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# Carbon dioxide extraction of ginseng root hair oil and ginsenosides

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**Abstract**

This investigation developed a semi-continuous flow process to extract crude oil from ginseng root hair. The extraction conditions were 308–333 K, 10.4–31.2 MPa, with the addition of ethyl alcohol as a co-solvent. Analysis of the content of the extracted crude oil and ginsenosides revealed that carbon dioxide extraction at 31.2 MPa, 333 K, 660 litres $CO_2$, with 6 mole% preload addition of ethanol achieved the closest result to hot water extraction, but remained inferior to ethanol extraction. Analytical results further demonstrated that the amount of crude oil extracted increased with pressure at constant temperature, and only increased with temperature when pressure exceeded 24.2 MPa. Furthermore, the maximum levels of crude oil and ginsenosides, extracted by supercritical carbon dioxide, were 0.1 g per gram of *Panax ginseng* and 1141 mg per kilogram absorbent, respectively.


*Keywords:* Ginseng root hair; Ginsenosides; Supercritical; Carbon dioxide

## 1. Introduction

The application of Supercritical Fluid Extraction (SFE), particularly in the field of natural products, has received considerable attention in recent years. This separation technique offers extraction comparable with that obtained by conventional extraction methods using organic solvents, but the process is more environmentally friendly. Since $CO_2$ has a relatively low critical pressure (7.38 MPa) and critical temperature (304 K), it can be considered a good solvent for processing natural products. Experimental applications with SFE using carbon dioxide have increased, for example, the extraction of evening primrose (Favati, King & Mazzanti, 1991), β-carotene (Subra, Castellani, Jestin & Aoufi, 1998) and peppermint oil (Goto, Masaki & Tsutomu, 1993). Meanwhile, there are several reports about aqueous extraction procedures for plant oils, for example, the extraction of clove buds (Clifford, Basile & Alsaidi, 1999). Supercritical carbon dioxide (SC-$CO_2$) is the most frequently used solvent, especially for thermolabile compounds, either alone or with a solubility modifier. Others, such as ethane, propane, ethylene, and dinitrogen oxide, and water can be used under sub- or supercritical conditions. It is well known that the addition of a co-solvent or modifier to a SC–$CO_2$ often leads to an enhancement in the solubility of a solute. The co-solvent effect, is defined as the ratio of the solubility obtained with a co-solvent to that obtained without a co-solvent. A co-solvent effect in excess of 10 mole% is often associated with hydrogen bonding between components in the SC–$CO_2$ phase (Lucien & Foster, 2000). Alcohols, ethers, and similar substances, have been employed as modifiers to increase the solubility of oils in SC-$CO_2$ ( Brunner & Peter, 1982; Chun, Shin & Lee, 1994).

Ginseng, the root of *Panax ginseng* C.A. Meyer, is one of the most common Chinese herbal drugs, and possesses cardiotonic and hypotensive effects (Chuang, Wa, Sheu, Chiou, Chang & Chen, 1995). Ginseng oil has many health benefits owing to its high level of unsaturated fatty acids. The active constituents of this plant are a complex mixture of saponins, often referred to as ginsenosides, and more than thirty known ginsenosides exist (Shibata, Tanaka, Shoji & Saito, 1985). Six major neutral saponins, namely the ginsenosides $Rb_1$, $Rb_2$, Rc, Rd, Re, $Rg_1$, have been reported to have a pharmacological effect on the human body. Chuang and Sheu (1994) reported that Soxhlet ethyl alcohol extraction of ginseng root hair produced 47,500 mg $kg^{-1}$ ginsenosides.

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There are literature reports on the extraction of ginseng root hair oil via ethanol, but few studies have considered SFE (Li, Li, Hong, Liu & Zhang, 1992). Therefore, this work attempts to extract oil of ginseng by sub- and supercritical $CO_2$ and to examine the effect of extraction conditions on the crude oil extracted and the quantity of the six ginsenosides.

