

Supercritical Fluid Extraction and Retrograde Condensation (SFE/RC) Applications in Biotechnology

by

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Chapter 13

**SUPERCRITICAL FLUID EXTRACTION AND RETROGRADE
CONDENSATION (SFE/RC) APPLICATIONS IN
BIOTECHNOLOGY**

Karl Schulz, Eloy E. Martinelli, and G. Ali Mansoori*

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ABSTRACT

The fundamentals and techniques of supercritical fluid extraction (SFE) and retrograde condensation (RC) processes are described. Supercritical (CO₂ and other biologically non-toxic supercritical fluids (SCFs) are found to be effective extracting agents of many compounds of interest in biotechnology. It is demonstrated that supercritical solubility calculation methods can be improved through the use of statistical mechanical theories of asymmetric mixtures. Such computation techniques could be successfully applied to improve engineering design. In addition, the effect of mixed supercritical solvents on the solubility of heavy solutes, at different pressures, temperatures, and solvent compositions is studied. The prospects for the commercial application of SFE/RC phenomena in biological processes are discussed and a review of important biotechnological applications of SFE/RC processes is presented.

I. INTRODUCTION

There has been a great deal of interest in recent years on separation techniques that could be of use in separating components of biological fluids in large industrial operations.¹⁻⁴ While there exist numerous separation techniques that have been used in the laboratory for bioseparation purposes, few such techniques are applicable for large-scale separations. Of the potential large-scale separation techniques bioseparation by partitioning^{2,3} and supercritical fluid extraction/retrograde condensation⁴ (SFE/RC) processes may be mentioned. There exist a number of biological separations in which either the partitioning or SFE/RC processes can be used. Partitioning is especially useful for separation of biologically active or live macromolecules, while the SFE/RC process is particularly applicable for separation of heat-sensitive biological fluids and compounds. In this chapter, the SFE/RC process as applied to biotechnical processes is presented and reviewed.

The SFE/RC phenomenon occurred in 1879, with Hannay and Hogarth's⁵ initial observation. A typical SFE/RC separation system is depicted in Figure 1. A high-pressure solvent gas tank is connected to an extraction vessel. In the extractor, the solute is put into contact with a supercritical solvent (i.e., with a solvent at conditions above its critical pressure and temperature). The gaseous mixture, saturated with the extracted substance, leaves the extraction chamber through an expansion valve where the pressure is greatly reduced. Because of the sudden expansion, the mixture temperature undergoes a significant drop; hence, a heat exchanger is usually necessary to rewarm the fluid to at least its initial temperature. Since the solubility of the solute in the gas is appreciably lessened due to the pressure decrease, some of the extracted material will be separated at this stage. Retrograde condensation, or depressurizing to precipitate the extract, can be accomplished through one large, or several smaller, pressure reduction steps. After each step, a heat exchanger and collection tank may be necessary. Upon completion of the extraction cycle, the solvent, now almost totally devoid of extracted material and low in pressure, is returned to its initial pressure and temperature, thus preparing it for further solute extraction.

An alternative to the use of an extraction chamber in SFE/RC systems is the jet extraction method,⁶ a technique developed primarily to facilitate extraction in the case of viscous mixtures of solids dispersed in liquids (which tend to clog the apparatus). In the jet extraction technique (see Figure 2), the starting viscous material is forced through a narrow capillary tube, often as fine as 0.2 mm i.d. The exit of this capillary tube is then inserted into a second capillary tube of slightly larger diameter. The second tube overlaps only a certain extent of the first tube. The extraction gas enters the larger capillary tube through the interstice between the two capillaries, thereby contacting the solute. Because the interstice is so small, the solvent achieves high velocity and a great deal of turbulence, thus effectively mixing

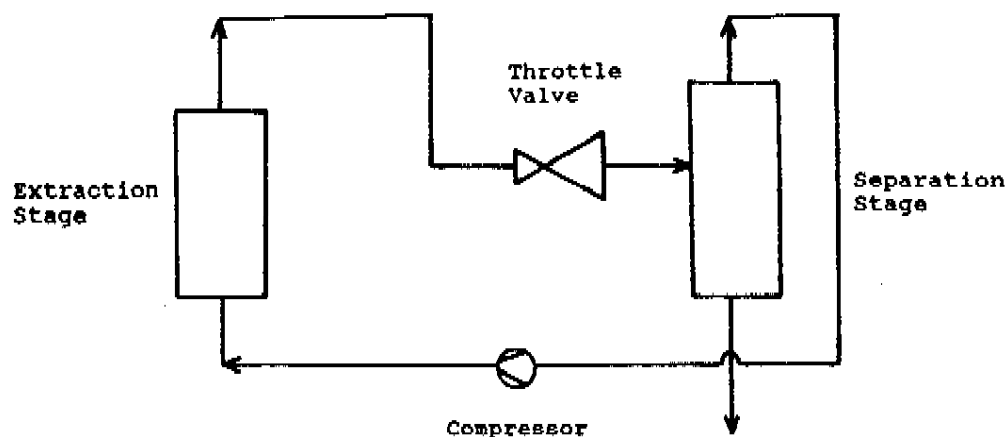


FIGURE 1. Schematic diagram of an SFE/RC cycle.

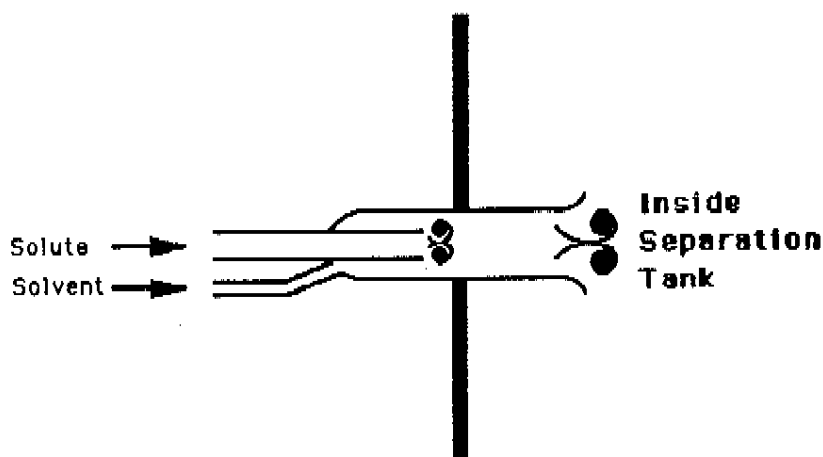


FIGURE 2. Jet mixing and extraction of viscous mixtures of solids dispersed in liquids.

itself with the solute in only a very short distance. The extraction is completed in this short distance, after which the undissolved material is collected in a separation tank while the gas phase passes through a second vessel where it undergoes retrograde condensation. Thereafter, the solvent gas is repressurized and recycled to the system.

An SCF can be an effective solvent for a condensed solute (liquid or solid). This requires a large molecular weight and size difference between the supercritical solvent and the condensed solute. To understand the behavior of SCFs, their physical properties must be compared to those of normal gases and liquids. The density of SCFs (about 0.3 to 0.7 g cm⁻³) is much greater than gas densities at normal conditions, but nearly equal to liquid densities at the same conditions (about 10⁻³ and 1 g cm⁻³, respectively). The viscosity of SCFs (10⁻⁶ to 10⁻³ g cm⁻¹ s⁻¹) lies between the gas and liquid viscosities (10⁻⁶ and 10⁻² g cm⁻¹ s⁻¹, respectively). The diffusion coefficient of SCFs ranges between 10⁻⁶ and 10⁻³ cm² s⁻¹, which is below 10⁻¹ (gas diffusivity) and similar to 10⁻⁵ (liquid diffusivity). Because the supercritical diffusivities are very small, molecular motion is relatively unimpeded, resulting in convective heat transfer as much as two orders of magnitude higher for SCFs than for liquids at normal conditions. The relatively low viscosities of SCFs make them easier to handle than liquids, and the loading capacity can be regulated through

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temperature and pressure control. A sudden temperature or pressure reduction often causes the loss of the special characteristics of the SCF. Therefore, a substance can be extracted at supercritical conditions of a solvent and precipitated by reducing the pressure or the temperature below the critical conditions of the solvent.

CO₂ has been found to be the most often used extraction solvent in the majority of SFE/RC systems in general and biosystems in particular. CO₂ is useful because it is not toxic when exposed to biological materials and is inexpensively produced, thus making it an ideal candidate for an extraction system. At room temperature and atmospheric pressure CO₂ is virtually useless as an organic solvent; however, at its supercritical conditions ($T > 304$ K and $P > 74$ bar) many molecules become quite soluble in CO₂. When CO₂ is used as a supercritical solvent, solubilities of solutes in it tend to increase markedly as the temperature rises above 323 to 333 K, especially when the pressure exceeds 400 bar.^{7,8} A possible reason for this strong solubility increase at high pressures is that above 400 bar the CO₂ solvent is nearly incompressible. When the temperature is increased, the solvent density undergoes only minor reductions, thus tending to decrease solute solubility. Simultaneously, the temperature increase induces a major vapor pressure increase, thus tending to increase solute solubility. Because the vapor pressure effect dominates the density effect, the net result is a large solute solubility increase. At pressures below 400 bar, the density of CO₂ will tend to decrease appreciably as the temperature rises. When this effect is combined with the increase in solute vapor pressure the result is only small solute solubility increases.

The solubility of a solute in CO₂ is hindered by the presence of polar functional groups in the molecular structure of the solute, such as carbonyl and hydroxyl groups. The solute solubility is more strongly hindered by hydroxyl groups, so much so that few solutes containing more than three hydroxyl groups per molecule exhibit appreciable solubility in supercritical CO₂. The high molecular weight of the solutes tend to inhibit solubility in CO₂, to a degree that only a few solutes with a molecular weight above 400 g mol⁻¹ show appreciable solubility in supercritical CO₂.

The use of cosolvents can sometimes increase solute solubility. Solute containing polar groups show solubility enhancement when cosolvents such as ethanol or methanol are used. This solubility increase can be explained in terms of the hydrogen bonding between the solute and the cosolvent. On the other hand, there exist cosolvents that lower solute solubility due to the formation of solid complexes between them. Cosolvents that enhance solute solubility are called entrainers. Usually it is hard to find the optimum cosolvent for a certain solute because each possible system must be individually studied.

Having reviewed the properties of SCFs, the solubility trends of solutes in supercritical solvents, and the hardware of the extraction system, the theoretical basis of SFE/RC phenomena and some current biotechnological applications of SFE/RC are examined.

II. THERMODYNAMIC MODELING OF SFE/RC PHENOMENA

Figure 3 illustrates the phenomena of SFE and RC from the thermodynamic point of view. A simple pressure vs. temperature (PVT) diagram of a fluid mixture exhibiting retrograde condensation is presented in Figure 3A. In Figure 3B, pressure vs. solubility of a heavy compound in the gas phase, a three different temperatures, is shown. According to Figure 3, above the critical solution temperature and at low pressures, solubility in the supercritical gas will decrease with an increase of pressure, at a given temperature. As pressure rises, solubility passes through a minimum and then increases rapidly until it reaches a maximum at a pressure slightly above retrograde.

