



PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
ESCUELA DE INGENIERÍA

**IDENTIFICATION OF THE EFFECT OF
ENTRAINERS (TRIOLEIN OR ETHANOL)
ON THE CAROTENOIDS EXTRACTION
FROM RED PAPRIKA (*Capsicum annuum* L.)
USING SUPERCRITICAL CARBON
DIOXIDE (SC-CO₂)**

KARINA A. ARAUS SARMIENTO

Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences

Advisor:

JOSÉ MANUEL DEL VALLE

Santiago de Chile, December, 2012

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To Jay Sea

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KARINA ALEJANDRA ARAUS SARMIENTO

ABSTRACT

Carotenoid pigments are used as colorants in food products and they are recognized for their antioxidant properties, helping in the prevention of a number of illnesses. Carotenoids have been traditionally extracted with organic solvents, which are currently being questioned by regulatory organizations, as traces of them, both in the extract as well as the residue, could jeopardize human health. Hence, there is a current trend from consumers favoring natural products with nutraceutical properties, which has incremented the interest to come up with extraction methods with resulting products having bio-active components, of natural origin by pharmaceutical and food industries.

Extraction of carotenoids from natural substrates by using SuperCritical carbon dioxide (SC-CO₂) can be a viable alternative to obtain natural colorants, preserving their functional nutraceutical properties, replacing artificial colorants, or eliminating organic solvents. This viability of extracting carotenoids with SC-CO₂ does have an important limitation: low solubility of carotenoids in SC-CO₂, with a subsequent low yields of extraction. This limitation, plus the cost of pressurizing liquid CO₂ above its critical pressure, makes this process not economically feasible for industry to invest at the commercial level. The addition of small amounts of another fluid into SC-CO₂, called entrainer, could increment the solubility of products of interest. The potential of using vegetable oils as entrainers in the extraction of solutes, such as carotenoids, could increase both their solubility and yield

in SC-CO₂, which could be associated with modifying and/or co-solvent effects of vegetable oil in the system.

The hypothesis of this work states that it is possible to distinguish between two functions of an entrainer (lipid or ethanol) when is added to SC-CO₂: as a co-solvent, improving the solubility of an specific solute and/or as a modifier, changing parameters that are related to the matrix of the substrate. In order to explain this phenomena, it is possible to separate both effects by measuring thermodynamic solubility of pure carotenoids in SC-CO₂ (binary systems) and mixtures of pure carotenoids in lipid or ethanol entrained SC-CO₂ (ternary systems) and comparing these systems to the apparent solubility of carotenoids in pure and entrained SC-CO₂ in a complex matrix of red pepper (*Capsicum annuum* L.).

The fundamental objective of this thesis is to contribute to a better understanding the effect of adding an entrainer on the extraction of carotenoids from rep pepper (*Capsicum annuum* L.) using SC-CO₂ and to clarify if this effect is based on changes in thermodynamic solubility (co-solvent effect and independent to the matrix) and/or apparent solubility (modifier effect, matrix dependent).

Thermodynamic solubility was measured in binary (SC-CO₂ + β -carotene) and ternary ((SC-CO₂ + β -carotene + triolein) and (SC-CO₂ + β -carotene + ethanol)) systems, as a function of pressure (P) at 17 to 34 MPa and temperature (T) at 313, 323, and 333 K. β -carotene was selected to validate experimental equipment, as there is extensive literature information in existence. Selecting triolein and ethanol as entrainers were derived from the fact that both compounds are considered GRAS (Generally Recognized as Safe), an important fact when using these ingredients in the food or pharmaceutical industry. Since the aim is to study the effect of edible vegetable oils as entrainers, triolein was selected, as it is a representative triglyceride of many of these oils.

Adapting methodology from static- to dynamic-analytical with re-circulation of the supercritical phase, reduced equilibrium time from 12 to 8 h and experimental measurements were better reproduced. Solubility of β -carotene in SC-CO₂ ranged from 0.17 to 1.09 $\mu\text{mol/mol}$. The three isotherms presented a dependency with P (above 17 MPa) with increased solubility. The effect of T over solubility depended in a crossover point predicted between 15.7 and 17.3 MPa. Above 17.3 MPa, solubility increased with T , while

solubility decreased with T at P values below 15.7 MPa. Literature work reported similar solubility for β -carotene as this work presents; hence, validating the system and experimental methodology.

Solubility of β -carotene in SC-CO₂ had a maximum of 1.845 $\mu\text{mol/mol}$ at 333 K and 33 MPa using contents of ethanol between 1.171 to 1.563×10^{-2} mol/mol; hence, increasing solubility of β -carotene by a factor of 3, with respect to pure SC-CO₂. Similarly, solubility of β -carotene in SC-CO₂ increased by a factor of 4 with respect to pure SC-CO₂ (3.3 $\mu\text{mol/mol}$ as maximum, at 333 K and 32 MPa) using contents of triolein between 0.008 to 0.387 mmol/mol.

Solubility of triolein in the ternary system (SC-CO₂ + β -carotene + triolein) presented deviations of ± 0.1 mmol/mol when compared to a binary system (SC-CO₂ + triolein) reported in literature, indicating that β -carotene did not affect the solubility of triolein; however, triolein incremented solubility of β -carotene as triolein is more soluble in SC-CO₂ than β -carotene. This increase in β -carotene solubility in SC-CO₂ is induced by the co-solvent effect of either triolein or ethanol. Increases in β -carotene solubility in the presence of ethanol, showed low dependency on equilibrium values of T and P , whereas solubility of β -carotene in the presence of triolein greatly increased with equilibrium values of P , concluding that increments on the solubility by co-solvent effect depends on the type of co-solvent and its concentration.

According to what is reported in the literature, increases of solubility in SC-CO₂ induced by the presence of a co-solvent, would be in part due to the increase in density (ρ) of the mixture SC-CO₂ and co-solvent; however, ρ of SC-CO₂ in the presence of ethanol or triolein estimated under similar experimental conditions of T and P by using Peng-Robinson equation of state (PR-EoS) with quadratic mixing rule resulted in maximums of 2.92 and 0.31% for ethanol and triolein, respectively, compared to the density of pure SC-CO₂. Results suggested these elevated values of solubility for β -carotene in SC-CO₂ do not depend in increases of SC-CO₂ ρ associated with dissolution of ethanol or triolein in the supercritical phase. Furthermore, co-solvent effect by triolein on the solubility of β -carotene was higher than with ethanol, even at lower concentrations. This effect can be

attributed to the preference of β -carotene to generate non-polar interactions with triolein, which would favor dissolution in SC-CO₂, compared to polar interaction between β -carotene and ethanol. Presence of triolein would also increment polarizability of SC-CO₂, phenomenon that would also contribute to increase of solubility, as it is a behavior observed in other non-polar co-solvents.

In order to study the effect of triolein upon the thermodynamic solubility of capsanthin isolated from extruded red pepper flakes (*Capsicum annuum* L.), measurements were carried out at 19 to 34 MPa for P and 313 or 333 K for T , for both binary (SC-CO₂ + capsanthin) and ternary (SC-CO₂ + capsanthin + triolein) systems.

Capsanthin solubility in the binary system increased with P (19 to 34 MPa) and T (313 to 333 K), to a maximum of 1.97 $\mu\text{mol/mol}$ at 32 MPa and 333 K. In the ternary system, the experimentally measured content of triolein was within the range of 0.156 to 0.41 mmol/mol, which incremented the solubility of capsanthin by a factor of 3 when compared to pure SC-CO₂, reaching a maximum of 5.27 $\mu\text{mol/mol}$ at 333 K and 32 MPa.

The content of triolein was equivalent to its solubility in a binary system (SC-CO₂ + triolein), reported in the literature and comparable with the results obtained in the ternary system (SC-CO₂ + β -carotene + triolein), measured under similar equilibrium conditions. The co-solvent effect of triolein on capsanthin was also observed for β -carotene, in which the solubility of the most soluble compound (triolein) remained constant, as the less-soluble compound (capsanthin or β -carotene) incremented due to the co-solvent effect of triolein. As it was discussed for β -carotene, a small increase in the density of SC-CO₂ in the presence of triolein would not explain the large increase in the solubility of capsanthin. The co-solvent effect of triolein on capsanthin could be the result of the non-polar interactions between three 18-carbon chains of fatty acid (oleic) that constitute triolein, with the non-polar 22-carbon chain which form the skeleton of carotenoids. Also, the possible increment in polarizability of CO₂ caused by triolein, which is 40 times larger than CO₂, might increase the solubility of carotenoids in the ternary system.

To evaluate the effect of vegetable oils on extraction yield and apparent solubility of capsanthin and total carotenoids, kinetics of extraction of oleoresin from extruded red

pepper flakes (*Capsicum annuum* L.) were evaluated, by using pure SC-CO₂ and in the presence of HOSO (High Oleic Sunflower Oil) at 0.53% (w/w) in CO₂, as a function of *T* at 313 K or 333 K and *P* at 28 MPa or 50 MPa. HOSO was selected due to its high content of triolein.

Extraction curves of accumulated yields from both total carotenoids and capsanthin *versus* consumption of pure SC-CO₂ and in the presence of HOSO, were mathematically obtained by using the Desorption-Dissolution-Diffusion (DDD) model, which considered microstructural characteristics of extruded red pepper and using apparent solubility as the only best-fitting parameter.

The raw material (red pepper flakes) was extruded, increasing the load density of the packed bed by a factor of 2.9 in comparison with red pepper flakes. The pre-conditioning of the flakes (elevating moisture content) prior to extrusion might favor elastic deformation and tortuosity (τ) of the substrate, combined with a large particle size; this might increase the resistance to mass transfer during the extraction process.

Extraction yield and apparent solubility of; capsanthin and total carotenoids increased, due to the effects of increasing *P* (28 MPa to 50 MPa), *T* (313 K to 333 K), and due to addition of HOSO. Specifically, comparing the best extraction condition (at 333 K and 50 MPa in the presence of HOSO) with the lesser favorable condition (at 313 K and 28 MPa in pure SC-CO₂), yields of capsanthin and total carotenoid content reached a maximum of 222.51 mg/kg of dried substrate (d.s) and 574.96 mg/kg d.s during the extraction, which represented an increment of 3.7 and 2.4 times, respectively. Comparing the apparent solubility of capsanthin and total carotenoids obtained at 333 K and 50 MPa in the presence of HOSO with 313 K and 28 MPa in pure SC-CO₂ were reached maximum values of 9.31×10^{-2} g/kg and 20.59×10^{-2} g/kg, increasing 21.2 and 7.6 times, respectively.

The native presence of a lipid fraction in red pepper oleoresin could behave as a co-solvent and/or modifier, helping in increasing the apparent solubility and yield of less-soluble components, such as carotenoids, in SC-CO₂. This effect was also favored by the increase in the concentration of lipid fraction by the addition of HOSO, mixture that augmented considerably the yield and apparent solubility of capsanthin and total carotenoids in SC-CO₂, as it was previously observed for β -carotene and capsanthin in the presence of

triolein, as this triglyceride has higher solubility than carotenoids in SC-CO₂. Results evidenced that the presence of HOSO would favor non-polar interactions, between triglycerides and carotenoids (carotenoids-HOSO-SC-CO₂), increasing the solubility of carotenoids in the supercritical phase (co-solvent effect) and/or it would physically modify the matrix of red pepper, facilitating desorption of carotenoids from matrix to supercritical phase (modifying effect).

Comparison between apparent and thermodynamic solubility of capsanthin showed differences for all conditions of P and T , including pure SC-CO₂ or in the presence of either triolein or HOSO. As an example, at 333 K and 28 MPa, thermodynamic and apparent solubility in pure SC-CO₂ were 1.65 $\mu\text{mol/mol}$ and 0.45 $\mu\text{mol/mol}$, whereas in the presence of triolein and HOSO the solubility was 4.29 $\mu\text{mol/mol}$ and 1.88 $\mu\text{mol/mol}$, respectively. Lower values for apparent solubility than thermodynamic solubility is attributed to potential interactions between solutes that constitute the mixture of native extracts with the matrix of red pepper and/or the presence of other solutes that could behave as anti-solvents, which would reduce the apparent solubility of capsanthin in SC-CO₂. Despite these differences in magnitude, the presence of triolein or HOSO incremented the solubility of capsanthin (apparent and thermodynamic) in SC-CO₂, where thermodynamic solubility depends exclusively on the co-solvent effect of triolein, whereas apparent solubility would depend on the co-solvent or anti-solvent effect of native solutes that compose the extract and their effect upon the matrix of red pepper regarding the release of target carotenoids. Also, differences can be attributed to the composition of triolein and HOSO (pure triglyceride *versus* a mixture of triglycerides), as well as the form of capsanthin, which is found principally in esterified form as it is extracted with SC-CO₂, which could generate interaction forces between solute-matrix and SC-CO₂, either pure or in the presence of entrainers.

As a general conclusion, the presence of triolein or HOSO allows increases of solubility and yield in the extraction of carotenoids from extruded red pepper flakes by using SC-CO₂. Studying kinetic of extraction it is not possible to discriminate between co-solvent and/or modifier effects of HOSO, differently to the study of phase equilibrium at high pressure, where the increment in solubility is attributed exclusively by the co-solvent effect

of triolein. The matrix of red pepper can be determinant in attributing a co-solvent and/or modifier effect to HOSO, which would also depend on the native composition (co-solvent or anti-solvent effect of solutes) of each vegetal substrate and the entrainer. However, the potential usage of vegetable oils as entrainers in the extraction of carotenoids from vegetal matrices using SC-CO₂, allowed to increase the yield of extraction considerably. At the same time, utilization of vegetable oils would permit to preserve adequately the extracted components with nutraceutical and functional properties, such as carotenoids, and their usage represent a viable alternative when using extraction processes to produce natural ingredients by SC-CO₂, characteristics that meet the demands for healthy nourishment, in tune with consumers and governmental programs.

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PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
ESCUELA DE INGENIERÍA

**IDENTIFICACIÓN DEL EFECTO DEL USO DE MODIFICADORES (TRIOLEÍNA
O ETANOL) EN LA EXTRACCIÓN DE CAROTENOIDES DE PIMENTÓN ROJO
(*Capsicum annuum* L.) USANDO DIÓXIDO DE CARBONO SUPERCRÍTICO
(CO₂-SC)**

Tesis enviada a la Dirección de Investigación y Postgrado en cumplimiento parcial de los
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RESUMEN

Los pigmentos carotenoides son usados como colorantes en productos alimenticios y son reconocidos por sus propiedades antioxidantes, que ayudan a la prevención de diversas enfermedades. Los carotenoides han sido tradicionalmente extraídos con solventes orgánicos, los que actualmente se encuentran cuestionados por organismos regulatorios internacionales, debido a que la presencia de trazas de solvente en el sustrato agotado y extracto puede ser perjudicial para la salud humana. Por otro lado, la tendencia actual de los consumidores por preferir productos naturales con propiedades nutraceuticas ha incrementado el interés de efectuar la extracción de componentes bioactivos a partir de sustratos naturales a nivel industrial, por parte de las industrias farmacéuticas y de alimentos.

La extracción de carotenoides a partir de sustratos vegetales usando dióxido de carbono SuperCrítico (CO₂-SC) puede ser una alternativa viable para obtener colorantes naturales, que preserven sus funciones nutraceuticas, en reemplazo de colorantes artificiales, o con presencia de solventes orgánicos. La viabilidad de la extracción de carotenoides con CO₂-SC presenta una importante limitación; la baja solubilidad de carotenoides en el CO₂-SC, que conlleva a la obtención de bajos rendimientos de extracción. Esta limitación, sumado al

costo de presurización del CO₂ líquido sobre su presión crítica, hacen que el proceso de extracción a nivel comercial sea poco rentable para que las industrias inviertan en esta tecnología de extracción. La adición de una pequeña cantidad de otro fluido al CO₂-SC, llamado agente de arrastre podría incrementar la solubilidad de solutos de interés. El potencial uso de aceite vegetal como agente de arrastre en la extracción de solutos con propiedades nutraceuticas como carotenoides, incrementaría tanto la solubilidad y rendimiento de extracción en el CO₂-SC, este incremento sería debido a efectos modificador y/o co-solvente ejercidos por parte del aceite vegetal.

La hipótesis de este trabajo indica que es posible distinguir entre las dos funciones de un agente de arrastre (lípidos o etanol) cuando es adicionado al CO₂-SC: como un co-solvente, mejorando la solubilidad de un soluto específico y/o como un modificador, cambiando parámetros relacionados con la matriz del sustrato. Con el fin de explicar estos fenómenos, es posible separar ambos efectos midiendo la solubilidad termodinámica de carotenoides puros en CO₂-SC (sistemas binarios) y mezclas de carotenoides puros en CO₂-SC usando un lípido o etanol como agente de arrastre (sistemas ternarios) y comparando estos sistemas con la solubilidad aparente de carotenoides en CO₂-SC puro como en presencia de un lípido o etanol en una matriz compleja de pimentón rojo (*Capsicum annuum* L.).

El objetivo fundamental de esta tesis es contribuir en un mejor entendimiento del efecto de la adición de un agente de arrastre (lípidos o etanol) sobre la extracción de carotenoides de pimentón rojo (*Capsicum annuum* L.) usando CO₂-SC y clarificar si este efecto se basa en cambios en la solubilidad termodinámica (efecto co-solvente e independiente de la matriz) y/o solubilidad aparente (efecto modificador, relacionado con la matriz).

Se midió la solubilidad termodinámica de los sistemas binario (CO₂-SC + β -caroteno) y ternarios ((CO₂-SC + β -caroteno + trioleína) y (CO₂-SC + β -caroteno + etanol)) en función de la presión (P) de 17 MPa a 34 MPa y temperaturas (T) de 313 K, 323 K, y 333 K. El β -caroteno fue seleccionado para validar el sistema experimental debido a la extensa información experimental existente en literatura. La elección de evaluar el efecto de etanol y trioleína como agentes de arrastre, radicó en que estos compuestos pertenecen a la categoría GRAS (*Generally Recognized as Safe*), de importancia para ingredientes a utilizar

en las industrias de alimentos y farmacéutica. Además, como el propósito es estudiar el efecto del uso de aceites vegetales como potenciales agentes de arrastre, se seleccionó trioleína debido a que es un triglicérido representativo de muchos de estos aceites.

La transformación de una metodología estática- a dinámica- analítica con recirculación de la fase supercrítica, redujo el tiempo necesario para alcanzar el equilibrio en el sistema de 12 a 8 h, e incrementó la reproducibilidad de las mediciones experimentales. La solubilidad de β -caroteno en CO_2 -SC alcanzó un rango de 0,17 a 1,09 $\mu\text{mol/mol}$. Las tres isothermas presentaron una dependencia con la P (sobre 17 MPa) la cual incrementó la solubilidad. El efecto de la T sobre la solubilidad dependió de la presencia de un punto de entrecruzamiento predicho entre 15,7 MPa y 17,3 MPa. Sobre 17,3 MPa la solubilidad incrementó con la T , mientras que la solubilidad disminuyó con T a P bajo 15,7 MPa. Los trabajos en literatura reportan solubilidad de β -caroteno en CO_2 -SC similar a lo informado en este trabajo; validando así el sistema y metodología experimental.

La solubilidad de β -caroteno en CO_2 -SC alcanzó un valor máximo de 1,845 $\mu\text{mol/mol}$ a 333 K y 33 MPa usando contenidos de etanol entre 1,171 a $1,563 \times 10^{-2}$ mol/mol; incrementando así en un factor de 3 la solubilidad de β -caroteno en comparación con CO_2 -SC puro. Similarmente, la solubilidad de β -caroteno en CO_2 -SC incrementó en un factor de 4 en comparación con CO_2 -SC puro (siendo 3,3 $\mu\text{mol/mol}$ la solubilidad máxima de β -caroteno, a 333 K y 32 MPa), usando contenidos de trioleína entre 0,008 a 0,387 mmol/mol.

La solubilidad de trioleína en el sistema ternario (CO_2 -SC + β -caroteno + trioleína) presentó desviaciones entre $\pm 0,1$ mmol/mol en comparación con valores de solubilidad en sistemas binarios (CO_2 -SC + trioleína) reportados en literatura, indicando que el β -caroteno no afectó la solubilidad de trioleína, en cambio, la trioleína incrementó la solubilidad de β -caroteno, debido a que es más soluble en CO_2 que el β -caroteno. Este incremento en la solubilidad de β -caroteno en CO_2 -SC fue inducido por un efecto co-solvente, tanto del etanol como de la trioleína. La mejora en solubilidad de β -caroteno, en presencia de etanol presentó una baja dependencia con la T y P de equilibrio, mientras que en presencia de trioleína la solubilidad de β -caroteno presentó un marcado incremento con la P de

equilibrio, concluyendo que la mejora en solubilidad del efecto co-solvente depende del tipo de co-solvente y su concentración.

Según lo reportado en literatura, el incremento de solubilidad en CO₂-SC inducido por la presencia de un co-solvente sería en parte atribuido a un incremento en la densidad (ρ) de la mezcla del CO₂-SC y co-solvente, sin embargo, la ρ de CO₂-SC en presencia de etanol y trioleína estimada bajo similares condiciones experimentales de T y P usando la ecuación de estado de Peng-Robinson (*PR-EoS*) con reglas de mezclado cuadráticas, incrementó un máximo de 2,92 y 0,31% para etanol y trioleína, respectivamente, en comparación con la ρ de CO₂-SC puro. Estos resultados sugieren que el elevado incremento en la solubilidad de β -caroteno en CO₂-SC no depende de un incremento en la ρ del CO₂ asociado con la disolución de etanol o trioleína en la fase supercrítica. Por otro lado, el efecto co-solvente sobre la solubilidad de β -caroteno fue mayor en presencia de trioleína que de etanol, incluso usando un menor contenido de trioleína que etanol. Esto puede deberse a la preferencia por parte del β -caroteno por generar interacciones no-polar con la trioleína, lo cual favorece la disolución de β -caroteno en CO₂-SC, comparado con interacciones polares entre β -caroteno y etanol. La presencia de trioleína también incrementaría la polarizabilidad del CO₂, fenómeno al cual se atribuiría el incremento en solubilidad, comportamiento anteriormente observado para otros co-solventes no-polares.

Con el fin de estudiar el efecto de trioleína sobre la solubilidad termodinámica de capsantina (principal carotenoide del pimentón rojo), aislada de hojuelas de pimentón rojo (*Capsicum annuum* L.) extruídas, se realizaron mediciones a P de 19 MPa a 34 MPa y T de 313 K ó 333 K, para ambos sistemas, binario (CO₂-SC + capsantina) y ternario (CO₂-SC + capsantina + trioleína).

La solubilidad de capsantina en el sistema binario incrementó con la P (19 MPa a 34 MPa) y T (313 K a 333 K), alcanzando un valor un máximo de 1,97 $\mu\text{mol/mol}$ a 32 MPa y 333 K. En el sistema ternario, el contenido de trioleína medido experimentalmente alcanzó un rango de 0,156 a 0,41 mmol/mol, el cual incrementó la solubilidad de capsantina en un factor de 3 en comparación con CO₂-SC puro, alcanzando un valor máximo de 5,27 $\mu\text{mol/mol}$ a 333 K y 32 MPa.

El contenido de trioleína resultó ser equivalente a su solubilidad en el sistema binario (CO₂-SC + trioleína) reportada en la literatura, y comparable, con los resultados obtenidos en el sistema ternario (CO₂-SC + β -caroteno + trioleína), medidos bajo similares condiciones de equilibrio. El efecto co-solvente de trioleína sobre capsantina, también fue observado para β -caroteno, dónde la solubilidad del compuesto más soluble (trioleína) permaneció constante, mientras que la solubilidad del compuesto menos soluble (capsantina o β -caroteno) incrementó debido al efecto co-solvente exhibido por la trioleína. Según lo discutido para β -caroteno, un leve incremento en la densidad del CO₂-SC en presencia de trioleína no podría explicar el gran incremento en la solubilidad de capsantina. El efecto co-solvente de trioleína sobre la capsantina, podría ser atribuido a las interacciones no-polares entre las tres cadenas de 18-carbonos del ácido graso (oleico) que conforman parte de la trioleína y la cadena de 22-carbonos no-polares que conforman el esqueleto de los carotenoides. Además, del posible incremento en la polarizabilidad del CO₂ causado por la trioleína, la cual es aproximadamente 40 veces mayor que la polarizabilidad del CO₂, lo cual podría incrementar la solubilidad de carotenoides en el sistema ternario.

Con el propósito de evaluar el efecto del aceite vegetal sobre el rendimiento de extracción y la solubilidad aparente, de capsantina y de los carotenoides totales, se midieron cinéticas de extracción de oleorresina de hojuelas de pimentón rojo (*Capsicum annuum* L.) extruídas, las que fueron evaluadas usando CO₂-SC puro y en presencia de 0,53% (p/p) en CO₂ de AGAO (Aceite de Girasol Alto Oleico), en función de la T a 313 K ó 333 K y P a 28 MPa ó 50 MPa. AGAO fue seleccionado por su alto contenido en trioleína.

Curvas de extracción de rendimiento acumulado del total de carotenoides y capsantina *versus* consumo de CO₂-SC puro y en presencia de AGAO fueron obtenidas matemáticamente utilizando el modelo Difusión-Disolución-Desorción (DDD), el cual consideró características microestructurales del pimentón rojo extruído, y utilizó como único parámetro de ajuste la solubilidad aparente.

La materia prima (hojuelas de pimentón rojo) fue extruída, incrementando la densidad de carga el lecho empacado en un factor de 2,9. El preacondicionamiento de las hojuelas (elevado contenido de humedad) previo a su extrusión podría favorecer la deformación elástica y la tortuosidad (τ) del substrato, esto combinado con un gran tamaño de partícula,

podría incrementar la resistencia de la transferencia de masa durante el proceso de extracción.

El rendimiento de extracción y la solubilidad aparente; de capsantina y de carotenoides totales incrementaron, debido a los efectos de incremento de P (28 MPa a 50 MPa), T (313 K a 333 K), y debido a la adición de AGAO. Específicamente, comparando las mejores condiciones de extracción (a 333 K y 50 MPa en la presencia de AGAO) con las condiciones menos favorables (a 313 K y 28 MPa en CO₂-SC puro), los rendimientos de capsantina y contenido total de carotenoides alcanzaron un máximo de 222,51 mg/kg sustrato seco (s.s) y de 574,96 mg/kg s.s durante la extracción, lo cual representó un incremento de 3,7 y 2,4 veces, respectivamente. Comparando la solubilidad aparente de capsantina y del contenido total de carotenoides obtenidos a 333 K y 50 MPa en presencia de AGAO con 313 K y 28 MPa en CO₂-SC puro se alcanzo un máximo de $9,31 \times 10^{-2}$ g/kg y de $20,59 \times 10^{-2}$ g/kg, incrementando 21,2 y 7,6 veces, respectivamente.

La presencia nativa de una fracción lipídica en la oleorresina de pimentón rojo, puede actuar como un co-solvente y/o de modificador, ayudando a incrementar la solubilidad aparente y rendimiento de extracción de componentes menos solubles, como carotenoides en CO₂-SC. Este efecto se favoreció por el incremento en la concentración de la fracción lipídica con la adición de AGAO, cuya mezcla incrementó considerablemente el rendimiento de extracción y la solubilidad aparente de capsantina y del total de carotenoides en CO₂-SC, tal como fue observado previamente para β -caroteno y capsantina en presencia de trioleína, debido a que dicho triglicérido tiene una mayor solubilidad en CO₂-SC que los carotenoides. El resultado evidencio que la presencia de AGAO podría favorecer las interacciones no-polares, entre triglicéridos y carotenoides (carotenoide-AGAO-CO₂-SC), aumentando su solubilidad en la fase supercrítica (efecto co-solvente), y/o modificaría físicamente la matriz de pimentón rojo, facilitando la desorción de los carotenoides desde la matriz hacia la fase supercrítica (efecto modificador).

La comparación entre la solubilidad aparente y termodinámica de capsantina mostró diferencias para todos las condiciones de P y T , inclusive usando CO₂-SC puro o en presencia de trioleína o AGAO. Por ejemplo, a 333 K y 28 MPa, la solubilidad termodinámica y aparente en CO₂-SC puro fue de 1,65 μ mol/mol y 0,45 μ mol/mol,

mientras que en presencia de trioleína y AGAO la solubilidad fue 4,29 $\mu\text{mol/mol}$ y 1,88 $\mu\text{mol/mol}$, respectivamente. La menor magnitud de la solubilidad aparente que la solubilidad termodinámica es atribuido a potenciales interacciones entre solutos que constituyen la mezcla nativa del extracto con la matriz del pimentón rojo, y/o a la presencia de otros solutos que pueden actuar como un anti-solvente, los cuales redujeron la solubilidad aparente de capsantina en $\text{CO}_2\text{-SC}$. A pesar, de las diferencias en magnitud la presencia de trioleína o AGAO, incrementó la solubilidad de capsantina (termodinámica o aparente) en $\text{CO}_2\text{-SC}$, dónde la solubilidad termodinámica depende exclusivamente de un efecto co-solvente de la trioleína, mientras, que la solubilidad aparente dependería del efecto co-solvente o anti-solvente de los solutos nativos que componen el extracto y del efecto que ejercería la matriz de pimentón sobre la liberación de los carotenoides de interés. También, las diferencias son atribuidas a la composición de trioleína y AGAO (triglicérido puro *versus* una mezcla de triglicéridos), y la forma de la capsantina, la cual se encuentra principalmente esterificada en el pimentón, cuando es extraída con $\text{CO}_2\text{-SC}$, lo cual podría generar diferentes fuerzas de interacción entre soluto-matriz y el $\text{CO}_2\text{-SC}$ puro o en presencia del co-solvente y/o modificador.

Como conclusión general, la presencia de trioleína o AGAO permite incrementar la solubilidad y rendimiento de extracción de carotenoides de pimentón rojo extruído usando $\text{CO}_2\text{-SC}$. Con estudios de cinética de extracción no es posible discriminar entre un efecto co-solvente y/o modificador del AGAO, a diferencia del estudio de equilibrio de fases a alta presión, dónde el incremento en solubilidad es inducido exclusivamente por un efecto co-solvente de la trioleína. La matriz de pimentón puede ser determinante en la atribución de un efecto co-solvente y/o modificador del AGAO, el cuál depende de la composición nativa (efecto co-solvente o anti-solvente de solutos) de cada substrato vegetal y del agente de arrastre. Sin embargo, el uso potencial de aceites vegetales como agentes de arrastre en la extracción de carotenoides a partir de matrices vegetales usando $\text{CO}_2\text{-SC}$, permitió incrementar considerablemente su rendimiento de extracción. Por otro lado, la utilización de aceites vegetales permitiría preservar adecuadamente compuestos extraídos con propiedades nutraceuticas y funcionales, como son los carotenoides, su uso representa una alternativa viable para procesos de extracción usando $\text{CO}_2\text{-SC}$ de ingredientes naturales,

cuyas características responden a las tendencias de alimentación saludable, actualmente en boga para consumidores y los programas gubernamentales.

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1. INTRODUCTION

Artificial colorants used in the food industry are subjects to extensive toxicological testing before they are allowed to be used in food products, because they are prepared using some compounds that are highly toxic, which could damage human health. As a consequence, the consumer has put pressure on the industry and regulatory entities to incorporate natural additives with nutraceutical properties that have health benefits. Currently, food and pharmaceutical industries have shown a keen interest in the extraction of natural pigments from biological substrates, including carotenoids. Therefore, natural colorants extraction is an appealing alternative as replacement to artificial colorants and additives.

Carotenoids are compounds found in vegetable matter such as fruits, vegetables, and seeds, as well as, unicellular and multicellular organisms, such as bacteria, yeast, and microalgae. Carotenoids are easily identified and recognized due to the coloration given to the substrate, from intense red to shades of orange and yellow. Structurally, carotenoids belong to the group of tetraterpene, having 40 carbons, with unsaturated double bonds. β -carotene and lycopene are examples of carotenes and they are composed only of carbon and hydrogen molecules. Lutein and capsanthin are examples of xanthophylls, they contain at least one oxygen incorporated into the molecular structure, in the form of hydroxyl or ketone functional groups (Stahl & Sies, 2005; Belitz *et al.*, 2009).

Carotenoids, used as colorants in food formulations, have also been linked to antioxidant properties. For example, protecting cells from degenerative oxidation, helping in reducing the risk of a number of cancer types and cardiovascular illnesses, due to their scavenging abilities to neutralize free radicals (Rao & Rao, 2007).

Red pepper (*Capsicum annuum* L.) oleoresin is a mixture of triglycerides and carotenoid pigments, which has been widely used as natural food colorant due to presence of carotenoids, specifically capsanthin, a xanthophyll that confers red color. Capsanthin is one of the main carotenoid pigments and exclusive of the red pepper reaching 35-50% of the total carotenoids, which is found in both free and esterified form with fatty acids (Curl, 1962; Locey & Guzinski, 2000). Capsanthin is a powerful radical scavenger acting as an antioxidant (Hirayama *et al.*, 1994; Matsufuji *et al.*, 1998) and have anticarcinogenic properties (Narisawa *et al.*, 2000).