## 2. Materials and methods

### 2.1. Reagents and materials

High-performance liquid chromotography (HPLC) grade ginsenosides $Rb_1$, $Rb_2$, Rc, Rd, Re, $Rg_1$ (Extrasynthese, France) were purchased from a local supplier. Deionized water, from a Milli-Q system (Millipore, USA), was used to prepare all sample solutions. Far-UV grade acetonitrile, 98.5% HPLC grade and 95% ethyl alcohol (Merck, Germany) were used without further purification. Ginseng root hair was purchased from a Chinese herbal market in Tai-chung city (Taiwan). Ginseng samples were ground using a mixer-grinder until they passed through a 140-mesh screen, and were then stored in a vacuum desiccator at room temperature before each experiment.

### 2.2. SFE and sample analysis

Fig. 1 depicts the flow diagram of equipment used for this study as described by Chang, Chiu, Chen and Chang (2000). The apparatus comprised primarily, a 100 ml syringe pump [4-1] (number in Fig. 1), a preheater [7], 300 ml extraction vessel [8], a separator [12], two absorbing vessels [13, 14], and a wet gas meter [16]. All tubes, connections and fittings were made of 316 grade stainless steel. Meanwhile, the 5 ml/min flow rate of $CO_2$ was controlled by the Isco syringe pump. To liquefy high pressure gaseous $CO_2$, when the gas entered the pump a solution of ethylene glycol at 277 K was circulated by a cool refrigerator. Then, a coiled stainless steel preheater [7], was used, which contained $CO_2$ liquid, immersed in a hot-water bath so that $CO_2$ could reach the desired temperature before entering the extractor [8]. The extractor was filled with 80 g Ginseng hair powder, and was then heated by a mantle. The temperature was controlled by a proportional-integral controller and measured using a K-type thermocouple [20]. In the case of the sequential co-solvent experiment, 6 mole% of ethanol (95% purity) acted as the co-solvent which was sequentially added in a co-solvent vaporizer [21] and mixed with 94 mole% carbon dioxide before entering the extractor. An absorbing system, that is one separator [12] and two absorbing vessels [13, 14], was filled with 1.4 l aqueous ethyl alcohol solution (acting as the absorbent) and maintained at 5.0 MPa and 297 K. The separator provided visual assurance that the saturated ginseng oil-laden supercritical $CO_2$, depleted from the extractor through a 1/16 inch I.D. nozzle, was sprayed into and mixed thoroughly with the absorbent. The absorption pressure was measured using a digital pressure transmitter (Druck, PDCR910) [18], while the extraction and following absorption were controlled by two back-pressure regulators [9-1∼9-2]. Total $CO_2$ volume was measured with a wet gas meter [16].

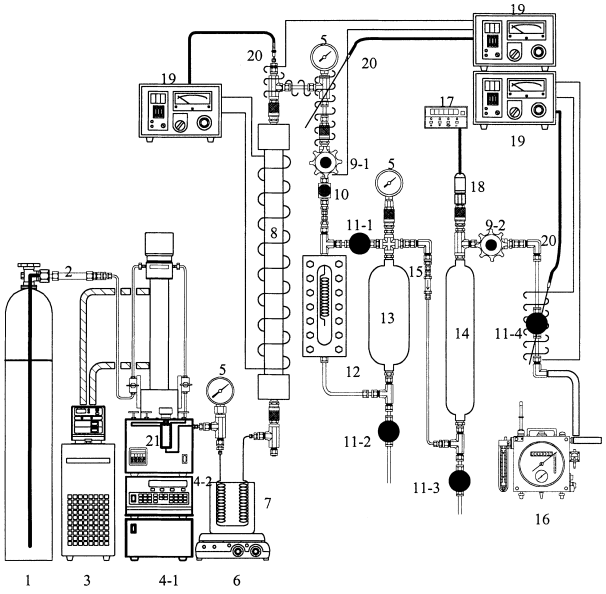


Fig. 1. The experimental equipment of supercritical fluid extraction.