In general, to model the thermodynamic behavior of SFE/RC phenomena, two different statistical mechanical procedures may be utilized:⁹ (1) the use of equations of state (EOSs) for pure fluids combined with appropriate conformal solution mixing rules, and (2) the use

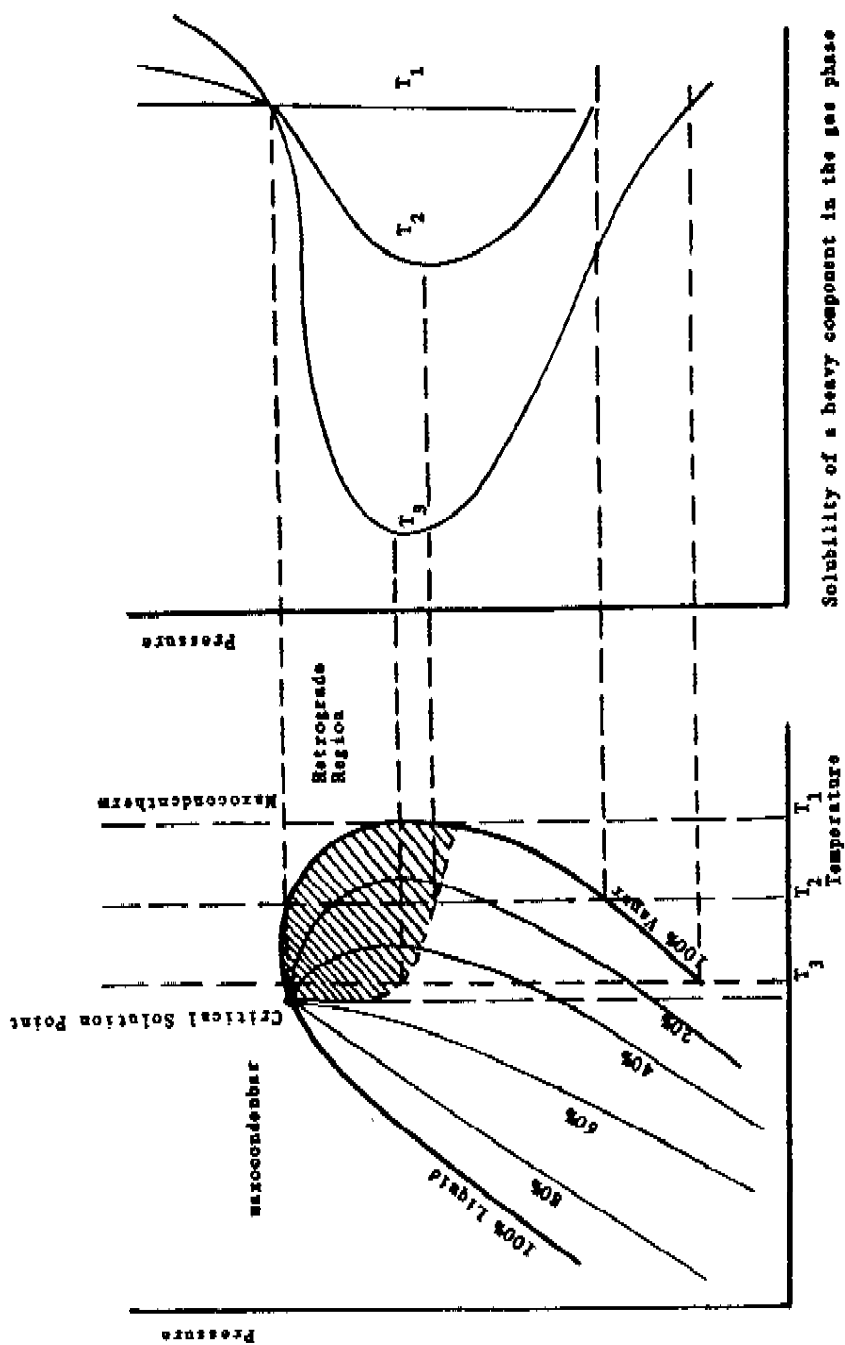


FIGURE 3. Pressure against temperature diagram of a representative fluid mixture exhibiting retrograde condensation, and pressure against solubility of a heavy component in the gas phase at three different temperatures.¹⁰⁻⁴³

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of detailed expressions for the intermolecular potential energy functions and rigorous statistical mechanics of mixtures.^{10,11} Conformal solution mixing rules and rigorous statistical mechanics of mixtures were developed substantially in the last decade;^{12,17} however, there is little information about intermolecular interaction parameters for the asymmetric molecules found in the SFE/RC processes. Therefore, utility of the second procedure is presently limited.

The concept of conformal solutions refers to substances whose intermolecular potential energy functions, ϕ_{ij} , are related to those of a reference pure fluid, indicated by the subscript (oo), according to:^{12,16}

$$\phi_{ij} = f_{ij} \phi_{oo}(r/h_{ij}^{1/3}) \quad (1)$$

where ϕ_{oo} is the potential energy function of the reference pure fluid, and f_{ij} and h_{ij} are the conformal interaction parameters of species i and j in the mixture.

For substances with an intermolecular potential energy function of the form

$$\phi_{ij} = E_{ij} [(L_{ij}/r)^n - (L_{ij}/r)^m] \quad (2)$$

and with exponents m and n equal to those for the reference substance, conformal parameters f_{ij} and h_{ij} will be related to the intermolecular potential energy parameter E_{ij} and to the intermolecular length parameter L_{ij} by the following equations:

$$f_{ij} = E_{ij}/E_{oo} \quad (3a)$$

$$h_{ij} = (L_{ij}/L_{oo})^3 \quad (3b)$$

The conformal solution theory for mixtures requires the definition of mixture conformal parameters f_{xx} and h_{xx} . These are related to the conformal parameters of the components of the mixture and to mixture composition:

$$f_{xx} = f_{xx}(f_{ij}, h_{ij}, x_i) \quad (4a)$$

$$h_{xx} = h_{xx}(f_{ij}, h_{ij}, x_i) \quad (4b)$$

Equations 4a and 4b are the conformal solution mixing rules. For different conformal solution theories of mixtures, different functional forms of the mixing rules will be obtained. The combining rules for unlike-interaction conformal parameters are also needed to formulate a mixture theory. The usual expressions are

$$f_{ij} = (1 - \tau_{ij}) (f_{ii}f_{jj})^{1/2} \quad (5a)$$

$$h_{ij} = (1 - \lambda_{ij}) [(h_{ii}^{1/3} + h_{jj}^{1/3})/2]^3 \quad (5b)$$

where τ_{ij} and λ_{ij} are fitting parameters.

In order to apply conformal solution mixing rules for the calculation of mixture thermodynamic properties, one needs to combine them with a pure-fluid EOS. Varieties of pure-fluid EOSs are available in the literature; however, for SFE/RC modeling, the use of semi-empirical EOSs is presently recommended because of the vast amount of database that is available for EOS parameters in the chemical and engineering industries. These equations are generally modifications of the van der Waals EOS:

$$Z = v/(v - b) - a/(vRT) \quad (6)$$

which was proposed by van der Waals¹⁸ in 1873.

The Peng-Robinson conformal solution (PRCS) EOS is found to be quite capable of predicting properties of asymmetric mixtures of interest in SFE/RC processes. The PRCS EOS is in the following form:⁹

$$Z = v/(v - b) - \{A/(RT) + C - 2[AC/(RT)]^{1/2}\}/[(v + b) + (b/v)(v - b)] \quad (7)$$

$$A = a_c (1 + \kappa)^2, C = a_c \kappa^2 / (RT_c) \quad (8)$$

$$a_c = a(T_c) = 0.45724 (RT_c)^2 / P_c \quad (9)$$

$$\kappa = 0.37464 + 1.54226 \omega - 0.26992 \omega^2 \quad (10)$$

$$b = 0.0778 RT_c / P_c \quad (11)$$

There exist three independent constants (A, b, and C) in this set of equations. Parameter A is proportional to (molecular volume)·(molecular energy), or $(A \propto f \cdot h)$, while parameters b and C are proportional to the molecular volume, or $b \propto h$, $C \propto h$. The conformal solution van der Waals mixing rules for the constants of the PRCS EOS are¹⁴

$$A = \sum_i \sum_j x_i x_j A_{ij} \quad (12)$$

$$b = \sum_i \sum_j x_i x_j b_{ij} \quad (13)$$

$$C = \sum_i \sum_j x_i x_j C_{ij} \quad (14)$$

According to Equations 5a and 5b, the combining rules for the unlike interaction parameters of this EOS are as:

$$A_{ij} = (1 - \tau_{ij})(A_{ii} A_{jj} / b_{ii} b_{jj})^{1/2} b_{ij} \quad (15)$$

$$b_{ij} = (1 - \lambda_{ij}) [(b_{ii}^{1/3} + b_{jj}^{1/3}) / 2]^3 \quad (16)$$

$$C_{ij} = (1 - \zeta_{ij}) [(C_{ii}^{1/3} + C_{jj}^{1/3}) / 2]^3 \quad (17)$$

As demonstrated previously, similar procedures can be used to derive conformal solution mixing rules for other EOSs.

In this chapter, the PRCS EOS is used in order to calculate the solubility of solids and liquids in supercritical pure and mixed solvents. The PRCS EOS can be utilized to perform accurate solubility calculations for heavy solutes in supercritical solvents.

The solubility of a condensed phase in a vapor phase can be expressed as:¹⁹

$$y_2 = (P_2^{sat}/P) (1/\Phi_2) \Phi_2^{sat} \exp \left\{ \int_{P_2^{sat}}^P (v_2^{solid}/RT) dP \right\} \quad (18)$$

where Φ_2^{sat} is the fugacity coefficient of the condensed phase at the saturation pressure, P_2^{sat} . The vapor pressure of solids is small, and the fugacity coefficient of pure solids at saturation pressure is almost unity. If it is also assumed that the solute specific volume is independent of pressure, the above equation reduces to:

$$y_2 = (P_2^{sat}/P) (1/\Phi_2) \exp \left\{ v_2^{solid} (P - P_2^{sat}) / RT \right\} \quad (19)$$

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In order to calculate the fugacity coefficient, the following equation,¹⁹ combined with an EOS and appropriate mixing rules, is generally used:

$$RT \ln \Phi_i = \int_V^{\infty} [(\partial P / \partial n_i)_{T, V, n_{j \neq i}} - (RT/V)] dV - RT \ln Z \quad (20)$$

With the PRCS EOS, Equation 7, and the correct version of the van der Waals mixing rules (Equations 12 to 14), the following expression for the fugacity coefficient is obtained:

$$\begin{aligned} \ln \Phi_i = & [(2 \sum_j x_j b_{ij} - b)/b] (Z - 1) - \ln(Z - B) - \\ & - [A^*/(8^{1/2} B)] \{ [2 \sum_j x_j A_{ij} + 2 RT \sum_j x_j C_{ij} - \\ & - 2 (A \sum_j x_j C_{ij} + C \sum_j x_j A_{ij}) (RT/AC)^{1/2}] / \beta^* - \\ & - (2 \sum_j x_j b_{ij} - b)/b \} \ln[(Z + (1 + 2^{1/2}) B)/(Z + (1 - 2^{1/2}) B)] \quad (21) \end{aligned}$$

where $\beta^* = A + CRT - 2(ACRT)^{1/2}$, $A^* = \beta^* P/(RT)^2$, and $B = bP/RT$.

III. MODEL SFE/RC SYSTEM CALCULATIONS

In the calculations reported here, solubilities of a number of model condensed solutes in supercritical solvents are predicted using the PRCS EOS for mixtures, in order to demonstrate the kind of general behavior that may be expected in SFE. PRCS EOS is shown to predict the phase behavior of model supercritical systems reported here accurately.⁹ The solubilities of heavy solutes in supercritical solvents are calculated using Equations 19 and 21.

