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Cosolvent effect of ethanol on the solubility of lutein in supercritical carbon dioxide

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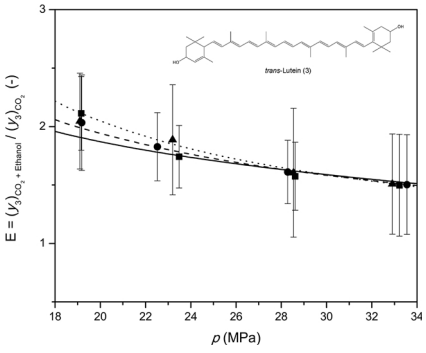
GRAPHICAL ABSTRACT



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ABSTRACT

This contribution presents new high-pressure solubility data of solid *trans*-lutein in pure carbon dioxide (CO_2), and mixtures of CO_2 and ethanol. The cosolvent effect of ethanol was determined by comparing lutein solubility between the ternary (CO_2 + ethanol + lutein) and binary (CO_2 + lutein) systems at (313, 323, and 333) K and from (18.70 to 33.55) MPa. 92% pure lutein was isolated from marigold flower petals. Solubility was measured using a dynamic-analytical method with recirculation and online analysis of the CO_2-rich phase. The solubility of lutein in pure and ethanol-modified CO_2 increased with system temperature and pressure. Solubilities in pure CO_2 ranged from $0.82\,10^{-6}$ mol mol^{-1} at (313 K and 18.70 MPa) to $2.45\,10^{-6}$ mol mol^{-1} at (333 K and 32.91 MPa). The highest solubility of lutein in CO_2 experimentally measured was $4.02\,10^{-6}$ mol mol^{-1} at (333 K and 32.91 MPa) when adding 0.0211 mol mol^{-1} of ethanol.

1. Introduction

The main xanthophyll in petals of marigold (*Tagetes erecta* L.) flowers is lutein (β,ε-carotene-3,3′-diol), which represents *ca.* 0.80–0.90 kg·kg^{-1} of all carotenoid constituents [1]. Lutein (Fig. 1) can be used as a natural yellow colorant in foods and feeds, and as an antioxidant in nutraceuticals because of its protective action against photic damage of human retina [2]. There exists a current industrial

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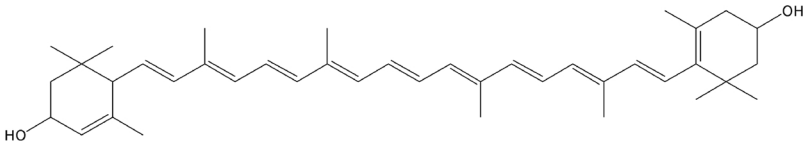

Fig. 1. Chemical structures of *trans*-lutein (β,ε-carotene-3,3′-diol).

interest in isolating lutein from natural matrices such as marigold petals using green solvents to comply with increasingly stricter regulations to manufacturing processes for foods and pharmaceuticals. Carbon dioxide (CO_2) above its critical conditions (temperature, 304.1 K; pressure, 7.38 MPa [3]), defined as SuperCritical (SC), can be utilized to recover organic-solvent-free extracts with minimal thermal damage and improved quality. The limitations of SC-CO_2 as a solvent for high molecular and/or polar solutes [3] such as xanthophylls, may limit industrial extraction processes for lutein.