Conventional extraction of carotenoids from vegetable substrates includes the use of organic solvents, such as petroleum ether, hexane, and other hydrocarbons; solvents that are detrimental to human health if present in excess of regulatory and permissible limits. Hence, complete removal of such solvents must be performed by using methods and techniques that would require long exposure time and high temperatures, which invariably would damage any component that is thermolabile, especially carotenoids, which in turn would diminish their functional properties. Currently, organic solvent extraction methods are being questioned. International legislations are becoming increasingly more restrictive in the trace amounts of residual solvent in oleoresins and extracts from natural sources (Barth *et al.*, 1995; Mukhopadhyay, 2000; Schoefs, 2004).

In response to consumers demand for natural choices of food and current legislation to eliminate the use of organic solvents, it is paramount to implement extraction processes that preserve the nutraceutical properties of carotenoids, as well as being friendly to the environment.

SuperCritical Fluid Extraction (SCFE), specifically, using carbon dioxide in supercritical state (SC-CO₂), is an excellent alternative method of extraction in comparison with conventional methods (King, 1990). An SCF is a substance at conditions of temperature (T) and pressure (P) above its critical values, which are defined as critical temperature (T_c) and critical pressure (P_c). Above critical conditions, CO₂, like any other fluid in supercritical state, is not a condensable substance. For example, it would not condense if T is lowered isobarically nor if P is increased isothermally, as long as conditions of P and T remain above the critical point. In terms of density (ρ), with values between gas and liquid states, a SCF can be managed very efficiently by simple changes of T and P ; thus, changing ρ and controlling the selectivity of the extraction process. Near the critical point, ρ is especially sensitive to small changes of T and P , within the region where the fluid is highly compressible. In relation to other properties, such as viscosity, surface tension, and diffusivity, which have intermediate values between gas and liquid states, these conditions would allow the SCF to easily penetrate the matrix, increasing the mass transfer of target compounds from matrix to supercritical phase, effectively depleting the raw material (Brunner, 1994; del Valle & Aguilera, 1999).

Critical point for SC-CO₂ is $T_c = 304.1$ K in terms of T and $P_c = 7.38$ MPa in terms of P (Brunner, 1994). Among the main characteristics that make CO₂ an ideal extraction solvent for natural components, while preserving their nutraceutical properties, one can include the following: (1) non corrosive, non flammable, non toxic, and does not damage the solute or matrix; (2) it is available in high purity and at a low cost; and (3) it can be operated at relatively low temperatures (del Valle & Aguilera, 1999). This fluid allows the selective extraction of some components when operated at conditions above T_c and P_c , while at standard conditions of P and T , in which CO₂ remains in gaseous state, the solute is effectively recovered, free from solvent. This method of extraction is ideal for the isolation of thermolabile compounds (Mukhopadhyay, 2000).

Solubility is defined as the maximum concentration of a compound that can be dissolved by a solvent, which is dependent on the P and T of the system. This equilibrium condition between the solute and solvent can be reached only if sufficient amount of solute (*e.g.*, saturation) is present in the system at steady conditions of P and T . Thermodynamic solubility is determined from measurements of high-pressure phase equilibrium of model system (SC-CO₂ + solute). On the other hand, in studies of dynamic extraction using SC-CO₂ the solubility for a compound extracted from a substrate is determined from the extraction curve (solubility controlled stage), specifically, from the initial slope of a plot of cumulative yield *versus* specific CO₂ consumption, which is called apparent solubility.

The solubility of solutes in SC-CO₂ is highly dependent on molecular weight, branching, number of rings, position and nature of functional groups, aromaticity, and polarity of solute (Dandge *et al.*, 1985; Palmer & Ting, 1995). SC-CO₂ has excellent qualities to solubilize solutes that have low molecular weight and non-polar nature, and under conditions of high pressure, CO₂ can extract components of slightly higher molecular weight and moderate polarity (del Valle & Aguilera, 1999). Despite these attributes, pure SC-CO₂ presents limitations for the solubility and subsequent extraction of compounds with high molecular weight and polarity. To overcome this limitation of pure SC-CO₂, the use of entrainers is a viable alternative to modify the solubility of solutes that are difficult to extract with pure SC-CO₂.

Studies have reported the feasibility of extraction of solutes using entrained + SC-CO₂. For pharmaceutical and food applications it is important to use an entrainer that has GRAS (Generally Recognized as Safe) status. Water, ethanol, and vegetable oils are recognized as entrainers with GRAS status (Moyler, 1993; Sanders, 1993). Ethanol has been used as an entrainer in various studies to improve the extraction yield of diverse compounds such as: cymene, 1.8-cineole, isomenthone, pulegone, and caryophyllene (terpenes) from flowers of khoa (*Satureja boliviana* Benth Briq) (Hatami *et al.*, 2011); α -carotene, β -carotene, β -cryptoxanthin, lycopene, lutein, and zeaxanthin (carotenoids) from japanese persimmon peel (*Diospyros kaki* Thunb.) (Takahashi *et al.*, 2006); palmitic, myristic, stearic, and arachidic (saturated fatty acids) and palmitoleic, oleic, linoleic, and linolenic (unsaturated fatty acids) acids of peach kernel from peaches (*Prunus persica* L.) (Mezzomo *et al.*, 2010); epigallocatechin gallate, epigallocatechin, epicatechin gallate, and epicatechin (catechins) from green tea (*Camellia sinensis* L.) (Park *et al.*, 2007); xanthines, theobromine, and caffeine (alkaloids) from cocoa beans (*Theobroma cacao* L.) (Li & Hartland, 1996); and arginine, lysine, glutamic, and phenylalanine (amino acids) from cottonseed (*Gossypium*) (Kuk & Hron, 1994). However, ethanol removal from the final extract requires high *T*, which can damage bioactive compounds, compared to the use of vegetable oil, which could remain in the final extract produced by supercritical extraction. The presence of an entrainer may generate specific interactions between target solute-entrainer-SC-CO₂ enhancing the solubility of the solute in entrainer + SC-CO₂ having a co-solvent effect (Ekart *et al.*, 1993). Also, an entrainer could act as a modifier by: (a) promoting structural changes in the matrix, such as swelling, that facilitates the release of the target solute from the substrate (Fahmy *et al.*, 1993); (b) generating interactions with functional groups of the substrate that facilitate the desorption of the target solute; and/or (c) competing with target solute for binding sites on the matrix (King & France, 1992). Evidence of the positive effect of the use edible vegetable oils as entrainer on carotenoids extraction from vegetable substrates with SC-CO₂ has been reported *e.g.*, extraction of carotenes such as: lycopene (Vasapollo *et al.*, 2004, Ciurlia *et al.*, 2009; Shi *et al.*, 2009; Saldaña *et al.*, 2010), α - and β -carotene (Sun & Temelli, 2006) and xanthophylls such as

lutein (Sun & Temelli, 2006), lutein esters (Ma *et al.*, 2008; Gao *et al.*, 2010; Palumpitag *et al.*, 2011), and astaxanthin (Krichnavaruk *et al.*, 2008).

On the other hand, a number of studies have reported the entrainer effect of ethanol on the solubility of natural components in SC-CO₂ having different molecular weights and polarity range (Gupta & Shim, 2007), but there is limited information about the use of ethanol and vegetable oil for the solubility of carotenoids. The solubility of lutein diesters using ethanol was studied by Naranjo-Modad *et al.* (2000) and Sovová *et al.* (2001) reported the β -carotene solubility using ethanol and vegetal oil from grape seeds.

As reported in the literature, the use of entrainer + SC-CO₂ can improve the solubility and extraction yield of carotenes and xanthophylls from substrates. Pretreatment, such as reduction of particle size, pelletization, and extrusion of substrate have also resulted in an increment in the extraction using SC-CO₂ (Gardner, 1982; del Valle *et al.*, 2003). Above pretreatments allow to enhance the mass transfer of the solute to the SC-CO₂ phase; thus, permitting easier access of CO₂, due to the rupture of barriers within the matrix. Regarding extrusion and pelletization, these pretreatments allow: (a) to form a new, porous microstructure, and (b) densification of substrate, thus increasing the bulk density of substrate in the packed bed (Gardner, 1982; del Valle & Uquiche, 2002; Uquiche *et al.*, 2004; Uquiche *et al.*, 2005; del Valle *et al.*, 2006). However, such high densification of substrate, as well as the restructured porous matrix can reduce the rate of extraction, which could be overcome by efficient transport properties of SC-CO₂ (Uquiche *et al.*, 2004).

Most commonly used mathematical models in SCFE describe the extraction of a solute from a packed bed, which are based on equations obtained from differential mass balance in a section of the bed, utilizing a number of assumptions that describe the mechanism of mass transfer and the relation in equilibrium between the concentration of the solute in the SCF film with the concentration of the solute in the solid. Diffusion (Goodarznia & Eikani, 1998), shrinking-core (Goto *et al.*, 1996), and Desorption-Dissolution-Diffusion (DDD) (Goto *et al.*, 1998) model, have been used as tools to simulate the extraction process *e.g.*, extraction of essential oil from herbs and oil from seeds using pure SC-CO₂, (Reverchon, 1996; del Valle *et al.*, 2006; Araus *et al.*, 2009). Uquiche *et al.* (2004) established a relationship that estimates the microstructural effect on the effective diffusivity of red

pepper and described the mass transfer inside the solid that was used to simulate the experimental cumulative extraction of oleoresin from red pepper pellets using the diffusion model.

1.1 Scope, hypothesis and objectives of the thesis

The hypothesis of this work states that it is possible to distinguish between two functions of an entrainer (lipid or ethanol) when is added to SC-CO₂: as a co-solvent, improving the solubility of an specific solute and/or as a modifier, changing parameters that are related to the matrix of the substrate. In order to explain this phenomena, it is possible to separate both effects by measuring thermodynamic solubility of pure carotenoids in SC-CO₂ (binary systems) and mixtures of pure carotenoids in lipid or ethanol entrained SC-CO₂ (ternary systems) and comparing these systems to the apparent solubility of carotenoids in pure and entrained SC-CO₂ in a complex matrix of red pepper (*Capsicum annuum* L.).

The fundamental objective of this thesis is to contribute to a better understanding the effect of adding an entrainer on the extraction of carotenoids from rep pepper (*Capsicum annuum* L.) using SC-CO₂ and to clarify if this effect is based on changes in thermodynamic solubility (co-solvent effect and independent to the matrix) and/or apparent solubility (modifier effect, matrix dependent).

To achieve the goal of this thesis, the following specific objectives were raised:

- i) To select the appropriate entrainer by measuring the thermodynamic solubility in binary (SC-CO₂ + β -carotene) and ternary (SC-CO₂ + β -carotene + triolein or ethanol) model systems as a function of equilibrium P and T ,
- ii) To extract, isolate, identify, and purify capsanthin from extruded red pepper (*Capsicum annuum* L.) flakes,
- iii) To measure the thermodynamic solubility of binary (SC-CO₂ + capsanthin) and ternary (SC-CO₂ + capsanthin + entrainer) systems as a function of equilibrium P and T , using the capsanthin extracted in (ii) and entrainer selected in (i),

- iv) To measure extraction kinetics and determine the apparent solubility of oleoresin, total carotenoids, and capsanthin from extruded red pepper using pure and entrained-SC-CO₂ as a function of P and T of extraction process, and
- v) To model cumulative extraction plots of total carotenoids and capsanthin from extruded red pepper (obtained in (iv)) using pure and entrained-SC-CO₂, employing the DDD model reported in the literature, which considered microstructural characteristics of extruded red pepper and using apparent solubility as the only best-fitting parameter.

1.2 Outline of the Thesis

The main purpose of this thesis is to understand and evaluate the effect of entrainers on the extraction of carotenoids from a vegetable substrate by the discrimination between the results of modifier and/or co-solvent effects. Hence, providing relevant data required for the development of extraction processes of carotenoids from a vegetable substrate using entrained-SC-CO₂.

Chapter 2 presents an exhaustive study about the possible contribution to the solubility of carotenoids in SC-CO₂, induced by the presence of an entrainer (triolein or ethanol) in order to understand and help to clarify the effect of these on the thermodynamic solubility by comparison of measurements of high-pressure phase equilibrium of binary (SC-CO₂ + β -carotene) and ternary (SC-CO₂ + β -carotene + entrainer) systems, evaluated as a function of entrainer type and equilibrium P and T . β -carotene was selected to validate the experimental measurements by means of comparisons with the existing information in the literature.

Chapter 3 contains a complete research of the co-solvent effect of triolein on the thermodynamic solubility of capsanthin in SC-CO₂ by studying the solubility results based on binary (SC-CO₂ + capsanthin) with ternary (SC-CO₂ + capsanthin + triolein) systems, as a function of equilibrium P and T . For this study, capsanthin was isolated from extruded red pepper flakes.

Chapter 4 contains a complete investigation of the main effects induced by the presence of high-oleic sunflower oil, used as an entrainer, on the apparent solubility and extraction yield of carotenoids from extruded red pepper using SC-CO₂. In order to understand and study the positive effect of the lipids on the apparent and thermodynamic solubilities of capsanthin in SC-CO₂ the obtained results were compared. Extraction curves of cumulated yields of total carotenoids and capsanthin using pure and entrained-SC-CO₂ were simulated using a Desorption-Dissolution-Diffusion (DDD) model, which was employed to describe the extraction process, using apparent solubility as the only best-fitting parameter.

Finally in Chapter 5, most relevant results and future prospects of the research are discussed.

2. SOLUBILITY OF β -CAROTENE IN ETHANOL- AND TRIOLEIN-MODIFIED CO₂

Abstract

Modification of an experimental device and methodology improved the speed and reproducibility of measurement of solubility of β -carotene in pure and modified SuperCritical (SC) CO₂ at (313 to 333) K. Solubilities of β -carotene in pure CO₂ at (17 to 34) MPa ranged between (0.17 to 1.09) $\mu\text{mol/mol}$ and agreed with values reported in the literature. The solubility of β -carotene in CO₂ modified with (1.2 to 1.6) % mol ethanol increased by a factor of 1.7 to 3.0 as compared to its solubility in pure CO₂ under equivalent conditions. The concentration of triolein in equilibrated ternary (SC-CO₂ + β -carotene + triolein) mixtures having excess triolein reached values of (0.008 to 0.387) mmol/mol corresponding to its solubility in pure SC-CO₂ under equivalent conditions. Under these conditions, the solubility of β -carotene in triolein-modified CO₂ increased by a factor of up to 4.0 in relation to its solubility in pure CO₂ at comparable system temperature and pressure, reaching an uppermost value of 3.3 $\mu\text{mol/mol}$ at 333 K and 32 MPa. Unlike in the case of ethanol, where enhancements in solubility were relatively independent of system conditions, solubility enhancements using triolein as co-solvent increased markedly with system pressure, and were larger than that obtained by using (1.2 to 1.4) % mol ethanol at about (24 to 28) MPa, depending on system temperature. The increase in the solubility of β -carotene in SC-CO₂ as a result of using ethanol or triolein as co-solvent apparently does not depend on the increase in density associated with the dissolution of the co-solvent in CO₂. Enhancements may be due to an increase in the polarizability of SC-CO₂, which possibly grows markedly as triolein dissolves in it when the system pressure becomes higher.

Keywords: Solubility, β -carotene, supercritical carbon dioxide, co-solvent, triolein, ethanol, correlation.

2.1 Introduction

Carotenoids are natural substances with strong biological activity and responsible for the color of many photosynthetic plants and non-photosynthetic organisms (Astorg, 1997). Being tetraterpene compounds, carotenoids have isoprene (2-methylbuta-1,3-diene, C₅H₈) as their basic constituent unit. Carotenoids are classified as carotenes (hydrocarbon compounds with molecular formula C₄₀H₅₆) or xanthophylls (carotenes substituted with hydroxyl, ketone, and other oxygen-containing functional groups). Among natural carotene, β -carotene –(all-E)-1,1'-(3,7,12,16-tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethyl-cyclohexene]– has the 22-C linear, methyl substituted chain common to all carotenoids, and is distinguished for having 9-C β -ionone rings at both ends (Fig. 2.1A). Food and pharmaceutical industries are interested in isolating β -carotene from biological matrices because of its strong antioxidant and pro-vitamin A activity.

An emerging technology for the isolation of non-polar and/or low-molecular-weight compounds from biological matrices is supercritical carbon dioxide (CO₂) extraction (Brunner, 1994). A SuperCritical (SC) Fluid (SCF) is a substance at a temperature and pressure above its corresponding critical values that has a near-liquid density and a near-gas viscosity and self-diffusivity, which allows highly selective and fast extractions. Moreover, because the physical properties of a SCF change pronouncedly with small modifications in temperature and pressure, especially near the critical point, system conditions can be easily adjusted to achieve a selective dissolution or precipitation of target solutes in continuous fractionation processes. From a technological viewpoint, fluids used in SCF Extraction (SCFE) processes are gases under normal conditions (25 °C and 1 atm), which are compressed to increase their density and solvent power, and subsequently (followed by a solute removal from the substrate) decompressed to release them from the extract and treated substrate. For optimal SCFE processes, the extraction pressure is adjusted to several (typically 4 to 10) times the critical pressure (P_c) of the gas, and the extraction temperature is generally slightly above its critical temperature (T_c).

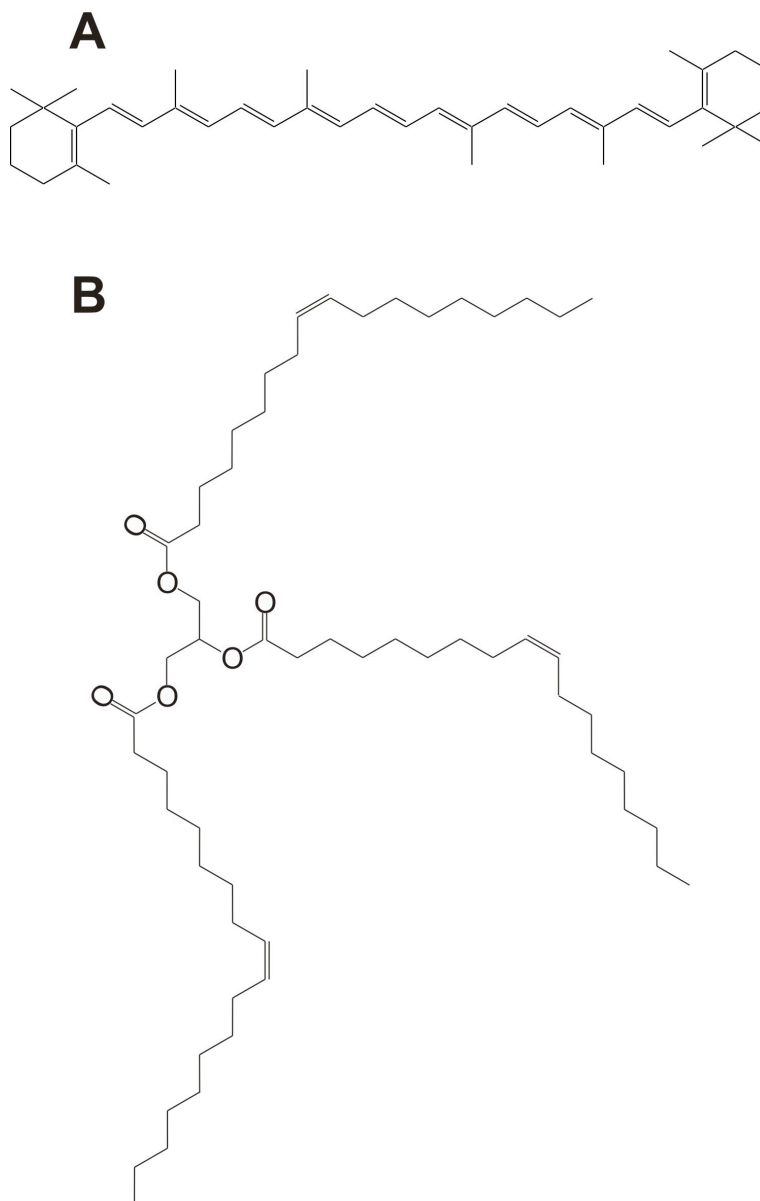


Figure 2.1 Chemical structures of (A) β -carotene, CAS [7235-40-7], $-(\text{all-E})-1,1'-(3,7,12,16\text{-tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl})\text{bis}[2,6,6\text{-trimethyl-cyclohexene}]$ and (B) triolein, CAS [122-32-7], $-1,2,3\text{-tri}[\text{cis-9-octadecenoyl}]\text{glycerol}$.

Thus, to avoid thermal damage of biological matrices, gases having a near-ambient T_c , such as CO₂ ($T_c = 304.1$ K, $P_c = 7.38$ MPa), are of special interest. Additional advantages making CO₂ the gas most commonly used in SCFE include inertness, non-flammability, and non-toxicity. Although SC-CO₂ is highly selective for the extraction of high-value

compounds in biological matrices (Brogle, 1982), drawbacks include the requirement of high-pressures (typically 30 to 75 MPa), and limited solvent power for polar and/or high-molecular-weight compounds such as carotenoids.

With the purpose of improving SCFE of natural carotenoids, entrainers are added to CO₂ that increase their solubility or facilitate their release from biological matrices. Different entrainers are used depending on the substrate and target solute but, to avoid losing the main advantages of using CO₂ as a solvent for high-value compounds in biological matrices, the added substance should have GRAS (Generally Recognized As Safe) status. A GRAS substance widely used as entrainer for the SCFE of biological matrices is ethanol, which has been applied to extract polar and/or heavy compounds such as carotenoids (Barth *et al.*, 1995; Baysal *et al.*, 2000; Valderrama *et al.*, 2003; Şanal *et al.*, 2005; Macías-Sánchez *et al.*, 2008), phospholipids (Dunford & Temelli, 1995; Montanari *et al.*, 1996), flavanols (Chang *et al.*, 2000; Park *et al.*, 2007) and other polyphenols (Adil *et al.*, 2007), saponins (Wang *et al.*, 2001), methylxanthines (Li & Hartland, 1996; Saldaña *et al.*, 2002; Park *et al.*, 2007) and other alkaloids (Song *et al.*, 1992), and pyrrole-containing pigments (Valderrama *et al.*, 2003; Macías-Sánchez *et al.*, 2008) from microalgae (Valderrama *et al.*, 2003; Macías-Sánchez *et al.*, 2008) and plant roots (Barth *et al.*, 1995; Wang *et al.*, 2001), fruits (Baysal *et al.*, 2000; Şanal *et al.*, 2005; Adil *et al.*, 2007), seeds (Dunford & Temelli, 1995; Montanari *et al.*, 1996; Saldaña *et al.*, 2002), beans (Li & Hartland, 1996; Saldaña *et al.*, 2002), and leaves (Song *et al.*, 1992; Chang *et al.*, 2000; Saldaña *et al.*, 2002; Park *et al.*, 2007). Because there is a limited amount of ethanol in biological matrices in their native state, using ethanol as CO₂ entrainer may require its removal from the extract following SCFE, which constitutes a limitation. Recent reports propose using vegetable oil (triglycerides) as CO₂ entrainers for the extraction of biological matrices, mainly of carotenoids from tomato (Vasapollo *et al.*, 2004; Shi *et al.*, 2009), carrot (Sun & Temelli, 2006), marigold (Ma *et al.*, 2008), and microalgae (Krichnavaruk *et al.*, 2008). A clear advantage of triglycerides over ethanol is that they are common constituents of biological matrices that are co-extracted with target solutes, and consequently their removal is not required following SCFE.

Several factors can explain improvements in SCFE when using modified instead of pure CO₂ as solvent, and high-pressure phase equilibrium studies in ternary (SC-CO₂ + solute + entrainer) systems can help interpret experimental data. Specific interactions of the entrainer with CO₂ and the target solute may increase the solubility of the solute in modified CO₂ as compared to pure CO₂ (Ekart *et al.*, 1993). Alternatively or in addition to this solubility-increasing (co-solvent) effect, the modifier may interact with functional groups on the surface of the biological matrix to help desorb the target solute (*e.g.*, by competing with the solute for binding sites on the matrix) (Fahmy *et al.*, 1993), or physically alter (*e.g.*, by swelling) the matrix (King & France, 1992) to facilitate inner mass transfer of the solute in the biological matrix (modifier effect). To discriminate the effect of entrainers as co-solvents (increasing the solubility of the target solute in CO₂) or modifiers (facilitating the release of the target solute from the solid matrix) in facilitating SCFE of biological matrices, it is necessary to measure one of these two effects separately. The co-solvent effect can be ascertained comparing the solubility of the target solute in co-solvent-modified CO₂ with its corresponding solubility in pure CO₂ under equivalent system temperature and pressure. There is ample high-pressure solubility data in ethanol-modified CO₂ of heavy and/or polar biological compounds including a pyrrolizidine alkaloid (Schaeffer *et al.*, 1988), phenolic acids (Ke *et al.*, 1996; Sovová, 2001; Chafer *et al.*, 2007), flavonoids (Berna *et al.*, 2001; Chafer *et al.*, 2002; Chafer *et al.*, 2004), a phenolic diterpene (Chafer *et al.*, 2005), tocotrienols (Birtigh *et al.*, 1995), sterols (Wong & Johnston, 1986), a triterpene (Catchpole *et al.*, 1998), and a carotenoid (Cygnarowicz *et al.*, 1990; Jay *et al.*, 1991; Sovová *et al.*, 2001), among other compounds. On the other hand, there is virtually no reported data of solubility in triglyceride-modified CO₂.

The aim of this work was to quantify the co-solvent effects of triolein and ethanol on the solubility of β -carotene in SC-CO₂ by measuring and comparing the model binary (SC-CO₂ + β -carotene) system with the ternary (SC-CO₂ + β -carotene + co-solvent) systems using system temperature ($313\text{ K} \leq T \leq 333\text{ K}$) and pressure ($15\text{ MPa} \leq P \leq 34\text{ MPa}$), and entrainer type and concentration as independent variables. We selected triolein –1,2,3-tri[*cis*-9-octadecenoyl]glycerol (Fig. 2.1B) as a model triglyceride because it is a representative compound for many vegetable oils.

2.2 Experimental

The solubility of β -carotene in binary (SC-CO₂ + solute) and ternary (SC-CO₂ + solute + co-solvent) systems was measured using a dynamic-analytical methodology in a modified version of the experimental apparatus described by de la Fuente *et al.* (2005) (Fig. 2.2). The experimental system consists of stirred, 50 cm³ (TharTech, Pittsburgh, PA) view-cell placed in a temperature-controlled air bath, which was complemented in this work with syringe pump (Teledyne ISCO 260D, Lincoln, NE) to load CO₂ into the system and adjust system pressure, and with a gear pump (GAH-T23, Eurotechnica, Bargteheide, Germany) to recirculate the CO₂-rich phase and aid system equilibration, and to feed samples of the CO₂-rich phase to the High Performance Liquid Chromatography (HPLC) system coupled to the equilibration system. The HPLC system consists of a L-7100 pump, L-7350 oven, and L-7455 photodiode array detector (Hitachi LaChrom, Tokyo, Japan).

For measurements, 0.6 g of type I ($\geq 95\%$ pure) β -carotene (component 2) from Sigma-Aldrich (Saint Louis, MO) were loaded in the equilibrium cell together with (in selected cases) 1.2 cm³ of HPLC grade ($\geq 99.9\%$ pure) ethanol (component 3) from Merck (Darmstadt, Germany) or 1 cm³ ($\geq 99\%$ pure) of triolein (component 4) from Sigma-Aldrich, which were loaded with an automatic pipette, and the air bath was adjusted to the required system temperature (313, 323, or 333) K. After loading the cell with β -carotene and, if needed, ethanol or triolein, the residual air was removed by displacement with low-pressure (5.7 MPa), food grade ($\geq 99\%$ pure) CO₂ (component 1) from AGA (Santiago, Chile) from a gas cylinder, and released with a vacuum pump (Welch Vacuum, Skokie, IL). After removing the air, high-pressure CO₂ was loaded into the equilibrium cell using the syringe pump to reach the desired system pressure (15 to 19) MPa. When pressure and temperature were at previously determined conditions, the cell was stirred and CO₂-rich phase was recirculated until equilibrium conditions were reached (8 h). After reaching equilibrium, a 20- μ L aliquot of the β -carotene-saturated, CO₂-rich phase was loaded into the loop of a six-port high-pressure injection valve (Rheodyne 7010, Rohnert Park, CA), and the β -carotene and triolein (when present) content(s) was (were) quantified by on-line HPLC analysis. Typically four measurements were made for each system condition. After that, CO₂ was

added to the cell using the syringe pump to increase system pressure as desired in order to complete the isotherm at (33 to 34) MPa.

The β -carotene content in the sampled aliquots was determined using a slightly modified version of the isocratic HPLC method of Robert *et al.* (2006). Separation was carried at 303 K using as the mobile phase 1 cm³/min of a 1:1 (v/v) mixture of HPLC-grade acetonitrile (Merck, Darmstadt, Germany) and HPLC-grade 2-propanol (Merck, Darmstadt, Germany), and a reverse-phase, 4.6-mm (inner diameter) wide, 25-cm long, C18 column (Waters symmetry column, Waters, Milford, MA) packed with silica particles of 5- μ m (diameter), used as stationary phase. β -carotene detection and quantification was done at 470 nm, using a calibration curve. For the calibration curve, standard solutions of β -carotene in HPLC-quality acetone (Merck, Darmstadt, Germany) were prepared and analyzed in the HPLC apparatus, where the procedure was a dissolution of the sample in ACS-reagent-quality petroleum ether from Merck (Darmstadt, Germany), solvent removal by bubbling of food grade ($\geq 99\%$ pure) N₂ from AGA, and re-dissolution in acetone.

The triolein content in aliquots of samples equilibrated with triolein-modified CO₂ was measured by HPLC with the same setup used for β -carotene measuring both compounds concurrently (at 470 nm and 205 nm for β -carotene and triolein, respectively), using a slightly modified version of the isocratic method of Lísá & Holčápek (2008). Triolein eluted after β -carotene, and was detected and quantified at a wavelength of 205 nm. The required calibration curve was prepared using standard solutions of triolein in acetone prepared similarly as those of β -carotene.

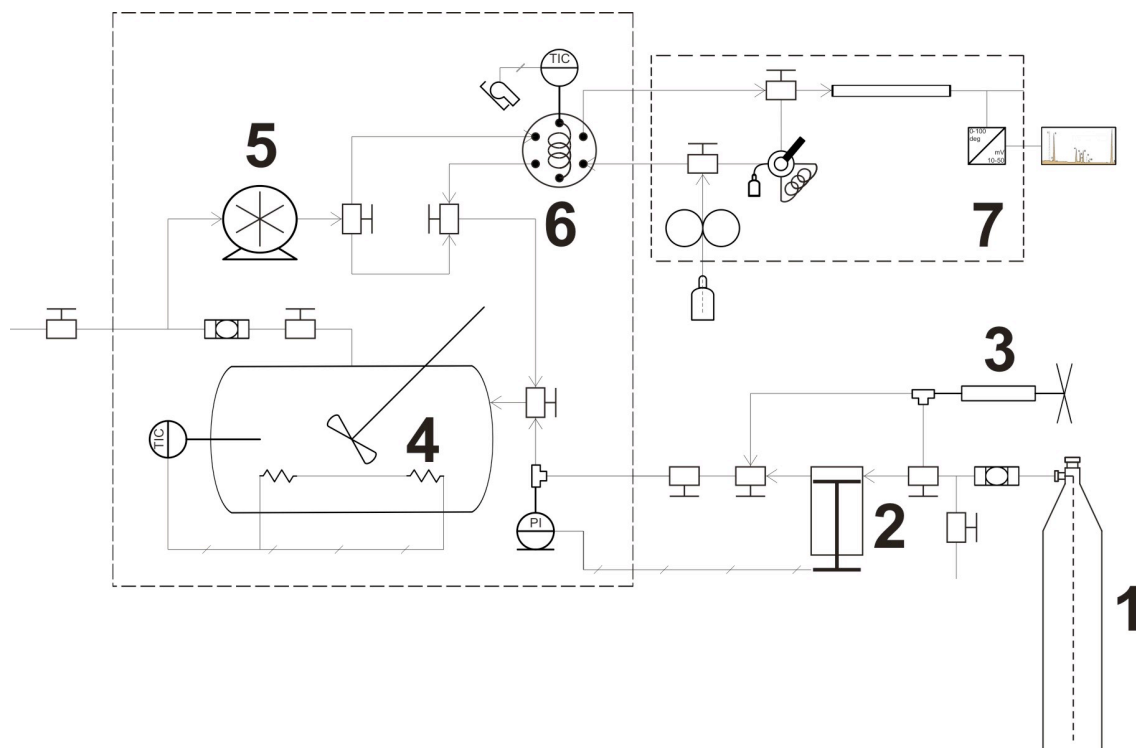


Figure 2.2 Schematic diagram of the experimental apparatus for solubility measurements. The main components of the system are the (1) CO_2 feed, (2) syringe pump, (3) manual compressor, (4) equilibrium cell with sapphire windows, (5) recirculation pump, (6) six-port high-pressure injection valve, and (7) HPLC.

2.3 Results and Discussion

Results are presented and discussed in four separate sections dealing with the solubility of β -carotene in pure CO_2 (Table 2.1), the solubility of β -carotene in ethanol-modified CO_2 (Table 2.2), the solubility of β -carotene in triolein-modified CO_2 (Table 2.3), and the modeling and analysis of consistency of the data. Experimental solubility data are compared with other values in literature for the same or similar systems. We used triolein as a representative compound for vegetable oils. Typical components of vegetable oils are triacylglycerols of 18-C fatty acids having different degrees of unsaturation (stearic acid, oleic acid, linoleic acid, and α - and γ -linolenic acids), and their solubility in high-pressure CO_2 depends on the total number of carbon atoms (typically 57) but not on the total number

of unsaturations (*e.g.*, most vegetable oils have similar solubility values) (del Valle & de la Fuente, 2006).