The solubility of 2,3-dimethylnaphthalene in supercritical CO₂ as a function of pressure at three different temperatures, compared with the calculations based on the PRCS EOS for mixtures, can be seen in Figure 4. According to this figure, the PRCS EOS is quite suitable for the model mixtures studied here. The same trends in Figure 4 are observed in Figure 5 for the solubility of 2,3-dimethylnaphthalene in supercritical ethylene. Interaction parameters τ_{ij} , λ_{ij} , and ζ_{ij} are constant, and they can be used as well for other temperatures consistent with the molecular theory of unlike interactions. Figures 6 to 8 are presented to study the effect of mixed supercritical solvents on the solubility of pure solutes. In these figures, the solvents consist of different mixtures of CO₂ and ethylene in contact with three different solutes. The solvent-cosolvent interaction parameters are taken into account to predict the solubility of solutes in mixed solvents. According to these figures, mixed solvents can either enhance or reduce the solubility of a solute, depending on the molecular interactions among them. This fact emphasizes the necessity of finding a proper solvent/cosolvent combination and their compositions for optimum design and operation of SFE/RC systems.

The phenomenon of the supercritical solubility peak, which has been observed experimentally for several condensed phase-dense gas systems, can be predicted using the PRCS EOS. Figures 9 to 12 demonstrate solubility peaks that are observed for 2,3-dimethylnaphthalene and 2,6-dimethylnaphthalene at temperatures above and below the critical temperature of the solvent. In addition, it is demonstrated that solubility decays are more pronounced at temperatures above the critical temperature of the solvent. The solubility peak was sharper when ethylene, instead of CO₂, was the supercritical solvent.

IV. CURRENT BIOTECHNOLOGICAL APPLICATIONS

The major requirement in the design of SFE/RC systems is the choice of a solvent that will cause a sharp change in solute solubility due to rather small changes in pressure or

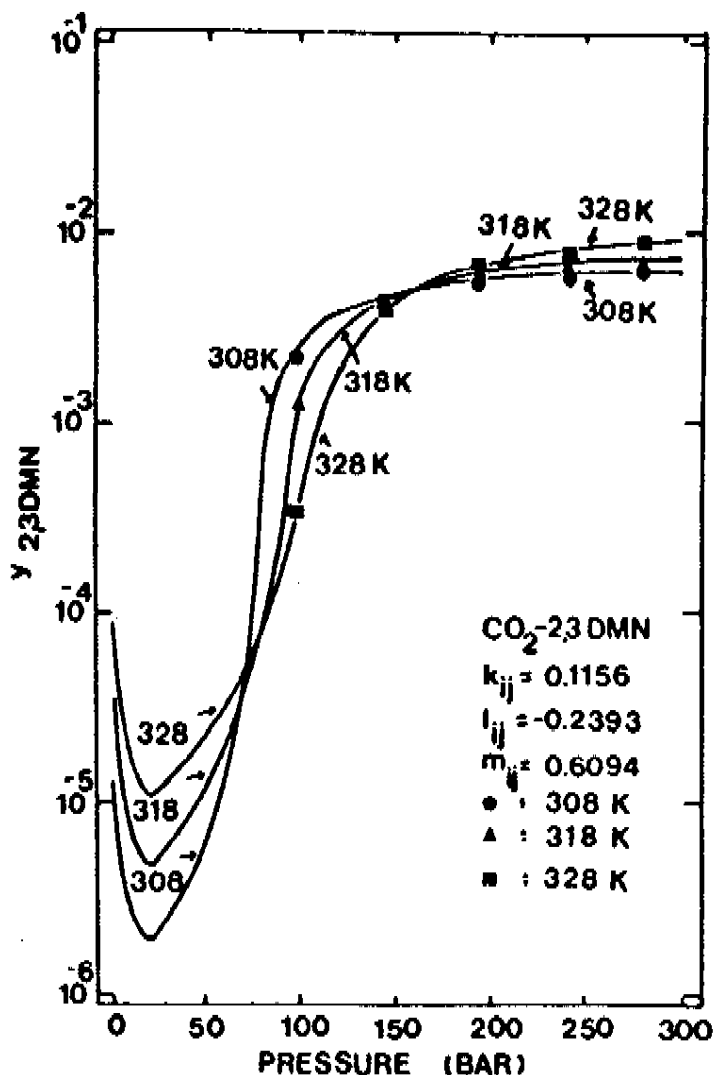


FIGURE 4. Solubility of 2,3-dimethylnaphthalene in supercritical CO₂ as a function of pressure, at different temperatures, as calculated by the PRCS EOS (solid lines), and compared to the experimental data.⁴³

temperature. Since an essentially infinite number of supercritical pure and mixed solvents can be chosen from the currently known compounds, there is little hope of ever generating a sufficient amount of experimental data to meet present, much less future, industrial needs. Nevertheless, there have been a number of key experimental measurements regarding biotechnological applications of SFE/RC phenomena which are reviewed in this section. Practical application of SFE/RC phenomena requires the knowledge of theoretical correlation and prediction techniques that could reduce the heavy burden of such experimental measurements.²⁰ This justifies the importance of the development of statistical mechanical techniques that can be used for predictive models of SFE/RC phenomena.

A. AMINO ACIDS

The influence of humid supercritical CO₂ and nitrogen on amino acids, namely L-glutamine, L-methionine, L-leucine, L-alanine, β -alanine, and L-lysine, was investigated.²⁵

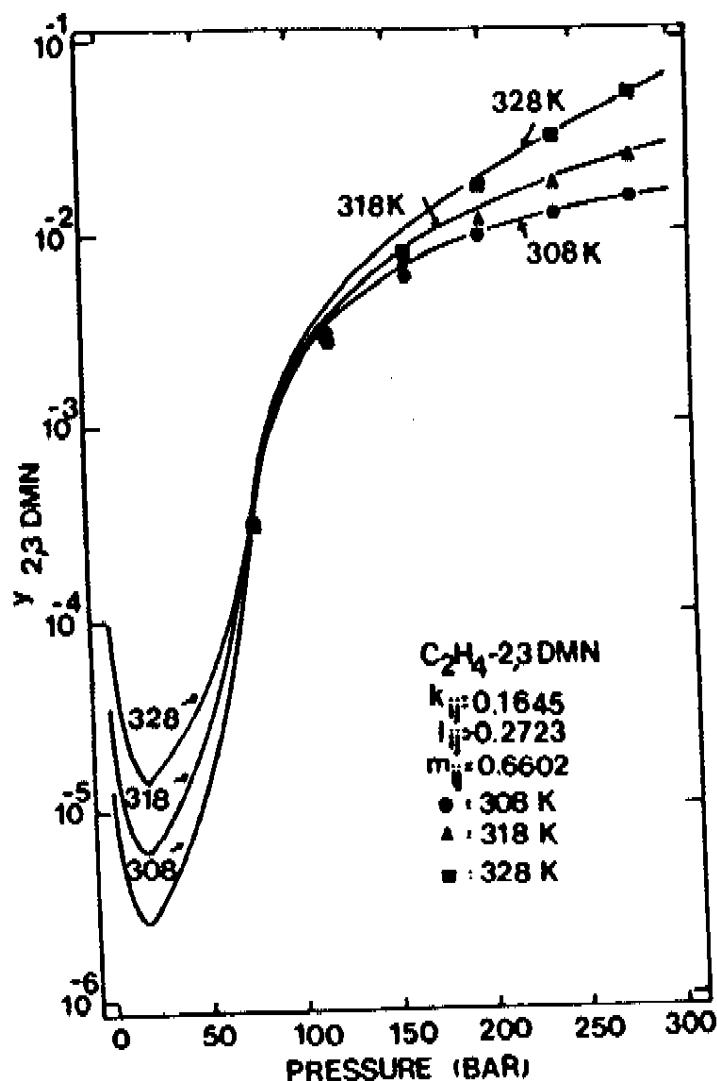
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FIGURE 5. Solubility of 2,3-dimethylnaphthalene in supercritical ethylene as a function of pressure, at different temperatures, as calculated by the PRCS EOS (solid lines), and compared to the experimental data.⁴³

After exposure to humid CO_2 at 300 bar and 353 K for 6 h, only L-glutamine showed a significant composition decay (15 to 23%). The L-glutamine loss was 10% when treated with nitrogen under the same conditions. The decomposition product of L-glutamine is 2-pyrrolidinone 5-carboxylic acid (PCA), which is an intermediate in the γ -glutamyl cycle of mammalian metabolism. The other amino acids did not show a significant decay in composition after exposure to humid CO_2 at 300 bar and 353 K for 6 h. When L-glutamine was exposed to supercritical CO_2 and nitrogen at room temperature and 300 bar for 6 h, no PCA was detected, thus implying that L-glutamine decomposes due to heating. Pressure seems to have little effect on the structural integrity of L-glutamine, since Vickery et al.²² have shown that an L-glutamine solution at atmospheric pressure will almost quantitatively convert to PCA and ammonia if exposed for 2 h at 373 K and pH 6.5. These results indicate that amino acids remain structurally sound after exposure to high pressure, and among tested amino acids, only L-glutamine is seriously damaged by heating.

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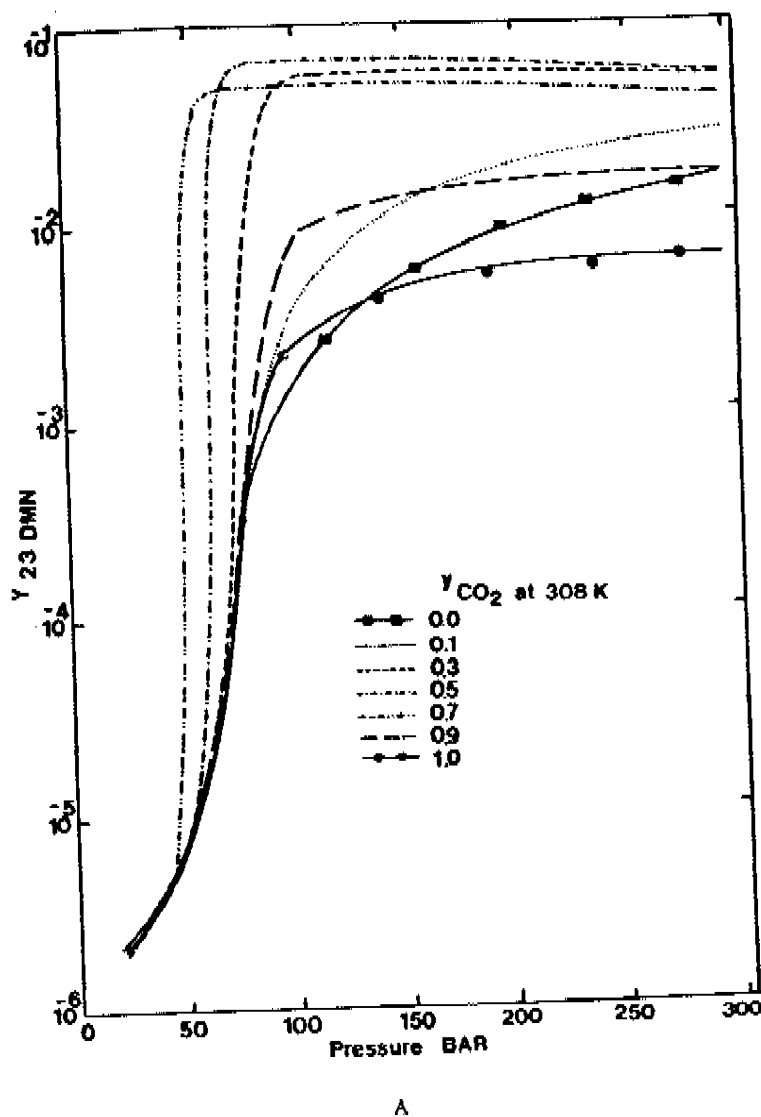


FIGURE 6. Solubility of 2,3-dimethylnaphthalene in different mixtures of CO_2 and ethylene according to the PRCS EOS (lines). In A, it is assumed that for CO_2 and ethylene $\tau_{12} = \lambda_{12} = \zeta_{12} = 0$. In B, the unlike interaction parameters of CO_2 and ethylene are assumed to be nonzero. They are calculated from CO_2 - C_2H_4 vapor-liquid equilibrium data** and the results show that $\tau_{12} = 0.1829$, $\lambda_{12} = 0.6053$, $\zeta_{12} = 0.2153$.