In order to increase the solubility (molar fraction, y_i) at operational pressure (p) and temperature (T) of xanthophylls in the SC-CO_2, so as to improve their extraction from biological substrates, it is common practice to add polar entrainers. To be acceptable by the industry, these entrainers must have GRAS (Generally Recognized As Safe) status, and possess affinity with CO_2, the biological matrix, and/or (the) target solute(s) [4]. Ethanol has been successfully added to SC-CO_2 to aid extraction of several carotenoids from a variety of biological substrates including plant material such as carrot roots [5], red pepper (paprika) fruits [6], tomato fruits [7,8], stinging nettle leaves [9], and apricot fruits [10], shells of marine animals such as crawfish [11] and blue crab [12], and unicellular microorganisms such as *Spirulina pacifica* [13], *Haematococcus pluvialis* [14,15], *Synechococcus* sp. [16], *Chlorella pyrenoidosa* [17], and *Chlorella vulgaris* [18]. Extracted carotenoid compounds include carotenes such as α-carotene [5], β-carotene [5–7,9,10,13,16], and lycopene [7,8], and free and esterified xanthophylls such as astaxanthin [11,12,14,15], capsanthin [6], capsorubin [6], β-cryptoxantin [6,13,16], lutein [9,17,18], and zeaxanthin [6,13,16]. The positive effect of ethanol added on the extraction of these carotenoids may be due to cosolvent (solubility-increasing) effects, resulting from specific interactions of ethanol with CO_2 and the target solute, or modifier (matrix-modifying) effects, resulting from specific interactions of ethanol with biding sites on the biological substrate that release bound carotenoids, or ethanol-induced swelling of the biological substrate that facilitates solute transport in the biological substrate [4].

The cosolvent effect of ethanol for the dissolution of a particular solute in CO_2 can be identified by comparing high-pressure phase equilibrium measurements, ternary (CO_2 + ethanol + solute) and binary (CO_2 + solute) systems. This was illustrated by Araus et al. [4] for β-carotene by comparing its solubility in (CO_2 + ethanol) and in pure CO_2 at the same temperature and pressure. An inherent difficulty in this type of studies is the high cost of pure carotenoid compounds, particularly from natural origin. Specifically, carrying out the present study would have been extremely expensive considering the requirement of two grams of solute, and the cost of > 97% pure lutein from Sigma-Aldrich (Saint Louis, MO) (650 USD for milligram) [19]. That is why some authors have isolated pure carotenoids from biological substrates to study their solubility in SC-CO_2 including free lutein from marigold [20], lutein diesters from sneezeweed (*Helenium autumnale*) [20] or marigold [21], lycopene from tomato [22], and capsanthin from red pepper [23].

The aim of this work was contributing to the understanding of the SC-CO_2 extraction of marigold petals using fluid mixtures of (CO_2 + ethanol) as the solvent, by characterizing the cosolvent effect of the ethanol. Free solid *trans*-lutein was isolated from marigold petals and a comparison was made of its solubility in the ternary (CO_2 + ethanol + lutein) system with its corresponding solubility in the binary (CO_2 + lutein) system at equivalent system temperatures (313, 323, and 333) K and pressures (18.70–33.55) MPa. This new information should help understanding the role of ethanol in facilitating release of the target solute from the biological matrix, and/or facilitating solute movement through the matrix.

2. Materials and methods

2.1. Materials

Free solid *trans*-lutein was isolated from petals of marigold flowers using the same method previously applied for the isolation of capsanthin from red pepper fruits [23]. Petals of flowers bought in a local market (Easy S.A.) were removed by hand, dried for 8 h in a tray drier set at 313 K–0.119 $kg \cdot kg^{-1}$ moisture, and coarsely ground in a pilot plant hammer mill. Dried and milled petals were fully extracted at room temperature using acetone. Following acetone removal by vacuum evaporation at < 329 K, saponification was performed at room temperature to completion (overnight) using a 1:4 (w/w) mixture of KOH and methanol. The organic phase was subsequently extracted with diethyl ether, neutralized by washing with water, dried over anhydrous Na_2SO_4, and filtered. Following desolventization by vacuum evaporation, the saponified lutein was isolated by open column chromatography using as the stationary phase a 1:1 (w/w) mixture of MgO and celite that was heat activated at 390 K for 2 h. Lutein was step-eluted using solvents of increasing polarity (mobile phase) [24]. The identification of free (de-esterified) *trans*-lutein was confirmed by HPLC analysis as described in Fig. S1, included in the Supplementary material section. The analysis indicated that the sample was *trans*-lutein 92% pure, which was verified by comparing its retention time and UV–vis spectrum with a standard, 97% pure *all-trans*-lutein from Sigma-Aldrich [19]. The chromatogram of lutein sample isolated in this work was similar to the one reported by Delgado-Vargas and Paredes-Lopez [27] for isolated lutein from petals of marigold flowers. In addition, the 8% impurities quantified in Fig. S1 was possibly associated to a mixture of carotenoids, among them *cis*-lutein [24–27]. The process used to recover *trans*-lutein would ensure that the solute was free of moisture. Table 1 summarizes the specifications of the chemicals used in this work.