After vacuum-drying at 110 mmHg and 328 K, the extract was prepared in absolute ethanol (99.8% purity) to form a sample. Then, the quantity of the crude extract was determined. The 218 nm absorption in a UV/Vis spectrophotometer (Hitachi, U-3000) measured the amount of oil extracted. The standard deviation of the UV measurements was within ±3%. The extraction yield of crude oil was expressed as the ratio of the amount of extracted oil to the amount of ginseng in the extractor.

Stock solutions of six ginsenosides $Rb_1$, $Rb_2$, Rc, Rd, Re, $Rg_1$ were prepared by dissolving measured quantities of standard compounds in 50% aqueous ethanol solution. Samples were collected from SFE, diluted to a certain concentration, taken into a 0.25-ml syringe and filtered through a 0.22-μm two-phase nylon membrane. A 100-μL volume of the filtered sample was injected

into the HPLC system and analyzed to determine the total ginsenoside content. The calibration curve was constructed via a linear regression of the peak area and the concentration of each standard compound. The measurement accuracy of this quantification was within ±3 μl/ml.

The HPLC system comprised a Perkin-Elmer 410 multisolvent delivery pump, a Perkin-Elmer Diode Array 235C absorbance detector at 198 nm, a Hitachi D-2500 Chromato-integrator controller and a Rhedoyne Model 9725 injector with a 100-μl loop. Chromatography was conducted with a reverse phase Nucleosil 100 $5C_{18}$ column (250×4.5 mm). Meanwhile, a gradient solvent program used two eluents of (A) $H_2O$-$CH_3CN$ (80:20 v/v) and (B) $H_2O$-$CH_3CN$ (15:85 v/v), listed in Table 1. The flow rate of the mobile phase was maintained at a constant 1.0 ml/min (Chuang & Sheu, 1994).

### 2.3. Soxhlet and ultrasonic extractions

The Soxhlet extraction used a 20 g ground sample, and a fourteen-fold volume of ethyl alcohol acted as the solvent; this ratio approximated the value employed in SFE extraction. The solid powder was transferred into a cellulose extraction thimble and inserted into a 500-ml reflux flask. Meanwhile, the Soxhlet apparatus was heated until the solvent boiled and then the 12-h extraction was conducted. Crude oil and ginsenosides were determined via the above procedures.

Ultrasonic extraction used the same ratio of solid and solvent. The mixture was transferred to a 350-ml flask, placed in an ultrasonic bath (Branson, 3200). Ultrasonification was carried out for 150 min and the extraction temperature was set at 333 K. The samples were stirred occasionally and rotated in the bath to ensure a well-mixed extraction. Alternately, the samples were mixed with a vortex mixer, periodically during ultrasonification. Finally, the extracted solutions were distributed among several 10-ml centrifuge tubes, and centrifuged at 1500 rpm. The upper layer, which contained the crude oil, was filtered and analyzed by the above procedures.

Table 1
High-performance liquid chromotography gradient elution programme for ginsenosides measurement

| Time (min) | A (%) | B (%) | Curve |
|---|---|---|---|
| Initial | 98 | 2 | |
| 15 | 96 | 4 | Linear |
| 25 | 85 | 15 | Linear |
| 40 | 75 | 25 | Linear |
| 50 | 0 | 100 | Linear |
| 62 | 0 | 100 | Linear |