The behavior of amino acids is very important for biological applications of supercritical systems because such molecules are the building blocks of life. The fact that the decomposition products of exposing amino acids to supercritical CO_2 are also naturally found in many food components makes SFE/RC application a potentially lucrative process for the food industry.

B. ESSENTIAL OILS FROM PLANTS

Limonene and eugenol, essential oils extracted from plants, are demonstrated to be soluble in supercritical CO_2 , exhibiting solubilities according to their respective molecular

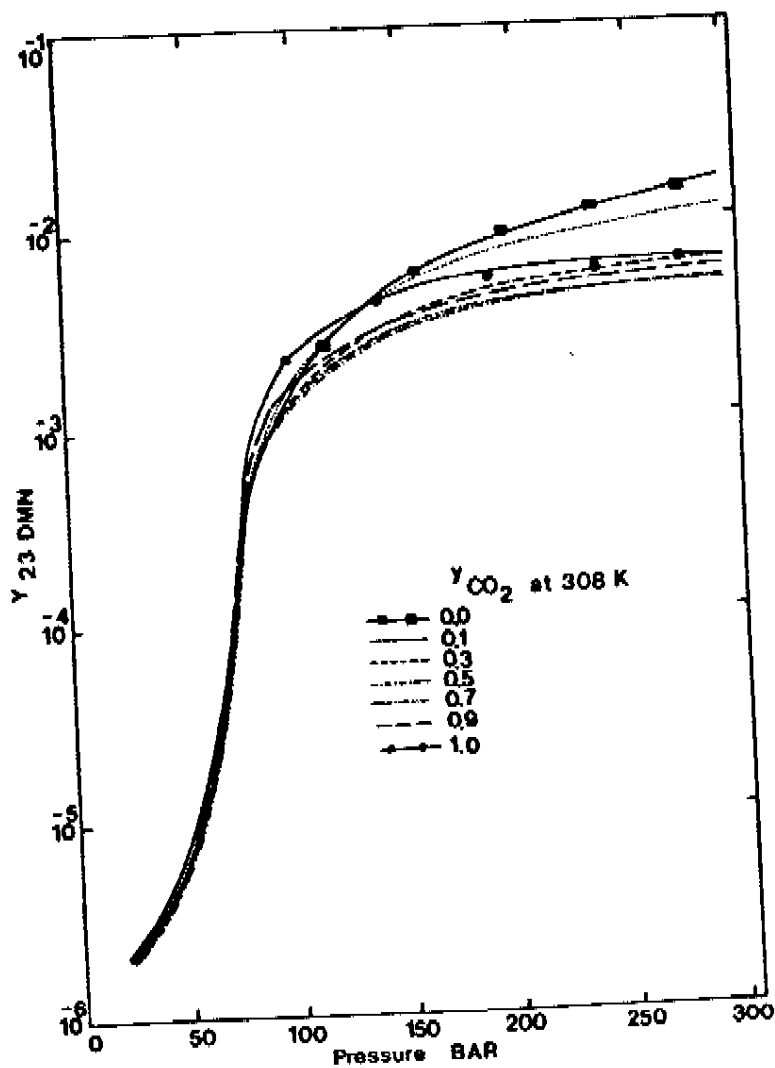
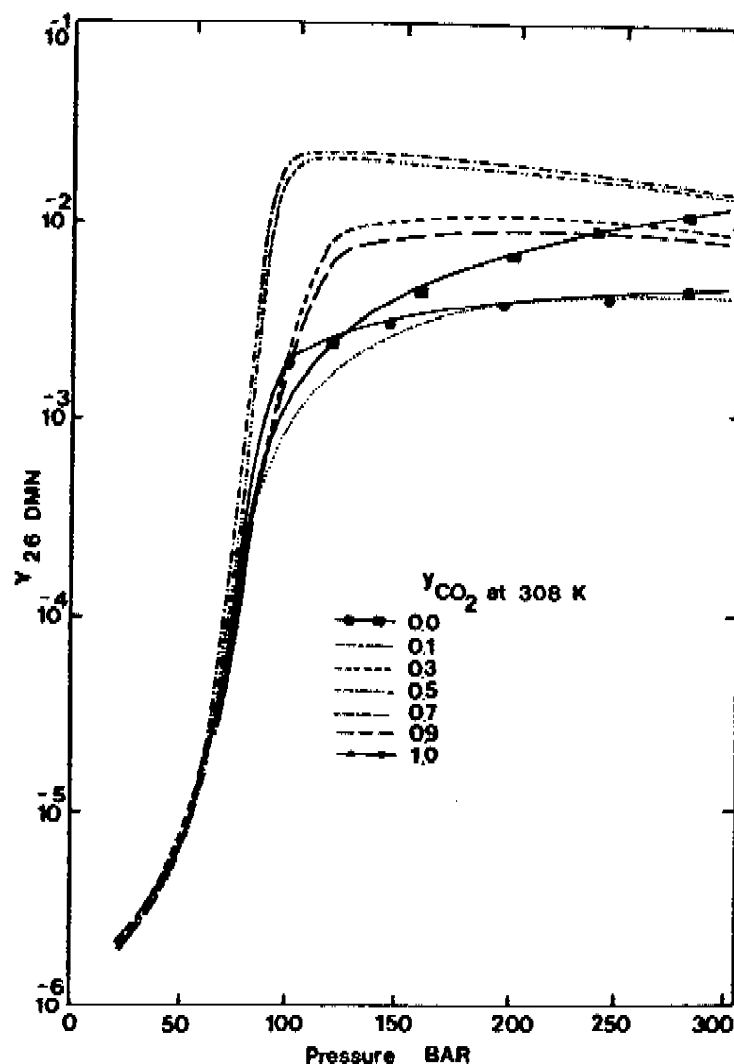
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FIGURE 6B.

polarities. Limonene contains no functional groups that could drastically increase molecular polarity, but eugenol has an ether and a hydroxyl group. The experimental evidence²³ indicates that eugenol is much less soluble in CO₂ than limonene, as shown in Figures 13 and 14. The sharp rise in solubility of both limonene and eugenol at low temperatures can be explained by the fact that CO₂ becomes a liquid and, therefore, attains a strong solvent power for these essential oils. This is one of the few cases where an organic molecule is found to be soluble in subcritical CO₂. Solubility data of these compounds in CO₂ (Figure 14) indicate that both limonene and eugenol isothermal solubilities pass through a minimum. They also show strong solubility increases as the pressure rises above the point of minimum solubility in supercritical CO₂. A third essential oil, caryophyllene (Figure 14), also exhibits a minimum solubility, it being more soluble in CO₂ than eugenol. The rate of solubility increase of caryophyllene is less extreme than that of eugenol, and the solubilities of eugenol and caryophyllene in CO₂ become equal at 80 bar.

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A

FIGURE 7. Solubility of 2,6-dimethylnaphthalene in different mixtures of CO_2 and ethylene according to the PRCS EOS (lines). In A, it is assumed that for CO_2 and ethylene $\tau_{12} = \lambda_{12} = \zeta_{12} = 0$. In B, the unlike interaction parameters of CO_2 and ethylene are assumed to be nonzero. These parameters are calculated from CO_2 - C_2H_4 vapor-liquid equilibrium data⁴⁴ and they are the same as those used in Figure 6.

C. HOPS

The extraction of hops in beer production has proved to be an important application of the SFE/RC process.²⁴ The desirable components of the hops are soft resins, consisting of humulones and lupulones. In the brewing process, humulones are isomerized to give beer its bitter flavor, thus it is necessary to control the beer taste by extracting only the soft resins while minimizing the production of undesirable by-products. Here, subcritical CO_2 has proven to be a superior extractor because it does not extract undesirable hard resins like supercritical CO_2 does.^{25,26} The SFE/RC of hops using liquid CO_2 requires more time than does SFE/RC with supercritical CO_2 because the liquid solvent extracts the hops components more selectively than does the supercritical solvent.

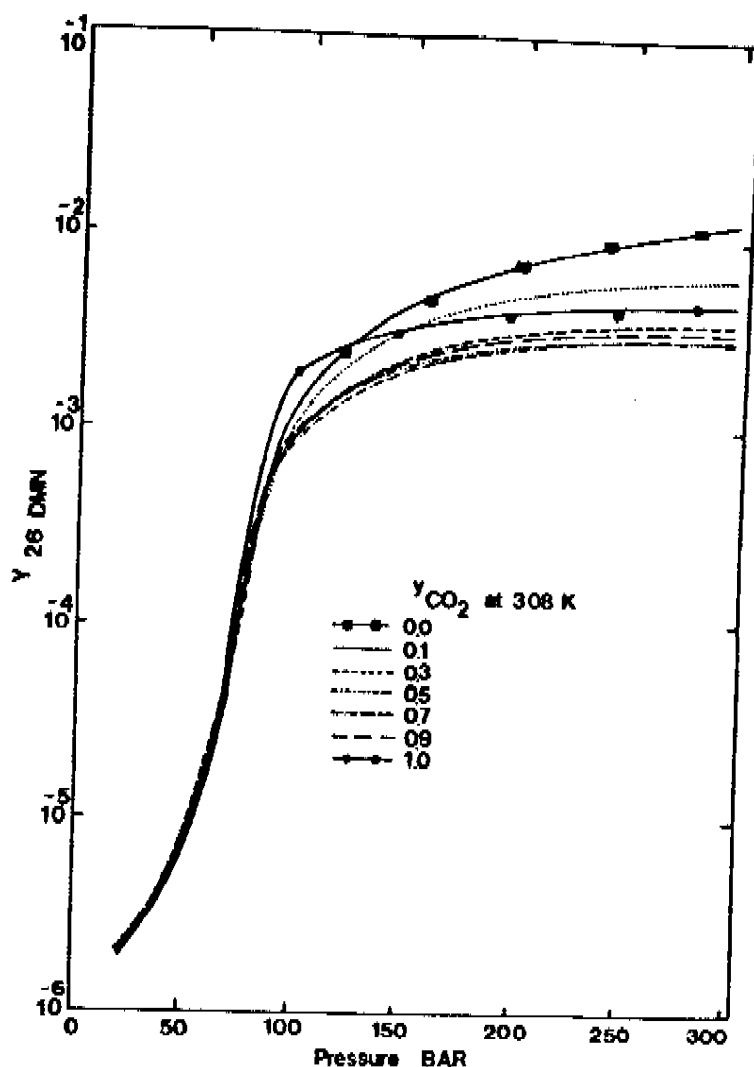
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FIGURE 7B.

Subcritical CO₂ extracts only the humulones, lupulones, and essential hop oils, while supercritical CO₂ extraction includes the undesirable hard resins in addition to those components extracted by subcritical CO₂. Many patents cover this technique, so its exploitability is somewhat limited.