2.2. Experimental methods

Solubility (y_i) of solid *trans*-lutein (i = 3) in pure CO_2 (i = 1) and fluid mixtures of (CO_2 + ethanol (i = 2)) was measured using the dynamic-analytical method and experimental procedure described by Araus et al. [4]. The apparatus consists of a high-pressure equilibrium view-cell (50 cm^3 capacity) coupled to a HPLC (Hitachi LaChrom, Tokyo, Japan) that includes a Photodiode Array Detector (PAD) to asses on-line the lutein content in the CO_2-rich phase. For each isotherm the equilibrium cell was loaded with approximately 0.6 g of isolated lutein, and for experiments with (CO_2 + ethanol), the alcohol was added directly to the cell by measuring a volume exactly 2.00 cm^3 of pure ethanol at 296 K (ρ_2 = 787.2 $kg \cdot m^{-3}$) that corresponds to 1.57 g. The average amount of CO_2 loaded, approximately 70 g for each data point, was calculated based on the previously measured 82 cm^3 [4] occupied by the gas in the cell and the recirculation solvent loop, and the volumetric properties of CO_2 estimated as a function of system temperature and pressure [28]. This amount of ethanol was fully dissolved in the CO_2-rich phase for all operation conditions considered, visually verified for each experiment that there were two phases solid + fluid at equilibrium, and confirmed by the phase behavior of the binary system CO_2

Table 1
Specification of chemical samples.

Chemical name	Source	Initial Mass Fraction Purity / kg kg^{-1}	Purification Method	Final Mass Fraction Purity / kg kg^{-1}	Analysis Method
Carbon dioxide	AGA-Chile S.A.	0.9999	None	0.9999	None
Ethanol	Sigma-Aldrich (Saint Louis, MO)	0.999	None	0.999	GC[a]
Lutein	*Tagetes erecta* L.	None	CC[b]	0.92	HPLC[c]
Acetone	J.T. Baker (Phillipsburg, NJ)	0.999	None	0.999	GC[a]
Acetonitrile	Merck (Darmstadt, Germany)	0.999	None	0.999	GC[a]
Diethyl ether	Merck (Darmstadt, Germany)	0.999	None	0.999	GC[a]
Methanol	Merck (Darmstadt, Germany)	0.999	None	0.999	GC[a]
Water	Merck (Darmstadt, Germany)	0.999	None	0.999	None
KOH	Merck (Darmstadt, Germany)	0.85–0.99	None	0.85-0.99	None
trans-Lutein[d]	Sigma-Aldrich (Saint Louis, MO)	0.97	None	0.97	None
Na_2SO_4	Sigma-Aldrich (Saint Louis, MO)	0.99	None	0.99	None

[a] Gas Chromatography (GC).
[b] Column chromatography (CC).
[c] High Performance Liquid Chromatography (HPLC).
[d] Analytical standard (CAS 127-40-2).

Table 2
Experimental molar fraction (y_3) and its combined expanded uncertainty ($U_{Comb}(y_3)$) [31] for solid *trans*-l€€€€€ (3) in supercritical CO_2 (1)-rich phase, as a function of system pressure (p) or density of pure CO_2 (ρ_1) at temperatures (T) of (313, 323, and 333) K.