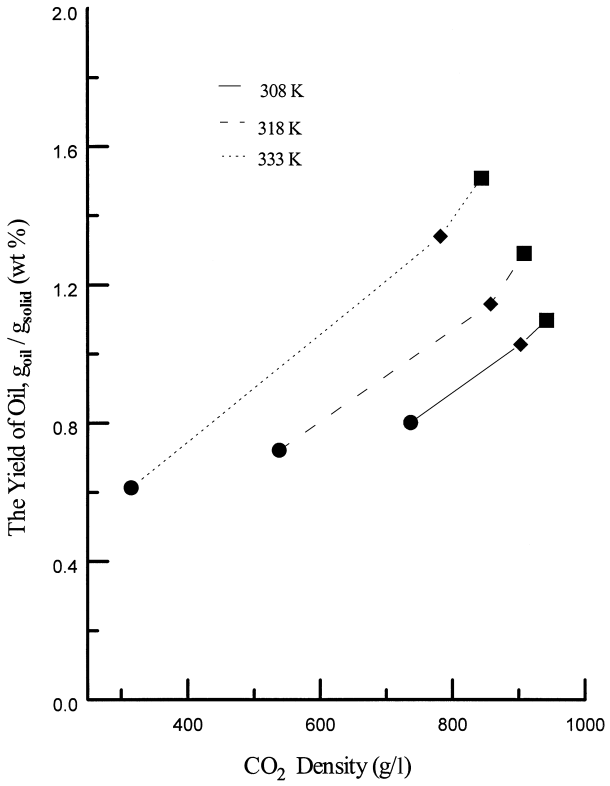


Fig. 2. The extracted oil yield in supercritical carbon dioxide as a function of the solvent density at different extraction conditions (● 10.4 MPa; ◆ 24.2 MPa; ■ 31.2 Mpa, — 308 K; - - - 318 K; · · · 333 K).

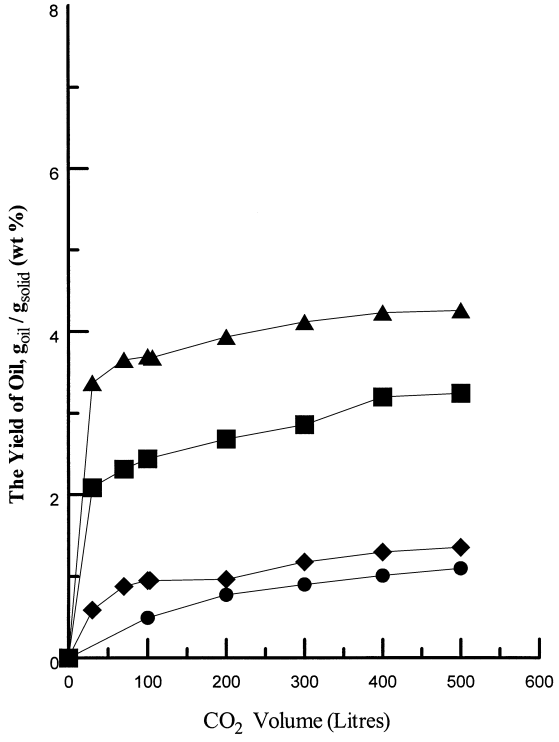


Fig. 3. The effect of co-solvent addition on the extracted Ginseng oil at 31.2 MPa, 308 K (● $CO_2$; ◆ $CO_2$ + 2.7 mole% preload; ▲ $CO_2$ + 6 mole% preload; ■ $CO_2$ + 6 mole% sequential).