D. JOJOBA OIL

Jojoba oil is extracted by SFE/RC using supercritical CO₂ as the solvent. This oil is obtained from crushed seeds, and it is mostly a wax ester mixture. When the solubility of jojoba oil as a function of temperature at pressures varying between 100 and 2600 bar is measured,²⁷ one can see that at 100 bar the solubility of jojoba oil in CO₂ is near zero (Figure 15). A sharp increase in solubility does not occur until the pressure rises above 200 bar. As the pressure continues rising, the solubility increases more quickly until the solubility peak is attained at 700 bar for temperatures above 313 K. After the peak, the solubility decreases slowly as the pressure rises further. At temperatures below 313 K, the maximum

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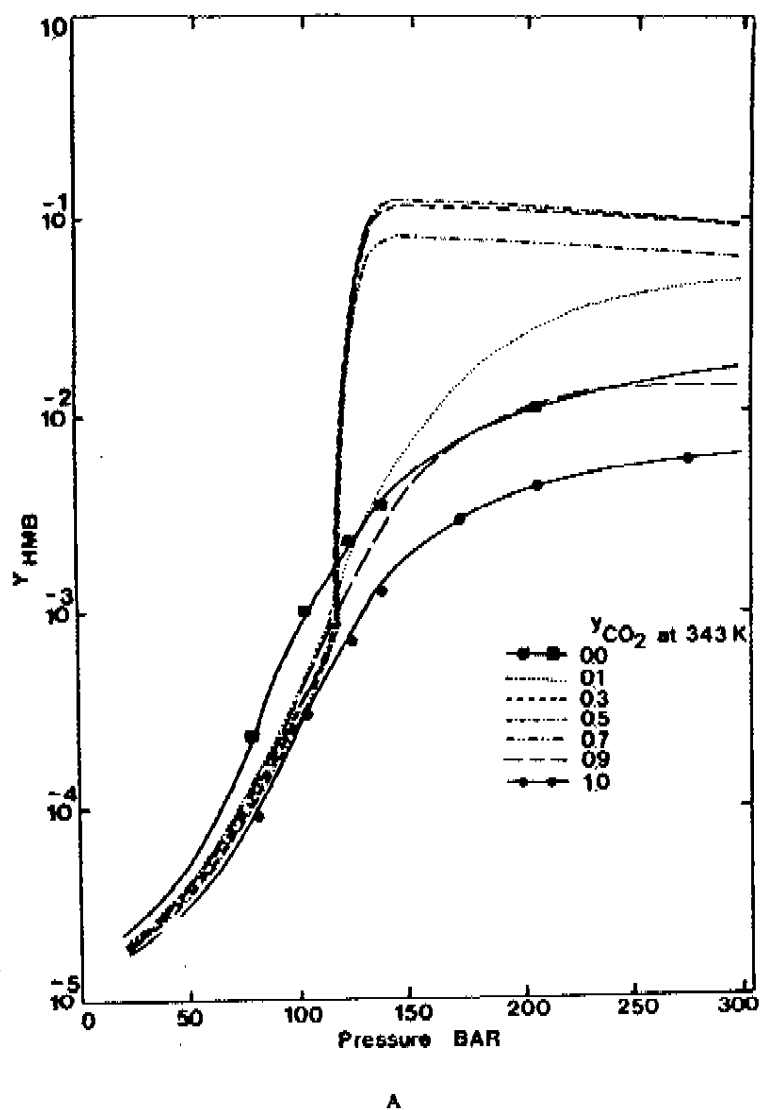


FIGURE 8. Solubility of hexamethylbenzene in different mixtures of CO₂ and ethylene according to the PRCS EOS (lines). In A, it is assumed that for CO₂ and ethylene $\tau_{12} \approx \lambda_{12} = \zeta_{12} = 0$. In B, the unlike interaction parameters of CO₂ and ethylene are assumed to be nonzero. These parameters are calculated from CO₂-C₂H₄ vapor-liquid equilibrium data⁴⁴ and they are the same as those used in Figure 6.

solubility of jojoba oil in supercritical CO₂ can be observed at pressures slightly below 700 bar. Beyond 700 bar, the solubility of jojoba oil decreases at all temperatures. For example, at 2600 bar and 333 K, this solubility is less than one third of the solubility at the same temperature and 700 bar.

E. LYSOZYME

Lysozyme, a protein found in chicken egg white, was treated with humid supercritical CO₂ and nitrogen, and showed no alterations, as was observed in the case of amino acid analysis. Studies were made at 300 bar, varying both temperature and time of exposure from 353 K to room temperature and 6 to 2 h, respectively.^{21,28}

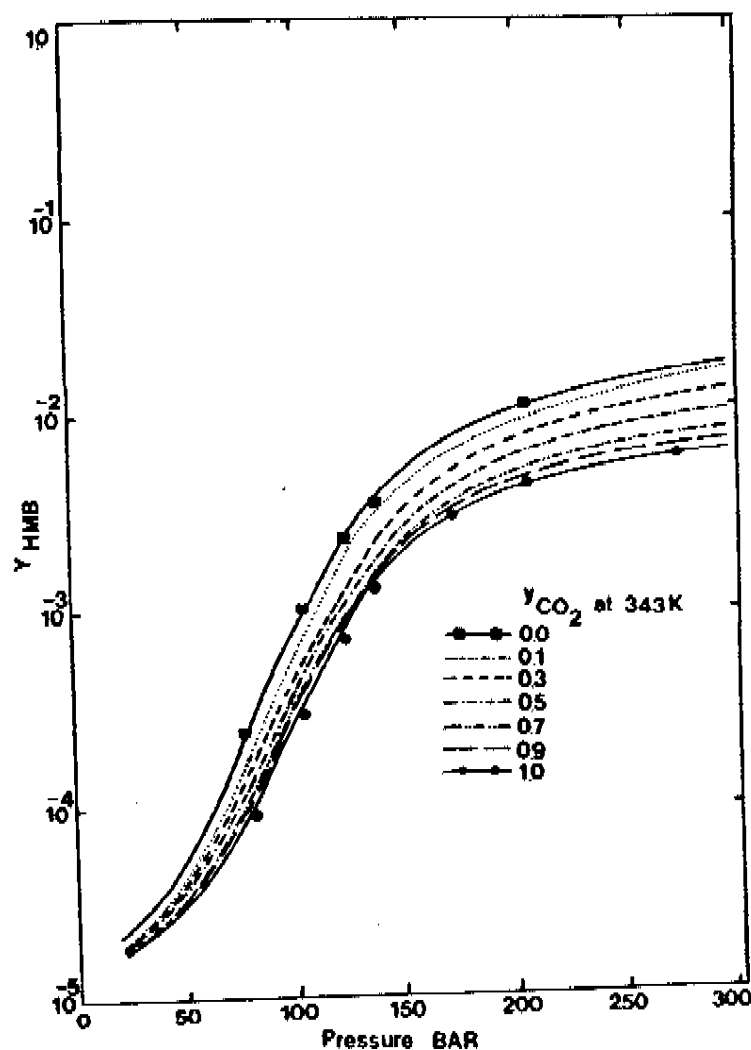
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FIGURE 8B.

As indicated in Table 1, only minimal decay in protein content was observed even at high temperatures (the same as in the case of amino acids). The lowest total protein composition after treatment was 86.6%, indicating only a small loss of protein. Digestibility of lysozyme by trypsin, an indicator of the structural integrity of lysozyme, was fairly independent of the type of solvent gas utilized in the experiment. When the humid gas was brought to 300 bar and 353 K, the digestibility of lysozyme by trypsin was markedly increased, compared to the rate of digestibility at room temperature and 300 bar. The amount of digestion at 300 bar and 353 K was similar to the amount observed heating lysozyme with 15% water at 353 K and atmospheric pressure, during either 2 or 6 h. Heating dry lysozyme at 353 K and atmospheric pressure showed a much smaller increase in trypsin digestibility compared to the increase observed with humidity at 353 K and 1 bar. Thus, the increased digestibility was due mainly to the presence of water and not to the supercritical conditions. Because lysozyme (and the other proteins studied) can withstand supercritical conditions, SFE/RC may prove to be an effective technique for the extraction of food products.

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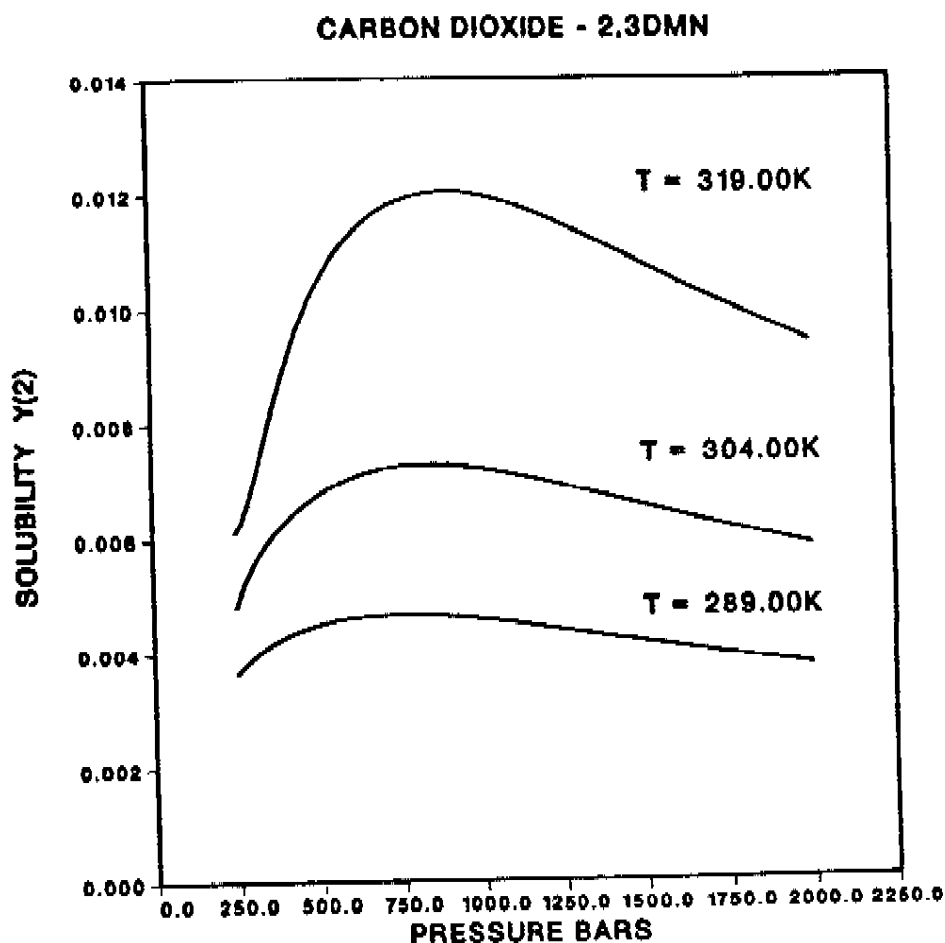


FIGURE 9. Solubility of 2,3-dimethylnaphthalene in CO₂ at supercritical, critical, and subcritical temperatures of CO₂ according to the PRCS EOS.

F. NICOTINE AND CAFFEINE

Nicotine, a major toxic agent found in tobacco, is shown to be soluble in supercritical CO₂.²⁹ At 323 K, the solubility peak of nicotine in CO₂ is 80 mg/g of solvent, at a gas density of about 0.650 g cm⁻³. At 343 K, the maximum solubility falls to about 65 mg nicotine per gram of CO₂, at a gas density of 0.480 g cm⁻³. Nicotine, unlike most other molecules that are soluble in supercritical gases, does not tend to exhibit larger solubilities as the temperature rises. The high solubility of nicotine in supercritical CO₂ is due to the fact that nicotine is a relatively nonpolar molecule possessing no carbonyl or hydroxyl functional groups that inhibit solubility. By contrast, caffeine, which is more polar than nicotine due to its two carbonyl groups per molecule, has a maximum solubility in CO₂ of <5 mg caffeine per gram of CO₂ even at 353 K and 390 bar. One industrial option in applying the SFE/RC process for extraction of caffeine consists of treating green coffee beans, presoaked in water, with CO₂ in a pressure vessel operated between 160 and 220 bar.²⁸ After adequate mixing, the caffeine-rich solvent is allowed to leave the pressure vessel and enter into a washing tower. In the washing tower, water at 343 to 363 K is used as an entrainer for the caffeine, stripping the CO₂ of the caffeine. The solvent is then recycled, and caffeine is recovered by distillation. The caffeine content of coffee beans can be reduced from 0.7 to 3% to as low as 0.2% βΨ using this industrial option.