T[a] / K	p[b] / MPa	ρ_1[c] / kg·m^{-3}	$y_3 \cdot 10^6$ / mol·mol^{-1}	U_{Comb} (y_3)[d]·10^6 / mol·mol^{-1}
313	18.70	825.60	0.82	0.18
	23.75	870.44	1.08	0.22
	28.28	899.98	1.14	0.20
	32.98	925.08	1.55	0.28
323	20.62	791.36	1.42	0.24
	23.77	822.84	1.66	0.23
	28.55	860.65	1.89	0.23
	32.41	884.84	2.05	0.21
333	19.17	709.39	1.26	0.14
	23.45	770.21	1.74	0.26
	28.59	819.02	2.33	0.28
	32.91	849.75	2.45	0.27

[a] u(T) = 0.1 K (standard uncertainty for temperature).
[b] u(p) = 0.01 MPa (standard uncertainty for pressure).
[c] [28].
[d] Values estimated with a 0.95 level of confidence.

+ ethanol [4,29].

The air bath where the cell was located was adjusted to the required system temperature. Following removal of residual air by displacement with low-pressure (0.2 MPa) food-grade CO_2 (> 99% pure) followed by application of negative pressure with a vacuum pump (Welch Vacuum, Skokie, IL), the cell was loaded with CO_2 to the required system pressure *ca* (18–34) MPa using a syringe pump (Teledyne ISCO 260D, Lincoln, NE). Once the required operating conditions were reached, the components within the cell were thoroughly mixed for 8 h by recirculating the CO_2-rich phase using a gear pump (GAH-T23, Eurotechnica, Bargteheide, Germany). After reaching equilibrium, the CO_2-rich phase was sampled and analyzed, and the pressure readjusted to a higher value by feeding CO_2 to the cell with the syringe pump. This procedure was repeated as required up to reaching the desired upper pressure. Typically three-to-four (occasionally two or five) replicate measurements were carried out for each experimental condition. The concentration of free lutein in CO_2-rich phase was quantified by using the HPLC gradient method of Giuffrida et al. [26]. The chromatographic separation of lutein was carried out in a C30 reversed-phase packed column (Carotenoid S-5, YMC Europe, Schermbeck, Germany) using 1.0 cm^3 min^{-1} of acetonitrile at 303 K. Lutein was identified at 445 nm by using the PAD and its molar fraction in the CO_2-rich phase was assessed from the chromatographic peak area compared with those of the standard solutions in acetone [4].

The standard and combined expanded uncertainties were estimated using the information available for the experimental procedure and data measured according to definitions of Chirico et al. [30]. The methodology used to estimate the combined expanded uncertainty of lutein molar fraction in the CO_2-rich phase $U_{Comb}(y_1)$ is detailed in Table 3 reported by Cabrera et al. [31]. The combined expanded uncertainty of ethanol, $U_{Comb}(y_2)$, was estimated by calculating independently the mole of CO_2 and ethanol fed to the cell, using the density (temperature and pressure dependent) and the total volume measured of each component. The standard uncertainties of the total volume of CO_2 and ethanol were 1 cm^3 and 0.1 cm^3, respectively. The uncertainty for the densities were determined with the same expression as a function of temperature (0.1 K) and pressure (0.01 MPa) used for the solute uncertainty calculations [31]. In both cases a coverage factor of 1.96 (95% of level of confidence for a normal distribution) was used.

3. Results and discussion

This section will present and discuss separately the results for the solubility of solid lutein in pure CO_2 and ethanol-modified CO_2.