2.3.1 Binary (SC-CO_2 + β -carotene) system

Table 2.1 reports experimental solubility isotherms of β -carotene in pure CO_2 at (313, 323, and 333) K and pressures ranging from 17 to 34 MPa, that were measured mainly to validate our modified system and experimental procedure considering the vast amount of information in the literature on the solubility of β -carotene in SC-CO_2 . The modifications resulted in a reduction of equilibration time and improved reproducibility of experimental results, as it can be concluded by comparing results in Table 2.1 and those obtained by de la Fuente *et al.* (2006) with lycopene, another carotene. Indeed, the 12 h equilibration period of de la Fuente *et al.* (2006) was reduced to 8 h in this work. Furthermore, standard errors were reduced from a maximum value of 37% in the work of de la Fuente *et al.* (2006), where standard errors did not depend on solubility to a great extent, to 24% in this work, where standard errors decreased sharply as the solubility increased, as expected.

Table 2.1 Experimental molar fraction of β -carotene (y_2) in pure SC-CO₂ as a function of system temperature (T) and pressure (P).

| T (K) | P (MPa) | $y_2 \times 10^6$ | T (K) | P (MPa) | $y_2 \times 10^6$ | T (K) | P (MPa) | $y_2 \times 10^6$ |
|---------|-----------|-------------------|---------|-----------|-------------------|---------|-----------|-------------------|
| 313.2 | 17.83 | 0.177 | 323.1 | 18.99 | 0.126 | 333.5 | 17.48 | 0.271 |
| 313.4 | 17.84 | 0.187 | 323.4 | 18.99 | 0.225 | 333.4 | 17.49 | 0.296 |
| 313.4 | 22.28 | 0.230 | 323.3 | 19.00 | 0.163 | 333.4 | 17.49 | 0.315 |
| 313.3 | 22.33 | 0.208 | 323.3 | 19.06 | 0.183 | 333.5 | 17.53 | 0.310 |
| 313.4 | 22.42 | 0.217 | 323.2 | 22.95 | 0.261 | 333.5 | 22.81 | 0.380 |
| 313.4 | 22.43 | 0.224 | 323.3 | 22.98 | 0.254 | 333.3 | 22.89 | 0.417 |
| 313.1 | 29.34 | 0.297 | 323.2 | 23.00 | 0.311 | 333.5 | 22.93 | 0.416 |
| 313.3 | 29.52 | 0.308 | 323.4 | 23.02 | 0.377 | 333.6 | 22.98 | 0.366 |
| 313.2 | 29.54 | 0.361 | 323.2 | 28.54 | 0.480 | 333.2 | 28.28 | 0.754 |
| 313.3 | 29.64 | 0.324 | 323.3 | 28.62 | 0.438 | 333.2 | 28.38 | 0.692 |
| 313.1 | 33.58 | 0.380 | 323.3 | 28.68 | 0.506 | 333.3 | 28.38 | 0.708 |
| 313.2 | 33.59 | 0.457 | 323.3 | 28.69 | 0.488 | 333.2 | 28.38 | 0.720 |
| 313.3 | 33.60 | 0.382 | 323.4 | 33.96 | 0.524 | 333.4 | 34.10 | 1.051 |
| 313.3 | 33.77 | 0.410 | 323.4 | 34.00 | 0.670 | 333.3 | 34.11 | 1.025 |
| | | | 323.4 | 34.11 | 0.652 | 333.3 | 34.14 | 1.071 |
| | | | | | | 333.3 | 34.15 | 1.087 |

The experimental variability in solubility measurements was compared with inherent errors. The absolute standard deviation of values of y_2 reported in Table 2.1 ranged from 7.1×10^{-9} mol/mol at 313 K and 18 MPa to 79×10^{-9} mol/mol at 323 K and 34 MPa, whereas the relative standard deviation or standard error ($100 \cdot \Delta y_2 / y_2$) ranged from (3.9 to 24) %. Relative inherent errors estimated using the methodology described in the Appendix A (24 to 41) % were within a narrower range than standard errors, and were mostly influenced by invariant errors in the volume of sampled SC-CO₂ phases and stock solutions analyzed by HPLC (20% in total). Another error of influence was the one of the HPLC integrator ($\leq 18\%$), but only for conditions of small solubility; the minimal error was 1.6% for the highest solubility measured. The contributions to the inherent error of density increased with system temperature and decreased with system pressure, ranging from (0.30 to 1.3) %.

Other errors were smaller and had no practical significance. Overall, absolute inherent errors ranged from 59×10^{-9} mol/mol at 313 K and 18 MPa and 251×10^{-9} mol/mol at 333 K and 34 MPa. Lower standard deviation than absolute inherent errors of experimental data bring added value to the reproducibility of experimental results.

Experimental data in Table 2.1 is presented in Fig. 2.3 and compared with the solubility of β -carotene in CO₂ reported by other authors (Cygnarowicz *et al.*, 1990; Sakaki, 1992; Škerget *et al.*, 1995; Johannsen & Brunner, 1997; Méndes *et al.*, 1999; Cocero *et al.*, 2000; Hansen *et al.*, 2001; Sovová *et al.*, 2001; Kraska *et al.*, 2002). For all three isotherms, solubility increased with system pressure due to the higher density and associated enhancement in the solvent power of CO₂ (Foster *et al.*, 1991; Brunner, 1994). The effect of system temperature in solubility is not as straightforward because data tendencies change with variations in the system pressure, as expected for a crossover point (Brunner, 1994; Foster *et al.*, 1991) that our model (see section 2.3.4) predicted was between 15.7 and 17.3 MPa within our experimental region. Below this crossover pressure, the solubility of β -carotene decreased as temperature increased (density and solvent power decreasing effect); above it, the solubility increased as temperature increased (vapor pressure and volatility increasing effect). At 34 MPa, the measured solubility of β -carotene in SC-CO₂ was 56% higher at 323 than that at 313 K, and 50% higher at 333 than that at 323 K.

Figure 2.3 also shows large differences between authors in terms of the solubility of β -carotene in CO₂, as noted before by Güçlü-Üstündağ & Temelli (2004) and de la Fuente *et al.* (2006). Sakaki (1992), Méndes *et al.* (1999), and Sovová *et al.* (2001) reported similar solubilities (to within ± 0.1 μ mol/mol) to those measured by us, Škerget *et al.* (1995) reported values above (> 0.1 μ mol/mol) ours, and Cygnarowicz *et al.* (1990) and Hansen *et al.* (2001) reported values below (> 0.1 μ mol/mol) ours. Johannsen & Brunner (1997), Cocero *et al.* (2000), and Kraska *et al.* (2002) observed different effects of temperature and/or pressure than us, with solubilities coinciding (to within ± 0.1 μ mol/mol) with those measured by us under selected conditions. The differences in solubility values between different sources in literature can be attributed to differences in the physical condition and purity of the sample, and/or limitations of the experimental technique (Güçlü-Üstündağ &

Temelli, 2004; del Valle & de la Fuente, 2006). Below its melting point (456 K) (O'Neil *et al.*, 2006), the solubility varies depending on whether β -carotene is in a crystalline or amorphous state (Sakaki, 1992). Sample impurities or degradation products from β -carotene may affect solubility by acting as co- or anti-solvents, thus affecting measured solubility (Cocero *et al.*, 2000). In this work, we measured the composition of the CO₂-rich phase by on-line analysis in a HPLC apparatus coupled to a static cell that operated using a recirculation method. On-line analysis of the saturated, CO₂-rich phase (Sakaki, 1992; Johannsen & Brunner, 1997; Hansen *et al.*, 2001; Kraska *et al.*, 2002) is generally better than off-line analysis (Cygnarowicz *et al.*, 1990; Škerget *et al.*, 1995; Méndes *et al.*, 1999; Cocero *et al.*, 2000; Sovová *et al.*, 2001) because the system conditions (temperature and pressure) remain unchanged during sampling, un-dissolved β -carotene does not entrain the CO₂-rich phase (overestimation of true solubility), and dissolved β -carotene does not precipitate in valves and tubing (underestimation of true solubility). When the CO₂-rich phase is recirculated (Cygnarowicz *et al.*, 1990) through a static cell (Škerget *et al.*, 1995; Johannsen & Brunner, 1997; Kraska *et al.*, 2002) true equilibrium conditions are attained and identified unlike in equilibration systems using a dynamic method (Sakaki, 1992; Méndes *et al.*, 1999; Cocero *et al.*, 2000; Sovová *et al.*, 2001; Hansen *et al.*, 2001). In our system, an equilibrium condition was assumed when the standard deviation of five consecutive solubility measurements was <5%. A period of 1.5 h was allowed between samples.

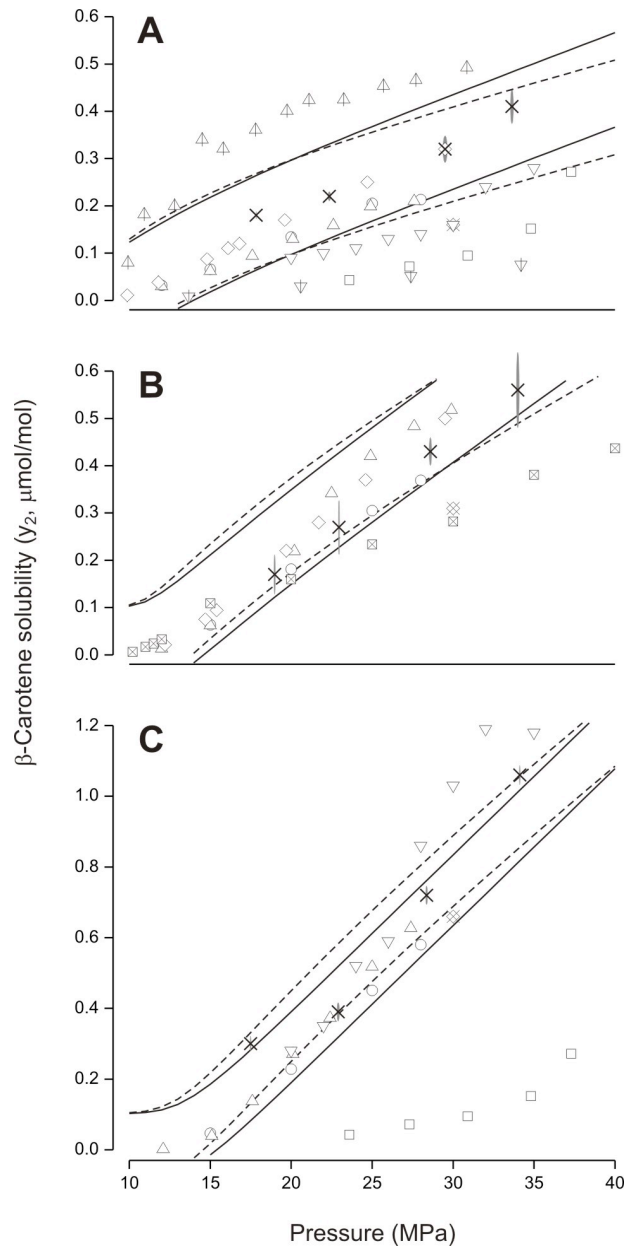


Figure 2.3 Comparison of solubility isotherms of β -carotene in pure SC-CO_2 reported in literature with those measured in this work, as a function of system pressure at (A) 313 K, (B) 323 K, and (C) 333 K: (\square) Cygnarowicz *et al.* (1990), (\diamond) Sakaki (1992), (\triangle) Škerget *et al.* (1995), (∇) Johannsen & Brunner (1997), (\triangle) Méndes *et al.* (1999), (\boxtimes) Cocero *et al.* (2000), (∇) Hansen *et al.* (2001), (\circ) Sovová *et al.* (2001), (\boxtimes) Kraska *et al.* (2002), and (\times) this work. Dotted lines represent the trend. Grey ovals represent standard deviations of pressure and solubility measurements of single data points, a measure of the dispersion of the data around average values.

2.3.2 Ternary (SC-CO₂ + β -carotene + ethanol) system

Table 2.2 reports experimental solubility isotherms of β -carotene in ethanol-modified CO₂ at (313, 323, and 333) K and pressures ranging from (18 to 34) MPa. We made these measurements mainly to validate our modified system and experimental procedure to measure solubilities in modified CO₂ considering the information in the literature on the solubility of β -carotene in ethanol-modified SC-CO₂ (Sovová *et al.*, 2001). In order to estimate the concentration of ethanol in the SC-CO₂ we assumed that ethanol dissolved completely in the SC-CO₂ in the initial mixture, which will remain in miscible and soluble conditions as small amounts of CO₂ are added to the system to compensate for sampling withdrawal. The total volume of the cell and other components in the solvent loop (82 cm³) was estimated from the experimentally observed pressure drop after sampling a measured amount of CO₂ using a mass balance for the system and the volumetric properties of CO₂ estimated as a function of system temperature and pressure using NIST database (Standard Database v8.0). Some assumptions used to develop the solubility calculations were no change in the modified CO₂ density compared with pure CO₂ density, and negligibility of the amount of ethanol removed while sampling aliquots for on-line analysis. Because system pressure was increased by adding CO₂ to the loop, the estimated concentration of ethanol decreased steadily as pressure was increased in any isotherm.

In this case, as in the binary system (SC-CO₂ + β -carotene), lower standard deviations of data in Table 2.2 than absolute inherent error bring validity to the reproducibility of our experimental measurements for ternary (SC-CO₂ + β -carotene + co-solvent) systems. The absolute standard deviation of values of y_2 reported in Table 2.2 ranged from 6.4×10^{-9} mol/mol at 313 K and 34 MPa to 58×10^{-9} mol/mol at 323 K and 34 MPa, whereas the standard error ranged from 0.78% at 333 K and 28 MPa to 8.8% at 313 K and 18 MPa. Corresponding values varied between 81×10^{-9} mol/mol (lowest solubility condition) and 405×10^{-9} mol/mol (highest solubility condition) in the case of the absolute inherent errors, and between 23% (highest solubility condition) and 24% (lowest solubility condition) in the case of the relative inherent errors estimated using the procedure presented in Appendix A.

Solubility of β -carotene in modified CO_2

Table 2.2 Experimental molar fraction of β -carotene (y_2) in SC- CO_2 modified with ethanol (y_3) as a function of system temperature (T) and pressure (P).

| T (K) | P (MPa) | $y_3 \times 10^2$ | $y_2 \times 10^6$ | T (K) | P (MPa) | $y_3 \times 10^2$ | $y_2 \times 10^6$ | T (K) | P (MPa) | $y_3 \times 10^2$ | $y_2 \times 10^6$ |
|---------|-----------|-------------------|-------------------|---------|-----------|-------------------|-------------------|---------|-----------|-------------------|-------------------|
| 313.4 | 17.92 | 1.329 | 0.354 | 323.2 | 18.71 | 1.414 | 0.596 | 333.2 | 18.26 | 1.563 | 0.611 |
| 313.4 | 17.96 | 1.328 | 0.356 | 323.2 | 18.76 | 1.414 | 0.577 | 333.2 | 18.28 | 1.563 | 0.652 |
| 313.4 | 17.99 | 1.327 | 0.294 | 323.2 | 18.77 | 1.414 | 0.646 | 333.2 | 18.33 | 1.563 | 0.677 |
| 313.4 | 17.99 | 1.327 | 0.325 | 323.2 | 18.81 | 1.414 | 0.656 | 333.1 | 22.81 | 1.416 | 0.905 |
| 313.2 | 23.48 | 1.251 | 0.436 | 323.3 | 22.77 | 1.334 | 0.747 | 333.1 | 23.07 | 1.416 | 0.911 |
| 313.2 | 23.55 | 1.250 | 0.437 | 323.3 | 22.78 | 1.334 | 0.756 | 333.1 | 23.07 | 1.416 | 0.961 |
| 313.2 | 23.55 | 1.250 | 0.491 | 323.3 | 22.78 | 1.334 | 0.768 | 333.1 | 23.08 | 1.416 | 0.959 |
| 313.2 | 23.56 | 1.250 | 0.462 | 323.3 | 22.83 | 1.334 | 0.722 | 333.1 | 23.10 | 1.416 | 0.903 |
| 313.5 | 27.44 | 1.216 | 0.524 | 323.3 | 27.62 | 1.272 | 1.087 | 333.5 | 27.97 | 1.335 | 1.487 |
| 313.4 | 27.44 | 1.216 | 0.539 | 323.3 | 27.67 | 1.272 | 1.118 | 333.5 | 28.02 | 1.335 | 1.496 |
| 313.5 | 27.45 | 1.216 | 0.519 | 323.3 | 27.71 | 1.272 | 1.099 | 333.5 | 28.06 | 1.335 | 1.492 |
| 313.2 | 27.48 | 1.214 | 0.548 | 323.3 | 27.72 | 1.272 | 1.203 | 333.5 | 28.12 | 1.335 | 1.514 |
| 313.4 | 33.72 | 1.172 | 0.664 | 323.3 | 33.38 | 1.221 | 1.352 | 333.3 | 33.60 | 1.272 | 1.755 |
| 313.3 | 33.73 | 1.172 | 0.662 | 323.3 | 33.40 | 1.221 | 1.390 | 333.3 | 33.64 | 1.272 | 1.792 |
| 313.4 | 33.75 | 1.172 | 0.655 | 323.3 | 33.46 | 1.221 | 1.262 | 333.3 | 33.65 | 1.272 | 1.845 |
| 313.4 | 33.85 | 1.171 | 0.671 | 323.3 | 33.48 | 1.221 | 1.381 | 333.3 | 33.67 | 1.272 | 1.781 |
| | | | | | | | | 333.3 | 33.69 | 1.272 | 1.840 |

Figure 2.4 presents experimental data in Table 2.2 as the enhancement (ratio between the solubility of a solute in modified and pure SC-CO₂ under the same system conditions) in the solubility of β -carotene brought about by the addition of a small amount (1.17 to 1.56) % mol of ethanol to CO₂. Values larger than 1 indicate that ethanol has a co-solvent effect (increases the solubility) for β -carotene. We observed that 1.35% mol/mol ethanol increases the solubility of β -carotene by a factor of 2 to 3, and that this enhancement has a low dependence on system temperature (small increase) and pressure (small decrease). The results were as expected, because other authors (Dobbs *et al.*, 1986; Dobbs *et al.*, 1987) observed that besides the specific solute and co-solvent, the co-solvent-induced enhancement in SC-CO₂ solubility depends on co-solvent concentration but does not depend on system temperature and pressure. Figure 2.4 also includes data of Sovová *et al.* (2001) for comparison. They observed a similar enhancement except at low pressure (15 MPa) their data were more scattered, probably because they had a larger variation in the amount of added ethanol whose mol fraction ranged between 0.00181 and 0.0227. Another explanation for the differences between the two studies is the use of azeotropic (96% aqueous) ethanol by Sovová *et al.* (2001) instead of anhydrous ethanol used in our study. One major question is whether the co-solvent-induced enhancement effect of ethanol on the solubility of β -carotene in SC-CO₂ is partially due to an increase in the density of the SCF resulting from the dissolution of ethanol. The actual density of the ethanol-modified SC-CO₂ (ρ_{mix}) was estimated using the Peng-Robinson equation of state (PR-EoS) with quadratic mixing rules (Brunner *et al.*, 2001). This was done using free software PE2000 with model parameters for CO₂ and binary (CO₂ + ethanol) system specified by Brunner *et al.* (2001) (binary interaction parameters $\kappa_{ij} = 0.11431$ and $\lambda_{ij} = 0.015021$). Based on the densities of ethanol-modified and pure SC-CO₂ estimated using PR-EoS $-(\rho_{\text{mix}})_{\text{PR}}$ and $(\rho_1)_{\text{PR}}$, respectively, the true density of the mixture was estimated using equation 2.1 (Ting *et al.*, 1993a):

$$\rho_{\text{mix}} = \left[1 + \frac{(\rho_{\text{mix}})_{\text{PR}} - (\rho_1)_{\text{PR}}}{(\rho_1)_{\text{PR}}} \right] \cdot (\rho_1)_{\text{NIST}} \quad (2.1)$$

where $(\rho_1)_{\text{NIST}}$ is the density of pure CO_2 calculated as a function of system temperature and pressure using NIST Standard Database (v8.0). The procedure was validated by estimating differences in density with experimental data of Pohler & Kiran (1997) using 0.1928 mol/mol ethanol at 323 K, 19.06 MPa, (+12% error), and by Zuñiga-Moreno & Galicia-Luna (2002) using 0.2317 mol/mol ethanol at 312.91 K, 18.932 MPa, (+10% error). Within our experimental region the density of the ethanol-modified SC- CO_2 was (0.18 to 2.92) % higher than the density of pure SC- CO_2 under the same system conditions (results not shown), and for each temperature it decreased as pressure increased, which was partially associated with the decrease in concentration of ethanol by dilution with SC- CO_2 . These antecedents suggest that the small increase in density of the SCF is not sufficient to justify the large (>100%) increase in the solubility of β -carotene associated with the dissolution of ethanol in SC- CO_2 experimentally observed.

Several explanations have been proposed in the literature for polar co-solvent (*e.g.* ethanol) induced effects increasing solute solubility in SC- CO_2 . Dobbs *et al.* (1987) proposed changes in dispersion, orientation, and acid-base partial solubility parameters of SC- CO_2 increasing the affinity between the solute and ethanol-modified SC- CO_2 as compared to pure SC- CO_2 . Ting *et al.* (1993b) proposed ethanol-mediated hydrogen bonding interactions between the solute and ethanol-modified SC- CO_2 resulting in the synthesis of complexes. Zhang *et al.* (2002) proposed an increase in local density around the solute and ethanol with ethanol associating preferentially with the solute.

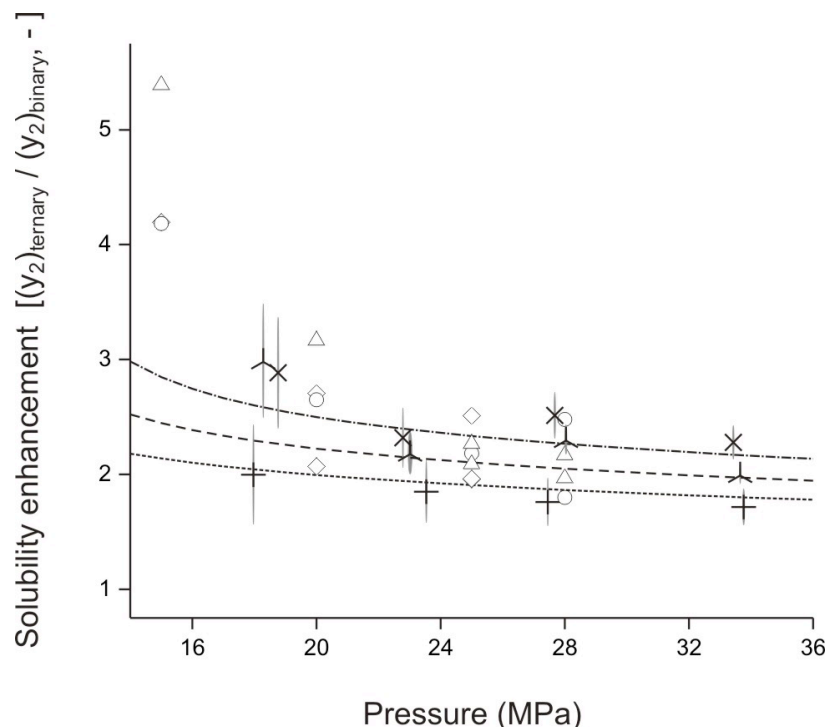


Figure 2.4 Enhancement in the solubility of β -carotene in ethanol-modified SC- CO_2 , as a function of system pressure at ($\diamond, +$) 313 K, (\circ, \times) 323 K, and ($\triangle, \blacktriangledown$) 333 K reported by ($\diamond, \circ, \triangle$) Sovová *et al.* (2001) and ($+, \times, \blacktriangledown$) measured in this work. Dotted lines represent the trend at (---) 313 K, (---) 323 K, and (---) 333 K. Grey ovals represent standard deviations of pressure and solubility measurements of single data points, a measure of the dispersion of the data around average values.

2.3.3 Ternary ($\text{SC-CO}_2 + \beta\text{-carotene} + \text{triolein}$) system

Table 2.3 reports experimental solubility isotherms of β -carotene in triolein-modified CO_2 at (313, 323, and 333) K and (15 to 34) MPa. Absolute standard deviations of values of y_2 reported in Table 2.3 ranged from 13×10^{-9} mol/mol at 333 K and 17 MPa to 218×10^{-9} mol/mol at 323 K and 34 MPa, whereas the standard errors calculated using the procedure described in Appendix A ranged from 29×10^{-9} mol/mol for the lowest solubility to 730×10^{-9} mol/mol for the highest. Corresponding values ranged from 1.2% at 333 K and 28 MPa to 50% at 323 K and 16 MPa in the case of standard errors of experimental measurements, and from 23% at 323 K and 34 MPa and 48% also at 323 K and 16 MPa in the case of

Solubility of β -carotene in modified CO_2

estimated relative inherent errors. The very large and anomalous value of the standard error at 323 K and 16 MPa was due to the very low solubility measured under these conditions. This low solubility resulted in a small response of the integrator of the HPLC that contributed 25% to the inherent standard error.

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Table 2.3 Experimental molar fraction of β -carotene (y_2) in SC- CO_2 modified with triolein (y_4) as a function of system temperature (T) and pressure (P).

| T (K) | P (MPa) | $y_4 \times 10^4$ | $y_2 \times 10^6$ | T (K) | P (MPa) | $y_4 \times 10^4$ | $y_2 \times 10^6$ | T (K) | P (MPa) | $y_4 \times 10^4$ | $y_2 \times 10^6$ |
|---------|-----------|-------------------|-------------------|---------|-----------|-------------------|-------------------|---------|-----------|-------------------|-------------------|
| 313.4 | 15.33 | 0.935 | 0.206 | 323.2 | 15.90 | 0.127 | 0.035 | 333.5 | 16.67 | 0.188 | 0.175 |
| 313.2 | 16.07 | 1.006 | 0.186 | 323.5 | 15.94 | 0.132 | 0.102 | 333.4 | 16.71 | 0.317 | 0.190 |
| 313.8 | 20.09 | 1.880 | 0.288 | 323.5 | 15.98 | 0.083 | 0.040 | 333.3 | 16.72 | 0.274 | 0.191 |
| 313.6 | 20.13 | 1.804 | 0.268 | 323.5 | 15.99 | 0.113 | 0.064 | 333.6 | 16.75 | 0.169 | 0.208 |
| 313.7 | 28.51 | 2.958 | 0.254 | 323.3 | 21.40 | 1.019 | 0.494 | 333.4 | 21.84 | 0.940 | 0.706 |
| 313.4 | 28.68 | 3.064 | 0.282 | 323.4 | 21.42 | 1.053 | 0.425 | 333.4 | 21.88 | 0.986 | 0.741 |
| 313.4 | 28.90 | 3.139 | 0.311 | 323.8 | 21.45 | 1.000 | 0.469 | 333.3 | 21.89 | 1.010 | 0.707 |
| 313.6 | 29.03 | 2.958 | 0.275 | 323.5 | 21.46 | 1.040 | 0.703 | 333.4 | 21.89 | 1.014 | 0.769 |
| 313.6 | 34.51 | 3.712 | 0.371 | 323.4 | 28.75 | 2.622 | 1.338 | 333.4 | 27.78 | 2.508 | 1.993 |
| 313.6 | 34.51 | 3.671 | 0.358 | 323.5 | 28.80 | 2.688 | 1.324 | 333.3 | 27.93 | 2.550 | 1.964 |
| 313.5 | 34.52 | 3.704 | 0.390 | 323.4 | 28.88 | 2.629 | 1.277 | 333.5 | 28.07 | 2.580 | 1.944 |
| | | | | 323.4 | 28.91 | 2.595 | 1.225 | 333.4 | 28.18 | 2.544 | 1.989 |
| | | | | 323.5 | 33.43 | 3.720 | 2.520 | 333.6 | 32.21 | 3.874 | 3.166 |
| | | | | 323.4 | 33.44 | 3.699 | 2.327 | 333.3 | 32.24 | 3.792 | 3.239 |
| | | | | 323.5 | 33.48 | 3.523 | 2.089 | 333.5 | 32.42 | 3.814 | 3.274 |
| | | | | 323.5 | 33.51 | 3.769 | 2.568 | 333.3 | 32.52 | 3.856 | 3.201 |

Table 2.3 also reports the concentrations of triolein in ternary (SC-CO₂ + β -carotene + triolein) mixtures as a function of system conditions. Absolute standard deviations of values of y_4 varied from 2.2 $\mu\text{mol/mol}$ at 313 K and 35 MPa to 107 $\mu\text{mol/mol}$ at 323 K and 34 MPa, whereas corresponding values of standard errors (0.59 to 30) % increased with system temperature and decreased with system pressure. Because measured concentrations of triolein were about two orders of magnitude larger than those of β -carotene, the contribution of integrator errors to relative inherent errors were comparatively small (0.038 to 1.4) %. This explains smaller and nearly invariant total relative inherent errors in estimates of triolein concentrations (21 to 23) %.

Figure 2.5 shows experimental data in Table 2.3 as the triolein-induced enhancement in the solubility of β -carotene in CO₂ brought about by the addition of small amounts ($0.0834 < y_4 \times 10^{-4} < 3.87$) of triolein. Unlike in the case of ethanol there was an excess of triolein in the equilibrium cell that did not dissolve in SC-CO₂ under system conditions to ensure a mixture saturated with triolein at equilibrium. The amount of triolein dissolved in the SC-CO₂ phase was experimentally measured (Table 2.3) and the concentrations were similar (to within ± 0.1 mmol/mol) to solubility values of pure triolein in SC-CO₂ reported by Chrastil (1982) and Nilsson & Hudson (1993) at 313 K, and by Nilsson *et al.* (1991) at 323 K and 333 K. Ribeiro & Bernardo-Gil (1995) and Chen *et al.* (2000) reported solubility values higher (> 0.1 mmol/mol) than the concentrations experimentally measured by us. The solubilities reported by Ribeiro & Bernardo-Gil (1995) were questioned by Güçlü-Üstündağ & Temelli (2000) in their review on the solubility of behavior of triglycerides in SC-CO₂. Based on these antecedents, the concentration of triolein in SC-CO₂ in our experiments corresponds to its solubility (saturation concentration) in pure SC-CO₂, and our data is within ± 0.1 mmol/mol of reliable values reported by others. Bamberger *et al.* (1988) observed that the solubility in SC-CO₂ of the least soluble triglyceride (component 2) in a mixture of triglycerides is enhanced, whereas that of the most soluble triglyceride (component 3) is the same as its solubility in pure SC-CO₂. It is apparent from our results that this is also valid when component 2 is replaced by β -carotene that is less soluble in SC-CO₂ than triolein (Table 2.3).

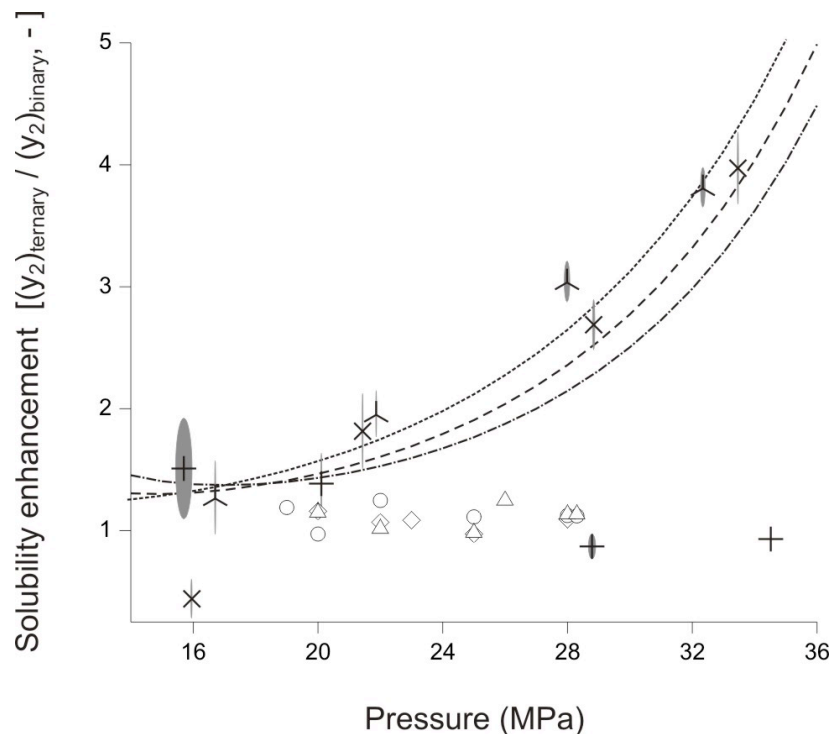


Figure 2.5 Enhancement in the solubility of β -carotene in triolein-modified SC- CO_2 , as a function of system pressure at ($\diamond, +$) 313 K, (\circ, \times) 323 K, and (\triangle, ∇) 333 K reported by ($\diamond, \circ, \triangle$) Sovová *et al.* (2001) and ($+, \times, \nabla$) measured in this work. Dotted lines represent the trend at (---) 313 K, (---) 323 K, and (-·-·-) 333 K.