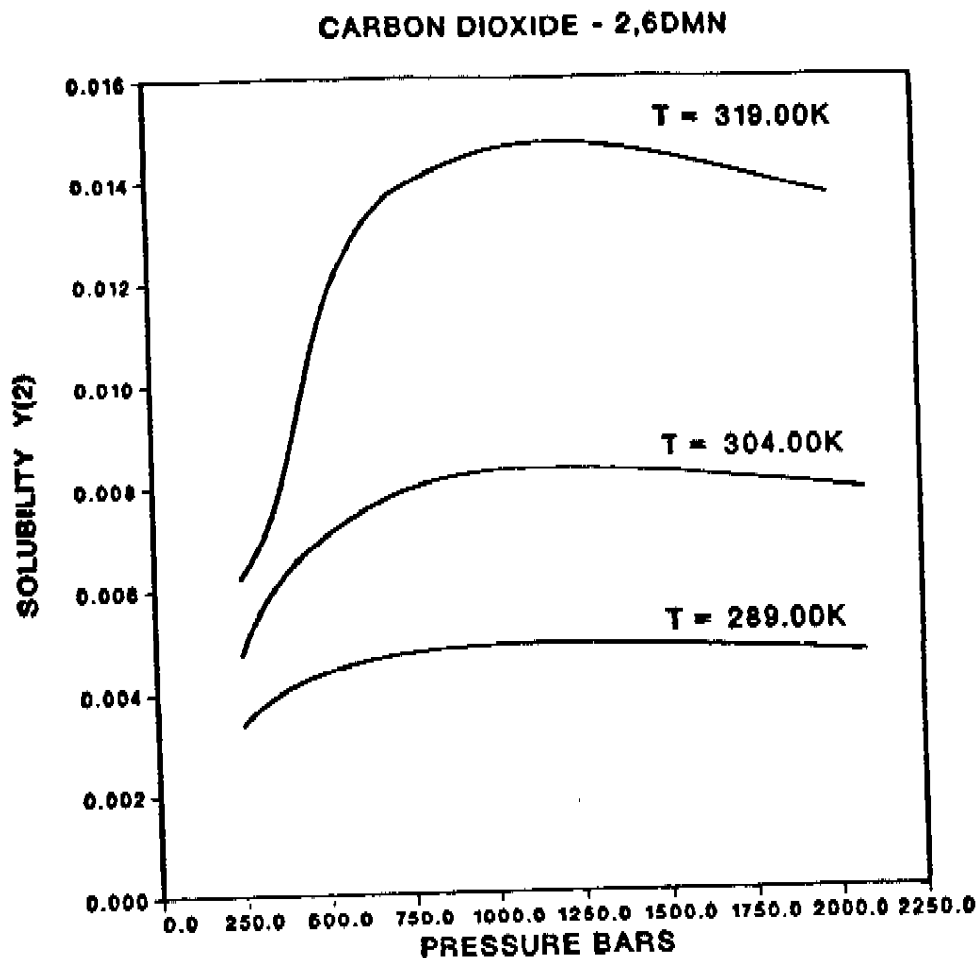


FIGURE 10. Solubility of 2,6-dimethylnaphthalene in CO₂ at supercritical, critical, and subcritical temperatures of CO₂ according to the PRCS EOS.

G. STEROIDS

Steroids, a class of compounds with various biological applications, have proven to be soluble in supercritical CO₂. There exist many different types of steroids, all of which have a cyclopentanoperhydrophenanthrene skeleton. The difference in the steroids' function lies in the variation of the groups attached to this skeleton. These compounds are quite stable and they survive after the death of the organism far longer than any other biological molecule. Steroids (cholesterol) cause heart disease. They contribute to the control of sexual development (progesterone), pain reduction (cortisone), and a number of other biological processes. Experiments have shown that sterols with the least number of hydroxy and carboxyl functional groups have the highest solubilities in CO₂.³⁰ Some examples are ergosterol and stigmasterol, each containing only one hydroxyl group. At 313 K, sitosterol, containing only one hydroxyl substituent, begins exhibiting solubility at 80 bar. Progesterone, containing two carbonyl groups, also becomes extractable at this pressure. Androsterone, with one hydroxyl and one carbonyl group, displays measurable solubility at 90 bar. Pregnanediol, containing two hydroxyl groups, requires a pressure of 120 bar to be extracted, while digitoxigenin, possessing two hydroxyl and one carbonyl group in an α,β -unsaturated lactone ring, does not become appreciably extractable until the pressure is raised to 150 bar.⁷

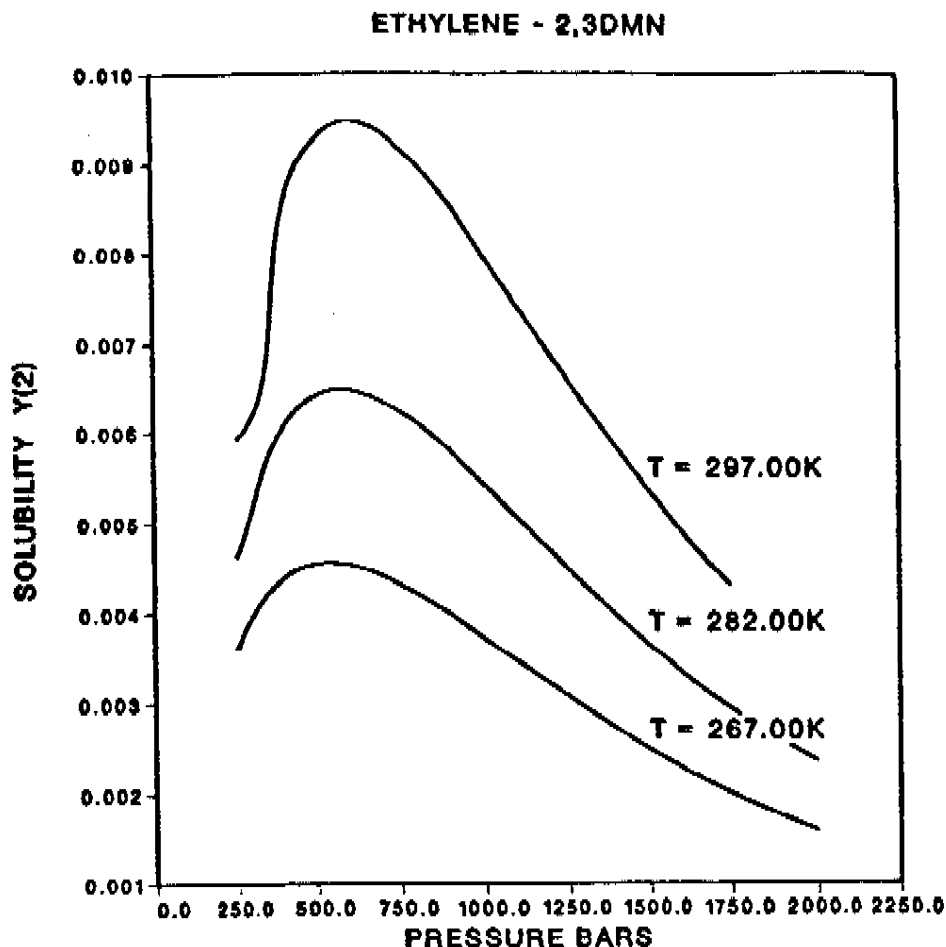


FIGURE 11. Solubility of 2,3-dimethylnaphthalene in ethylene at supercritical, critical, and subcritical temperatures of ethylene according to the PRCS EOS.

H. THUJONE

Wormwood, which has a bitter taste, is toxic because of its thujone content. SFE/RC with supercritical CO_2 can remove thujone from wormwood, leaving most of the bitter flavor which is mainly due to absinthine.³¹ Thujone has only one carbonyl functional group per molecule, quite low polarity, and thus it is rather easy to extract. Absinthine and artabsine, which are also present in wormwood, have lower solubilities in supercritical CO_2 compared to thujone. By examining the solubility curves of thujone and artabsine (Figure 16), it can be observed that thujone exhibits a solubility in CO_2 much greater than artabsine. At around 90 bar, thujone is ten times more soluble than artabsine, but for all other pressures, the solubility ratio increases rapidly. Artabsine has one hydroxyl and one carbonyl functional group per molecule, and is therefore more polar and less soluble in supercritical CO_2 than thujone. Absinthine is nearly unextractable by SFE/RC using CO_2 as the solvent because it contains two hydroxyl and two carbonyl functional groups per molecule. The high molecular weight of absinthine (497 g mol^{-1}) also hinders its solubility in supercritical CO_2 . Experiments have shown that use of the SFE/RC process for extraction of wormwood can be quickened by increasing the mass ratio of CO_2 to wormwood in the process cycle. At mass ratios of six parts CO_2 to one part wormwood or greater, thujone is nearly 100% extracted. While at a mass ratio of two parts carbon dioxide to one part thujone, only about 80% of the thujone will be dissolved in the gas phase.

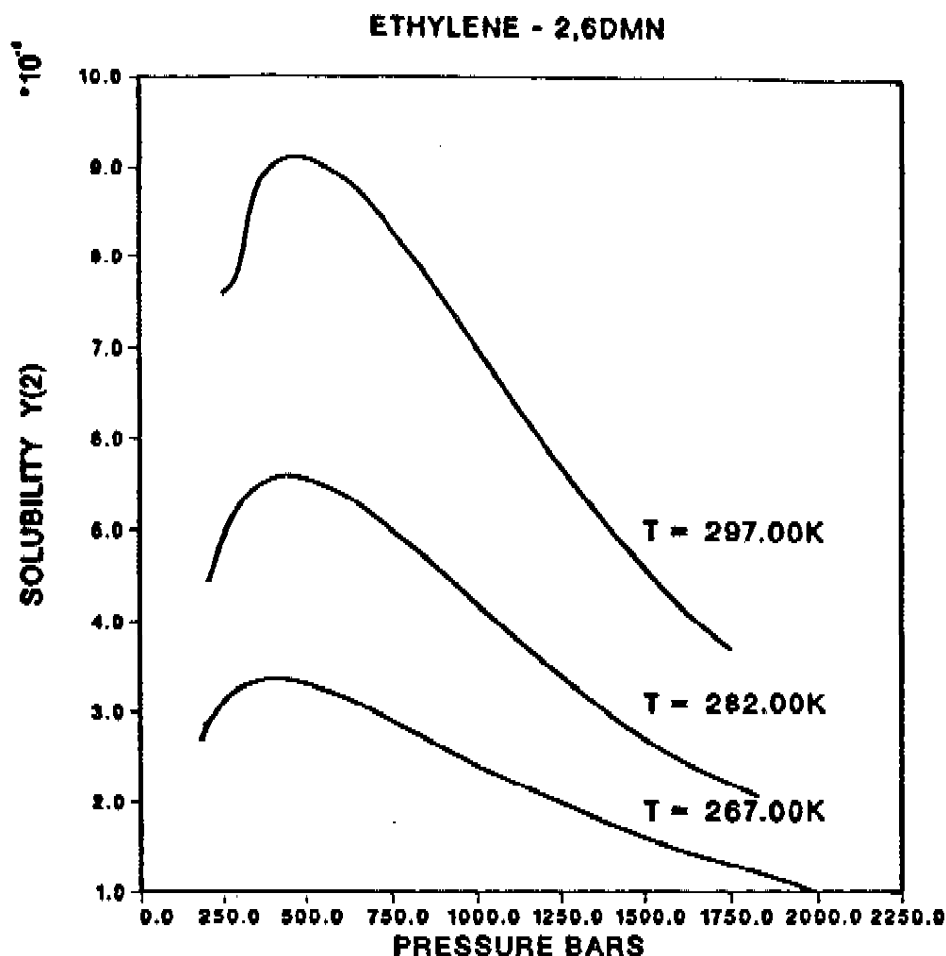
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FIGURE 12. Solubility of 2,6-dimethylnaphthalene in ethylene at supercritical, critical, and subcritical temperatures of ethylene according to the PRCS EOS.