3.1. Solubility of solid lutein in pure CO_2

Table 2 and Fig. 2 present the solubility of solid lutein in pure CO_2-

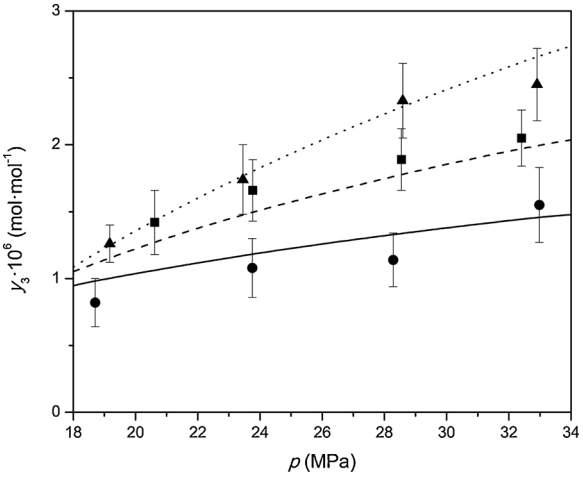

Fig. 2. Experimental molar fraction (solubility, y_3) of solid *trans*-lutein (3) in pure CO_2 (1) as a function of system pressure (p) with their corresponding uncertainties represented by the error bars, at 313 K (●, —); 323 K (■, — —) and 333 K (▲, – –). Lines represent the correlation of Méndez-Santiago and Teja for binary systems according to Equations (1).

rich phase as a function of temperature and pressure. Uncertainties of lutein solubilities reported in Table 2 were $U_{Comb}(y_3) \leq \pm 0.28 \cdot 10^{-6}$ mol·mol^{-1}, using a level of confidence of 95% (i.e., coverage factor of 1.96). The solubility increased with both temperature and pressure, by a factor of about 3 between extreme conditions, from 313 K and 18.70 MPa ($y_3 = 0.82 \cdot 10^{-6}$ mol mol^{-1}) to 333 K and 32.91 MPa ($y_3 = 2.45 \cdot 10^{-6}$ mol mol^{-1}).

Using the same experimental apparatus and methodology, authors observed equivalent trends and slightly higher values, (0.65–1.97)·10^{-6} mol mol^{-1} for capsanthin [23], and values about 2–3 times smaller (0.17–1.06)·10^{-6} mol mol^{-1} for β-carotene [4] within the same experimental region. Solubility increased with system pressure as a result of an increase in the solvent power of CO_2 with its density [3]. Solubility also increased with system temperature as a result of the increase in the volatility (vapor pressure) of the solute [3]. Concurrent increases in the solvent power of CO_2 and the volatility of the solute would facilitate transfer of the solute from the solid to the supercritical phase. Nevertheless, Fig. 2 suggests that the positive effect of system temperature on solubility decreases with system pressure.

The correlation of Méndes-Santiago and Teja [32], $T \cdot [\ln(p \cdot (y_3^{Calc})_{CO_2}) - C_3] = A_3 + B_3 \cdot \rho_1$, was selected to calculate the solubility of lutein in pure CO_2, $(y_3^{Calc})_{CO_2}$, as a function of CO_2 density (ρ_1), estimated using NIST database [28], and three adjustable parameters of the model (A_3, B_3 and C_3) which were fitted to the experimental data of Table 2. The result, presented in Equation (1), indicated a mean deviation of ± 0.13·10^{-6}mol·mol^{-1}.

$$(y_3^{Calc})_{CO_2} \cdot 10^6 = \frac{0.1013}{p} \cdot \exp\left[\frac{-9589 + 3.069 \cdot \rho_1}{T} + 13.90\right] \quad (1)$$

Lines in Fig. 2 correspond to predictions of Eq. (1). The extrapolation of these lines suggest a estimated crossover pressure of (15.7–17.4) MPa for the binary (CO_2 + lutein) system (not shown), that is close to the crossover pressure reported for other carotenoids, approximately 16 MPa for lycopene [22], (15.7–17.3) MPa for β-carotene [4], and 16.7 MPa for capxanthin [23]. At the so-called crossover pressure [33] the effects of the temperature on reducing the density of CO_2 and increasing the vapor pressure of lutein counterbalance each other so that the solubility of lutein in SC-CO_2 remains constant when the system is heated isobarically. Below the crossover pressure, solubility decreases as temperature increases because of the marked decrease in CO_2 density associated with its increased compressibility as it approaches critical conditions.