As a consequence of the dissolution of increasing amounts of triolein in SC- CO_2 as pressure increases, the triolein-induced enhancement increased pronouncedly with system pressure (Fig. 2.5) unlike the ethanol-induced enhancement (Fig. 2.4). This is as expected, because co-solvent-induced enhancement depends on the co-solvent and its concentration but not on system temperature and pressure (Dobbs *et al.*, 1986; Dobbs *et al.*, 1987). Predictions within our experimental region suggest a decrease in enhancement at lower temperatures (see lines in Fig. 2.5), but this is mainly due to the decrease in triolein solubility with temperature at pressures below 40 MPa (the binary SC- CO_2 + triolein system exhibits an unusually high crossover pressure), as suggested by our predictions and those made using the correlation proposed by Güçlü-Üstündağ & Temelli (2000).

One effect associated with the dissolution of increasing amounts of triolein in SC- CO_2 is an increase in the density and the associated solvent power of the SCF phase. Similar to the

case of ethanol-modified SC-CO₂, the actual density of the saturated solution of triolein-modified SC-CO₂ (ρ_{mix}) was estimated using PR-EoS with quadratic mixing rules. Values of PR-EoS parameters a_c , b , and κ adopted for triolein were calculated from estimates of critical properties (T_c , P_c) and acentric factor (ω). T_c (954 K) and ω (1.69) were estimated using the method of Dohrn & Brunner (1994), and P_c (0.654 MPa) was best-fitted to experimental values of the density of pure (liquid) triolein (Formo *et al.*, 1979). Binary interaction parameters for mixtures of CO₂ and triolein were fitted to solubility isotherms measured in this work at (15 to 34) MPa. These values were $k_{14} = 0.098$ and $l_{14} = 0.128$ at 313 K, $k_{14} = 0.099$ and $l_{14} = 0.216$ at 323 K, and $k_{14} = 0.086$ and $l_{14} = 0.192$ at 333 K. Within our experimental region the density of the triolein-modified SC-CO₂ was (0.006 to 0.31) % higher than the density of pure SC-CO₂ under the same system conditions (results not shown), with the percent difference increasing with pressure at (323 and 333) K, but not at 313 K. These results are not consistent with those of Chen *et al.* (2000), who experimentally observed a decrease in the density of SC-CO₂ as a result of dissolution of triolein. However, the results of Chen *et al.* (2000) are questionable considering the differences in the solubility of triolein as compared with those measured by us and others (Güçlü-Üstündağ & Temelli, 2000). The extremely small increase in the density of SC-CO₂ as a result of the dissolution of triolein is not sufficient to justify the very large (up to about 400%) increase in the solubility of β -carotene experimentally observed. This is in agreement with Dobbs *et al.* (1987), who observed that in the dense SCF region (reduced density above 1.3, as in our experimental region) the modification in the density of the SC-CO₂ resulting from the addition of a co-solvent, which may even diminish, contributes only slightly to co-solvent-induced enhancements in solubility.

The results in this study show that triolein has a condition-of-state-dependent co-solvent effect on the solubility of β -carotene in SC-CO₂ that increases due to dissolution of triolein. This effect is larger than measured using a larger molar fraction (0.012 to 0.016) of ethanol at high temperature (≥ 323 K) and pressure (≥ 27 MPa). This may be due to an increase in the polarizability (dispersion forces) of SC-CO₂ brought about by dissolved triolein as observed for other non-polar co-solvents (Dobbs *et al.*, 1986). β -Carotene is a non-polar

solute and would not benefit from specific (polar) interactions such as hydrogen bonds favoring dissolution of polar solutes in ethanol-modified SC-CO₂. In these experiments, the system had excess triolein, which was unavoidable due to its limited solubility in CO₂, and the presence of a separate liquid phase, unlike when using ethanol as co-solvent, may have a positive impact on the solubility of β -carotene in SC-CO₂.

Our results do not agree with those of Sovová *et al.* (2001), who reported higher solubilities of β -carotene in ethanol- than vegetable-oil-modified SC-CO₂ (see Fig. 2.4 and Fig. 2.5). Sovová *et al.* (2001) also added an antioxidant (Butylated HydroxyToluene, BHT) to the system to minimize chemical changes of β -carotene during measurements but BHT may affect solubilities by acting as co- or antisolvent (Güçlü-Üstündağ & Temelli, 2004). Their measurements were made using off-line analysis of binary (SC-CO₂ + β -carotene) samples equilibrated using a dynamic method without recirculation of the SCF phase that is less precise than our method. However, the method and solute mixture analyzed by Sovová *et al.* (2001) were the same as those used to measure solubilities of β -carotene in pure (Fig. 2.3) and ethanol-modified (Fig. 2.4) SC-CO₂, which agreed with the solubilities we measured. Another explanation of the differences between the results of Sovová *et al.* (2001) and ours is the replacement of triolein by grape seed oil, but no large differences in solubility are expected between the two unless the grape seed oil contains relatively large amounts of components exhibiting large differences in solubility as compared to triglycerides such as free fatty acids (del Valle & de la Fuente, 2006). Sovová *et al.* (2001) reported an increase in the concentration of vegetable oil in modified SC-CO₂ with system pressure as expected, but these concentrations differed from those estimated by us for triolein-saturated SC-CO₂. Differences in co-solvent-induced enhancements in the solubility of β -carotene between ethanol and triolein observed in our study qualitatively agree with other results reported in literature. Saldaña *et al.* (2010) measured the apparent solubility of β -carotene in pure, ethanol-modified, and canola oil modified SC-CO₂ at 313 K and 40 MPa by dynamic extraction of tomato skin and pulp. These apparent solubilities were 0.62 $\mu\text{mol/mol}$ in pure SC-CO₂, 0.91 $\mu\text{mol/mol}$ in ethanol-modified SC-CO₂, and 1.8 $\mu\text{mol/mol}$ in canola oil modified SC-CO₂. Corresponding values extrapolated to 313 K and 40 MPa from our

experimental results were 0.47 $\mu\text{mol/mol}$ (pure SC-CO_2), 0.81 $\mu\text{mol/mol}$ (SC-CO_2 modified with 0.012 mol/mol ethanol), and 8.8 $\mu\text{mol/mol}$ (SC-CO_2 modified with 0.63 mmol/mol triolein). Both sets of results suggest that β -carotene solubility increases in the following order: pure $\text{SC-CO}_2 < \text{ethanol-modified SC-CO}_2 < \text{triolein-modified SC-CO}_2$. Apparent solubilities depend on co- and anti-solvency effects due to other components (*e.g.*, triglycerides) in tomato skin and pulp, as well as on the modifier effects of ethanol and canola oil (helping swelling and/or desorbing β -carotene to/from the solid matrix). It is however apparent that triolein acts as a co-solvent of β -carotene increasing its solubility in SC-CO_2 .

2.3.4 Correlation of experimental solubility data for binary and ternary systems

Experimental solubilities of β -carotene in pure and ethanol- or triolein-modified SC-CO_2 were correlated using empirical corrections to a density-based model originally proposed by Méndez-Santiago & Teja (1999). The model of Méndez-Santiago & Teja (1999) gives $\ln(E)$ versus ρ and T , where $E (=P_{y_2} / P^{\text{sub}})$ is a thermodynamic enhancement that, unlike the co-solvent-induced enhancements defined and discussed in sections 2.3.2 and 2.3.3, gives the increase in partial pressure of the solid solute (component 2, β -carotene in this case) in a gaseous phase resulting from its solvation in SC-CO_2 , as compared with its normal tendency to transfer to a non-interacting gaseous phase defined by its sublimation pressure (P^{sub}). Méndez-Santiago & Teja (2000) added empirical corrections to account for the effect of the co-solvents (terms having y_3 and y_4 as independent variables depending on the added co-solvent; ethanol or triolein, respectively). Given that the values of P^{sub} are unknown for many solutes (including β -carotene), an additional empirical correction assumes a Clausius-Clapeyron-type dependency of P^{sub} on absolute temperature (Méndez-Santiago & Teja, 1999; Sauceau *et al.*, 2003). Sauceau's *et al.* (2003) final correlation equation (equation 2.2) is as follows:

$$T \ln(E') = A + B \rho + C T + D y_3 + F y_4 \quad (2.2)$$

where $E' (=P_{y_2} / P^{\text{std}})$ is a modified thermodynamic enhancement using a reference or standard pressure ($P^{\text{std}} = 0.1013 \text{ MPa}$ or 1 atm) to replace P^{sub} . Equation 2.2 can be used to

test the self-consistency of the data because it predicts that solubility isotherms collapse to a single line when plotting $\Psi(E', T, y_2, y_3, y_4)$ versus ρ , where

$$\Psi = T \left[\ln(E') - C \right] - D y_3 - F y_4 \quad (2.3)$$

Indeed, replacing this definition (equation 2.3) in equation 2.2, the correlation for the solubility can be expressed as follows (equation 2.4):

$$\Psi = A + B \rho \quad (2.4)$$

Equation 2.2 was best-fitted to the data in Table 2.1 (D and F both nulls) and all data (Table 2.2 and Table 2.3) so as to obtain equation 2.5 (valid for the solubility of β -carotene in pure CO₂) and equation 2.6 (valid for the solubility of β -carotene in ethanol- and/or triolein-modified CO₂), respectively:

$$y_2 \times 10^6 = \frac{0.1013}{P} \exp \left(\frac{-13167 + 4.190 \rho + 20.65}{T} \right) \pm 0.07021 \quad (2.5)$$

$$y_2 \times 10^6 = \frac{0.1013}{P} \exp \left(\frac{-13174 + 3.828 \rho + 1.741 y_3 + 0.01145 y_4 + 21.64}{T} \right) \pm 0.2458 \quad (2.6)$$

$$y_4 \times 10^4 = \frac{0.1013}{P} \exp \left(\frac{-11996 + 6.270 \rho + 17.78}{T} \right) \pm 0.4296 \quad (2.7)$$

In equations 2.2 and 2.5-2.7, P is the pressure (MPa), T is the absolute temperature (K), ρ is the density of the CO₂ (kg/m³) at T and P , y_2 is the molar fraction of β -carotene in the SC-CO₂ phase (dimensionless), y_3 is the molar fraction of ethanol in the SC-CO₂ phase (dimensionless), and y_4 is the molar fraction of triolein in the SC-CO₂ phase (dimensionless). Equation 2.7 gives the solubility of triolein in CO₂ and was obtained by best-fitting equation 2.2 to data for y_4 in Table 2.3, and neglecting the co-solvent effect of dissolved β -carotene on the solubility of triolein in SC-CO₂ (terms D and F both nulls), as experimentally observed.

As predicted by equation 2.4, Fig. 2.6 demonstrates the self-consistency of solubility data for β -carotene in pure and co-solvent-modified SC-CO₂. Indeed all isotherms collapsed to a

single best-fit line in a plot of Ψ (estimated using best-fit parameters in equation 2.5) *versus* ρ for data in Table 2.1 (Fig. 2.6A). In the case of the whole data set (Tables 2.1 to 2.3), all isotherms for pure SC-CO₂ (Table 2.1), all isotherms for ethanol-modified SC-CO₂ (Table 2.2), and the 333 K isotherm for triolein modified SC-CO₂ (Table 2.3) collapsed to a single best-fit line in a plot of Ψ (estimated using best-fit parameters in equation 2.6) *versus* ρ (Fig. 2.6B). Solubility data at 313 and 323 K for triolein-modified SC-CO₂ in Table 2.3 was not considered in the correlation (equation 2.6) because of the anomalous high-pressure (>28 MPa) behavior at 313 K and low-pressure (<17 MPa) behavior at 323 K, where there was no enhancement in the solubility of β -carotene as a result of the dissolution of triolein the SC-CO₂ phase. (The data associated with these three conditions appear as outliers in Fig. 2.6B.) We do not have an explanation for this anomalous behavior.

An assumption of equation 2.2 is that the density of SC-CO₂ solutions matched the density of pure SC-CO₂ at the same system conditions. Re-fitting of equation 2.2 using corrected densities of ethanol- and triolein-modified SC-CO₂ mixtures resulted in very small differences in Fig. 2.6 (only perceptible for measurements using ethanol-modified SC-CO₂) and no improvement in the correlation (similar dispersion of data and error estimates). The effect of dissolved β -carotene in the density of the mixtures was not estimated, but we assumed it was even smaller than the one of dissolved triolein because of its even smaller solubility. The contribution of errors in density estimates to relative inherent errors in solubility estimates ranged (0.29 to 1.4) % in experiments with ethanol- and triolein-modified SC-CO₂, and because these values are smaller than increases in the density of SC-CO₂ resulting from the dissolution of triolein (0.006 to 0.31) %, and comparable to those resulting from the dissolution of ethanol (0.18 to 2.92) %, it is possibly unnecessary to account for these differences in modeling the effect of solvent density in β -carotene solubility. We believe equation 2.6 is more convenient than the correlation using corrected densities (data not shown) because of the complexities associated with the estimation of corrected densities using the procedure adopted in this contribution (see equation 2.1). However, readers should use equation 2.6 with caution to estimate the solubility of β -carotene in ethanol-modified SC-CO₂ (the term $D y_3$ is nearly invariant in our data set)

Solubility of β -carotene in modified CO_2

because we did not evaluated in this study the effect of ethanol concentration ($0.0117 < y_3 < 0.0156$) on β -carotene solubility.

On the other hand, the concentration of triolein varied within the wide range ($8.34 \times 10^{-6} < y_4 < 3.87 \times 10^{-4}$) relevant for extractions at (313 to 333) K and (16 to 35) MPa.

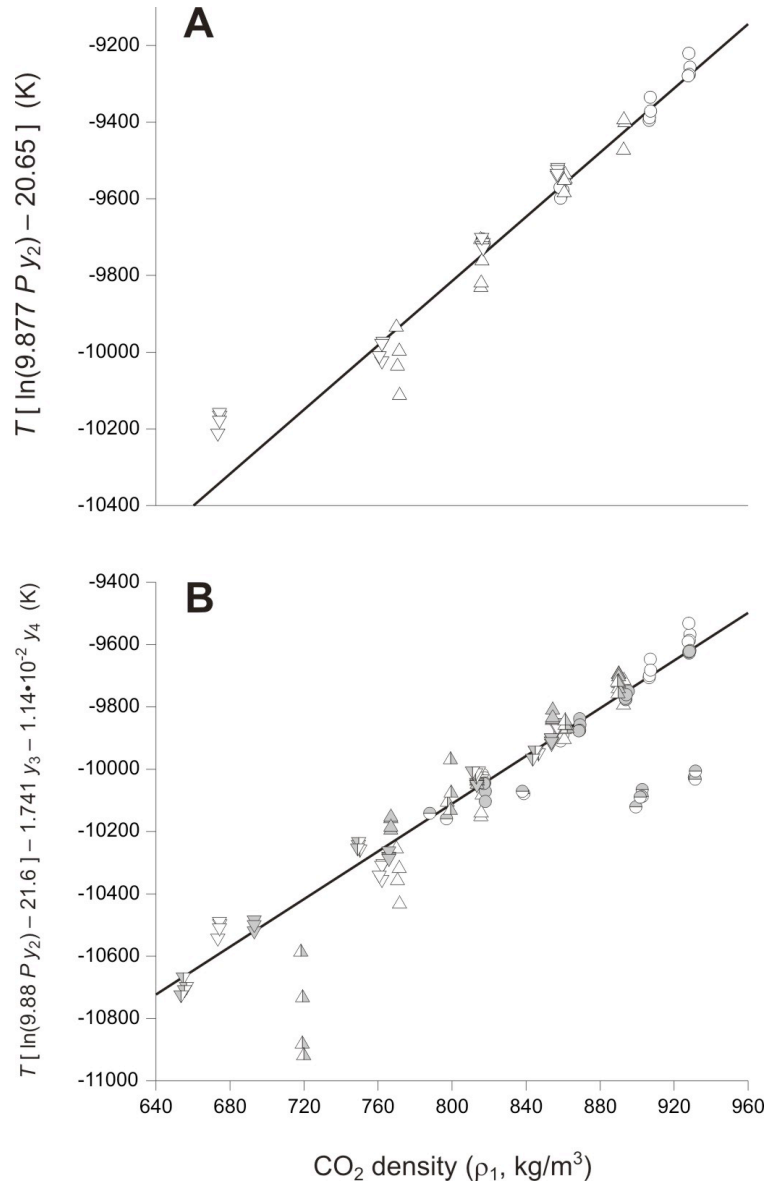


Figure 2.6 Comparison of experimental solubility of β -carotene with correlated results from the Méndez-Santiago & Teja model, as a function of CO₂ density (**A**) experimental data using exclusively pure SC-CO₂ and (**B**) all experimental data including pure and modified SC-CO₂, the line corresponds to correlation of data points using relationship 2.4. (○, △, ▽) pure, (●, ▲, ▼) ethanol-modified, and (⊖, ⬤, ▽) triolein-modified SC-CO₂ at (○, ●, ⊖) 313 K, (△, ▲, ⬤) 323 K, and (▽, ▼, ▽) 333 K.

2.4 Conclusions

The modifications of the experimental device and methodology we introduce in de la Fuente *et al.* (2005) allowed improved measurements of solubility in pure and modified SC-CO₂ both in terms of speed and reproducibility. The solubility of β -carotene in SC-CO₂ and ethanol-modified SC-CO₂ compared well with reliable results in the literature. New results are reported on the solubility of β -carotene in triolein-modified SC-CO₂. The solubility of triolein in the ternary (SC-CO₂ + β -carotene + triolein) system was the same as in the binary (SC-CO₂ + triolein) system because triolein is more soluble in SC-CO₂ than β -carotene. On the other hand, saturation of SC-CO₂ with triolein resulted in triolein-associated enhancement in the solubility of β -carotene that increased with system pressure and decreased with system temperature below 34 MPa (slightly below the crossover pressure for the binary SC-CO₂ + triolein). For the same molar concentrations of ethanol and triolein in SC-CO₂ the co-solvent effect for β -carotene of triolein was larger than the one of ethanol because there are no polar interactions with ethanol favoring dissolution of β -carotene. Co-solvent-associated enhancements are not due to a great extent to the increase in the density and solvent power of the SC-CO₂ resulting from the dissolution of ethanol nor triolein. Rather these enhancements are due possibly to an increase in the polarizability of CO₂.

3. EFFECT OF TRIOLEIN ADDITION ON THE SOLUBILITY OF CAPSANTHIN IN SUPERCRITICAL CARBON DIOXIDE

Abstract

This manuscript presents new phase equilibrium data for capsanthin in pure and triolein-entrained SuperCritical (SC) carbon dioxide (CO₂). The aim of the work was to determine the co-solvent effect of triolein on capsanthin by comparing solubility results in a ternary (SC-CO₂ + capsanthin + triolein) system and binary (SC-CO₂ + capsanthin) system at (313 or 333) K and (19 to 34) MPa. For this, authors capsanthin isolated from red pepper (*Capsicum annuum* L.) and tested using a dynamic-analytical method in an apparatus with recirculation and online analysis of the CO₂-rich phase. Within the experimental region, the solubility of capsanthin in pure SC-CO₂ increased with system temperature at isobaric conditions and also increased with pressure at isothermal conditions. Solubilities ranged from a minimal of 0.64 $\mu\text{mol/mol}$ at 313 K and 19 MPa to a maximal of 1.97 $\mu\text{mol/mol}$ at 333 K and 32 MPa. The concentration of triolein in the ternary system was equivalent to its solubility in pure SC-CO₂ depending on system temperature and pressure conditions. Crossover pressure was determined experimentally at 29.6 MPa, below which solubility of triolein decreased with temperature (effect of density). Above the crossover pressure, solubility of triolein increased with temperature (vapor pressure effect). Values of solubility within this range were 0.16 mmol/mol at 19 MPa and 313 K to 0.41 mmol/mol at 33 MPa and 333 K. Independent of system temperature and pressure, capsanthin solubility in triolein-entrained SC-CO₂ increased by a factor of about 3 (triolein-induced enhancement factor) as compared to its solubility in pure CO₂, under similar conditions of pressure and temperature. The maximal solubility of capsanthin in SC-CO₂ experimentally observed in this study was 5.27 $\mu\text{mol/mol}$ at 333 K and 33 MPa in the presence of 4.10 mmol/mol triolein.

Keywords: Solubility, capsanthin, supercritical carbon dioxide, co-solvent, triolein, correlation.

3.1 Introduction

Red pepper (*Capsicum annuum* L.) is used widely as a natural food colorant due to its high carotenoid content. The main carotenoid is a reddish di-hydroxy, ketocarotenoid or xanthophyll, capsanthin [(all-E,3R,3'S,5'R)-3,3'-dihydroxy- β , κ -caroten-6'-one], which is found exclusively in red pepper and amounts to 35% to 50% of all carotenoids in the fruit (Curl, 1962; Locey & Guzinski, 2000). Capsanthin (Fig. 3.1) has the 22-C methyl substituted, linear, non-polar core common to all carotenoids connecting a typical 9-C β -ionone ring on the left and an unusual 9-C cyclic end group on the right, which are substituted by oxygen-containing functional groups, similar to all xanthophylls. The same as in the case of other carotenoids, capsanthin consumption offers humans advantages because of its nutraceutical properties: it is a powerful radical scavenger (Hirayama *et al.*, 1994; Matsufuji *et al.*, 1998) exhibiting antioxidant and anticarcinogenic activities (Narisawa *et al.*, 2000).

SuperCritical carbon dioxide (SC-CO₂) is an appropriate solvent for the extraction of bioactive compounds from biological substrates, but it has limited capacity to dissolve compounds with high molecular weight, regardless of their polarity (Brunner, 1994) such as carotenoids. Previous work demonstrated the need to add an entrainer to SC-CO₂ to improve the extraction of carotenoids from biological substrates, presumably to increase their solubility in the supercritical phase (Vasapollo *et al.*, 2004; Sun and Temelli, 2006; Krichnavaruk *et al.*, 2008; Ma *et al.*, 2008; Ciurlia *et al.*, 2009; Shi *et al.*, 2009; Gao *et al.*, 2010; Palumpitag *et al.*, 2011). Entrainers must have Generally Recognized As Safe (GRAS) status to be acceptable in the food and pharmaceutical industries. In addition, they must have affinity with CO₂, the biological matrix, and/or (the) target solute(s) (chapter 2). There are reports on the use of various vegetable oils to improve the SC-CO₂ extraction of carotenoids from diverse biological substrates, including the extraction of lycopene from tomato (Vasapollo *et al.*, 2004; Ciurlia *et al.*, 2009; Shi *et al.*, 2009; Saldaña *et al.*, 2010), α - and β -carotene and lutein from carrot (Sun & Temelli, 2006), lutein from marigold (Ma *et al.*, 2008; Gao *et al.*, 2010; Palumpitag *et al.*, 2011), and astaxanthin from microalgae (Krichnavaruk *et al.*, 2008) using almond kernel (Vasapollo *et al.*, 2004), peanut

(Vasapollo *et al.*, 2004), hazelnut (Vasapollo *et al.*, 2004; Ciurlia *et al.*, 2009), sunflower seed (Vasapollo *et al.*, 2004; Gao *et al.*, 2010), olive fruit (Krichnavaruk *et al.*, 2008; Shi *et al.*, 2009; Palumpitag *et al.*, 2011), low-erucic (canola) or normal rape seed (Sun & Temelli, 2006; Gao *et al.*, 2010), soybean (Krichnavaruk *et al.*, 2008, Ma *et al.*, 2008; Gao *et al.*, 2010; Palumpitag *et al.*, 2011), and palm (Palumpitag *et al.*, 2011) oils as entrainers. More than one factor can explain improvements in SC-CO₂ extractions when using entrainers. Specific interactions of the entrainer with CO₂ and the target solute may increase its solubility in entrained as compared to pure CO₂ (chapter 2). Alternatively or in addition to this co-solvent (solubility-increasing) effect, the entrainer can help desorb the solute by competing with it for binding sites on the matrix, or facilitate solute movement in the solid matrix by swelling it (modifier effects) (chapter 2). An independent measurement of the effect of an entrainer as co-solvent or as modifier may help clarify if the improvement in the SC-CO₂ extraction is due to an increase in the solubility of the target solute in CO₂, facilitated release of the target solute from the biological matrix, and/or facilitated movement of the target solute through the matrix.

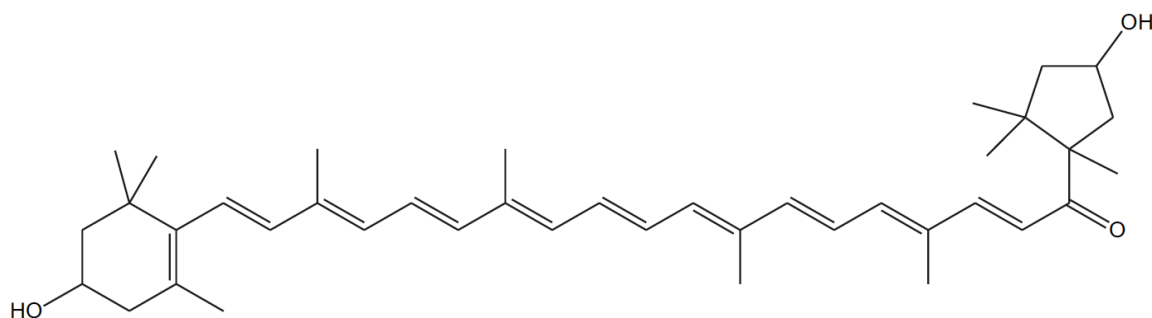


Figure 3.1 Chemical structure of capsanthin, CAS [465-42-9]: all-E,3R,3'S,5'R)-3,3'-dihydroxy- β,κ -caroten-6'-one.

High-pressure phase equilibrium studies in binary (CO_2 + solute) and ternary (CO_2 + solute + entrainer) systems can help interpret experimental data on SC- CO_2 extraction studies by quantifying co-solvent effects. This was presented in chapter 2, where it was discussed the co-solvent effects of triolein, a representative triglyceride in vegetable oil, on the solvation of carotenoids by comparing the solubility of β -carotene in entrained and pure SC- CO_2 at the same temperature and pressure. An inherent difficulty in this type of studies is the

unavailability or high cost of pure carotenoid compounds, particularly from natural origin. For example, the two grams of solute required to carry out phase equilibrium studies such as the present one would cost USD 18,000 in the case of lutein or USD 15,000 in the case of lycopene, as compared to USD 16 to 20 in the case of synthetic β -carotene (Sigma-Aldrich). On the other hand, the Sigma-Aldrich catalog does not list capsanthin among its offered chemicals. Thus, authors are compelled to purify carotenoids from extracts of biological substrates as done to study the solubility in high-pressure CO₂ of free lutein from marigold (*Tagetes erecta*) (Jay *et al.*, 1991), lutein diesters from unsaponified extracts of *Helenium autumnale* (sneezeweed) (Jay *et al.*, 1991) or *T. erecta* (Naranjo-Modad *et al.*, 2000), and lycopene from tomato (de la Fuente *et al.*, 2006).

The purpose of this work was to characterize co-solvent effects in the supercritical extraction of red pepper with vegetable-oil-entrained SC-CO₂. For that, we measured solubilities of capsanthin (a representative carotenoid in red pepper) in the ternary (SC-CO₂ + capsanthin + triolein) model system, and compared them with corresponding solubilities in the binary (SC-CO₂ + capsanthin) system at equivalent temperatures (313 or 333) K and pressures (19 to 34) MPa.

3.2 Experimental

Capsanthin was extracted and isolated from dried, milled red pepper from Invertec Foods (Rengo, Chile). The substrate (1 kg) was extracted to exhaustion at room temperature (22 °C) using High Performance Liquid Chromatography (HPLC) grade acetone (10 L) (J.T. Baker, Phillipsburg, NJ, USA) (Philip & Francis, 1971), that was removed subsequently from the oleoresin by vacuum evaporation at <56 °C. The desolventized extract was saponified overnight using 20% analytical-grade KOH (50 mL) (Merck, Darmstadt, Germany) in HPLC-grade methanol (Merck, Darmstadt, Germany) at room temperature (22 °C). The organic phase, containing saponified carotenoids, was then extracted with analytical-grade diethyl ether (60 mL) (Merck, Darmstadt, Germany), washed with HPLC-grade water (50 mL) (Merck, Darmstadt, Germany) up to reaching neutral pH, dried over anhydrous Na₂SO₄ (Sigma-Aldrich, Saint Louis, MO, USA), filtered, and desolventized by evaporation. Complete saponification of oleoresin carotenoids (absence of esters) was

verified by HPLC analysis (Weissenberg *et al.*, 1997) as discussed in the last paragraph of this section. Saponified carotenoids were isolated by open column chromatography on heat-activated (1:1 w/w, activated at 120 °C for 2 h) mixture of MgO and celite (stationary phase) and carotenoid pigments were eluted using step elution with solvents of increasing polarity (mobile phase) (Rodríguez-Amaya, 1999). The identity of capsanthin was confirmed by HPLC analysis (Weissenberg *et al.*, 1997), and its purity estimated by spectrophotometry (Jaren-Galan *et al.*, 1999). The capsanthin isolate used in phase equilibrium measurements had 86% (w/w) purity.

Solubility of capsanthin in pure and triolein-entrained SC-CO₂ was measured using the dynamic-analytical method and experimental procedure described elsewhere (chapter 2). The system consisted of a high-pressure equilibrium view-cell coupled to a HPLC apparatus for on-line analysis of capsanthin and, in selected cases, triolein in the CO₂-rich phase. The equilibrium cell was loaded with approximately 0.6 g of isolated capsanthin and, in experiments with entrained CO₂, with approximately 600 µl of 99% pure triolein (Sigma-Aldrich, Saint Louis, MO, USA). The air bath where the cell was located was adjusted to the required system temperature, and the cell was deaerated and loaded with food-grade (>99% pure) CO₂ (AGA, Santiago, Chile) to the required system pressure (*ca.* 18 MPa) using a syringe pump. Once operating conditions were reached, the components within the cell were thoroughly mixed for 8 h by recirculating the CO₂-rich phase using a gear micropump. After reaching equilibrium the CO₂-rich phase was sampled and analyzed, and the pressure readjusted to a higher value by feeding CO₂ to the cell with the syringe pump. This was repeated three times up to reaching the required upper pressure (*ca.* 34 MPa). Two-to-four replicate measurements were carried out for each experimental condition.

The concentration of capsanthin and/or triolein in the CO₂-rich phase was quantified by on-line HPLC analysis in a Hitachi LaChrom (Tokyo, Japan) apparatus using the isocratic methods of Weissenberg *et al.* (1997) and Líska & Holčapek (2008), respectively, with minor modifications. The chromatographic separation of both components was carried out in a reversed-phase C18 Waters Symmetry column (5 µm particle size, 4.6 mm i.d. × 25 cm) packed with C18 aliphatic-bonded silica particles (Waters, Milford, MA) using 0.5

cm³/min of HPLC-grade acetonitrile (Merck, Darmstadt, Germany) at 30 °C. UV-detection was done at 450 nm for capsanthin and 205 nm for triolein. Molar fractions of capsanthin and/or triolein in the CO₂-rich phase were calculated from chromatographic peak areas and those of standard solutions of capsanthin and triolein in HPLC-grade acetone (J.T. Baker, Phillipsburg, NJ, USA) as previously described (chapter 2).

3.3 Results and Discussion

The solubility of capsanthin in SC-CO₂ increased with system pressure ($19 \text{ MPa} \leq P \leq 33 \text{ MPa}$) and was higher at 333 K than 313 K (Table 3.1, Fig. 3.2). Furthermore there was a positive interaction between the two factors. Consequently, the solubility increased by a factor of slightly more than 3 between 313 K and 19 MPa ($y_2 = 0.65 \pm 0.02 \text{ } \mu\text{mol/mol}$) and 333 K and 32 MPa ($y_2 = 1.968 \pm 0.005 \text{ } \mu\text{mol/mol}$). These trends respond to the increase in the solvent power of SC-CO₂ as a result of the increase in density associated with the increase in system pressure, on one hand, and the increase in the volatility of the solute as a result of the increase in vapor pressure associated with the increase in system temperature, on the other hand (Brunner, 1994). In supercritical fluids, also referred to as dense gases (Stahl *et al.*, 1988), the two factors (increase in solvent power of CO₂ and increase in solute volatility) facilitate transfer of solutes from solid matrices to the fluid phase. Indeed, SC-CO₂ extraction is described as a separation process that shares some features with both vapor-pressure-driven or distillation processes, and solute-solvent-interaction-driven or extraction processes (Zosel, 1978). The decrease in the positive effect of system temperature on solubility at low pressure is due to the increase in the compressibility of the fluid when its pressure approaches the critical value ($P_c = 7.38 \text{ MPa}$ in the case of CO₂). At a so-called crossover pressure (Foster *et al.*, 1991) the two effects (reduction of density, increase of vapor pressure) compensate and the solubility does not change when heating the system isobarically.