I. THYMOL

The essential oil of thyme (a plant of the mint family whose leaves are used for seasoning) is called thymol. Structurally, thymol consists of benzene ring with hydroxyl, isopropyl, and methyl substituents. The fact that thymol contains only one strongly polar functional group (OH) allows thymol to be easily extractable by SFE/RC with CO₂ as the solvent. The oil extract obtained by SFE/RC of thyme with CO₂ has been compared to that from steam distillation and to commercially available red thyme oil.³² The results indicate that CO₂ at 40 bar and 323 K extracts as many as 50 different compounds from thyme. The extract contains 36.6% thymol and 19% stearic acid and other high molecular weight compounds, while steam distillation produces an extract containing 48.3% thymol and only 0.27% stearic acid and heavier molecular weight compounds. Analysis of red thyme oil indicated no heavy compounds and 30.9% thymol. Thus, the SFE/RC of thyme with supercritical CO₂ produces an extract containing a larger percentage of high molecular weight compounds than the red thyme oil or the oil from the steam distillate.

J. TRIGLYCERIDES

Triglycerides are composed of three organic acids attached to glycol. Triglycerides are considered neutral fats easily mobilized as an energy source in mammals. The extraction of

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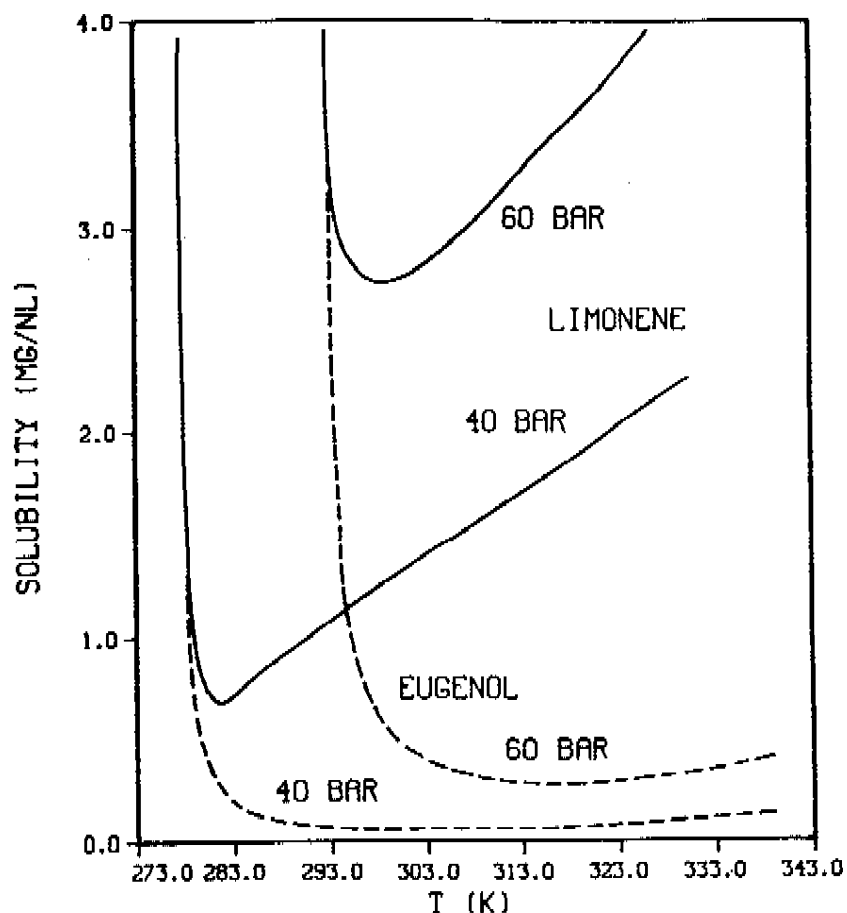


FIGURE 13. Solubility of limonene and eugenol in CO_2 as a function of temperature at 40 and 60 bar.²³

triglycerides with supercritical CO_2 is found to be a promising procedure for the modification of butter oils in order to lower their cholesterol level and improve their spreadability at refrigeration temperatures.³³ Triacylglycerols from plant tissue have been shown to be extractable by supercritical CO_2 . Samples of dried coconut flesh, or Copra, containing triacylglycerols were extracted in pressurized batches weighing 700 g using supercritical CO_2 at various conditions. The results of this analysis are reported in Table 2.

Examination of the results in Table 2 indicate that at the conditions of lowest pressure and temperature (300 bar and 313 K), a CO_2 /extract mass ratio of 64.9 was required for an exposure time of 8 h to achieve a triacylglycerol yield of around 60%. The experiment utilizing the highest pressure and temperature (900 bar and 333 K) required a CO_2 /extract ratio of only 6.2 for an exposure time of 1 h in order to achieve the same yield. This case is 83 times more effective in its extraction of triacylglycerols than the 300 bar, 313 K example and helps prove the general rule that solubility increases with an increase in pressure and temperature.³⁴

A major source of triglycerides produced on a world scale is soybean oil. Several important observations can be made when the solubility data of soybean oil in CO_2 (Figure 17) are studied. Examination of the data indicates that the solubility of soybean oil in CO_2 increases as the temperature rises and when the pressure is above 280 bar.³⁵ The solubility increase becomes more pronounced with rising temperature, reaching a peak of about 65

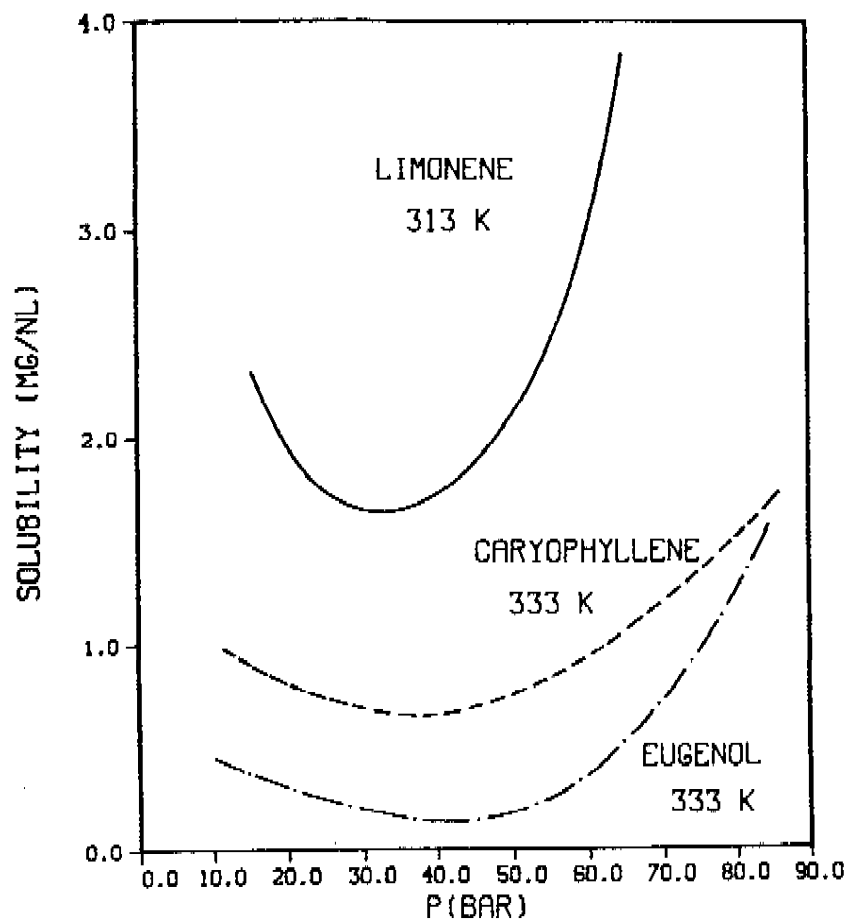
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FIGURE 14. Solubility of limonene at 313 K, eugenol at 333 K, and caryophyllene at 333 K, in CO_2 , as a function of pressure.²³

mg nl^{-1} at 333 K and more than 140 mg nl^{-1} at 353 K (Figure 17). The solubility of soybean oil in CO_2 exhibits the crossover effect at 280 bar. The existence of the crossovers was also observed in the theoretical and experimental results of the model systems reported in Figures 4 and 5. The existence of solubility maxima of soybean oil in CO_2 is also observed for each isotherm studied. According to Figure 17, the solubility maxima are reached at higher pressures as the temperature is raised. The solubility maxima for soybean oil in CO_2 and for jojoba oil in the same solvent occur at a gas phase density between 1.04 and 1.08 g cm^{-3} .

Presently, industrial extraction of soybean oil is mainly carried out by using liquid hexane as the solvent. Soybean oil extracted by SFE/RC with CO_2 as the supercritical solvent tends to be lighter in color and exhibits lower oxidation stability than the oil extracted by hexane, although extraction yields are similar. The lack of oxidation inhibiting phosphatides in the supercritical CO_2 extract seems to be the cause of the instability of the extract.²⁶

K. VIABILITY OF BACTERIA UNDER HIGH PRESSURE

A set of experiments have been performed to test the viability of certain bacteria in a supercritical CO_2 environment. These experiments were conducted at room temperature and 20 min of exposure time at pressures of 500, 1500, and 2500 bar.²⁷ The results showed that *Pseudomonas aeruginosa* and *Candida albicans* were completely destroyed after exposure

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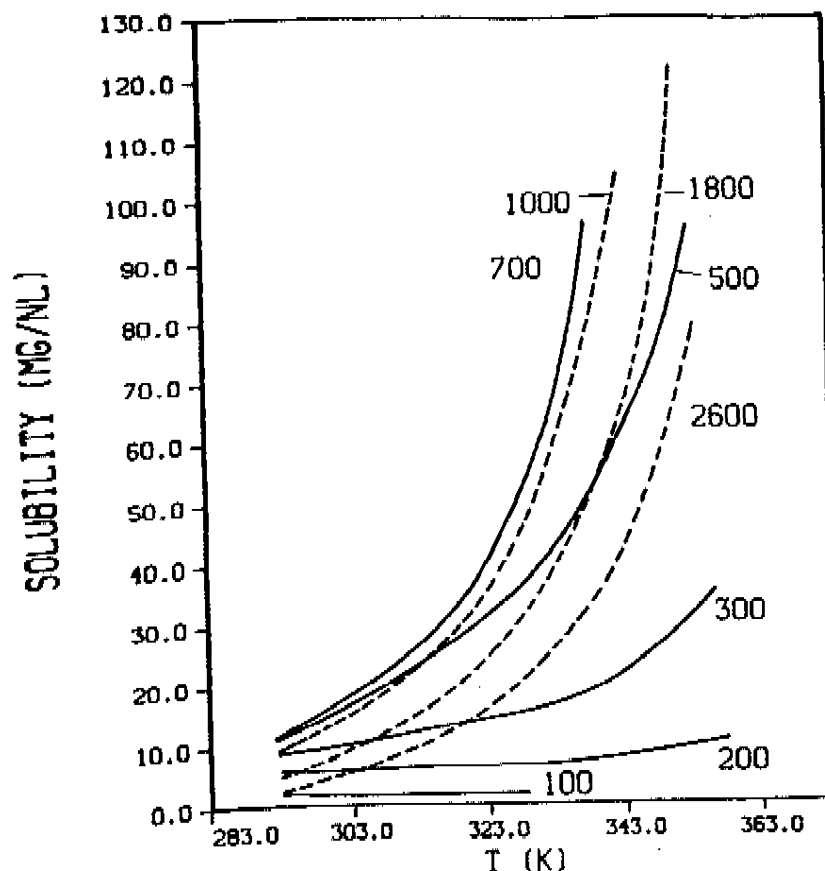