Isotherms reported in Table 2 (313, 323, and 333) K are complementary with those of Jay et al. [20], (288, 308, and 328) K, for the solubility of 88% pure lutein isolated from marigold. The analysis of both data sets show that the solubility values of lutein in the CO_2 are consistent and comparable. According to Güçlü-Üstündağ and Temelli [34], the solute purity and the nature of the impurities could affect the measured solubility values, because impurities may act as cosolvents or antisolvents, as observed by these authors for the solubility of β-carotene in SC-CO_2. The 8% impurities estimated for the isolated lutein were qualitatively identified as a mixture of carotenoids, based on HPLC chromatograms reported by Giuffrida et al., [26] and Delgado-Vargas and Paredes-Lopez [27]. The agreement between reported results in this contribution and literature data [20] might be indicative of minor cosolvent or antisolvent effects of impurities limited to 8–12 % corresponding to a mixture of carotenoids [26,27].

3.2. Solubility of solid lutein in ethanol-modified CO_2

Table 3 reports the solubility of solid lutein in mixtures of (CO_2 + ethanol) as a function of system temperature and pressure. Uncertainties of data in Table 3 estimated using a level of confidence of 95% were $U_{Comb}(y_2) \leq \pm 0.0032$ mol·mol^{-1} for ethanol concentration, and $U_{Comb}(y_3) \leq \pm 0.42 \cdot 10^{-6}$ mol·mol^{-1} for lutein solubilities. Results showed that the effects of system temperature and pressure on the solubility behavior of lutein in the mixtures (CO_2 + ethanol) were similar to those in pure CO_2, but with larger values because of the ethanol cosolvency effects. According to the solute purity reported, values in Table 3 should be considered as indicative values for the solubility of pure lutein in SC-CO_2 modified with ethanol.

To characterize the cosolvent effect of ethanol on the solubility of lutein in SC-CO_2 the solubility enhancement (E), was computed according to the Eq. (2).

$$E(T, p) = \frac{(y_3)_{(CO_2+Ethanol)}(T, p)}{(y_3)_{CO_2}(T, p)} \quad (2)$$

The experimental solubilities measured of solid lutein in (CO_2 + ethanol) (Table 3) were divided by estimated solubilities of lutein in pure CO_2, computed using Eq. (1) for the experimental values of T, p, and ρ_1 [28]. Fig. 3 summarizes results of these computations. Enhancement decreased from 2.1 at (323 K and 19.16 MPa) to a constant asymptotic value of approximately 1.5 for the three isotherms as pressure increased. This effect was partly due to the decreasing in the ethanol concentration, calculated from the amount of ethanol fed to the cell starting the isotherm, because of its dilution with the CO_2 added to increase the cell pressure, with a decrease from the lower to upper pressure of 10.2% at 313 K, 12.9% at 323 K, and 16.3% at 333 K. The variability for the computed values of enhancement (Fig. 3) was larger than the variability for the experimental values of solubility in pure CO_2 (Fig. 2) because of the uncertainties in the estimated solubilities of lutein in pure CO_2 using Eq. (1). Although the limited increases in the density of CO_2 resulting from the dissolution of approximately 0.02 mol mol^{-1} ethanol probably cannot explain the increase in solubility of lutein in the fluid mixtures (CO_2 + ethanol), as compared to pure CO_2 [4], ethanol may cause an increase in local density near solute molecules, by preferentially associating with lutein molecules [35]. Alternatively, ethanol dissolved might modify the dispersion, orientation, and/or acid-base partial solubility parameters of (CO_2 + ethanol) as compared to pure CO_2, thus, increasing their affinity with lutein molecules [36]. Finally, ethanol-mediated hydrogen bonds may facilitate the synthesis of high-solubility lutein complexes [37]. Values and trends for enhancements in the solubility of lutein in CO_2 by the addition of (0.0194–0.0252) mol mol^{-1} of ethanol were similar to those observed for the solubility of β-carotene in (CO_2 + ethanol), where the enhancement increased from 1.7 at (313 K and 34 MPa) to 2.6 at (333 K and 18 MPa) [4]. It was a surprise that the cosolvent effect of ethanol