Experimental data in Table 3.1 was modeled (trend lines in Fig. 3.2) using equation 3.1 by best-fitting the semi-empirical equation developed by Méndez-Santiago & Teja (1999) using the theory of dilute solutions:

$$y_2 \times 10^6 = \frac{0.1013}{P} \exp\left(\frac{3.468 \rho_1 - 10769}{T} + 16.16\right) \pm 0.057 \quad (3.1)$$

where y_2 is the molar fraction of capsanthin, P is the pressure (MPa) and T the absolute temperature (K) of the system, and ρ_1 is the density of CO₂ (kg/m³) at P and T estimated using NIST Standard Database (v8.0). Lines in Fig. 3.2 correspond to predictions of equation 3.1. These lines suggest a crossover pressure at approximately 16.7 MPa for the binary (SC-CO₂ + capsanthin) system.

Table 3.1 Experimental data of molar fraction of capsanthin (y_2) in saturated supercritical CO₂ as a function of system temperature (T) and pressure (P).

| T /K | P /MPa | $y_2 \times 10^6$ | T /K | P /MPa | $y_2 \times 10^6$ |
|--------|----------|-------------------|--------|----------|-------------------|
| 313.3 | 18.61 | 0.639 | 333.2 | 18.67 | 0.760 |
| 313.3 | 18.63 | 0.671 | 333.2 | 18.70 | 0.740 |
| 313.3 | 22.15 | 0.731 | 333.3 | 23.64 | 1.349 |
| 313.4 | 22.25 | 0.637 | 333.2 | 23.68 | 1.312 |
| 313.4 | 27.53 | 0.899 | 333.3 | 23.62 | 1.383 |
| 313.3 | 27.77 | 0.797 | 333.2 | 27.91 | 1.649 |
| 313.3 | 32.83 | 1.060 | 333.2 | 27.93 | 1.618 |
| 313.4 | 32.96 | 1.079 | 333.2 | 27.95 | 1.668 |
| | | | 333.2 | 32.00 | 1.964 |
| | | | 333.2 | 32.05 | 1.966 |
| | | | 333.2 | 32.04 | 1.974 |

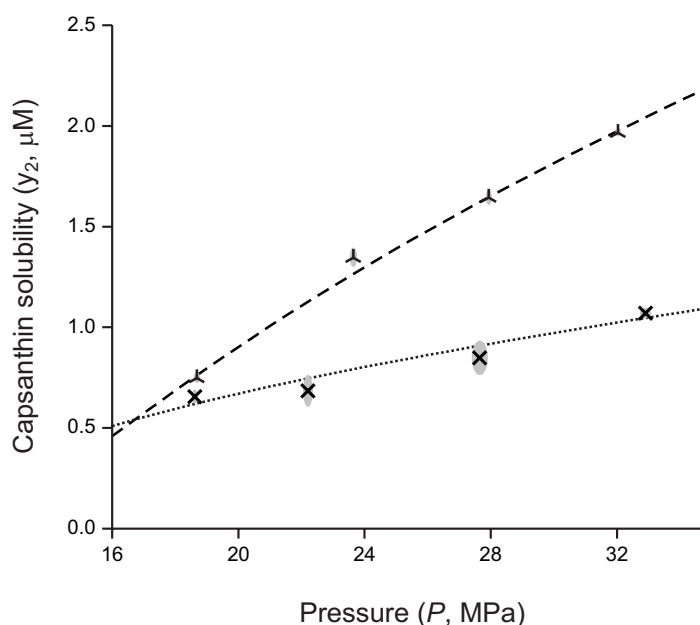


Figure 3.2 Solubility isotherms for capsanthin in supercritical CO₂ as a function of system pressure. Symbols represent the average of two-to-four experimental measurements at (····X····) 313 K or (—▲—) 333 K; lines represent predictions using equation 3.1; and gray ovals represent the standard deviation of pressure and solubility measurements of single data points, a measure of the dispersion of the data around average values.

The effects of system temperature and pressure on the solubility of capsanthin in triolein-entrained SC-CO₂ were similar to those in pure SC-CO₂, but values were larger (Table 3.2) because of a cosolvency effect. Triolein dissolves in SC-CO₂ more easily than capsanthin, and bridges molecules of capsanthin in CO₂, possibly because it participates in non-polar interactions between 18-C monounsaturated, fatty (oleic) acid chains in the triglyceride and the 22-C methyl substituted, linear, non-polar core seen in Fig. 3.1. The remainder of this section discusses the co-solvent effect of triolein on capsanthin in detail.

Table 3.2 Experimental data of molar fraction of capsanthin (y_2) in triolein-entrained (molar fraction y_3) supercritical CO₂ as a function of system temperature (T) and pressure (P).

| T /K | P /MPa | $y_3 \times 10^4$ | $y_2 \times 10^6$ | T /K | P /MPa | $y_3 \times 10^4$ | $y_2 \times 10^6$ |
|--------|----------|-------------------|-------------------|--------|----------|-------------------|-------------------|
| 313.2 | 18.85 | 1.631 | 1.868 | 333.3 | 18.58 | 1.556 | 1.912 |
| 313.2 | 18.85 | 1.614 | 1.959 | 333.2 | 18.57 | 1.563 | 1.865 |
| 313.3 | 18.82 | 1.602 | 1.896 | 333.2 | 18.55 | 1.637 | 1.822 |
| 313.2 | 23.68 | 3.153 | 2.058 | 333.2 | 23.55 | 2.265 | 3.326 |
| 313.2 | 23.57 | 3.016 | 2.235 | 333.2 | 23.31 | 2.237 | 3.364 |
| 313.3 | 23.63 | 2.875 | 2.218 | 333.2 | 28.25 | 3.203 | 4.182 |
| 313.2 | 28.24 | 3.446 | 2.782 | 333.2 | 28.21 | 3.351 | 4.311 |
| 313.2 | 28.26 | 3.499 | 2.754 | 333.2 | 28.20 | 3.268 | 4.366 |
| 313.1 | 33.89 | 3.742 | 3.312 | 333.2 | 32.84 | 4.117 | 5.257 |
| 313.4 | 33.91 | 3.991 | 3.503 | 333.2 | 32.81 | 4.086 | 5.274 |
| 313.3 | 33.73 | 4.020 | 3.521 | | | | |
| 313.4 | 33.58 | 4.003 | 3.425 | | | | |

The concentration of triolein in saturated mixtures with CO₂ and capsanthin was approximately the same as in a binary (SC-CO₂ + triolein) system as previously observed in chapter 2 for the ternary (SC-CO₂ + β -carotene + triolein) system. Although saturation concentrations of triolein were larger in the present study than values presented in chapter 2, the largest experimental differences between the two studies were about 0.11 mmol/mol for experimental measurements at 333 K and (21.8 to 23.4) MPa.

This value is slightly above the limit of 0.1 mmol/mol obtained in chapter 2 to claim that the saturation concentration of triolein in the ternary (SC-CO₂ + carotenoid + triolein) system was equivalent to the solubility of pure triolein in SC-CO₂ reported by others (Chrastil, 1982; Nilsson *et al.*, 1991; Nilsson & Hudson, 1993). The explanation given in chapter 2 for this was that in ternary mixtures with SC-CO₂ the solubility of the most soluble compound (triolein in this case) remains constant, whereas the one of the least soluble compound (capsanthin in this case) increases (triolein exhibits co-solvent effect).

The experimental data in Table 3.2 was modeled using equation 3.2 by best-fitting the equation of Méndez-Santiago & Teja (1999):

$$y_3 \times 10^4 = \frac{0.1013}{P} \exp\left(\frac{3.870 \rho_1 - 8424}{T} + 8.798\right) \pm 0.256 \quad (3.2)$$

where y_3 is the molar fraction of triolein dissolved in the mixture. Plotting data and equation 3.2 suggests a crossover pressure at approximately 29.6 MPa for the dissolution of triolein in SC-CO₂ (data not shown).

The best way to analyze the effect of triolein on the solubility of capsanthin in SC-CO₂ is using the so-called solubility enhancement, a parameter defined as the ratio between the solubility of capsanthin in triolein-entrained SC-CO₂ and its corresponding solubility in pure SC-CO₂ under equivalent system conditions (P , T). For that, experimental data in Table 3.2 was divided by values predicted using equation 3.1 for the experimental values of P , T , and ρ_1 (estimated as a function of P and T using NIST Standard Database (v.8.0)). Figure 3.3 summarizes the results of these computations.

Triolein has a co-solvent effect in that it increases the solubility of capsanthin in SC-CO₂ by a factor of about 3 within the experimental region studied in this work (Fig. 3.3). Although it appeared as if this co-solvent effect was higher at 313 than 333 K and did not depend on pressure, these further precisions are not warranted given wide variability of experiment results. A large part of this variability is intrinsic to uncertainties in using equation 3.1 to estimate the solubility of capsanthin in pure CO₂. The conclusion of Fig. 3.3 does not coincide with the one presented in chapter 2 on the co-solvent effect of triolein on the solubility of β -carotene in SC-CO₂. Instead, was found a pronounced increase in the co-solvent effect with pressure that it was attributed to dissolution of triolein in SC-CO₂, as suggested by model predictions (lines) in Fig. 3.3. This experimental trend was defined solely by experimental data at 333 K; in chapter 2 was observed limited co-solvent effect at 313 K and high pressure (≥ 28 MPa) or 323 K and low pressure (≤ 17 MPa). From a physicochemical standpoint, this solubility enhancement can be due to the increase in the density and solvent power of the fluid phase, and/or the increase in the polarizability of the fluid phase brought about by dissolution of triolein in SC-CO₂ (Dobbs *et al.*, 1986). In

chapter 2 was estimated that the change in density of SC-CO₂ was very small upon saturation with triolein, and consequently it could not explain the large effect of dissolved triolein in the solubility of β -carotene in triolein-entrained SC-CO₂. On the other hand, a large increase in polarizability is surmised considering that the polarizability of triolein (Correa & Saramago, 2004) is *ca.* 40-times as large as the polarizability of CO₂ (Bose & Cole, 1970).

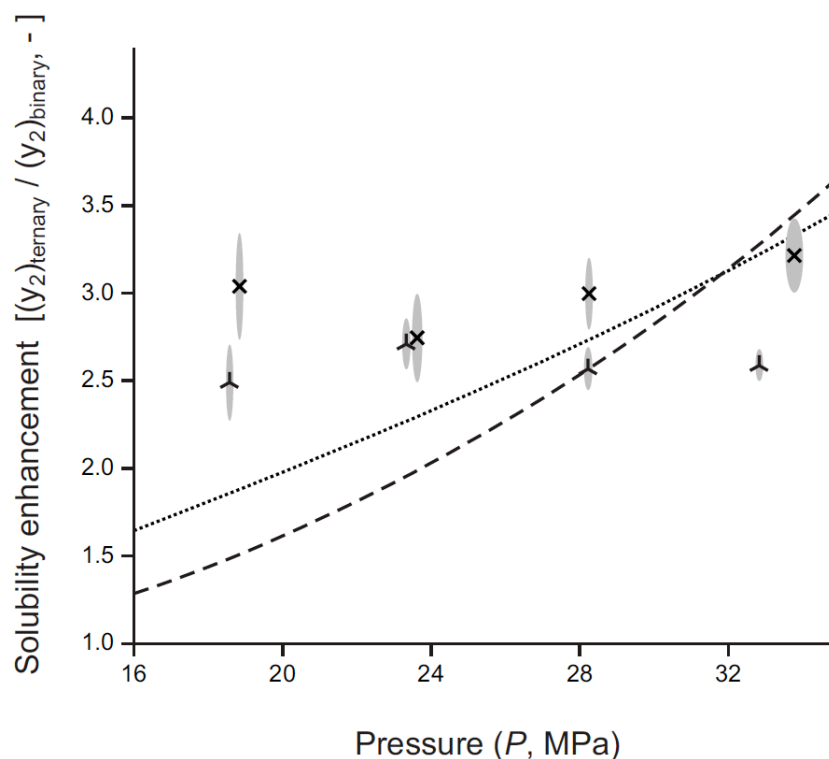


Figure 3.3 Enhancement of capsanthin solubility in supercritical CO₂ (SC-CO₂) resulting from the saturation of the SC-CO₂ phase with triolein as a function of system temperature and pressure. Symbols represent the average of two-to-four experimental measurements at (•••X•••) 313 K or (—A—) 333 K; lines represent predictions using equations (3.2) and (3.3) to estimate the solubility of capsanthin in triolein-entrained SC-CO₂ and equation 3.1 to estimate the solubility of capsanthin in pure SC-CO₂ at same system conditions; and gray ovals represent the standard deviation of pressure and solubility measurements of single data points.

Experimental data in Table 3.1 and Table 3.2 was modeled using equation. 3.3 by best-fitting a semi-empirical extension of the equation the by Méndez-Santiago & Teja (1999) for solvent mixtures (Méndez-Santiago & Teja, 2000):

$$y_2 \times 10^6 = \frac{0.1013}{P} \exp \left(\frac{3.407 \rho_1 + 0.009558 y_3 - 10769}{T} + 16.32 \right) \pm 0.499 \quad (3.3)$$

Authors used equation 3.3 for two purposes, namely to include trend lines in Fig. 3.3, and to analyze the consistency of experimental data, and to compare the solubility of capsanthin in SC-CO₂ with another report in literature. The lines in Fig. 3.3 were computed by replacing equations 3.1-3.3 in the definition of solubility enhancement. For that, values of y_3 estimated using equation 3.2 were replaced in equation 3.3, and the resulting solubility of capsanthin in triolein-saturated SC-CO₂ was divided by the corresponding solubility in pure SC-CO₂ estimated using equation 3.1. Lines in Fig. 3.3 represent average predicted values and do not include indications of dispersion. Caution is suggested in interpreting the lines in Fig. 3.3 because the standard error of predictions of solubility enhancement is large, decreasing from approximately 1.0 at 313 or 333 K and 16 MPa to 0.49 at 313 K and 35 MPa or to 0.25 at 333 K and 35 MPa. Thus, the two isotherms for solubility enhancement largely overlap. It is interesting commenting that the isotherms in Fig. 3.3 cross at approximately 31.8 MPa, which is close to the crossover pressure for triolein solubility in SC-CO₂, as expected.

Equation 3.3 can be rearranged into complex function of y_2 and other system conditions (P , T , y_3) as a linear function of ρ_1 (Fig. 3.4). The collapse of all isotherms to a single line is the basis of the consistency analysis proposed by Méndez-Santiago & Teja (2000) for solute solubility in co-solvent-entrained SC-CO₂, as exemplified in Fig. 3.4, which shows the collapse of the two isotherms for the binary and ternary systems to a single line, suggesting improved data consistency as compared to the previous work (chapter 2). Data for the binary systems (open triangles) behaved better than the data for the ternary systems (black and white symbols), possibly because of the limitations of the assumption that the co-solvent effect is proportional to the concentration of dissolved triolein in the SC-CO₂ phase: the co-solvent effect experimentally observed at low pressures was higher than predicted (see Fig. 3.3). On the other hand, although it is apparent that measurements of Jay *et al.* (1991) at different temperatures also collapse to a single line, their solubility values were considerably lower than measured by us. In comparing the solubility of β -carotene in SC-

CO₂ at 313 K and 30 MPa reported in literature, de la Fuente *et al.* (2006) claimed that the method used by Jay *et al.* (1991) caused errors (measured solubilities too low). Jay *et al.* (1991) used on-line analysis of the SC-CO₂ rich phase to monitor attainment of equilibrium, but it is possible that the signal of the UV-Vis detector they used may be saturated for high concentrations of dissolved carotenoids (de la Fuente *et al.*, 2006). Another source of differences is associated with sample purity, that may change between studies. There was no report of capsanthin purity in the study of Jay *et al.* (1991). On the other hand, the presence of 14% impurities in our study may be responsible for some of the anomalies detected in the present work. Sample impurities could have a co- or antisolvent effect thus respectively increasing or decreasing the solubility of capsanthin measured by us as compared to the true solubility of capsanthin in pure or triolein-entrained SC-CO₂.

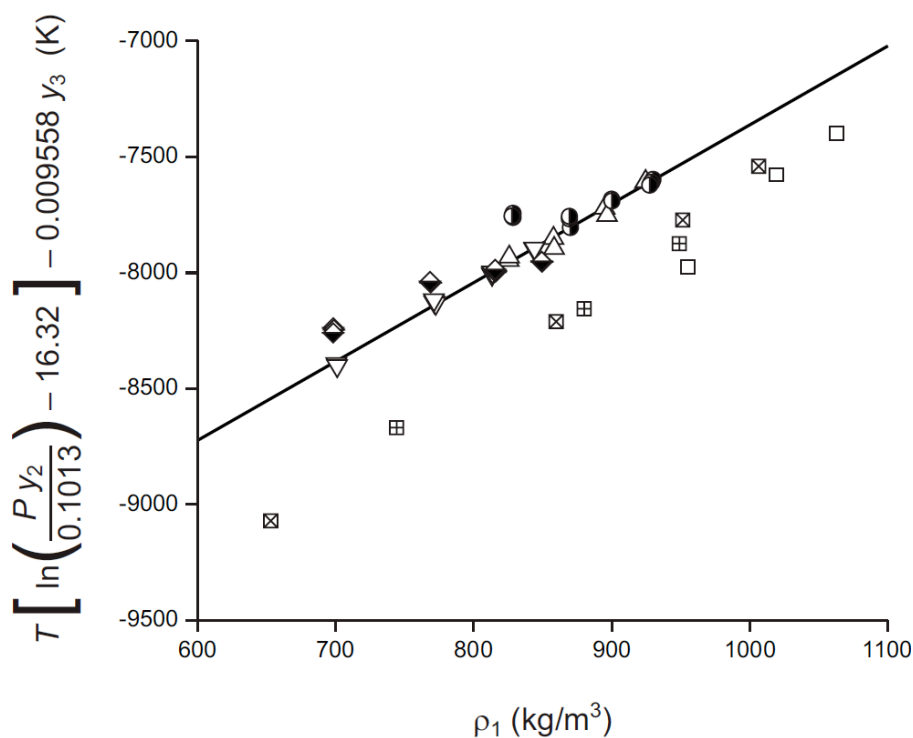


Figure 3.4 Comparison of experimental measurements of the solubility of capsanthin in supercritical CO₂ (SC-CO₂) (symbols) with predictions using equation 3.3 (line). Symbols represent experimental data points using (Δ, ▽) pure or (●, ◆) triolein-entrained SC-CO₂ at (Δ, ●) 313 K or (▽, ◆) 333 K. The plot includes also experimental data points of Jay *et al.* (1991) at (□) 288 K, (⊠) 308 K, or (⊞) 328 K.

Comparison of standard deviations of experimental measurements and inherent errors associated with uncertainties of readings in apparatus' instruments and analytical measurements suggested an adequate reproducibility of results using the adopted experimental procedure. Indeed, the absolute standard deviations of experimental data ($<0.072 \mu\text{mol/mol}$ for capsanthin in the binary system, $<0.098 \mu\text{mol/mol}$ for capsanthin in the ternary system, and $<0.014 \text{ mmol/mol}$ for triolein in the ternary system) were always smaller than corresponding absolute inherent errors estimated as outlined in chapter 2 ($<0.50 \mu\text{mol/mol}$ for capsanthin in the binary system, $<1.3 \mu\text{mol/mol}$ for capsanthin in the ternary system, and $<0.114 \text{ mmol/mol}$ for triolein in the ternary system). Thus, trends suggested by the data and previously analyzed correspond to true trends in the solubility of capsanthin in SC-CO₂ as a function of system temperature and pressure, and dissolution of triolein in SC-CO₂.

3.4 Conclusions

This study contributes new phase equilibrium data for capsanthin isolated from red pepper in pure and triolein-entrained SC-CO₂. In the binary (SC-CO₂ + capsanthin) system solubility increases with system pressure and, for pressures above 16.7 MPa, with system temperature. In the ternary (SC-CO₂ + capsanthin + triolein) system the concentration of triolein in the CO₂-rich phase equals the solubility of triolein in pure SC-CO₂. This is so because the solubility of triolein in SC-CO₂ is about 100-times larger than that of capsanthin in SC-CO₂. On the other hand, dissolution of triolein in SC-CO₂ increases the solubility of capsanthin by a factor of about 3, independent of system temperature and pressure. Because this co-solvent effect of triolein was previously observed for a carotene (β -carotene), it can be claimed that it is due to non-polar interactions between the 18-C fatty acid chains in triolein and the 22-C non-polar core of carotenoids. Experimental reproducibility was adequate in that the absolute standard deviation of solubility between replicates was smaller than absolute inherent error associated with uncertainties of readings in apparatus' instruments and analytical measurements.

4. SUPERCRITICAL CO₂ EXTRACTION OF CAROTENOIDS FROM EXTRUDED RED PEPPER USING HIGH-OLEIC SUNFLOWER OIL AS ENTRAINER

Abstract

The objective of this work was to evaluate the use of extrusion pretreatment and high-oleic sunflower oil as entrainer to improve the supercritical CO₂ extraction of carotenoids from red pepper. Extractions were carried out at 313 or 333 K, 28 or 50 MPa, and using 67 kg CO₂/kg dry substrate (d.s) of pure CO₂ or entrained with 0.53% (w/w) of high-oleic sunflower oil in CO₂ at a constant superficial velocity (1 mm/s). Extraction rate and yield of oleoresin, carotenoid, and capsanthin increased with the increase of temperature, pressure, and addition of high-oleic sunflower oil. The addition of entrainer, in the form of sunflower oil with high-oleic acid content, proved to act as a co-solvent and/or modifier. Experimental data on cumulative extraction yield of total carotenoids and capsanthin were modeled using a Desorption-Dissolution-Diffusion (DDD), model that assumes spherical, porous, and isotropic particles. Model parameters included the external film coefficient and axial dispersion coefficient characterizing external mass transfer phenomena, which were estimated using dimensionless correlation for packed beds operating with supercritical fluids, and the effective diffusivity (D_e) of the extract inside the porous matrix was related to the microstructural features of the extruded substrate, and using apparent solubility as the only best-fitting parameter. Experimental data of total carotenoids and capsanthin extraction performed with pure and entrained-SC-CO₂ were described by the DDD model.

Keywords: Red pepper, extrusion, supercritical CO₂ extraction, capsanthin, entrainer, high-oleic sunflower oil, modeling.

4.1 Introduction

Red pepper (*Capsicum annuum* L.) is a good source of capsanthin and capsorubin, that represent 65-80% of all carotenoids in the fruit, and contribute red color (Weissenberg *et al.*, 1997; Jaren-Galan *et al.*, 1999). Capsanthin, the main carotenoid, is a powerful antioxidant (Hirayama *et al.*, 1994; Matsufuji *et al.*, 1998) and has anticarcinogenic properties (Narisawa *et al.*, 2000). Although red pepper carotenoids can be extracted using organic solvents, global legislation's tightening regulations regarding the use of toxic substances as solvents, have propelled the search of "green" alternatives for the extraction of high-value target solutes from biological substrates, such as supercritical carbon dioxide (CO₂). SuperCritical (SC) CO₂ produces solvent-free extracts with minimal thermal degradation, thus keeping the native bioactivity (Palmer & Ting, 1995; del Valle & Aguilera, 1999).

Although SC-CO₂ can easily solubilize low-molecular-weight and non-polar solutes, its solvent power decreases when the molecular weight of the solute increases, unbranched molecules (denser stereochemistry for compounds of equal molecular weight), and/or bulky (aromatic) and/or polar functional groups appear (Dandge *et al.*, 1985). In the case of low-solubility compounds such as carotenoids, SC-CO₂ can be complemented with entrainers that may increase the solubility (co-solvent effect) and/or help to release the solute from the solid matrix (modifier effect). Indeed, entrainers may generate specific CO₂-entrainer-solute interactions enhancing the solubility of the solute in entrained as compared to pure SC-CO₂, may compete with the solute for binding sites on the vegetable matrix, thus facilitating its desorption, and/or may swell the biological matrix thus facilitating solute movement within the substrate (chapter 2). For pharmaceutical and food applications, it is important to use entrainers having GRAS (Generally Recognized as Safe) status, such as vegetable oils (Moyler, 1993). Vegetable oils have been used as entrainers for the SC-CO₂ extraction of carotenoids from various substrates, including lycopene from tomato (Vasapollo *et al.*, 2004; Ciurlia *et al.*, 2009; Shi *et al.*, 2009; Saldaña *et al.*, 2010), α - and β - carotene and lutein from carrot (Sun & Temelli, 2006), lutein esters from marigold flowers (Ma *et al.*, 2008; Gao *et al.*, 2010; Palumpitag *et al.*, 2011), and astaxanthin from

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microalgae (Krichnavaruk *et al.*, 2008) using peanut and almond kernel (Vasapollo *et al.*, 2004), hazelnut (Vasapollo *et al.*, 2004; Ciurlia *et al.*, 2009), sunflower seed (Vasapollo *et al.*, 2004; Gao *et al.*, 2010), olive fruit (Krichnavaruk *et al.*, 2008; Shi *et al.*, 2009; Palumpitag *et al.*, 2011), rape seed (Sun & Temelli, 2006; Gao *et al.*, 2010), soybean (Krichnavaruk *et al.*, 2008; Ma *et al.*, 2008; Gao *et al.*, 2010; Palumpitag *et al.*, 2011), and palm (Palumpitag *et al.*, 2011) oils as entrainers, which were mixed with the substrate in the extraction vessel prior to extraction (Vasapollo *et al.*, 2004; Ma *et al.*, 2008; Ciurlia *et al.*, 2009; Palumpitag *et al.*, 2011) or fed continuously together with SC-CO₂ using a complimentary co-solvent pump (Sun & Temelli, 2006; Krichnavaruk *et al.*, 2008; Gao *et al.*, 2010). Shi *et al.* (2009) do not inform how they added the entrainer in their study.

Besides the use of an entrainer to improve the extraction of low-solubility solutes, substrate pretreatment can be devised to improve SC-CO₂ extraction of biological substrates. Extrusion allows the rupture of tissues and cell walls and releases solutes from the inner part of cells, on one hand, and restructure the substrate into a high-density matrix with interconnected pores, on the other. The destruction of inner mass transfer barriers and densification caused by extrusion can maximize the volumetric productivity of a SC-CO₂ extraction plant (weight of extract per unit volume of the extractor and per unit time) (del Valle *et al.*, 2003). A seminal contribution to the use of densification for the SC-CO₂ extraction of vegetable substrate is the work of Hallberg *et al.* (1989) with hops. Gardner (1982) inform that α -acid recovery from hop cones in CO₂ extraction experiments increases from 75-80% when using a hammer mill, to 95% when using an extruder, which they claimed was due to improved, shear-induced cell wall rupture. They also reported an increase in bulk density from 150-250 kg/m³ in milled hops to 400 kg/m³ in extruded hops. Uquiche *et al.* (2004) densified red pepper (*Capsicum annuum* L.) in a pellet mill, and later Uquiche *et al.* (2005) compacted Jalapeño pepper using an extruder prior to SC-CO₂ extraction of carotenoid-rich and capsaicin-rich oleoresins, respectively. These two pretreatments differ in that pelletized mill flakes of dried material are forced into cylindrical openings and shearing results from friction of the substrate against the walls of the channel, whereas in the extruder moistened particles of milled material are forced into a spiraling and contracting channel by a screw that turns within a heated barrel. In the second

case, plasticized material emerges through the constricting opening placed at the end of the barrel (die) and water flashes to an extent dependent on the moisture content and final temperature of the material.

Mathematical models are difficult to implement, as many parameters must be considered to address microstructure-extractability and internal mass transfer of solute in the extraction, for example. However, this information plays an essential role for delivering real information for designing and scaling-up the SC extraction process (del Valle & Urrego, 2012). In this study, we use the Desorption-Dissolution-Diffusion (DDD) model reported by Goto *et al.* (1998) to describe the extraction processes, correlating the effective diffusivity or transfer rate of solute with microstructural features that quantify the effect of the inner barriers into porous substrate, such as inner porosity of particle and connectivity of the network of pore, and using apparent solubility as the only best-fitting parameter.

The aim of this work was to study and model the effects of SC-CO₂ extraction temperature and pressure, and use of High-Oleic Sunflower Oil (HOSO) on apparent solubility and yield of oleoresins, total carotenoids, and capsanthin extracted from extruded red pepper flakes.

4.2 Materials and Methods

4.2.1 Substrate for extraction

Commercial flakes of red pepper containing 5.1% dry basis (d.b.) water and 3.03% d.b. oleoresin were obtained from an agroindustrial company (Invertec Foods, Rengo, Chile). Moisture content was determined gravimetrically by drying in an oven for 15 h at 105 °C, whereas the oleoresin content was determined gravimetrically by extraction at room temperature (22 °C) using HPLC-grade acetone (J.T. Baker, Phillipsburg, NJ, USA) (Philip, & Francis, 1971). The material was ground in a disc mill (4E Grinding Mill, The Straub Company, Philadelphia, PA) to pass a 14-mesh screen (particle diameter ≤ 1.18 mm), moistened to 30% d.b. with tap water and extruded in a laboratory single-screw apparatus Haake PolyDrive 0-120 Nm (Thermo Electron, Karlsruhe GmbH, Germany). The extrusion conditions were screw speed of 46 rpm and die temperature of 80 °C. The extruded vegetable substrate was dried to 9.37% d.b. in a convection oven set at 40 °C. The 4 mm

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extrudate was manually cut into 4 mm long pieces so that particles were shaped as spheres with a Sauter diameter (d_{ps}) of 4 mm.

4.2.2 Characterization of substrate

The bulk density of the extruded material in a packed bed (ρ_b) was determined using the gravimetric procedure of Uquiche *et al.* (2004). The true density of the substrate (ρ_s), on the other hand, was determined by N₂ pycnometry in a Ultrapyc 1200e (Quantachrome, Boynton Beach, FL). The inner porosity (ϵ_p) of the extrudate was determined by Hg porosimetry in a Macropores Unit 120 and Porosimeter 2000 (Carlos Erba Instrument, Milano, Italy) and computed using Milestone 200 software.

The tortuosity (τ) of extrudate was estimated by fractal-texture analysis of binary light-microscopy images, as done by Uquiche *et al.* (2004). Following sample fixing, paraffin embedding, slicing, and staining, images of thin sections were obtained by means of an Olympus BX50 light microscope (Tokyo, Japan) that was equipped with a JVC TK-1280E video camera (Yokohama, Japan) by using Studio DC10 plus software (Pinnacle System Inc., Braunschweig, Germany).

4.2.3 Supercritical extraction and analysis of extracts

Supercritical extractions were carried out at 313 or 333 K and 28 or 50 MPa in a Spe-ed SFE unit (Applied Separations, Allentown, PA) after loading 24.8 g dry substrate (d.s.) into the 50-cm³ extraction vessel (14 mm inner diameter). In these experiments, extraction pressure was manually controlled with an air-booster pump and the temperature of the air convection oven containing the extraction vessel was automatically controlled. Depending on the extraction temperature and pressure, 4.2 to 5.1 L NPT/min of food grade (99.8% pure) CO₂ (AGA S.A., Santiago, Chile) was fed to the extraction vessel so as to maintain a constant superficial velocity $U = 1$ mm/s. A 20 min static extraction period was followed by a dynamic extraction period; the expansion valve was kept at 120 °C. In all experiments, the total mass of CO₂ consumed was 1.65 kg. In extractions involving an entrainer, HOSO (Camilo Ferrón Chile S.A, Chile) was pumped into the feed line of CO₂ at a mass flow rate of 45 mg/min of oil by using a HPLC pump (Knauer model K-501, Berlin, Germany).

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A number of oleoresin samples were taken in pre-weighted glass vials (60 cm³ capacity), covering the entire extraction of about 200 min approximately. Sampling was carried out every 5 min or so at the beginning of the run, but spaced to longer intervals towards the end of the run (see Figures for sampling frequency, converted to Kg CO₂/kg d.s.). Mass of oleoresin aliquots was assessed gravimetrically by difference with cleaned and dried vials after removing co-extracted water in a desiccator with silica gel. A sample of oleoresin was dissolved in chloroform p.a. (Merck, Darmstadt, Germany) and total carotenoid content of oleoresin aliquots (mg carotenoids/g oleoresin) was quantified at 460 nm using a SP-2000 UV-Vis spectrophotometer Thermo Fisher Scientific (Waltham, MA, USA) (Uquiche *et al.*, 2012). The standard for the analysis of carotenoids (β -carotene type I, $\geq 95\%$ pure) was obtained from Sigma-Aldrich (Saint Louis, MO, USA).

Oleoresin samples were analyzed by HPLC following saponification of esterified carotenoids in the oleoresin, as described previously in chapter 3. Identification and quantification of capsanthin (mg capsanthin/g oleoresin) were done by HPLC (Hitachi LaChrom, Tokyo, Japan) using the modified method of Weissenberg *et al.* (1997). Calibration curves were prepared with standard solutions of capsanthin (86% pure) which was extracted, isolated, and purified from red pepper as in our previous work (chapter 3) in HPLC grade acetone (J.T. Baker, Phillipsburg, NJ, USA) and quantification of capsanthin was determined from the calibration curves. Separation of carotenoids was performed by reversed-phase column (YMCTM Carotenoid, C30, 5 μ m particle size, 250 \times 4.6 mm i.d.) (Waters symmetry column, Waters, Milford, MA) at 30 °C and flow rate 2 mL/min under isocratic conditions. The composition of the mobile phase used was 75:25 (v/v) of acetonitrile and 2-propanol, respectively, solvents of HPLC grade (Merck, Darmstadt, Germany). Capsanthin was monitored at 450 nm in a UV diode array detector (Hitachi LaChrom, Tokyo, Japan) and was identified by comparison with standard and from their retention time.