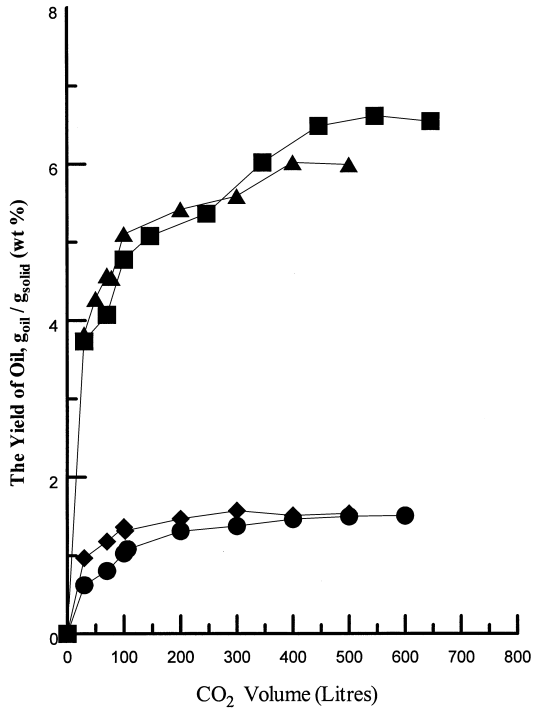


Fig. 4. The effect of co-solvent addition on the extracted Ginseng oil at 31.2 MPa, 333 K (● $CO_2$; ◆ $CO_2$ + 2.7mole % preload; ▲ $CO_2$ + 6mole % preload; ■ $CO_2$ + 6mole % sequential).

## 3. Results and discussion

McHugh and Krukonis (1986) indicate that the solubility of solute in supercritical fluid is related to the solvent density and solute volatility. In the present study, major factors that influence the extraction yield of ginseng oil are carbon dioxide density, the diffusion coefficient of ginseng oil in carbon dioxide, and the volatility of ginseng oil.

Fig. 2 displays the amount of crude oil extracted in SC-$CO_2$, plotted as a function of solvent density under various extraction pressures and temperatures. Supercritical carbon dioxide density was calculated by using the Isco (1991) software. Our result indicates that carbon dioxide density was analogously proportional to the yield of ginseng oil. From 24.2 to 31.2 MPa, higher temperature increased oil yield, probably because vapour pressure of the extracted materials dominates the extraction. A contradictory effect was observed at lower pressure (at 10.4 MPa), indicating that solvent density is a major factor enhancing the quantity of oil extracted. Consequently, supercritical carbon dioxide at 333 K and 31.2 MPa is considered the most efficient operating condition. Similar results have been reported for an SFE process involving natural plants (Friedrich, List & Heakin, 1982; Garcia, Lucas, Rincon, Alvarez,

Table 2
Extraction of six major ginsenosides and crude oil from ginseng root hair[a]

| Solvent (mole%) | T (K) | $Rb_1$ (mg/kg) | $Rb_2$ (mg/kg) | Rc (mg/kg) | Rd (mg/kg) | Re (mg/kg) | $Rg_1$ (mg/kg) | Ginsenosides ($mg/kg_{absorbent}$) | Oil yield ($g/g_{solid}$),(%) |
|---|---|---|---|---|---|---|---|---|---|
| $CO_2$ | 308 | 14 | ND | 38 | ND | 21 | ND | 73 | 1.1 |
| $CO_2$ | 333 | 24.2 | 3 | ND | 35 | 41 | 4.4 | 108 | 1.51 |
| *Preload addition* | | | | | | | | | |
| $CO_2$ + 2.7% $EtOH_{(aq)}$ | 308 | | | | | | | | 1.35 |
| $CO_2$ + 2.7% $EtOH_{(aq)}$ | 333 | 14.3 | 3.8 | 2 | 25 | 42.7 | 2.9 | 91 | 1.54 |
| $CO_2$ + 6% $EtOH_{(aq)}$ | 308 | 31 | 131 | 42 | 212 | 284 | 99 | 800 | 4.26 |
| $CO_2$ + 6% $EtOH_{(aq)}$ | 333 | 126 | 57 | 118 | 662 | 125 | 52 | 1141 | 5.99 |
| *Sequential addition* | | | | | | | | | |
| $CO_2$ + 6% $EtOH_{(aq)}$ | 308 | 90.3 | 12 | 12 | 77 | 27 | 18.9 | 236 | 3.24 |
| $CO_2$ + 6% $EtOH_{(aq)}$ | 333 | 131 | 15 | 57.4 | 81 | 23.5 | 13 | 322 | 6.54 |
| $CO_2$ + 6% $EtOH_{(aq)}$ (absorbent:95% $EtOH_{(aq)}$) | 333 | 48.6 | 19 | 17 | 28 | 29 | 10 | 153 | 10.74 |
| *Soxhlet extraction* | | | | | | | | | |
| 95% $EtOH_{(aq)}$ | B.P. | 239 | 186.4 | 1057 | 257 | 257 | 68 | 2028 | 25.11 |
| 46% $EtOH_{(aq)}$ | B.P. | 278 | 154 | 208 | 917 | 42 | 76 | 1677 | 27.39 |
| $H_2O$ | B.P | 46.7 | 53 | 81 | 419.5 | 78 | 21.7 | 1700 | 23.67 |
| *Ultrasonic extraction* | | | | | | | | | |
| 95% $EtOH_{(aq)}$ | 333 | 218 | 133 | 193 | 896 | 192 | 67 | 1701 | 13.78 |
| 46% $EtOH_{(aq)}$ | 333 | 184 | 124 | 165 | 116 | 109 | 30 | 729 | 16.88 |
| $H_2O$ | 333 | 15.7 | 58 | 56 | 379 | 63 | 21.7 | 622.4 | 10.34 |