Table 3
Experimental molar fraction (y_i) of the vapor phase and its combined expanded uncertainty ($U_{Comb}(y_i)$) [31] for the ternary system consisting of CO_2 (1), ethanol (2) and solid *trans*-l€€€€€ (3), as a function of system pressure (p) or density of pure CO_2 (ρ_1) at temperatures (T) of (313, 323, and 333) K.

T[a] / K	p[b] / MPa	ρ[c] / kg·m^{-3}	$y_2 \cdot 10^2$ / mol·mol^{-1}	U_{Comb} (y_2)[d]·10^2 / mol·mol^{-1}	$y_3 \cdot 10^6$ / mol·mol^{-1}	U_{Comb} (y_3)[d]·10^6 / mol·mol^{-1}
313	19.18	831.53	2.16[e]	0.27	2.04	0.28
	22.52	861.37	2.08	0.26	2.09	0.16
	28.28	900.10	2.00	0.25	2.15	0.14
	33.55	927.64	1.94	0.24	2.22	0.30
323	19.16	773.11	2.32[e]	0.29	2.45	0.18
	23.48	820.65	2.19	0.28	2.58	0.14
	28.61	861.27	2.09	0.26	2.81	0.16
	33.22	889.49	2.02	0.25	3.02	0.31
333	19.10	708.47	2.52[e]	0.32	2.55	0.28
	23.20	767.14	2.33	0.30	3.32	0.34
	28.54	818.39	2.19	0.28	3.69	0.42
	32.91	849.93	2.11	0.27	4.02	0.30

[a] u(T) = 0.1 K (standard uncertainty for temperature).
[b] u(p) = 0.01 MPa (standard uncertainty for pressure).
[c] [28].
[d] Values estimated with a 0.95 level of confidence.
[e] Calculated from the amount of ethanol (2) fed to the cell starting the isotherm.

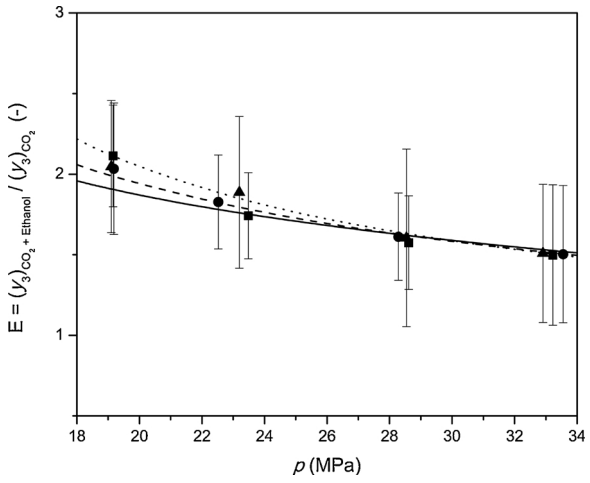

Fig. 3. Enhancement in the solubility (molar fraction) of solid *trans*-lutein (3) in fluid mixtures of CO_2 (1) + ethanol (2), compared with solubility in pure CO_2 (1), E= $(y_3)_{(CO_2+Ethanol)}/(y_3)_{CO_2}$ defined in Equation (2), as a function of pressure (p), at 313 K (●, —); 323 K (■, — —) and 333 K (▲, – –). Symbols represent values calculated with experimental measurements. Lines represent predictions using solubility correlations of Méndez-Santiago and Teja for binary [32] and ternary [38] systems, equations (1) and (3). The error bars represent the uncertainties estimated for the enhancement based on the uncertainties of the experimental solubility data.