4.2.4 Mathematical model

Experimental data on cumulative extraction yield (mg extract/kg d.s) of total carotenoids and capsanthin were modeled using the Desorption-Dissolution-Diffusion (DDD) model

(Goto *et al.*, 1998). This model supposes desorption and dissolution of a solute inside the pores of a porous and homogeneous particle, and diffusion until reaching the surface.

The following simplifying assumptions were used to implement this model: (i) the extraction is an irreversible desorption process; (ii) the substrate is spherically-shaped (radius R), porous particle where a single pseudo-solute is uniformly distributed initially; (iii) the system is isothermal; (iv) the physical properties of the SC Fluid (SCF) remain constant during the extraction; (v) axial dispersion (D_L) phenomena is considered. The model is based on a differential mass balance for SCF in the void spaces of the extractor (equation 4.1) coupled with a differential equation for SCF inside the pores of solid particle (equation 4.2) through a flux term (J) of pseudo-solute transfer from the particle surface to the SCF given by equation 4.3. It was assumed that equilibrium is established instantaneously in the pores due to relatively fast desorption, which can be characterized by a constant partition coefficient of solute ($K = C_s/C_p$) between the solid matrix and fluid phase within the pores. The solute concentration in the solid phase loaded into the extractor vessel at zero time of extraction C_{so} and C_{po} the concentration of dissolved solute within substrate pores are determined by the initial total concentration of solute (C_o) and the equilibrium relation between both phases (Goto *et al.*, 1998):

$$\frac{\partial C_f}{\partial t} + u \frac{\partial C_f}{\partial z} - D_L \frac{\partial^2 C_f}{\partial z^2} = \frac{1-\epsilon}{\epsilon} J \quad (4.1)$$

$$\frac{\partial C_p}{\partial t} = \frac{D_e}{\epsilon_p + K(1-\epsilon_p)} \left(\frac{\partial^2 C_p}{\partial r^2} + \frac{2}{r} \frac{\partial C_p}{\partial r} \right) \quad (4.2)$$

$$J|_{z,t} = \frac{3 k_f}{R} \left(C_p|_{r=R,z,t} - C_f|_{z,t} \right) \quad (4.3)$$

where C_f is the concentration in the SFC phase in the bed, t is the extraction time, z is the axial coordinate of the extractor vessel, and r is the radial position within the particle.

These equations were solved numerically using a fourth-order Runge-Kutta method implemented in MATLAB 6.0 (Math Works, Natick, MA) using C_{po} (concentration of solute in SCF or apparent solubility) as the single adjustable parameter of the model for

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each cumulative extraction plot. The following initial and boundary conditions were considered for numerical solution:

$$C_f = 0 \quad (t = 0, \quad 0 \leq z \leq L) \quad (4.4a)$$

$$uC_f - D_L \frac{\partial C_f}{\partial z} = 0 \quad (z = 0, \quad t \geq 0) \quad (4.4b)$$

$$\frac{\partial C_f}{\partial z} = 0 \quad (z = L, \quad t \geq 0) \quad (4.4c)$$

$$C_p|_{r,z,t=0} = C_{po} \quad (4.4d)$$

$$\left. \frac{\partial C_p}{\partial r} \right|_{r=0,z,t} = 0 \quad (4.4e)$$

$$-D_e \left. \frac{\partial C_s}{\partial r} \right|_{r=R,z,t} = k_f \left(C_p|_{r=R,z,t} - C_f|_{z,t} \right) \quad (4.4f)$$

where L is the height of the packed bed.

4.2.5 Model parameters

The film mass transfer coefficient (k_f) was estimated from the dimensionless correlation of Puiggené *et al.* (1997), the axial dispersion coefficient (D_L) was estimated using the correlation of del Valle *et al.* (2011), which required estimates of the physical properties of loaded SC-CO₂ phase. Density (ρ) and viscosity (μ) of the SC-CO₂ were estimated using the NIST database for pure CO₂. The binary diffusion coefficient (D_{12}) was estimated as a function of the reduced temperature ($T_r = T/T_c$, $T_c = 304.3$ K) and reduced density ($\rho_r = \rho/\rho_c$, $\rho_c = 468$ kg/m³) of the solvent, and the molecular weight (MW_2) and critical volume (V_{c2}) of a representative mixture or solute using the correlation of Catchpole & King (1994). V_{c2} was estimated for the representative solute in extracts using Joback's modification of Lydersen's group contribution method (Poling *et al.*, 2000). Table 4.1 summarizes experimental conditions and physical properties of SC-CO₂ and extracts, and model parameters for all experiments.

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Table 4.1 Experimental conditions, physical properties of SC-CO₂, and model parameters.

| T K | P MPa | HOSO %(w/w) | ρ kg m ⁻³ | μ Pa s $\times 10^5$ | $^*D_{12}$ m ² s ⁻¹ $\times 10^9$ | $^{**}D_{12}$ m ² s ⁻¹ $\times 10^9$ | *D_L m ² s ⁻¹ $\times 10^6$ | $^{**}D_L$ m ² s ⁻¹ $\times 10^6$ | *k_f m s ⁻¹ $\times 10^6$ | $^{**}k_f$ m s ⁻¹ $\times 10^6$ | *D_e m ² s ⁻¹ $\times 10^{10}$ | $^{**}D_e$ m ² s ⁻¹ $\times 10^{10}$ | $^*C_{po}$ g kg ⁻¹ $\times 10^2$ | $^{**}C_{po}$ g kg ⁻¹ $\times 10^2$ | *K (-) | $^{**}K$ (-) |
|----------|------------|----------------|------------------------------|-----------------------------|--|---|---|--|--|---|--|---|--|---|--------------|-----------------|
| 313 | 28 | 0 | 898.33 | 9.10 | 3.49 | 4.07 | 4.02 | 4.02 | 10.45 | 11.59 | 2.17 | 2.53 | 2.71 | 0.44 | 30.55 | 72.96 |
| 313 | 50 | 0 | 991.15 | 11.77 | 2.81 | 3.28 | 4.01 | 4.01 | 8.40 | 9.32 | 1.75 | 2.04 | 6.18 | 1.45 | 12.14 | 20.04 |
| 333 | 28 | 0 | 813.76 | 7.39 | 4.48 | 5.24 | 4.02 | 4.02 | 13.01 | 14.43 | 2.79 | 3.26 | 3.17 | 0.60 | 28.86 | 58.75 |
| 333 | 50 | 0 | 933.36 | 9.99 | 3.42 | 4.0 | 4.02 | 4.02 | 10.06 | 11.16 | 2.13 | 2.49 | 7.14 | 2.16 | 11.16 | 14.26 |
| 313 | 28 | 0.53 | 898.33 | 9.10 | 3.49 | 4.07 | 4.02 | 4.02 | 10.45 | 11.59 | 2.17 | 2.53 | 4.96 | 2.01 | 16.69 | 15.94 |
| 313 | 50 | 0.53 | 991.15 | 11.77 | 2.81 | 3.28 | 4.01 | 4.01 | 8.40 | 9.32 | 1.75 | 2.04 | 13.60 | 5.68 | 5.52 | 5.11 |
| 333 | 28 | 0.53 | 813.76 | 7.39 | 4.48 | 5.24 | 4.02 | 4.02 | 13.01 | 14.43 | 2.79 | 3.26 | 7.23 | 2.50 | 12.64 | 14.17 |
| 333 | 50 | 0.53 | 933.36 | 9.99 | 3.42 | 4.0 | 4.02 | 4.02 | 10.06 | 11.16 | 2.13 | 2.49 | 20.59 | 9.31 | 3.87 | 3.31 |

(*) Total carotenoids; (**) Capsanthin

4.3 Results and Discussion

This section is divided into three subsections, in which we present the characterization of the extruded substrate, the effects of temperature and pressure on the extraction kinetics of oleoresin, total carotenoids, and capsanthin from extruded red pepper using pure and HOSO-entrained SC-CO₂ are discussed, and the experimental data of cumulative extraction yield *versus* specific CO₂ mass are compared with predictions made using DDD model for total carotenoids and capsanthin.

4.3.1 Characterization of the extruded substrate

There was a 2.9-fold increase in the amount of substrate loaded into the extraction vessel when flakes ($\rho_b = 173 \text{ kg/m}^3$) were compared to extruded red pepper ($\rho_b = 496 \text{ kg/m}^3$), similar to that reported by Gardner (1982), Hallberg *et al.* (1989), del Valle *et al.* (2003), and Uquiche *et al.* (2004, 2005). The porosity of the bed (ϵ_b) was estimated as a function of ρ_b , ρ_s , and ϵ_p according to equation 4.5:

$$\epsilon_b = \frac{1 - \rho_b / \rho_s - \epsilon_p}{1 - \epsilon_p} \quad (4.5)$$

which discounts from the total void volume in the packed bed the void volume within substrate particles. Where the ϵ_b was 0.57 which depends on the values $\rho_s = 1421 \text{ kg/m}^3$ and $\epsilon_p = 0.181$, the d_{ps} of extruded red pepper was 4 mm.

Extrusion causes an increase in the densification of red pepper flakes due to the new microstructure adopted by extruded red pepper, where void spaces were occupied with cellular tissue and constituents of substrate that were released by breakage and deformations of thermoplastic material. Figure 4.1 clearly shows the change in microstructure of flakes (Fig. 4.1A) *versus* extruded red pepper (Fig. 4.1B). The microscopic images confirm the damage of the cell wall, and destruction of inner barriers to mass transfer of target solute within the matrix due to high-shear stresses caused during extrusion, in accordance with the reports for pelletized red pepper (Uquiche *et al.*, 2004) and extruded Jalapeño pepper (Uquiche *et al.*, 2005). Specifically, carotenoids are stored in

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the structure as thylakoid membranes and organelle as plastids, which are ruptured, revealing the carotenoid pigments (Cseke *et al.*, 2006), increasing the availability of carotenoids to SC-CO₂ for extraction from vegetable substrate.

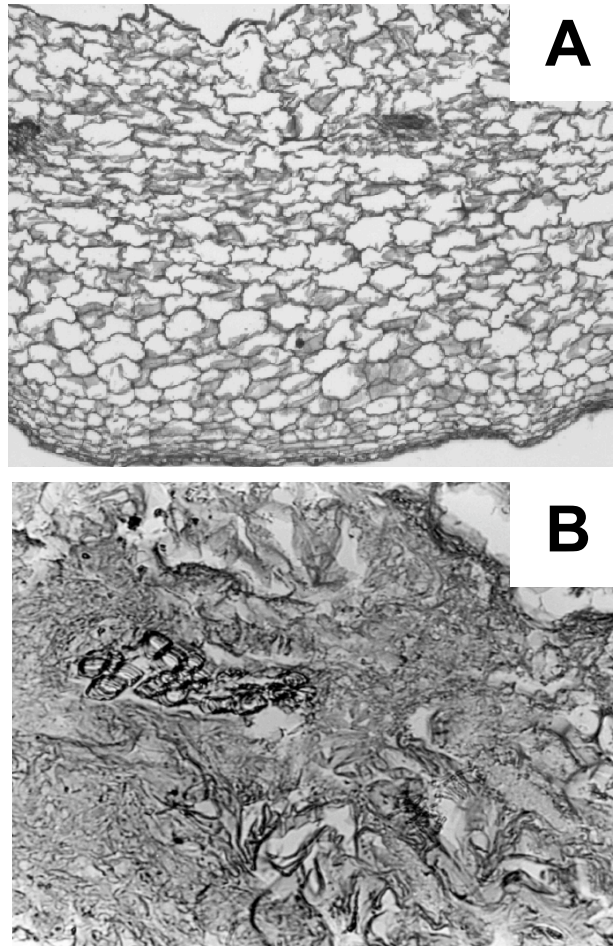


Figure 4.1 Light microscopy images of flakes (A) and extruded red pepper (B).

The internal mass transfer of solute in a porous vegetable substrate can be related to an effective diffusivity (D_e), which was estimated as a function of the binary diffusion coefficient (D_{12}) of a representative solute (component 1) in CO₂ (component 2) and of the relation between ϵ_p and τ or microstructural factor (F), which quantifies the resistance in internal mass transfer of solute by the effect of the inner barriers in the porous substrate, using equation 4.6 (Cussler, 1997):

$$D_e = D_{12} \frac{\varepsilon_p}{\tau} \quad (4.6)$$

Tortuosity (τ), a value that quantifies the intricate passage of a solute through the porous network (Anderson *et al.*, 1996), was determined as a function of fractal parameters D_m and d which are used to describe the heterogeneity of the porous space, and the connectedness of a network of pores, respectively. In this work, D_m , d , and τ were 1.81, 1.50, and 2.91, respectively, which were higher for the extrudate used in this work compared to those for pelletized material reported by Uquiche *et al.* (2004). In both pretreatment cases, the value of ρ_b was increased, but using extrusion and high moisture, may impeded densification when compared to pelletization, causing elastic deformation and discouraging plastic deformation; thus, forming scaffolds of interconnected pores network (del Valle *et al.*, 2003). Inner porosity for extruded and pelletized materials were $\varepsilon_p = 0.181$ (this work) and $\varepsilon_p = 0.17$ (Uquiche *et al.* (2004)), respectively, which would explain slower internal mass transfer in extruded material when compared to pelletized version.

4.3.2 Effect of temperature and pressure of pure and HOSO-entrained SC-CO₂ on the extraction kinetics

We studied the effects of temperature and pressure on cumulative yield of oleoresin, total carotenoids, and capsanthin *versus* specific CO₂ mass for extraction of extruded red pepper using pure SC-CO₂ at 313 and 333 K and 28 and 50 MPa (Fig. 4.2). Talking into consideration that two competing effects would influence the yield of the extraction process, we found that, at isothermal conditions, the prevailing effect is increased solvent power due to higher densities of CO₂ at higher pressures, while at isobaric conditions, the prevailing effect is increased vapor pressure/volatility of the solute with temperature. As an illustration of the first effect, increasing the pressure from 28 to 50 MPa, the yield increased from 8.62 to 9.78 g oleoresin/kg d.s. at 313 K or from 8.68 to 10.63 g oleoresin/kg d.s. at 333 K (data not shown). The effect of volatility is demonstrated by an increase in yield from 8.62 to 8.68 g oleoresin/kg d.s. at 28 MPa or from 9.78 to 10.63 g oleoresin/kg d.s. at 50 MPa when temperature is increased from 313 to 333 K (data not shown). The extraction

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yield of oleoresin, total carotenoids, and capsanthin increased to a greater extent due to the effect of pressure than temperature (Fig. 4.2), demonstrating the effect of solvent power of CO₂ enhancing the extraction yield and mass transfer of solute to supercritical phase (Mukhopadhyay, 2000) within the range under study. The increase in the extraction yield of oleoresin, total carotenoids, and capsanthin from extruded red pepper as result of increased pressure has been described in the literature. For example, there was a 3-fold increase in the total carotenoids concentration in the oleoresin extracted from paprika upon increasing the pressure from 13.8 to 41.4 MPa (Jaren-Galan *et al.*, 1999). Maximum recovery of carotenoid extracted from ground pungent paprika was reached by increasing the pressure until 40 MPa (Gnayfeed *et al.*, 2001; Daood *et al.*, 2002) and the increase of pressure from 30 to 50 MPa enhanced the extraction rate of carotenoids from paprika (Ambrogi *et al.*, 2002). Using 40 MPa instead of 20 MPa resulted in the maximum extraction yield of oleoresin from ground red pepper (Tepic *et al.*, 2009). At higher values for pressure and temperature (35 MPa and 333 K), the maximum extraction yield and rate of oleoresin extracted from red pepper were reached (Kwon *et al.*, 2011). Uquiche *et al.* (2004) also reported an improvement in oleoresin extraction from pelletized red pepper. The yield had a sharp increase due to the effect of pressure, raising from approximately 35 to 55.3 g oleoresin/kg substrate when the pressure was increased from 32 to 54 MPa at 313 K. However, there are differences attributable to the pretreatment of raw material on yield and kinetics of extraction, when extrusion in this work was compared with pelletization of red pepper reported previously (Uquiche *et al.*, 2004), as higher particle size was used for extrudate than pellets, which reduced the specific surface area to mass transfer, complicating the path of solute within the porous solid, increasing the resistance to mass transfer. Also, the high moisture content of vegetable substrate used for extrusion might induce elastic deformation, causing slower velocity and mass transfer of solute through the pores, due to higher heterogeneity of the interconnected pores network and ϵ_p , compared with pretreatment by pelletization. The degree of ripeness of the fruit affects the oleoresin content of the plant material. The distribution of the oily matter within the matrix and the integrity of the oleoresin during the storage period are also factors that must be taken into consideration when comparing work by different authors.

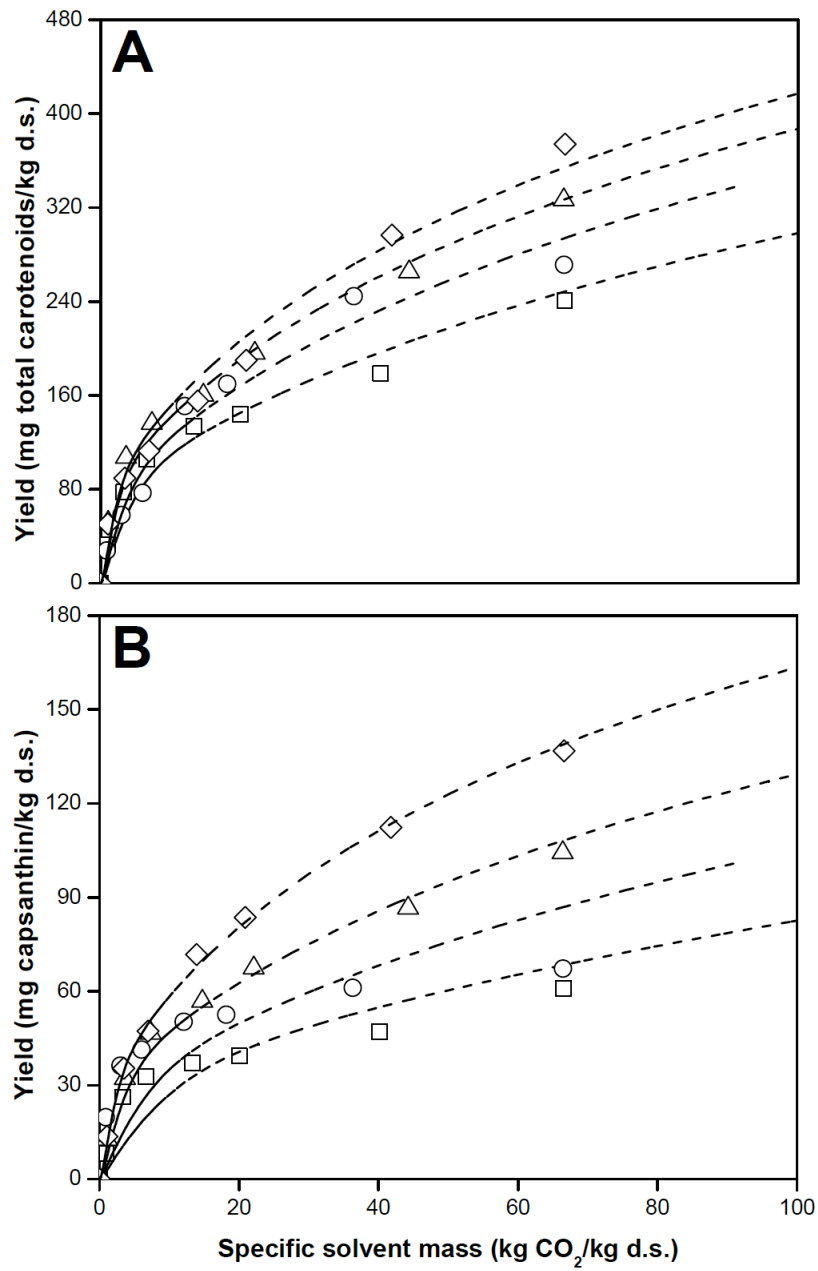


Figure 4.2 Effect of temperature and pressure on cumulative yield of total carotenoids (**A**) and capsanthin (**B**) *versus* specific CO₂ mass extracted from extruded red pepper using pure SC-CO₂ at 28 MPa using (□) 313 K and (○) 333 K; 50 MPa using (△) 313 K and (◇) 333 K. Dotted line represents the DDD prediction model (---).

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A typical extraction curve is divided into three stages. The first portion represents the solubilization of relatively free solutes in SC-CO₂, which also assumes to be in enough quantities to saturate the incoming fluid, using low flow rate allowing sufficient residence time. This saturation is known as apparent solubility (C_{po}) and it is obtained from the slope of the extraction yield *versus* CO₂ consumption curve. Specifically, for this work the apparent solubility was obtained as one of the adjustable model parameters (see section 4.3.3), as these experimental values were not in agreement with the effects of pressure and temperature and this behavior was associated with the uncertainty of the experimental apparatus and analytical measurements. In the second stage, the slope of the extraction curve decreases due to insufficient quantities of free solute on the particle surface having SC-CO₂ to diffuse into the substrate for solubilize the solutes, where there are restrictions to inner mass transfer of solutes through the matrix, which are exerted by the lattice to desorb the solute attached in the substrate. The third and final period corresponds to the maximum yield that can be obtained at the experimental conditions of extraction imposed in the system (Brunner, 1994). Breaking down below extraction curve into stages (Fig. 4.2A and 4.2B), it can be observed that the initial rate at favorable conditions (*e.g.*, 50 MPa and 333 K) is higher than milder conditions (*e.g.*, 28 MPa and 313 K), indicating that the static period at zero time allows a larger amount of solubilized solute in the supercritical phase. As extraction progresses, the rate of the curve is slower, being limited by internal mass transfer resistances within the vegetable substrate (Fig. 4.2), which was accentuated by the sample pretreatment. Despite releasing compounds from the matrix by the effect of reduction of barriers to mass transfer in red pepper, the pretreatment might reduce the transport properties of SC-CO₂, making it difficult for the solvent to flow through the packed bed, loaded with a porous highly densified substrate, shaped with interconnected pores network, highly heterogeneous, and large particle size. Finally, in the extraction yield *versus* extraction time curves (not shown), the combination of experimental conditions used indicate that the extraction time (3 h) employed was not enough to deplete the vegetable substrate.

Figure 4.3 illustrates the effect of HOSO as an entrainer on the amount of oleoresin, total carotenoids, and capsanthin extracted from extruded red pepper with respect to extraction

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time. The extraction kinetic of the actual oleoresin generated from the raw material itself was difficult to derive due to the small yield obtained, which coupled with entrainer oil, only amounted to 9.9% of extract. The continuous addition of HOSO, we understand, would eventually dilute the native oleoresin to a minimum, but it would be impossible to estimate exactly when this would occur. It is feasible to establish a differentiation between triglycerides by analytical means, of course, but this was beyond the scope of the current work. Nevertheless, a plot of total carotenoids in the extract mixture was generated. Despite the advantages of using entrainers to enhance the efficiency of extraction of solutes that have limited solubility in pure SC-CO₂, the way in which an entrainer is added does not always ensure a constant concentration in the system during extraction, unlike the extraction process where the entrainer is added directly to the vegetable substrate, in which case its concentration changes during the dynamic period. An fundamental aspect to consider is to avoid possible phase separation of the mixture entrained-SC-CO₂ (Page *et al.*, 1992). We predicted the solubility of vegetable oil in SC-CO₂ using the equation of del Valle *et al.* (2012) to ensure a monophasic mixture of HOSO and SC-CO₂ in the supercritical phase.

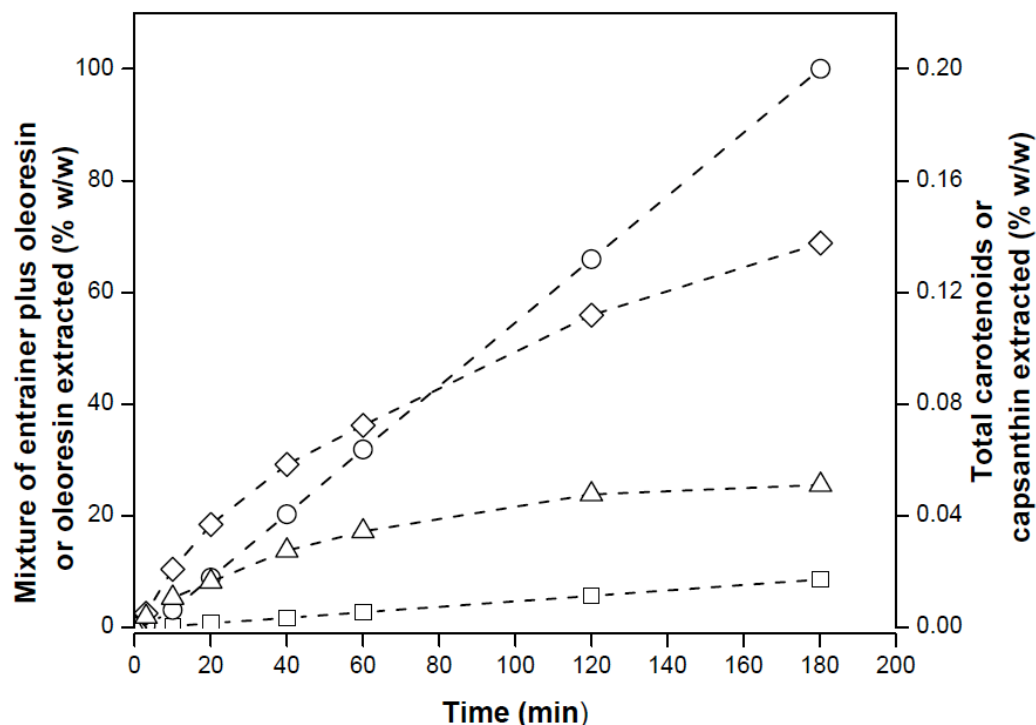


Figure 4.3 Effect of continuous addition of HOSO on the amount of oleoresin (\square), total carotenoids (\diamond), and capsanthin (\triangle) compared with the all recovery of the mixture HOSO plus oleoresin (\circ), extracted from extruded red pepper *versus* extraction time using pure SC-CO₂ at 313 K and 50 MPa. Dotted lines represent the trend.

The extraction yield obtained after 3 h with HOSO-entrained SC-CO₂ for total carotenoids ranged from 349.60 to 574.96 mg/kg d.s and capsanthin was from 120.27 to 222.51 mg/kg d.s at experimental conditions tested (Fig. 4.4), with yields of 2.4-fold higher for total carotenoids and 3.7-fold higher for capsanthin when comparing HOSO-entrained SC-CO₂ with pure SC-CO₂. Cold acetone extractions for this raw material gave 770.22 mg total carotenoids/kg d.s and 316.62 mg capsanthin/kg d.s. Figure 4.4 shows an increase in extraction yield and extraction rate due to both pressure and temperature using HOSO-entrained SC-CO₂, with trends similar to those obtained with pure SC-CO₂ for extraction yield of total carotenoids and capsanthin (Fig. 4.2).

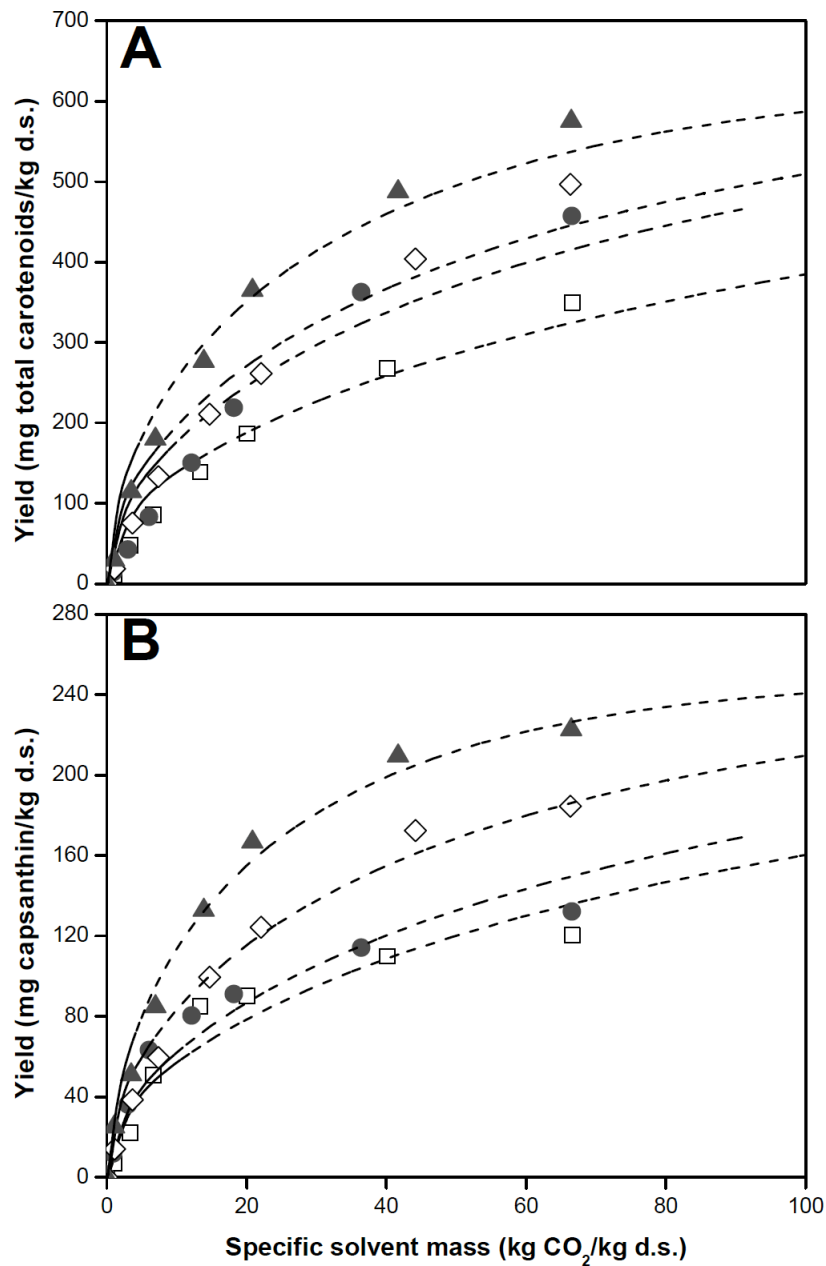


Figure 4.4 Effect of temperature and pressure on cumulative yield of total carotenoids (**A**) and capsanthin (**B**) *versus* specific CO₂ mass extracted from extruded red pepper using HOSO-entrained SC-CO₂ at 28 MPa using (□) 313 K and (●) 333 K; 50 MPa using (◇) 313 K and (▲) 333 K. Dotted line represents the DDD prediction model (---).

A global view of comparative curves on the extraction yield for components extracted from extruded red pepper using pure and HOSO-entrained SC-CO₂ *versus* specific CO₂ mass, at

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333 K and 50 MPa is shown in Figure 4.5. A parallel increase in cumulative yield of oleoresin, total carotenoids, and capsanthin was observed as extraction progressed. Oleoresin is a complex mixture of lipid matter and pigments (Jaren-Galan *et al.*, 1999). Lipid compounds have higher solubility in SC-CO₂ than carotenoids, which help to increase the solubility of less soluble compounds. The entrainer effect of more soluble components on less soluble components in SC-CO₂ has been reported for mixtures of triglycerides *e.g.*, the tripalmitin solubility in SC-CO₂ was increased by the presence of trilaurin or trimyristin, which are triglycerides having lower molecular weight and higher solubility in pure SC-CO₂ than tripalmitin (Bamberger *et al.*, 1988). Extraction of carotenoids, whether they are bound or free in the matrix, seems to be highly dependant on native oleoresin and certainly influenced with the addition of HOSO. In general, solubility of extractable components is increased by higher concentrations of entrainer (Dobbs *et al.*, 1986; Dobbs *et al.*, 1987).