FIGURE 15. Solubility of jojoba oil in CO_2 as a function of temperature at various pressures.²⁷

TABLE 1
Results from the Treatment of Lysozyme with CO_2

Solvent	Untreated	Protein content (%)		
		RmT*, 6 h	353 K, 2 h	353 K, 6h
CO_2	90.3	89.7	93.0	92.9
N_2	90.3	93.1	90.4	86.6

* RmT: room temperature.

to CO_2 at 500 bar. *Bacillus subtilis* showed 30% logarithmic viability at all three pressures. *Escherichia coli*, which lives in human intestines, showed a 25% logarithmic viability at 500 bar, 10% at 1500 bar, and around 0% at 2500 bar. *Coliphage* showed less sensitivity to pressure with 75% logarithmic viability at 2500 bar. Implications of these experiments could be far-reaching. For example, if it was found that certain bacteria in milk were pressure sensitive, a new and possibly more economical milk sterilization technique could be developed. Other applications would be the destruction of harmful bacteria in a wide variety of foodstuffs, such as seeds or goods earmarked for long-term packaging. These studies

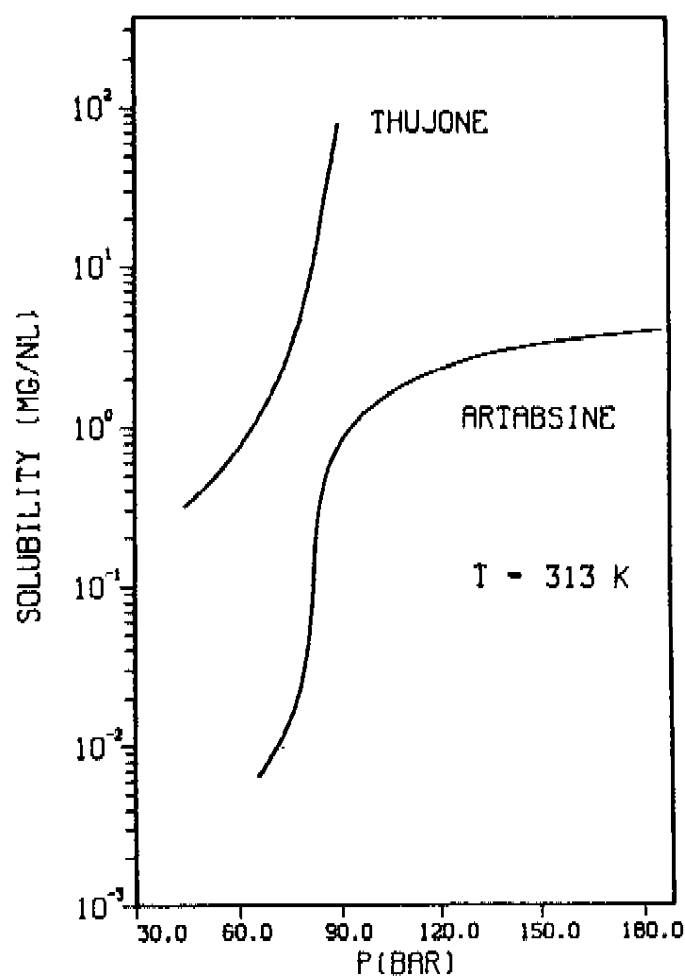
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FIGURE 16. Solubility of thujone and artabsine in supercritical CO_2 as a function of pressure at 313 K.³¹

TABLE 2
Results of the Extraction of Triacylglycerols from Copra

P (bar)	T (K)	g CO_2 /g extract	t (h)	Yield (%)
300	313	64.9	8	59.9
600	313	34.1	5	58.6
600	333	12.8	2	61.4
900	333	6.2	1	60.8

could also indicate whether the SFE/RC process is applicable to certain products (mainly foods) without altering their useful microbiological contents. At the present time, the industrial potential of such a process is unknown. Numerous bacteriological experiments need to be performed on each specific bacteria strain in order to test the pressure sensitivity of these organisms.

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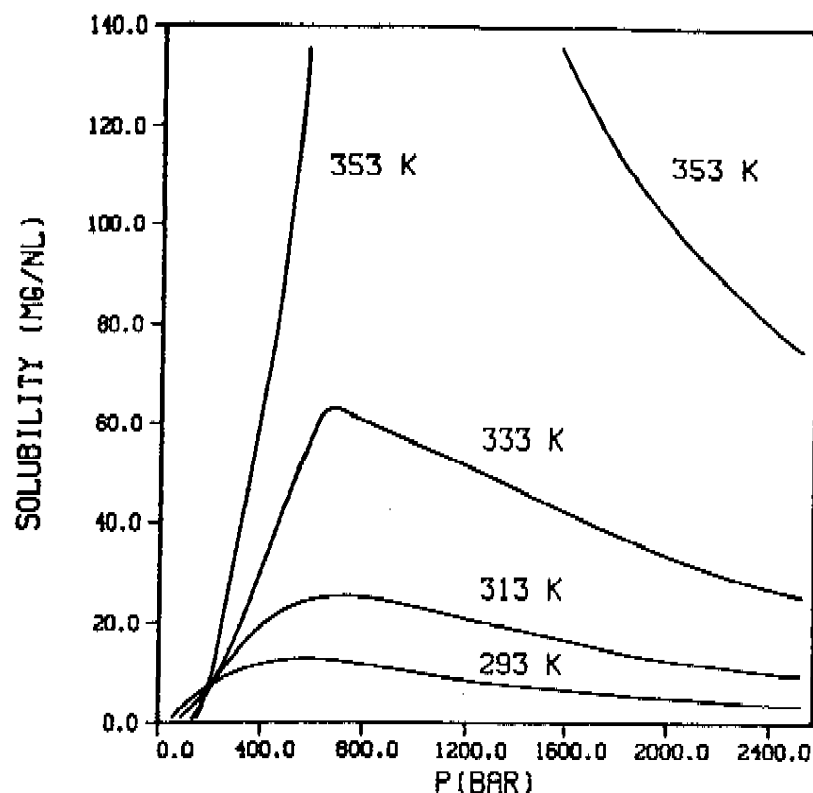


FIGURE 17. Solubility of soybean oil in CO_2 as a function of pressure at various temperatures.²¹

V. CONCLUSIONS AND RECOMMENDATIONS

In Table 3 the SFE/RC biotechnological applications discussed above are summarized. According to the above discussion and the summary reported in Table 3, biological molecules exhibit different degrees of solubility in supercritical solvents, especially CO_2 . These solubility properties can be exploited by extracting, in some cases selectively, the specific molecules by carefully controlling the pressure and temperature. The industrial and commercial implications of these investigations are still to be determined. One major possibility is the purification of substances vital to human health. Since in many SFE/RC cases efficient extraction can be carried out at normal to low temperatures (below 318 K), many thermolabile compounds found in natural systems could be, for the first time, extracted without a loss. Possible applications are the extraction of hormones, the regulation of nutrients in the blood, and the separation of toxic contaminants. Further research is necessary in this direction in order to determine the solubility and temperature sensitivity of fluids found in the human body. Another possible research route is the study of humid supercritical CO_2 solvents. It is possible that water can enhance solubilities in such systems as those extracting nicotine or oleic acid.

In order to tackle the problems of utilizing the SFE/RC phenomena in biotechnology and related disciplines, the following general tasks would have to be accomplished:

1. Experimental studies for measurement of solubilities, compositions, and solute-solvent interaction characteristics by utilizing high pressure SFE/RC cycles, gas chromatography, high pressure liquid chromatography, gel permeation chromatography, etc.

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TABLE 3
Biological SFE/RC Applications Summary

Solute	Solvent	T(K)	P(bar)	Notes
Amino acids	CO ₂	298—353	300	SFE/RC conditions have no effect on their structural integrity
Caffeine	N ₂ CO ₂	313—353	150—400	
Essential oils from plants (limonene, eugenol and caryophyllene)	CO ₂	278—333	15—80	Maximum solubility at highest temperature Soluble in subcritical CO ₂
Hops	CO ₂	<304	<74	Extraction with subcritical CO ₂ is more selective than SFE
Joboa oil	CO ₂	293—353	100— 2600	
Lysozyme	CO ₂	298—353	300	Maximum solubility occurs at around 700 bar for all temperatures
Nicotine	N ₂	323—343	70—250	Small decay in protein content due to SFE conditions
	CO ₂			Higher solubilities at lower temperatures
Steroids	CO ₂	313	80—250	Steroids have the lowest solubilities Thujone can be selectively extracted from wormwood due its much higher solubility
Thujone (artabsin, absinthine)	CO ₂	313	50—200	
Thymol	CO ₂	>304	>74	SFE process is more effective than steam distillation technique
Triglycerides (triacylglycerols, soybean oil)	CO ₂	313—333	300— 1000	Solubility greatly increases above 333 K and 400 bar

- Due to the high pressure conditions of SFE/RC processes and the exhaustive number of solvent and cosolvent compositions that need to be tried, the extensive experimental studies alone will not be practical from an economic point of view. As a result, development of computational schemes for the prediction of solubilities of solutes (pure or mixture) in pure or mixed supercritical solvents will be needed to reduce the burden of experimental measurements. Such schemes^{13,38} would be quite useful to search for solvents to be used in a particular supercritical separation problem or feasibility study of separating new candidate biological compounds by supercritical fluids.
- Engineering design and economic comparative study of separation, purification, and stripping of heat-sensitive biological fluids and close-molecular weight compounds through SFE/RC and other techniques will establish the feasibility of each process for commercial applications.³⁹
- Experimental studies and modeling of flow and diffusion in pipes and conduits of pastes and slurries in the presence of supercritical solvents.

Overall, the extraction and separation of biotechnological material using the SFE/RC processes is a growing field of research and development with unlimited potential. The future commercialization of such technology could prove to be quite rewarding.

ACKNOWLEDGMENT

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NOMENCLATURE

A, a, b, C	EOS parameters
E	Intermolecular energy parameter
f	Conformal solution energy parameters
h	Hour
h	Conformal solution volume parameter
L	Intermolecular length parameter
mg	Milligram
K	Degree Kelvin
n	Number of moles
NL	Normal liter (at 1 atm and 25°C)
P	Pressure
PRCS	Peng-Robinson conformal solution EOS
r	Intermolecular distance
R	Universal gas constant
RC	Retrograde condensation
SFE	Supercritical fluid extraction
t	Time
T	Absolute temperature
V	Total volume
v	Specific volume
x	Mole fraction
y	Solubility (solute mole fraction in the gas phase)
Z	Compressibility factor ($Z = Pv/RT$)

Subscripts and Superscripts

2	Condensed phase (solute)
i, j	Component (or molecule) identifiers
c	Critical property
sat	Saturation

Greek Letters

τ, λ, ζ	Binary unlike-interaction parameters
ϕ	Intermolecular potential energy function
Φ	Fugacity coefficient
ω	Acentric factor
Σ	Summation over the number of components in the mixture

GLOSSARY OF TERMS

Critical Point: The condition at which a gas and a liquid are at equilibrium, and have identical composition and densities.

Critical Temperature: Temperature at the critical point, above which the liquid and vapor phases are indistinguishable.

Critical Pressure: Pressure of a fluid at its critical point.

Entrainer: Fluid that enhances the solubility of a dense phase.

Supercritical Gas: A gas at conditions above its critical temperature and pressure.

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