[a] Supercritical fluid extraction: 31.2 MPa, 660-l $CO_2$ and 46% aqueous ethyl alcohol solution as the absorbent.

Garcia & Garcia, 1996; Stahl, Schultz & Mangold, 1980).

Figs. 3 and 4 illustrate the influence of co-solvent addition on the extracted ginseng oil at 308 and 333 K, respectively. The extraction curve is characterized by an asymptotic approach to the horizontal, becoming saturated at the end of the extraction. Table 2 lists the experimental data for all investigations. The addition of co-solvent increased the solubility of ginseng oil in SF-$CO_2$, as well as the dissolution of six ginsenosides. The enhancement of oil solubility, due to the effect of the co-solvent, may be a physical interaction of solutes with the mixed solvent, possibly owing to a increase in the solvent density, as well as a specific chemical interaction, such as hydrogen bonding (Palmer & Ting, 1995). At 31.2 MPa, 333 K, the addition of 95% ethanol from 0 to 6 mole % resulted in at least a three-fold increase of ginsenoside content. Table 2 also displays that the yield of the extracted oil and ginsenosides content increased with temperature.

In almost all cases, the yield is highest for Soxhlet ethanol extraction. This result implies that, unlike carbon dioxide, ethanol (or hot water) is a non-selective solvent and is good for extracting fat-soluble substances, such as free fatty acids, pigments and unsaponifiable substances, together with triglycerides (Stahl, Schultz & Mangold, 1980; Wang, Hong, Chen & Tsai, 1997). Table 2 indicates that Soxhlet ethanol extraction produces the largest amounts of total ginsenosides and crude oil, with ultrasonic ethanol extraction following. Our experimental results further indicate that preload addition of co-solvent in SFE enables the extraction of 1141 mg/kg ginsenosides, which is close to Soxhlet water extraction and ultrasonic ethanol extraction.

## 4. Conclusion

Supercritical carbon dioxide extraction, with the addition of 6 mole % ethanol, allows markedly higher extraction yields than pure carbon dioxide extraction. Under some operating conditions, SFE of six ginsenosides is similar to conventional ethanol extraction. It was found herein that comparable results, in terms of six ginsenoside contents, could be obtained using SFE. Furthermore, SFE could save extraction time (SFE: 4 h; Soxhlet: 12 h), organic solvent consumption (SFE: 80 ml; Soxhlet and Ultrasonic: 280 and 350 ml), and diminish extraction temperature (SFE: 333 K; Soxhlet: solvent boiling point). It is proposed that SFE could be combined with conventional liquid extraction to obtain ginseng root hair oil. An industrial scale plant design and cost evaluation are required to confirm this conclusion.

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## References

Brunner, G., & Peter, S. (1982). State of art of extraction with compressed gas. *Ger. Chem. Eng., 5*, 181–195.