On the other hand, the highest extraction yield of total carotenoids and capsanthin were reached at 50 MPa and 333 K, the highest pressure and temperature tested. Therefore, this experimental condition was compared with the extraction yield of total carotenoids and capsanthin obtained using pure SC-CO₂ (Fig. 4.5). There was an improvement in the extraction of total carotenoids and capsanthin that is HOSO-induced. The results obtained indicate the co-solvent and/or modifier effect of HOSO on the extraction yield from extruded red pepper; this effect may be justified by the presence of HOSO in the system, which benefits the interactions between target solutes-entrainer-CO₂ increasing its solubility in the supercritical phase, and/or modify the vegetable matrix, and/or compete to occupy the active sites facilitating the adsorption of oil into matrix where the solute is bound, increasing its desorption and mass transfer to the entrained supercritical phase (chapter 2). We used HOSO as an entrainer, having triolein as the main triglyceride (Ruiz-Gutiérrez *et al.*, 1998). Triolein has a high solubility in SC-CO₂ compared with carotenoids (Chrastil, 1982; Cygnarowicz *et al.*, 1990; Jay *et al.*, 1991; Nilsson *et al.*, 1991; Sakaki, 1992; Nilsson & Hudson, 1993; Škerget *et al.*, 1995; Johannsen & Brunner, 1997; Mendes *et al.*, 1999; Cocero *et al.* 2000; Naranjo-Modad *et al.*, 2000; Hansen *et al.*, 2001; Sovová *et al.*, 2001; Kraska *et al.*, 2002; Güçlü-Üstündağ & Temelli, 2004; de la Fuente *et al.*,

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2006), which can generate non-polar type of interactions between monounsaturated fatty (oleic acid) acid chains in the triglyceride and hydrocarbon of non-polar central skeleton of capsanthin, increasing its extraction yield. A similar effect was observed for non-polar and polar carotenoids extracted from vegetable substrates using vegetable oil-entrained-SC-CO₂ (Vasapollo *et al.*, 2004; Sun & Temelli, 2006; Krichnavaruk *et al.*, 2008; Ma *et al.*, 2008; Ciurlia *et al.*, 2009; Shi *et al.*, 2009; Gao *et al.*, 2010; Palumpitag *et al.*, 2011). In order to illustrate this notion, it is necessary to summarize the components that are found in this mixture; linolein as the main triglyceride in red pepper oleoresin (Fernandez-Ronco *et al.*, 2011), with linoleic, palmitic, oleic and myristic acids in lower quantities (Philip & Francis, 1971; Matsufuji *et al.*, 1998), capsanthin, which is found in free and esterified form (Minguez-Mosquera & Hornero-Mendez, 1994; Matsufuji *et al.*, 1998; Jaren-Galan *et al.*, 1999) and, of course, HOSO which contribute large amounts of triolein (Ruiz-Gutiérrez *et al.*, 1998), to this mixture that is highly favorable to the solubilization of lipophilic compounds.

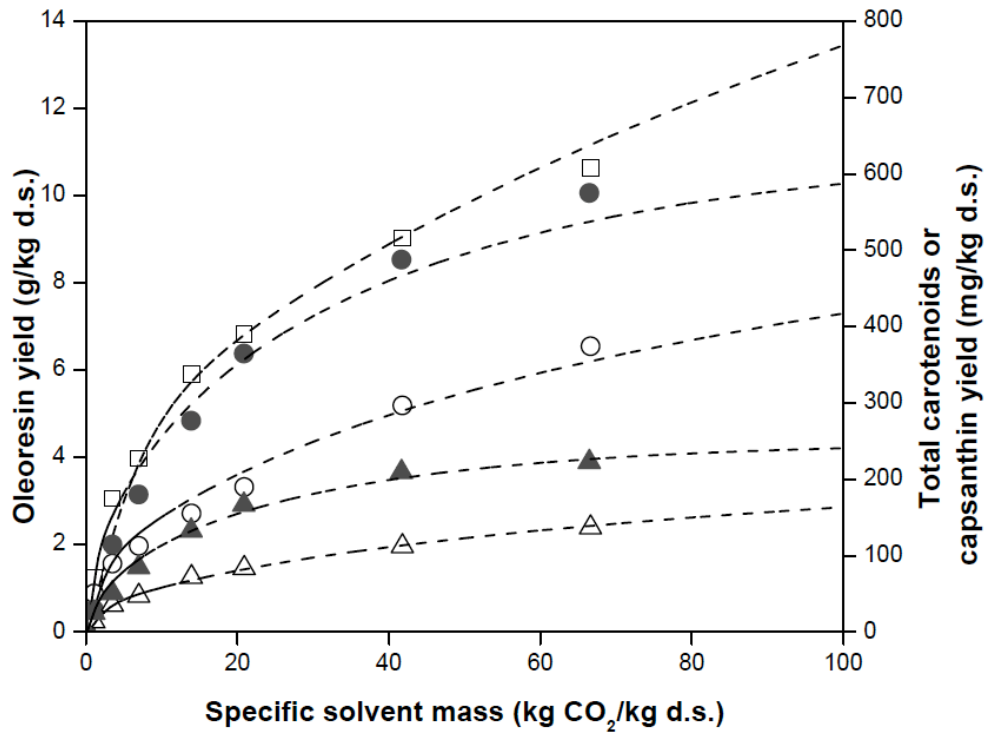


Figure 4.5 Comparative curves on the global cumulative yield of (□) oleoresin, (○) total carotenoids, and (△) capsanthin using pure SC-CO₂; (●) total carotenoids and (▲) capsanthin using HOSO-entrained SC-CO₂ versus specific CO₂ mass extracted from extruded red pepper at 333 K and 50 MPa. Dotted line represents the DDD prediction model (---).

4.3.3 Modeling of extraction kinetics of total carotenoids and capsanthin extracted from extruded red pepper using pure and HOSO-entrained SC-CO₂

For modelling of experimental data for total carotenoids and capsanthin extraction using pure and entrained-SC-CO₂ (Fig. 4.2, 4.4-4.5) we used values of solute concentration in the solid phase loaded into the extractor vessel at zero time of extraction of $C_{s0} = 0.74$ and $C_{s0} = 0.29 \text{ kg/m}^3$ for total carotenoids and capsanthin, respectively. These values were determined using the initial total concentration of solute (C_0) for total carotenoids as $C_{s0} = 638.89$ and capsanthin as $C_{s0} = 247.22 \text{ mg/kg d.s.}$, which were determined from experimental tests at high pressure and temperature (50 MPa and 333 K) using HOSO-entrained SC-CO₂ until full depletion of extract from extruded red pepper (about 6 h of extraction). We adopted values of C_0 as constant, independent of temperature and pressure

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of CO₂, and pretreatment of the raw material. Table 4.1 reported the experimental conditions of extraction, physical properties of SC-CO₂, model parameters, and best-fit values of C_{p0} for each experimental data set.

D_{12} for capsanthin and total carotenoids was estimated as a function of T_r and ρ_r of SC-CO₂ and V_{c2} and MW_2 of a representative solute of supercritical extract, as was reported in Section 4.2.5. To determinate D_{12} of capsanthin we considered xanthophyll as non-esterified capsanthin. Values of V_{c2} and MW_2 for capsanthin were estimated as 2006.50 cm³/mol and 584.89 g/mol, respectively. Regarding the estimation of V_{c2} and MW_2 for total carotenoids, we considered the proportional weight percentage of the main carotenoids that constitute the oleoresin extracted from red pepper (Minguez-Mosquera & Hornero-Mendez, 1994; Matsufuji *et al.*, 1998; Tepic *et al.*, 2009), as follows; β -carotene ($V_{c2} = 1957.50$ cm³/mol and $MW_2 = 536.89$ g/mol) and capsanthin ($V_{c2} = 2006.50$ cm³/mol and $MW_2 = 584.89$ g/mol) in free form, reaching 30%, capsanthin-myristate ($V_{c2} = 2797.50$ cm³/mol and $MW_2 = 795.25$ g/mol) in monoester form, reached 30%, and capsanthin-di-myristate ($V_{c2} = 3588.50$ cm³/mol and $MW_2 = 1005.62$ g/mol) in diester form, reaching 40%, resulting in overall values of combined $V_{c2} = 2868.66$ cm³/mol and $MW_2 = 808.51$ g/mol. D_{12} for capsanthin and total carotenoids ranged from 3.28 to 5.24×10⁻⁹ m²/s and from 2.81 to 4.48×10⁻⁹ m²/s (Table 4.1), respectively. Similar procedure for the determination of V_{c2} and MW_2 of oleoresin capsicum (mixture form of carotenoids, capsaicinoids, fatty acids, mono-di and tri-glycerides) was reported by Fernandez-Ronco *et al.* (2011), parameters that were estimated using the group contribution method of Constantinou & Gani (1994). The fraction of carotenoids in the oleoresin was composed of free carotenoids such as: capsanthin, capsorubin, β -carotene, and zeaxanthin, having V_{c2} and MW_2 for total carotenoids as 2031.1 cm³/mol and 571.60 g/mol respectively (Fernandez-Ronco *et al.*, 2011). These values are lower than those estimated by us mainly due to the difference in the composition of carotenoids, which make up the oleoresin.

D_e in the porous matrix for total carotenoids and capsanthin was estimated using equation 4.6, as a function of D_{12} (Table 4.1), ϵ_p and τ (values reported in Section 4.3.1). D_e for free capsanthin (D_e ranged from 2.04 to 3.26×10⁻¹⁰ m²/s) were larger than D_e for total carotenoids (D_e ranged from 1.75 to 2.79×10⁻¹⁰ m²/s) due to smaller size of molecules and

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functional groups of free capsanthin, which permit higher migration rates or movement of carotenoids in the fluid phase trapped in the porous matrix, in comparison with total carotenoids (mixture mainly composed of mono- and di-esters of capsanthin).

Also, we report in Table 4.1 phase equilibrium parameters, such as C_{po} (apparent solubility, concentration of total carotenoids or capsanthin in the supercritical phase) for each experimental data set, as single adjustable model parameters and values of K (partition coefficient) of total carotenoids or capsanthin between the vegetable substrate and supercritical phase, which were determined by the ratio between C_{so} and C_{po} .

Temperature and pressure of SC-CO₂, and presence of HOSO had a positive effect on C_{po} (Table 4.1). This can be exemplified in the extraction of total carotenoids using pure and entrained-SC-CO₂ (e.g., 2.71 *versus* 4.96×10^{-2} g/kg at 313 K and 28 MPa), where an increase in the partition of solutes between the vegetable matrix and supercritical phase was observed. C_{po} for total carotenoids using pure SC-CO₂ improved 2.3-fold due to an increase in density of SC-CO₂ when the pressure was increased from 28 to 50 MPa at 313 K and 1.2-fold by the increase in vapor pressure of total carotenoids when the temperature was increased from 313 to 333 K at 28 MPa, with a similar effect observed in extractions performed with entrained-SC-CO₂. The values of C_{po} for total carotenoids using HOSO-SC-CO₂ increased from 1.8-fold at 313 K and 28 MPa to 2.9-fold at 333 K and 50 MPa compared with pure SC-CO₂ (Table 4.1) and this improvement in C_{po} for total carotenoids was HOSO-induced. The results indicate the co-solvent and/or modifier effect of HOSO on the apparent solubility of carotenoids from extruded red pepper. C_{po} for capsanthin using pure and entrained-SC-CO₂ followed the similar trend as total carotenoids, with increases in apparent solubility with temperature and pressure. In the presence of HOSO, the maximum value on C_{po} of capsanthin was 4.6-fold at 313 K and 28 MPa in comparison with C_{po} using pure SC-CO₂ (Table 4.1), the improvement of C_{po} for capsanthin was also HOSO-induced, which could act as co-solvent and/or modifier.

In the chapter 3 we reported thermodynamic solubility (C_{sat}) of capsanthin extracted from red pepper in pure and triolein-SC-CO₂, where the results showed that triolein contributes to co-solvent-induced enhancement in the solubility of capsanthin in SC-CO₂. At 28 MPa, the solubility of capsanthin increased from 0.85 to 2.77 $\mu\text{mol/mol}$ at 313 K and 1.65 to 4.29

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μmol/mol at 333 K, when comparing values without and with triolein. Using these data, we predicted C_{sat} at 50 MPa, 313 K or 333 K and without or with triolein. Predicted values of C_{sat} ranged from 1.44 to 8.27 μmol/mol at 313 K and 3.21 to 24.97 μmol/mol at 333 K, again comparing solubilities of capsanthin without and with triolein.

We found differences between values of solubility of capsanthin C_{sat} with C_{po} using pure and entrained-SC-CO₂. The capsanthin solubility (C_{sat}) was higher than C_{po} in pure and entrained-SC-CO₂ at temperatures of 313 or 333 K and pressures of 28 or 50 MPa. For example, C_{sat} was 1.65 μmol/mol in pure SC-CO₂ and 4.29 μmol/mol in triolein-SC-CO₂ (chapter 3) while C_{po} was 0.45 μmol/mol in pure SC-CO₂ and 1.53 μmol/mol in HOSO-SC-CO₂, compared at 333 K and 28 MPa. Considering that C_{sat} is determined from measurements of high-pressure phase equilibrium of model system (SC-CO₂ + solute), which did not include matrix effect and the mixture of different solutes, differences between C_{sat} and C_{po} are related with potential interactions between solutes that constitute the mixture and/or with the vegetable matrix and/or the presence of other solutes that can act as anti-solvent, which could reduce the C_{po} in pure SC-CO₂. It is apparent that C_{po} is a fraction of C_{sat} of capsanthin in pure SC-CO₂ (del Valle & Urrego, 2012). The explanation can be analogous to chromatographic separation process, where the mixture of components or extract is transported and dissolved by the mobile phase or SC-CO₂, which is carried through the stationary phase or vegetable matrix. The molecular interactions of the components in the mixture depends on their chemical affinity with the stationary phase; thus, solutes with strong forces are difficult to dissolve in the mobile phase; however, solutes with weak interactions are easily transported by the mobile phase. Both phases, competing for solute would define the partition of compounds within the system. If the aim is to increase the desorption of specific solutes, appropriate entrainers can be used, encouraging the competition between the entrained mobile phase and solutes for the active adsorption sites on the stationary phase (Brunner, 1994; Brunner & Johannsen, 2006).

The presence of an entrainer enhances the capsanthin solubility of C_{sat} and C_{po} . Despite that these values are different, the improvement on C_{sat} exclusively depends on the co-solvent effect of entrainer, while the value of C_{po} would depend on the prevailing effect of the solute (co- or anti-solvent), as well as the effect of the matrix. It is important to mention

that del Valle & Urrego (2012) found that C_{sat} was higher than C_{po} in the majority of the work they reviewed, except for a handful of cases, in which Saldaña *et al.* (2010) was one of those when comparing the extraction of carotenoids in tomato skin and pulp. Also, the differences can be attributed to the composition of entrainers used, pure triglyceride *versus* mixture of triglycerides (vegetable oil), and the form of capsanthin, which is found mainly esterified on the vegetable matrix when it was extracted with SC-CO₂ and then subsequently saponified, however, this could generate different interaction forces between solute-matrix- and pure or entrained-SC-CO₂.

We determined model parameters using different composition of mixture for total carotenoids with MW_2 of 584.89, 808.51, and 1005.62 g/mol and V_{c2} of 2006.50, 2868.66, and 3588.50 cm³/mol, respectively (data not shown), observing an increase in D_{12} with decreases on MW_2 of mixture of total carotenoids when compared at equal pressure and temperature of SC-CO₂. The value of D_{12} would vary about 23% for the range of MW_2 between 584.89 and 1005.62 g/mol. This uncertainty in choosing the proper MW_2 would result in a variability in the yield of about 14% in the prediction obtained by the mathematical model. Hence, it is important to carefully select the MW_2 of the main components that constitute the mixture of solutes presented in the extract to determine the exact yield. However, the shape of the curve is not altered and describes well the extraction process, based on the parameters considered for this modeling.

Considering that only one adjusted parameter was used, there was good correlation between the cumulative extraction curve modelled and experimental data in extractions of total carotenoids and capsanthin from extruded red pepper performed with pure and entrained-SC-CO₂ (Fig. 4.2, 4.4-4.5).

4.4 Conclusions

Our results showed an increase in the amount of substrate load in the extraction vessel by extrusion of flakes of red pepper. Pre-extrusion conditioning of raw material can favor the elastic deformation and discourage plastic deformation. This combined with a large particle size reduce the internal mass transfer of solutes in porous matrix in extraction with SC-CO₂. Extraction yield of oleoresin, total carotenoids, and capsanthin in entrained-SC-CO₂

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was higher in the presence of HOSO than the yield of extraction obtained in pure SC-CO₂ at equal pressure and temperature. Our results showed a marked increase in the extraction rate, apparent solubility, and yield of extraction of capsanthin, which were HOSO-induced. The enhancements in the presence of HOSO-entrained SC-CO₂ on the extraction rate, apparent solubility, and yield of total carotenoids and capsanthin extracted from extruded red pepper can not be exclusively explained by the co-solvent effect of the entrainer, but also could depend on the prevailing effect of the solute (co- or anti- solvent), as well as the effect of the matrix. Experimental data for the supercritical extraction performed with pure and entrained-SC-CO₂ were described by the model, which considered a single adjustable model parameter. This model considered a relationship that estimated the microstructural effects on the effective diffusivity of vegetable substrate that predicted the extraction processes of carotenoids from extruded red pepper with pure and entrained-SC-CO₂. We contribute with new experimental data and information on the effect of HOSO as an entrainer in the extraction from extruded red pepper of oleoresin, total carotenoids, and capsanthin in SC-CO₂ and modeling the experimental data.

5. GENERAL CONCLUSIONS AND FUTURE PROSPECTS

Conclusions from this work have been divided into three sections: the first being study and measurements of thermodynamic solubility of β -carotene in pure SC-CO₂ as a function of equilibrium T and P , in the presence of triolein or ethanol. As the final purpose is the study of the effect of vegetable oils as potential entrainers, selecting triolein as a representative triglyceride was considered a valid choice. The second section presents the conclusions arrived upon this research involving triolein and its effect on the thermodynamic solubility of capsanthin in SC-CO₂, which was isolated from extruded red pepper flakes. The third section includes conclusions arrived from the apparent solubility and extraction yield of total carotenoids and capsanthin in the oleoresin of red pepper extracted with pure SC-CO₂, as a function of T and P , in the presence of High-Oleic Sunflower Oil (HOSO). A final section is dedicated to a few recommendations for future work.

Thermodynamic solubility of β -carotene using SC-CO₂ (313-333 K and 17-34 MPa) increased with P above 17 MPa, while T effect was observed around the cross-over point (between 15.7 MPa and 17.3 MPa) in that: above 17.3 MPa solubility increased with T , while below 15.7 MPa solubility decreased with T . Experimental data showed good reproducibility (deviation of ± 0.1 $\mu\text{mol/mol}$) when compared with literature; thus, validating the system used and the experimental methodology.

Phase equilibrium studies revealed that solubility of β -carotene in SC-CO₂ increased in the presence of triolein or ethanol; however, the lipid component showed a better co-solvent effect than the alcohol (factor of 4 *versus* a factor of 3), even under lower molar ratios of triolein/CO₂ with respect to ethanol/CO₂, at the same equilibrium conditions.

The system also showed that the presence of β -carotene did not affect the solubility of triolein in a ternary system (SC-CO₂ + β -carotene + triolein), when compared with the binary alternative (SC-CO₂ + triolein), the latter being supported by literature. However, the solubility of β -carotene did increase in the supercritical phase in the presence of the lipid component, as its affinity for the supercritical phase is also higher than β -carotene.

Estimation of ρ of SC-CO₂-triolein mixtures using equation of state by Peng-Robison (PR-EoS) with quadratic mixing rule demonstrated that increases in solubility due to co-solvent

General conclusions and future prospects

effect of triolein or ethanol were independent of the increase in ρ of the mixture associated to their dissolution in the supercritical phase, as these increments in ρ were 0.31% and 2.98% higher than pure CO₂, for triolein and ethanol, respectively.

Increments of solubility of β -carotene, induced by the co-solvent effect of triolein or ethanol, were dependent on the type of co-solvent and their content, but independent of equilibrium T and P . In fact, results showed a preference to form non-polar interactions between triolein and β -carotene, while disfavoring polar interactions with ethanol. The net result of this affinity was reflected in higher solubility of β -carotene in the triolein system. The presence of triolein would also increase the polarizability of CO₂, a phenomenon that has been observed and reported by others regarding non-polar agents.

The effect of triolein addition on the thermodynamic solubility of capsanthin, extracted from extruded red pepper flakes, by using SC-CO₂ (313 K or 333 K and 19-34 MPa) was investigated. Solubility of capsanthin increased with P (19 MPa to 33 MPa) and T (313 K to 333 K). Research demonstrated the co-solvent effect of triolein in SC-CO₂ by a factor of 3, when it was compared to the binary system (SC-CO₂ + capsanthin), under equivalent equilibrium conditions.

Solubility of triolein in a ternary system (SC-CO₂ + capsanthin + triolein) turned out to be equivalent to that in the binary system (SC-CO₂ + triolein), reported in the literature and, comparable with results obtained in the ternary system for β -carotene (SC-CO₂ + β -carotene + triolein), under equivalent equilibrium conditions. In fact, these results confirmed the behavior of ternary systems in which the more soluble component (triolein) induced higher solubility values for the less soluble compounds (capsanthin or β -carotene) due to the co-solvent effect of the lipid component. Non-polar interaction between the aliphatic chains of solute and co-solvent (22 and 18 carbons, respectively) would explain this enhanced solubility. Polarizability is another factor that would contribute to this enhancement role, as polarizability of triolein is about 40 times higher than that of CO₂.

Kinetic extraction of oleoresin, total carotenoids, and capsanthin obtained from extruded red pepper flakes, by using pure SC-CO₂ or in the presence of HOSO (0.53% w/w) at 313

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K or 333 K and 28 MPa or 50 MPa, showed an increase in both rate and yield for values of T and P above 313 K and 28 MPa, respectively.

Extrusion of red pepper flakes, increasing the density of the substrate, allowing a larger charge of the packed bed along with pre-conditioning of the flakes by increasing its moisture content prior to extrusion, favoring the elastic deformation of the substrate, combined with a larger particle size might increase the resistance to mass transfer during the extraction process.

DDD modeling predicted the curves of extraction for total carotenoids and capsanthin with respect to SC-CO₂ consumption for both pure CO₂ and entrained with HOSO. Information about microstructure characteristics of extruded red pepper was fed to the model, and apparent solubility was used as adjusting parameter.

In the presence of HOSO, extraction yield of capsanthin and total carotenoids increased by 3.7 and 2.4 folds, respectively, when compared to pure SC-CO₂. Apparent solubility increased by 4.6 and 2.9 folds for capsanthin and total carotenoids, respectively, when HOSO was used, compared to pure SC-CO₂. In all cases, under equivalent conditions of T and P .

The presence of native lipid content in the oleoresin of red pepper could also behave as co-solvent and/or modifier, aiding in the increase of apparent solubility and yield of components with less solubility in SC-CO₂, such as carotenoids. Thus, the increase in the lipid fraction of the mixture by adding HOSO would favor both yield and rate of capsanthin and total carotenoids considerably, and the presence of HOSO would enhance non-polar interactions between triglycerides and carotenoids (solute-HOSO-SC-CO₂), augmenting their solubility in the supercritical phase (co-solvent effect) and/or physically modifying the matrix of pepper and/or by actively competing for active sites in the matrix, facilitating desorption of carotenoids from the matrix to the supercritical phase (modifier effect).

Experimental data showed differences between thermodynamic and apparent solubility in both, pure and entrained (HOSO and triolein) SC-CO₂, in all conditions of P and T . Apparent solubility was lower than thermodynamic solubility, results that can be attributed to potential interactions between solutes and native compounds found in the matrix of red pepper, which could also act as anti-solvent, reducing the overall apparent solubility of

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capsanthin in SC-CO₂. However, these differences in magnitude always showed that, for a given set of conditions, entrained SC-CO₂ was always higher in thermodynamic solubility (due to co-solvent effect), whereas apparent solubility would depend on the co- or anti-solvent effect of native solutes that composed the extract and the effect of the matrix towards the release of target carotenoids. Some of these differences must be attributed to the composition of triolein and HOSO (pure *versus* mixture of triglycerides) as well as the state of capsanthin, which is found in esterified form in the pepper, which could generate further interactions between solute-matrix and SC-CO₂ either pure or entrained (co-solvent and/or modifier).

Finally, few recommendations that aroused from this investigation herein, and suggestions that could be considered for future work are as follows:

Present research involved extraction kinetics and phase equilibrium of capsanthin by using pure SC-CO₂ and in the presence of entrainers in order to discriminate between co-solvent and/or modifier effect. In relation to thermodynamic and apparent solubility, it is suggested, as complementary research, the idea of equilibrium of partition where a target component should be subjected to phase equilibrium directly from its own matrix, which would help to discriminate about the modifying effect of an entrainer. This partition equilibrium data could also be used to enhance mathematical models and predictive data.

Another suggested work could be the extraction of pepper oleoresin (or other concentrates of interest) by pure or entrained SC-CO₂ and re-depositing the extract on its own depleted residue and/or inert matrix for subsequent extraction, which could clarify further the effect of the entrainer in the matrix and/or the oleoresin.

It is also recommended to continue phase equilibrium measurements of oleoresins by using pure or HOSO entrained-SC-CO₂. In general terms, solubility of target components in the extract are determined by assuming a single pseudo-solute in the supercritical phase; however, this assumption does not take into consideration possible interactions (negative or positive) of other components, also present in the oleoresin, that could influence the solubility of the target compound. The study of complex systems at high pressure would allow advances of knowledge and understanding about the effect of entrainers in supercritical extraction processes to better evaluate differences in thermodynamic and

General conclusions and future prospects

apparent solubility for target components (*e.g.*, free or esterified capsanthin or other solutes) when confronted with entrainers (*e.g.*, triolein, HOSO or other entrainers).

Estimation of ρ of supercritical mixtures using ethanol or triolein as entrainers was derived from Peng-Robison equation of state (PR-EoS) with quadratic mixing rule; however, this equation does not predict their behavior near the critical point of SC-CO₂. Experimental determination of ρ of these mixtures in this area is affected by very small quantities of entrainers. Thus, by having such data, increases in solubility of capsanthin would be explained by ρ changes alone.

Better yields and ease of extraction occurred as a result of using vegetable oils. This was the case for carotenoids and capsanthin from red pepper; therefore, it would be necessary to study the extraction of other carotenoids, such as lycopene, lutein, β -cryptoxanthin and zeaxanthin, among others. The usage of other triglycerides (in pure form or in the form of vegetable oils) as entrainers is also suggested, as well as other types and forms of raw material, such as algae, flowers, by-products, and agro-industrial refuse having considerable bio-active compounds of high functionality, which can be efficiently extracted using SC-CO₂.

6. REFERENCES

- Adil, I.H.; Cetin, H.I.; Yener, M.E.; Bayindirli, A. (2007). Subcritical (carbon dioxide plus ethanol) extraction of polyphenols from apple and peach pomaces, and determination of the antioxidant activities of the extracts. *The Journal of Supercritical Fluids*, 43: 55-63.
- Ambrogi, A.; Cardarelli, D.; Eggers, R. (2002). Fractional extraction of paprika using supercritical carbon dioxide and on-line determination of carotenoids. *Journal of Food Science*, 67: 3236-3241.
- Anderson, A.N.; McBratney, A.B.; FitzPatrick, E.A. (1996). Soil mass, surface, and spectral fractal dimensions estimated from thin section photographs. *Soil Science Society of America Journal*, 60: 962-969.
- Araus, K.; Uquiche, E.; del Valle, J.M. (2009). Matrix effects in supercritical CO₂ extraction of essential oils from plant material. *Journal of Food Engineering*, 92: 438-447.
- Astorg, P. (1997). Food carotenoids and cancer prevention: An overview of current research. *Trends in Food Science & Technology*, 8: 406-413.
- Bamberger, T.; Erickson, J.C.; Cooney, C.L.; Kumar, S.K. (1988). Measurement and model prediction of solubilities of pure fatty-acids, pure triglycerides, and mixtures of triglycerides in supercritical carbon-dioxide. *Journal of Chemical and Engineering Data*, 33: 327-333.
- Barth, M.M.; Zhou, C.; Kute, K.M.; Rosenthal, G.A. (1995). Determination of optimum conditions for supercritical fluid extraction of carotenoids from carrot (*Daucus carota* L.) tissue. *Journal of Agricultural and Food Chemistry*, 43: 2876-2878.
- Baysal, T.; Ersus, S.; Starmans, D.A.J. (2000). Supercritical CO₂ extraction of beta-carotene and lycopene from tomato paste waste. *Journal of Agricultural and Food Chemistry*, 48: 5507-5511.
- Belitz, H.D.; Grosch, W.; Schieberle, P. (2009). Food chemistry. (4th ed.). Springer-Verlag, Berlin.
- Berna, A.; Cháfer, A.; Montón, J.B.; Subirats, S. (2001). High-pressure solubility data of system ethanol (1) plus catechin (2) plus CO₂ (3). *The Journal of Supercritical Fluids*, 20: 157-162.

References

- Birtigh, A.; Stoldt, J.; Brunner, G. (1995). New method for supercritical fluid regeneration. *The Journal of Supercritical Fluids*, 8: 162-166.
- Bose, T.K.; Cole, R.H. (1970). Dielectric and pressure virial coefficients of imperfect gases. II. CO₂-Argon mixtures. *Journal of Chemical Physics*, 52: 140-148.
- Brogle, H. (1982). CO₂ as a solvent: Its properties and applications. *Chemistry and Industry*, 19: 385-390.
- Brunner, G. (1994). Gas extraction: An introduction to fundamentals of supercritical fluids and the application to separation processes. Springer, New York.
- Brunner, G.; Pfohl, O.; Petkov, S. (2001). Phase Equilibria 2000 (PE 2000). Technical University of Hamburg-Harburg.
- Brunner, G.; Johannsen, M. (2006). New aspects on adsorption from supercritical fluid phases. *The Journal of Supercritical Fluids*, 38: 181-200.
- Catchpole, O.J.; King, M.B. (1994). Measurement and correlation of binary diffusion coefficients in near critical fluids. *Industrial & Engineering Chemistry Research*, 33: 1828-1837.
- Catchpole, O.J.; Grey, J.B.; Noermark, K.A. (1998). Solubility of fish oil components in supercritical CO₂ and CO₂ plus ethanol mixtures. *Journal of Chemical and Engineering Data*, 43: 1091-1095.
- Chafer, A.; Berna, A.; Monton, J.B.; Munoz, R. (2002). High-pressure solubility data of system ethanol (1) plus epicatechin (2) plus CO₂ (3). *The Journal of Supercritical Fluids*, 24: 103-109.
- Chafer, A.; Fornari, T.; Berna, A.; Stateva, R.P. (2004). Solubility of quercetin in supercritical CO₂ plus ethanol as a modifier: Measurements and thermodynamic modelling. *The Journal of Supercritical Fluids*, 32: 89-96.
- Chafer, A.; Fornari, T.; Berna, A.; Ibanez, E.; Reglero, G. (2005). Solubility of solid carnosic acid in supercritical CO₂ with ethanol as a co-solvent. *The Journal of Supercritical Fluids*, 34: 323-329.
- Chafer, A.; Fornari, T.; Stateva, R.P.; Berna, A.; Garcia-Reverter, J. (2007). Solubility of the natural antioxidant gallic acid in supercritical CO₂+ethanol as a co-solvent. *Journal of Chemical and Engineering Data*, 52: 116-121.

References

- Chang, C.M.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.
- Chen, C.C.; Chang, C.M.J.; Yang, P.W. (2000). Vapor-liquid equilibria of carbon dioxide with linoleic acid, alpha-tocopherol and triolein at elevated pressures. *Fluid Phase Equilibria*, 175: 107-115.
- Chrastil, J. (1982). Solubility of solids and liquids in supercritical gases. *The Journal of Physical Chemistry*, 86: 3016-3021.
- Ciurlia, L.; Bleve, M.; Rescio, L. (2009). Supercritical carbon dioxide co-extraction of tomatoes (*Lycopersicum esculentum* L.) and hazelnuts (*Corylus avellana* L.): A new procedure in obtaining a source of natural lycopene. *The Journal of Supercritical Fluids*, 49: 338-344.
- Cocero, M.J.; Gonzalez, S.; Perez, S.; Alonso, E. (2000). Supercritical extraction of unsaturated products. Degradation of beta-carotene in supercritical extraction processes. *The Journal of Supercritical Fluids*, 19: 39-44.
- Constantinou, L.; Gani, R. (1994). New group contribution method for estimating properties of pure compounds. *American Institute Chemical Engineering Journal*, 40: 1697-1710.
- Correa, R.; Saramago, B. (2004). On the calculation of disjoining pressure isotherms for nonaqueous films. *Journal of Colloid and Interface Science*, 270: 426-435.
- Cseke, L.J.; Lu, C.R.; Kornfeld, A.; Kaufman, P.B.; Kirakosyan, A. (2006). How and why these compounds are synthesized by plants. In: Cseke, L.J.; Kirakosyan, A.; Kaufman, P.; Warber, S.L.; Duke, J.A.; Brielmann, H.L. (2nd ed.). Natural products from plants. CRC Press Taylor and Francis Group, Boca Raton, U.S.
- Curl, A.L. (1962). Red pepper carotenoids. The carotenoids of red bell peppers. *Journal of Agricultural and Food Chemistry*, 10: 504-509.
- Cussler, E.L. (1997). Diffusion: Mass transfer in fluid systems. (2nd ed.). Cambridge University Press, Cambridge. UK.
- Cygnarowicz, M.L.; Maxwell, R.J.; Seider, W.D. (1990). Equilibrium solubilities of beta-carotene in supercritical carbon dioxide. *Fluid Phase Equilibria*, 59: 57-71.

