0004	0.0010	0.45	333	Nilsson
0.0031	0.0024	0.53	333	Nilsson
0.0054	0.0051	0.61	333	Nilsson
0.0096	0.0132	0.73	333	Nilsson

Table 11. Experimental and predicted data of linoleic acid solubility in SC-CO_2

y (g/g)		$\rho x10^{-3}$ (kg/m^3)	T (K)	Author
exp.	pred.			
0.0045	0.0058	0.74	313	This work
0.0222	0.0193	0.87	313	This work
0.0263	0.0248	0.90	313	This work
0.0027	0.0027	0.63	323	This work
0.0158	0.0142	0.79	323	This work
0.0269	0.0267	0.86	323	This work
0.0011	0.0011	0.53	333	This work
0.0176	0.0129	0.74	333	This work
0.0189	0.0276	0.82	333	This work