Chang, C. J., Chiu, K. L., Chen, Y. L., & Chang, C. Y. (2000). Separation of catechins from green tea using carbon dioxide extraction. *Food Chemistry, 68*, 109–113.

Chuang, W. C., Wa, H. K., Sheu, S. J., Chiou, S. I., Chang, I. C., & Chen, Y. P. (1995). A comparative study on commercial samples of ginseng radix. *Planta Med., 61*, 459–465.

Chuang, W. C., & Sheu, S. J. (1994). Determination of ginsenosides in ginseng crude extracts by high performance liquid chromatography. *J. Chromatogr. A, 685*, 243–251.

Chun, M. K., Shin, H. W., & Lee, H. (1994). Supercritical fluid extraction of taxol and baccatin from needle of taxus cuspidate. *Biotechnology Techniques, 8*, 547–550.

Clifford, A. A., Basile, A., & Alsaidi, S. H. R. (1999). A comparison of the extraction of clove buds with supercritical carbon dioxide and superheated water. *Fresenius' J. Anal. Chem., 364*(7), 635–637.

Favati, F., King, J. W., & Mazzanti, M. (1991). Supercritical carbon dioxide extraction of evening primrose oil. *Journal of the American Oil Chemists Society, 68*, 422–427.

Friedrich, J. P., List, G. R., & Heakin, A. J. (1982). Petroleum-free extraction of oil from soybeans with supercritical $CO_2$. *Journal of the American Oil Chemists Society, 59*, 288–292.

Garcia, A., Lucas, A. D., Rincon, J., Alvarez, A., Garcia, I., & Garcia, M. A. (1996). Supercritical carbon dioxide extraction of fatty and waxy material from rice bran. *Journal of the American Oil Chemists Society, 73*(8), 1127–1131.

Goto, M., Masaki, S., & Tsutomu, H. (1993). Extraction of peppermint by supercritical carbon dioxide. *J. Chem. Eng. of Jpn, 26*, 401–407.

Isco (1991). Pressure/density/temperature relationship for IBM-PC: SFE Utility Program. Version 2.5.1, Isco Inc., Lincoln, NE 68505.

Li, Y. H., Li, X. L., Hong, I., Liu, J. Y., & Zhang, M. Y. (1992). Determination of panaxadiol and panaxatriol in ginseng and its preparations by capillary supercritical fluid chromatography (SFC). *Biomedical Chromatography, 6*(2), 88–90.

Lucien, F. P., & Foster, N. R. (2000). Solubilities of solid mixtures in supercritical carbon dioxide: a review. *Journal of Supercritical Fluids, 17*, 111–134.

McHugh, M. A. & Krukonis, V. J. (1986). *Supercritical fluid extraction: principle and practice*, Boston: Butterworth Publishers.

Palmer, M. V., & Ting, S. S. T. (1995). Applications for supercritical fluid technology in food processing. *Food Chemistry, 52*, 345–352.

Shibata, S., Tanaka, O., Shoji, J. & Saito, H. (1985). Chemistry and pharmacology of panax. In H. Wagner et al. *Economic and medicinal plant research* (Vol. 1, p. 217). London: Academic Press.

Stahl, E., Schultz, E., & Mangold, H. K. (1980). Extraction of seed oils with liquid and supercritical carbon dioxide. *Journal of Agricultural and Food Chemistry, 28*, 1153–1157.

Subra, P., Castellani, S., Jestin, P., & Aoufi, A. (1998). Extraction of β-carotene with supercritical fluids experiments and modeling. *Journal of Supercritical Fluids, 12*, 261–269.

Wang, Y. H., Hong, C. Y., Chen, C. F., & Tsai, T. H. (1997). Determinations of trilinolein and 1,2-dilinoleoyl-3-oleoyl-glycerol in various panax ginseng by HPLC. *J. Liq. Chrom. & Rel. Technol, 20*(6), 899–905.