References

- Dandge, D.K.; Heller, J.P.; Wilson, K.V. (1985). Structure solubility correlations: Organic compounds and dense carbon dioxide binary systems. *Industrial & Engineering Chemistry Product Research and Development*, 24: 162-166.
- Daood, H.G.; Illes, V.; Gnayfeed, M.H.; Meszaros, B.; Horvath, G.; Biacs, P.A. (2002). Extraction of pungent spice paprika by supercritical carbon dioxide and subcritical propane. *The Journal of Supercritical Fluids*, 23: 143-152.
- de la Fuente, J.C.; Valderrama, J.O.; Bottini, S.B.; del Valle, J.M. (2005). Measurement and modeling of solubilities of capsaicin in high-pressure CO₂. *The Journal of Supercritical Fluids*, 34: 195-201.
- de la Fuente, J.C.; Oyarzun, B.; Quezada, N.; del Valle, J.M. (2006). Solubility of carotenoid pigments (lycopene and astaxanthin) in supercritical carbon dioxide. *Fluid Phase Equilibria*, 247: 90-95.
- del Valle, J.M.; Aguilera, J.M. (1999). Revisión: Extracción con CO₂ a alta presión. Fundamentos y aplicaciones en la industria de alimentos. *Food Science and Technology International*, 5: 1-24.
- del Valle, J.M.; Uquiche, E.L. (2002). Particle size effects on supercritical CO₂ extraction of oil-containing seeds. *Journal of the American Oil Chemists Society*, 79: 1261-1266.
- del Valle, J.M.; Jimenez, M.; Napolitano, P.; Zetzl, C.; Brunner, G. (2003). Supercritical carbon dioxide extraction of pelletized Jalapeno peppers. *Journal of the Science of Food and Agriculture*, 83: 550-556.
- del Valle, J.M.; de la Fuente, J.C. (2006). Supercritical CO₂ extraction of oilseeds: Review of kinetic and equilibrium models. *Critical Reviews in Food Science and Nutrition*, 46: 131-160.
- del Valle, J.M.; Germain, J.C.; Uquiche, E.; Zetzl, C.; Brunner, G. (2006). Microstructural effects on internal mass transfer of lipids in prepressed and flaked vegetable substrates. *The Journal of Supercritical Fluids*, 37: 178-190.
- del Valle, J.M.; de la Fuente, J.C.; Uquiche, E.; Zetzl, C.; Brunner, G. (2011). Mass transfer and equilibrium parameters on high-pressure CO₂ extraction of plant essential oils. In: Aguilera, J.M.; Simpson, R.; Welte-Chanes, J.; Bermudez-Aguirre, D.; Barbosa-Canovas, G. (Eds.). *Food engineering interfaces*. Springer, New York, NY.

References

- del Valle, J.M.; Uquiche, E.L.; de la Fuente, J.C. (2012). A refined equation for predicting the solubility of vegetable oils in high-pressure CO₂. *The Journal of Supercritical Fluids*, 67: 60-70.
- del Valle, J.M.; Urrego, F.A. (2012). Free solute content and solute-matrix interactions affect apparent solubility and apparent solute content in supercritical CO₂ extractions. A hypothesis paper. *The Journal of Supercritical Fluids*, 66: 157-175.
- Dobbs, J.M.; Wong, J.M.; Johnston, K.P. (1986). Nonpolar co-solvents for solubility enhancement in supercritical fluid carbon dioxide. *Journal of Chemical & Engineering Data*, 31: 303-308.
- Dobbs, J.M.; Wong, J.M.; Lahiere, R.J.; Johnston, K.P. (1987). Modification of supercritical fluid phase behavior using polar co-solvents. *Industrial & Engineering Chemistry Research*, 26: 56-65.
- Dohrn, R.; Brunner, G. (1994). An estimation method to calculate T_b, T_c, P_c and ω from the liquid molar volume and the vapor pressure. *Proceedings of the Third International Symposium on Supercritical Fluids*, Strasbourg, France.
- Downham, A.; Collins, P. (2000). Colouring our foods in the last and next millennium. *International Journal of Food Science and Technology*, 35: 5-22.
- Dunford, N.T.; Temelli, F. (1995). Extraction of phospholipids from canola with supercritical carbon dioxide and ethanol. *Journal of the American Oil Chemists Society*, 72: 1009-1015.
- Ekart, M.P.; Bennett, K.L.; Ekart, S.M.; Gurdial, G.S.; Liotta, C.L.; Eckert, C.A. (1993). Co-solvent interactions in supercritical fluid solutions. *AIChE Journal*, 39: 235-248.
- Fahmy, T.M.; Paulaitis, M.E.; Johnson, D.M.; McNally, M.E.P. (1993). Modifier effects in the supercritical fluid extraction of solutes from clay, soil, and plant materials. *Analytical Chemistry*, 65: 1462-1469.
- Fernandez-Ronco, M.P.; Gracia, I.; De Lucas, A.; Rodriguez, J.F. (2011). Measurement and modeling of the high-pressure phase equilibria of CO₂-oleoresin Capsicum. *The Journal of Supercritical Fluids*, 57: 112-119.
- Formo, M.W.; Jungermann, E.; Norris, F.A.; Sonntag, N.O.V. (1979). Bailey's industry oil and fat products. (4th ed.). John Wiley and Sons, New York.

References

- Foster, N.R.; Gurdial, G.S.; Yun, J.S.L.; Liong, K.K.; Tilly, K.D.; Ting, S.S.T.; Singh, H.; Lee, J.H. (1991). Significance of the crossover pressure in solid supercritical fluid phase-equilibria. *Industrial & Engineering Chemistry Research*, 30: 1955-1964.
- Gao, Y.; Liu, X.; Xu, H.; Zhao, J.; Wang, Q.; Liu, G.; Hao, Q. (2010). Optimization of supercritical carbon dioxide extraction of lutein esters from marigold (*Tagetes erecta* L.) with vegetable oils as continuous co-solvents. *Separation and Purification Technology*, 71: 214-219.
- Gardner, D.S. (1982). Industrial-scale hop extraction with liquid CO₂. *Chemistry & Industry*, 12: 402-405.
- Gnayfeed, M.H.; Daood, H.G.; Illes, V.; Biacs, P.A. (2001). Supercritical CO₂ and subcritical propane extraction of pungent paprika and quantification of carotenoids, tocopherols, and capsaicinoid. *Journal of Agricultural and Food Chemistry*, 49: 2761-2766.
- Goodarznia, I.; Eikani, M.H. (1998). Supercritical carbon dioxide extraction of essential oils: Modeling and simulation. *Chemical Engineering Science*, 53: 1387-1395.
- Goto, M.; Roy, B.C.; Hirose, T. (1996). Shrinking-core leaching model for supercritical-fluid extraction. *The Journal of Supercritical Fluids*, 9: 128-133.
- Goto, M.; Roy, B.C.; Kodama, A.; Hirose, T. (1998). Modeling supercritical fluid extraction process involving solute-solid interaction. *Journal of Chemical Engineering of Japan*, 31: 171-177.
- Güçlü-Üstündağ, Ö.; Temelli, F. (2000). Correlating the solubility behavior of fatty acids, mono-, di-, and triglycerides, and fatty acid esters in supercritical carbon dioxide. *Industrial & Engineering Chemistry Research*, 39: 4756-4766.
- Güçlü-Üstündağ, Ö.; Temelli, F. (2004). Correlating the solubility behavior of minor lipid components in supercritical carbon dioxide. *The Journal of Supercritical Fluids*, 31: 235-253.
- Gupta, R.B.; Shim, J.J. (2007). Solubility in supercritical carbon dioxide. CRC Press Taylor and Francis Group, Boca Raton, New York.
- Hallberg, W.; Jekat, H.; Schütz, E.; Stork, K.; Vollbrecht, H.R. (1989). Method of extracting hop pellets with liquified carbon dioxide. United States Patent, 4828867.

References

- Hansen, B.N.; Bruno, T.J. (1993). Solubility measurement by direct-injection of supercritical-fluid solutions into a HPLC system. *The Journal of Supercritical Fluids*, 6: 229-232.
- Hansen, B.N.; Harvey, A.H.; Coelho, J.A.P.; Palavra, A.M.F.; Bruno, T.J. (2001). Solubility of capsaicin and beta-carotene in supercritical carbon dioxide and in halocarbons. *Journal of Chemical and Engineering Data*, 46: 1054-1058.
- Hatami, T.; Rahimi, M.; Veggi, P.C.; Portillo-Prieto, R.; Meireles, M.A.A. (2011). Near-critical carbon dioxide extraction of khoa (*Satureja boliviana* Benth Briq) using ethanol as a co-solvent: Experiment and modeling. *The Journal of Supercritical Fluids*, 55: 929-936.
- Hirayama, O.; Nakamura, K.; Hamada, S.; Kobayasi, Y. (1994). Singlet oxygen quenching ability of naturally occurring carotenoids. *Lipids*, 29: 149-150.
- Jaren-Galan, M.; Nienaber, U.; Schwartz, S.J. (1999). Paprika (*Capsicum annuum*) oleoresin extraction with supercritical carbon dioxide. *Journal of Agricultural and Food Chemistry*, 47: 3558-3564.
- Jay, A.J.; Steytler, D.C.; Knights, M. (1991). Spectrophotometric studies of food colors in near-critical carbon dioxide. *The Journal of Supercritical Fluids*, 4: 131-141.
- Johannsen, M.; Brunner, G. (1997). Solubilities of the fat-soluble vitamins A, D, E, and K in supercritical carbon dioxide. *Journal of Chemical and Engineering Data*, 42: 106-111.
- Ke, J.; Mao, C.; Zhong, M.H.; Han, B.X.; Yan, H.K. (1996). Solubilities of salicylic acid in supercritical carbon dioxide with ethanol co-solvent. *The Journal of Supercritical Fluids*, 9: 82-87.
- King, J.W. (1990). Analytical supercritical fluid extraction. In: Lee, M.L.; Markides, K.E. (Eds.). Analytical supercritical fluid chromatography and extraction. Chromatography Conferences: Provo, Utah.
- King J.W.; France J.E. (1992). Basic principles of analytical supercritical fluid extraction. In: Wenclawiak B. (Eds.). Analysis with supercritical fluids: Extraction and chromatography. Springer-Verlag, Berlin.
- Kraska, T.; Leonhard, K.O.; Tuma, D.; Schneider, G.M. (2002). Correlation of the solubility of low-volatile organic compounds in near- and supercritical fluids. Part 1: applications to adamantane and beta-carotene. *The Journal of Supercritical Fluids*, 23: 209-224.

References

- Krichnavaruk, S.; Shotipruk, A.; Goto, M.; Pavasant, P. (2008). Supercritical carbon dioxide extraction of astaxanthin from *Haematococcus pluvialis* with vegetable oils as co-solvent. *Bioresource Technology*, 99: 5556-5560.
- Kuk, M.S.; Hron, R.J. (1994). Supercritical carbon dioxide extraction of cottonseed with co-solvents. *Journal of the American Oil Chemists Society*, 71: 1353-1356.
- Kwon, K.T.; Uddin, M.S.; Jung, G.W.; Sim, J.E.; Lee, S.M.; Woo, H.C.; Chun, B.S. (2011). Solubility of red pepper (*Capsicum annum*) oil in near- and supercritical carbon dioxide and quantification of capsaicin. *Korean Journal of Chemical Engineering*, 28: 1433-1438.
- Li, S.F.; Hartland, S. (1996). A new industrial process for extracting cocoa butter and xanthines with supercritical carbon dioxide. *Journal of the American Oil Chemists' Society*, 73: 423-429.
- Lisa, M.; Holcapek, M. (2008). Triacylglycerols profiling in plant oils important in food industry, dietetics and cosmetics using high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *Journal of Chromatography A*, 1198: 115-130.
- Locey, C.L.; Guzinski, J.A. (2000). Paprika. In: Lauro, G.J.; Francis F.J. (Eds.). *Natural food colorants*. Marcel Dekker, New York.
- Ma, Q.; Xu, X.; Gao, Y.; Wang, Q.; Zhao, J. (2008). Optimisation of supercritical carbon dioxide extraction of lutein esters from marigold (*Tagetes erect* L.) with soybean oil as a co-solvent. *International Journal of Food Science & Technology*, 43: 1763-1769.
- Macías-Sánchez, M.D.; Serrano, C.M.; Rodríguez, M.; de la Ossa, E.M.; Lubian, L.M.; Montero, O. (2008). Extraction of carotenoids and chlorophyll from microalgae with supercritical carbon dioxide and ethanol as co-solvent. *Journal of Separation Science*, 31: 1352-1362.
- Matsufuji, H.; Nakamura, H.; Chino, M.; Takeda, M. (1998). Antioxidant activity of capsanthin and the fatty acid esters in paprika (*Capsicum annum*). *Journal of Agricultural and Food Chemistry*, 46: 3468-3472.
- Méendes, R.L.; Nobre, B.P.; Coelho, J.P.; Palavra, A.F. (1999). Solubility of beta-carotene in supercritical carbon dioxide and ethane. *The Journal of Supercritical Fluids*, 16: 99-106.

References

- Méndez-Santiago, J.; Teja, A.S. (1999). The solubility of solids in supercritical fluids. *Fluid Phase Equilibria*, 158: 501-510.
- Méndez-Santiago, J.; Teja, A.S. (2000). Solubility of solids in supercritical fluids: Consistency of data and a new model for co-solvent systems. *Industrial & Engineering Chemistry Research*, 39: 4767-4771.
- Mezzomo, N.; Mileo, B.R.; Friedrich, M.T.; Martinez, J.; Ferreira, S.R.S. (2010). Supercritical fluid extraction of peach (*Prunus persica*) almond oil: Process yield and extract composition. *Bioresource Technology*, 101: 5622-5632.
- Minguez-Mosquera, M.I.; Hornero-Mendez, D. (1994). Changes in carotenoid esterification during the fruit ripening of *Capsicum annuum* Cv. Bola. *Journal of Agricultural and Food Chemistry*, 42: 640-644.
- Montanari, L.; King, J.W.; List, G.R.; Rennick, K.A. (1996). Selective extraction of phospholipid mixtures by supercritical carbon dioxide and co-solvents. *Journal of Food Science*, 61: 1230-1234.
- Moyler, D.A. (1993). Extraction of flavours and fragrances with compressed CO₂. In: King, M.B.; Bott, T.R. (Eds.). *Extraction of natural products using near-critical solvents*. Blackie Academic & Professional. Glasgow, London.
- Mukhopadhyay, M. (2000). *Natural extracts using supercritical carbon dioxide*. CRC Press Taylor and Francis Group, Boca Raton, New York.
- Naranjo-Modad, S.; Lopez-Munguia, A.; Vilarem, G.; Gaset, A.; Barzana, E. (2000). Solubility of purified lutein diesters obtained from *Tagetes erecta* in supercritical CO₂ and the effect of solvent modifiers. *Journal of Agricultural and Food Chemistry*, 48: 5640-5642.
- Narisawa, T.; Fukaura, Y.; Hasebe, M.; Nomura, S.; Oshima, S.; Inakuma, T. (2000). Prevention of N-methylnitrosourea-induced colon carcinogenesis in rats by oxygenated carotenoid capsanthin and capsanthin-rich paprika juice. *Proceedings of the Society for Experimental Biology and Medicine*, 224: 116-122.
- Nilsson, W.B.; Gauglitz, E.J.; Hudson, J.K. (1991). Solubilities of methyl oleate, oleic acid, oleyl glycerols, and oleyl glycerol mixtures in supercritical carbon dioxide. *Journal of the American Oil Chemists' Society*, 68: 87-91.

References

- Nilsson, W.B.; Hudson, J.K. (1993). Solubility of simple and mixed triacylglycerols in supercritical CO₂. *Journal of the American Oil Chemists' Society*, 70: 749-754.
- NIST, Fluid Thermodynamic and Transport Properties, version 8.0. <<http://www.nist.gov/srd/nist23.htm>>.
- O'Neil, M.J.; Heckelman, P.E.; Koch, C.B.; Roman, K.J. (2006). The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. (14th ed.). Merck & Co, Inc., New Jersey.
- Page, S.H.; Sumpter, S.R.; Lee, M.L. (1992). Fluid phase equilibria in supercritical fluid chromatography with CO₂-based mixed mobile phases: A review. *Journal of Microcolumn Separations*, 4: 91-122.
- Palmer, M.V.; Ting, S.S.T. (1995). Applications for supercritical fluid technology in food processing. *Food Chemistry*, 52: 345-352.
- Palumpitag, W.; Prasitchoke, P.; Goto, M.; Shotipruk, A. (2011). Supercritical carbon dioxide extraction of marigold lutein fatty acid esters: Effects of co-solvents and saponification conditions. *Separation Science and Technology*, 46: 605-610.
- Park, H.S.; Lee, H.J.; Shin, M.H.; Lee, K.W.; Lee, H.; Kim, Y.S.; Kim, K.O.; Kim, K.H. (2007). Effects of co-solvents on the decaffeination of green tea by supercritical carbon dioxide. *Food Chemistry*, 105: 1011-1017.
- Philip, T.; Francis, F.J. (1971). Oxidation of capsanthin. *Journal of Food Science*, 36: 96-97.
- Pohler, H.; Kiran, E. (1997). Volumetric properties of carbon dioxide plus ethanol at high pressures. *Journal of Chemical and Engineering Data*, 42: 384-388.
- Poling, B.E.; Prausnitz, J.M.; O'Connell, J.P. (2000). The properties of gases and liquids. (5th ed.). McGraw-Hill, New York, NY.
- Puiggené J.; Larrayoz, M.A.; Recasens, F. (1997). Free liquid-to-supercritical fluid mass transfer in packed beds. *Chemical Engineering Science*, 52: 195-212.
- Rao, A.V.; Rao, L.G. (2007). Carotenoids and human health. *Pharmacological Research*, 55: 207-216.
- Reverchon, E. (1996). Mathematical modeling of supercritical extraction of sage oil. *AIChE Journal*, 42: 1765-1771.

References

- Ribeiro, M.A.; Bernardogil, M.G. (1995). Solubilities of triolein in supercritical CO₂. *Journal of Chemical and Engineering Data*, 40: 1188-1192.
- Robert, P.; Romero, N.; Ortiz, J.; Masson, L.; Barrera-Arellano, D. (2006). Effect of rosa mosqueta (*Rosa rubiginosa*) extract on the performance of Chilean hazelnut oil (*Gevuina avellana* Mol.) at high temperature. *Journal of the American Oil Chemists Society*, 83: 691-695.
- Rodríguez-Amaya, D.A. (1999). Guide to carotenoid analysis in food. ILSI Press, Washington, DC.
- Ruiz-Gutiérrez, V.; Morgado, N.; Prada, J.L.; Pérez-Jiménez, F.; Muriana, F.J.G. (1998). Composition of human VLDL triacylglycerols after ingestion of olive oil and high oleic sunflower oil. *The Journal of Nutrition*, 128: 570-576.
- Sakaki, K. (1992). Solubility of beta-carotene in dense carbon dioxide and nitrous oxide from 308 to 323 K and from 9.6 to 30 MPa. *Journal of Chemical and Engineering Data*, 37: 249-251.
- Saldaña, M.D.A.; Zetzi, C.; Mohamed, R.S.; Brunner, G. (2002). Extraction of methylxanthines from guarana seeds, mate leaves, and cocoa beans using supercritical carbon dioxide and ethanol. *Journal of Agricultural and Food Chemistry*, 50: 4820-4826.
- Saldaña, M.D.A.; Temelli, F.; Guigard, S.E.; Tomberli, B.; Gray, C.G. (2010). Apparent solubility of lycopene and beta-carotene in supercritical CO₂, CO₂ + ethanol and CO₂ + canola oil using dynamic extraction of tomatoes. *Journal of Food Engineering*, 99: 1-8.
- Şanal, İ.S.; Bayraktar, E.; Mehmetoğlu, Ü.; Çalımlı, A. (2005). Determination of optimum conditions for SC-(CO₂ plus ethanol) extraction of beta-carotene from apricot pomace using response surface methodology. *The Journal of Supercritical Fluids*, 34: 331-338.
- Sanders, N. (1993). Food legislation and the scope for increased use of near-critical fluid extraction operations in the food, flavouring and pharmaceutical industries. In: King, M.B.; Bott, T.R. (Eds.). *Extraction of natural products using near-critical solvents*. Blackie Academic & Professional, Glasgow, London.
- Sauceau, M.; Letourneau, J.J.; Richon, D.; Fages, J. (2003). Enhanced density-based models for solid compound solubilities in supercritical carbon dioxide with co-solvents. *Fluid Phase Equilibria*, 208: 99-113.

References

- Schaeffer, S.T.; Zalkow, L.H.; Teja, A.S. (1988). Solubility of monocrotaline in supercritical carbon dioxide and carbon dioxide-ethanol mixtures. *Fluid Phase Equilibria*, 43: 45-56.
- Schoefs, B. (2004). Determination of pigments in vegetables. *Journal of Chromatography A*, 1054: 217-226.
- Shi, J.; Yi, C.; Xue, S.J.; Jiang, Y.M.; Ma, Y.; Li, D. (2009). Effects of modifiers on the profile of lycopene extracted from tomato skins by supercritical CO₂. *Journal of Food Engineering*, 93: 431-436.
- Sigma-Aldrich. <http://www.sigmaaldrich.com/chile.html> (accessed on October 13, 2011).
- Škerget, M.; Knez, Z.; Habulin, M. (1995). Solubility of beta-carotene and oleic acid in dense CO₂ and data correlation by a density based model. *Fluid Phase Equilibria*, 109: 131-138.
- Song, K.M.; Park, S.W.; Hong, W.H.; Lee, H.; Kwak, S.S.; Liu, J.R. (1992). Isolation of vindoline from *Catharanthus roseus* by supercritical fluid extraction. *Biotechnology Progress*, 8: 583-586.
- Sovová, H. (2001). Solubility of ferulic acid in supercritical carbon dioxide with ethanol as co-solvent. *Journal of Chemical and Engineering Data*, 46: 1255-1257.
- Sovová, H.; Stateva, R.P.; Galushko, A.A. (2001). Solubility of beta-carotene in supercritical CO₂ and the effect of entrainers. *The Journal of Supercritical Fluids*, 21: 195-203.
- Stahl, E.; Quirin, K.-W.; Gerard, D. (1988). Dense gases for extraction and refining. Springer-Verlag, Berlin.
- Stahl, W.; Sies, H. (2005). Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta-Molecular Basis of Disease*, 1740: 101-107.
- Sun, M.; Temelli, F. (2006). Supercritical carbon dioxide extraction of carotenoids from carrot using canola oil as a continuous co-solvent. *The Journal of Supercritical Fluids*, 37: 397-408.
- Takahashi, M.; Watanabe, H.; Kikkawa, J.; Ota, M.; Watanabe, M.; Sato, Y.; Inomata, H.; Sato, N. (2006). Carotenoids extraction from Japanese persimmon (Hachiya-kaki) peels by supercritical CO₂ with ethanol. *Analytical Sciences*, 22: 1441-1447.
- Tepic, A.; Zekovic, Z.; Kravic, S.; Mandic, A. (2009). Pigment content and fatty acid composition of paprika oleoresins obtained by conventional and supercritical carbon dioxide extraction. *Cyta-Journal of Food*, 7: 95-102.

References

- Ting, S.S.T.; Macnaughton, S.J.; Tomasko, D.L.; Foster, N.R. (1993a). Solubility of naproxen in supercritical carbon dioxide with and without co-solvents. *Industrial & Engineering Chemistry Research*, 32: 1471-1481.
- Ting, S.S.T.; Tomasko, D.L.; Macnaughton, S.J.; Foster, N.R. (1993b). Chemical physical interpretation of co-solvent effects in supercritical fluids. *Industrial & Engineering Chemistry Research*, 32: 1482-1487.
- Uquiche, E.; del Valle, J.M.; Ortiz, J. (2004). Supercritical carbon dioxide extraction of red pepper (*Capsicum annuum* L.) oleoresin. *Journal of Food Engineering*, 65: 55-66.
- Uquiche, E.; del Valle, J.M.; Ihl, M. (2005). Microstructure-extractability relationships in the extraction of prepelletized Jalapeño peppers with supercritical carbon dioxide. *Journal of Food Science*, 70: E379-E386.
- Uquiche, E.; Fica, X.; Salazar, K.; del Valle, J.M. (2012). Time fractionation of minor lipids from cold-pressed rapeseed cake using supercritical CO₂. *Journal of the American Oil Chemists' Society*. 89: 1135-1144.
- Valderrama, J.O.; Perrut, M.; Majewski, W. (2003). Extraction of astaxantine and phycocyanine from microalgae with supercritical carbon dioxide. *Journal of Chemical and Engineering Data*, 48: 827-830.
- Vasapollo, G.; Longo, L.; Rescio, L.; Ciurlia, L. (2004). Innovative supercritical CO₂ extraction of lycopene from tomato in the presence of vegetable oil as co-solvent. *The Journal of Supercritical Fluids*, 29: 87-96.
- Wang, H.C.; Chen, C.R.; Chang, C.M.J. (2001). Carbon dioxide extraction of ginseng root hair oil and ginsenosides. *Food Chemistry*, 72: 505-509.
- Weissenberg, M.; Schaeffler, I.; Menagem, E.; Barzilai, M.; Levy, A. (1997). Isocratic non-aqueous reversed-phase high-performance liquid chromatographic separation of capsanthin and capsorubin in red peppers (*Capsicum annuum* L), paprika and oleoresin. *Journal of Chromatography A*, 757: 89-95.
- Wong, J.M.; Johnston, K.P. (1986). Solubilization of biomolecules in carbon dioxide based supercritical fluids. *Biotechnology Progress*, 2: 29-39.

References

- Zhang, X.; Han, B.; Hou, Z.; Zhang, J.; Liu, Z.; Jiang, T.; He, J.; Li, H. (2002). Why do co-solvents enhance the solubility of solutes in supercritical fluids? New evidence and opinion. *Chemistry – A European Journal*, 8: 5107-5111.
- Zosel, K. (1978). Separation with supercritical gases: Practical applications. *Angewandte Chemie-International Edition in English*, 17: 702-709.
- Zuñiga-Moreno, A.; Galicia-Luna, L.A. (2002). Compressed liquid densities of carbon dioxide plus ethanol mixtures at four compositions via a vibrating tube densimeter up to 363 K and 25 MPa. *Journal of Chemical and Engineering Data*, 47: 149-154.

7. APPENDIX A

The inherent errors in estimates of y_2 or y_4 reported in Results and Discussion (section 2.3) were calculated using the following procedure. Values of y_i were estimated using equation 2.8, based on the chromatographic response (peak area A_i) of $V_{IV} = 20\text{-}\mu\text{L}$ -aliquot (the volume of the loop of the injection valve, component 6 in Fig. 2.2) as compared to the chromatographic response (peak area A_{Si}) of a $V_S = 20\text{-}\mu\text{L}$ -aliquot (the volume of the loop of the HPLC injector, included in component 7 in Fig. 2.2) of a stock solution of known concentration (C_{Si}) of the solute of interest (β -carotene or triolein) used for calibration purposes, as proposed by Hansen & Bruno (1993):

$$y_i = \left(\frac{A_i/V_{IV}}{A_{Si}/V_S} \right) \cdot C_{Si} \cdot v_1 \cdot MW_1 \quad (2.8)$$

where v_1 is the specific volume of CO_2 (molecular weight $MW_1 = 44.01 \text{ g/mol}$) under test conditions. The inherent error associated with the use of equation 2.8 can be estimated using equation 2.9:

$$\Delta y_i = \sum_j \left| \frac{\partial y_i}{\partial \xi_j} \right| \cdot \Delta \xi_j \quad (2.9)$$

where ξ_j ($j = 1, 2, \dots, 6$) is an independent variable in equation 2.8 (A_i , A_S , V_{IV} , V_S , C_{Si} , and v_1). Considering that $\partial y_i / \partial \xi_j = y_i / \xi_j$ for A_i , V_S , C_{Si} , and v_1 , and $\partial y_i / \partial \xi_j = -y_i / \xi_j$ for A_{Si} and V_{IV} (cf. equation 2.8), equation 2.10 applies for the absolute inherent error (Δy_2) and equation 2.11 applied for the relative inherent error ($\Delta y_2 / y_2$):

$$\Delta y_i = \sum_j y_i \cdot \left(\frac{\Delta \xi_j}{\xi_j} \right) \text{ and} \quad (2.10)$$

$$\frac{\Delta y_i}{y_i} = \sum_j \frac{\Delta \xi_j}{\xi_j} \quad (2.11)$$

Because the molar concentration of the stock solution used to calibrate the HPLC was adjusted by dissolving a weighed amount of solute (m_i) with acetone in a volumetric flask ($V_{VF} = 50 \text{ cm}^3$), C_S can be estimated using equation 2.12:

$$C_{Si} = \frac{m_i}{V_{VF} \cdot MW_i} \quad (2.12)$$

where MW_i is the molecular weight of the solute ($MW_2 = 536.87$ g/mol for β -carotene, $MW_4 = 885.43$ g/mol for triolein). Proceeding the same as previously, and considering that from equation 2.12 $\partial C_S / \partial m_i = C_S / m_i$ and $\partial C_S / \partial V_{VF} = -C_S / V_{VF}$, the relative inherent error in estimates of C_{Si} is given by equation 2.13:

$$\frac{\Delta C_{Si}}{C_{Si}} = \frac{\Delta m_i}{m_i} + \frac{\Delta V_{VF}}{V_{VF}} \quad (2.13)$$

On the other hand, because v_1 is a property of state that depends on system temperature and pressure, the following definitions apply for Δv_1 and $\Delta v_1 / \Delta v_1$:

$$\Delta v_1 = \left| \frac{\partial v_1}{\partial T} \right| \cdot \Delta T + \left| \frac{\partial v_1}{\partial P} \right| \cdot \Delta P \text{ and} \quad (2.14)$$

$$\frac{\Delta v_1}{v_1} = \left| \frac{1}{v_1} \cdot \frac{\partial v_1}{\partial T} \right| \cdot \Delta T + \left| \frac{1}{v_1} \cdot \frac{\partial v_1}{\partial P} \right| \cdot \Delta P \quad (2.15)$$

Replacing the definitions of the isothermal compressibility (β_{T1} , equation 2.16) and volumetric expansibility (α_{v1} , equation 2.17) of CO_2 in equation 2.15, the final expression for the relative inherent error of y_2 (equation 2.18) was obtained by replacing equation 2.13 and equation 2.15 in equation 2.11:

$$\beta_{T1} = -\frac{1}{v_1} \left(\frac{\partial v_1}{\partial P} \right)_T = \left| \frac{1}{v_1} \cdot \frac{\partial v_1}{\partial P} \right| \quad (2.16)$$

$$\alpha_{v1} = \frac{1}{v_1} \left(\frac{\partial v_1}{\partial T} \right)_P = \left| \frac{1}{v_1} \cdot \frac{\partial v_1}{\partial T} \right| \text{ and} \quad (2.17)$$

$$\frac{\Delta y_i}{y_i} = \frac{\Delta A_i}{A_i} + \frac{\Delta A_{Si}}{A_{Si}} + \frac{\Delta V_{IV}}{V_{IV}} + \frac{\Delta V_S}{V_S} + \left(\frac{\Delta m_i}{m_i} + \frac{\Delta V_{VF}}{V_{VF}} \right) + (\beta_{T1} \cdot \Delta T + \alpha_{v1} \cdot \Delta P) \quad (2.18)$$

For computations, we adopted the following values for the experimental error: $\Delta A_i = \Delta A_{Si} = 3500$ AU, $\Delta A_{IV} = \Delta V_S = 2$ μ L, $\Delta m_i = 100$ μ g, $\Delta V_{VF} = 80$ μ L, $\Delta T = 0.5$ K, and $\Delta P = 0.2$ MPa.

LIST OF PAPERS

This thesis is composed of the following three papers, which are presented in the text as chapters:

PAPER 2. Araus, K.A.; Canales, R.I.; del Valle, J.M.; de la Fuente, J.C. (2011). **Solubility of β -carotene in ethanol- and triolein-modified CO₂.** *The Journal of Chemical Thermodynamics*. 43, 1991-2001.

PAPER 3. Araus, K.A.; del Valle, J.M.; Robert, P.S.; de la Fuente, J.C. (2012). **Effect of triolein addition on the solubility of capsanthin in supercritical carbon dioxide.** *The Journal of Chemical Thermodynamics*. 51, 190-194.

PAPER 4. Uquiche, E.; Araus, K.A.; del Valle, J.M. (2012). **Supercritical CO₂ extraction of carotenoids from extruded red pepper using high-oleic sunflower oil as entrainer.** *The Journal of Supercritical Fluids*. Submitted.

PROCEEDINGS

Parts of the work have also been presented in national and international congresses under the following references:

- Araus, K.; Canales, R.; del Valle, JM.; de la Fuente, JC. (2009). ***Solubilidad de β -caroteno en mezclas de dióxido de carbono supercrítico + trioleína***. XVII Congreso Chileno de Ingeniería Química. Escuela de Ingeniería Química-Pontificia Universidad Católica de Valparaíso (EIQ-PUCV). Viña del Mar-Valparaíso, Chile.
- Araus, K.; Canales, R.; Robert, P.; del Valle, JM.; de la Fuente, JC. (2010). **High-pressure solubility of β -carotene and capsanthin in pure and modified carbon dioxide with ethanol or triolein**. 12th International Conference on Properties and Phase Equilibria for Product and Process Design (PPEPPD 2010). Jiangsu, China.
- Araus, K.; Canales, R.; Robert, P.; del Valle, JM.; de la Fuente, JC. (2010). **Solubility of nutraceutical carotenoid compounds in pure supercritical CO₂ and modified with triolein or ethanol**. 14th International Biotechnology Symposium. Rimini, Italy.
- Araus, K.; Uquiche, E.; del Valle, JM. (2011). **Oleoresin extraction from extruded red pepper (*Capsicum annuum* L.) using supercritical CO₂ modified with high-oleic sunflower oil**. XXV Interamerican Congress of Chemical Engineering, XVIII Chilean Congress of Chemical Engineering. Santiago, Chile.