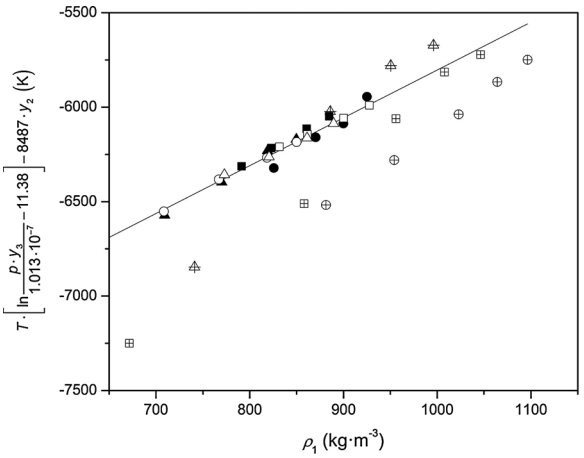

Fig. 4. Molar fraction (solubility, y_3) of solid *trans*-lutein (3) in fluid mixtures of CO_2 (1) + ethanol (2), and pure CO_2 (1) represented in a two-dimensional arrangement of $T\cdot[\ln(p\cdot y_3) - J_3] - H_3\cdot y_2$ as a function of CO_2 density (ρ_1). Symbols represent experimental results. Binary system CO_2 (1) + lutein (3): This work (Table 2) at 313 K (●); 323 K (■) and 333 K (▲); Jay et al. [20] at (⊕) 288 K, (⊞) 308 K, or (⟁) 338 K. Ternary system CO_2 (1) + ethanol (2) + lutein (3): This work (Table 3) at 313 K (○); 323 K (□) and 333 K (△). Straight line represents the Méndez-Santiago and Teja [38] correlation based on Equation (3).

was not larger for lutein than for less polar β-carotene, considering that the two −OH substituents in lutein (Fig. 1) should facilitate the engagement of lutein in acid-base and hydrogen bond interactions as compared to β-carotene.

The extension of the original equation of Méndez-Santiago and Teja [38] for mixtures of CO_2 and co-solvents, $T\cdot[\ln(p\cdot(y_3^{Calc})_{(CO_2+Ethanol)}) - J_3] = F_3 + G_3\cdot\rho_1 + H_3\cdot y_2$, was used to calculate the molar fraction of solid lutein, $(y_3^{Calc})_{(CO_2+Ethanol)}$, in mixtures ($CO_2$ + ethanol). The four adjustable parameters (F_3, G_3, H_3, and J_3) were fitted to data in Table 3, resulting in Eq. (3) with a mean deviation of ± 0.15·10^{-6}*mol·mol*$^{-1}$.

$$(y_3^{Calc})_{(CO_2+Ethanol)}\cdot 10^6 = \frac{0.1013}{p}\cdot\exp\left[\frac{-8335 + 2.532\cdot\rho_1 + 8487\cdot y_2}{T} + 11.38\right] \quad (3)$$

Eqs. (1) and (3) were used to estimate the enhancements, represented by lines in Fig. 3, at (313, 323, and 333) K. The molar fraction of ethanol was estimated with Eq. (4).

$$y_2 = \frac{m_2/MW_2}{m_2/MW_2 + V\cdot\rho_1\cdot 10^{-3}/MW_1}, \quad (4)$$

where, $m_2 = 1.574g$ is the weight of ethanol loaded into the cell, $MW_2 = 46.07\text{g·mol}^{-1}$ is the molecular weight of ethanol, $MW_2 = 46.07\text{g·mol}^{-1}$ is the molecular weight of CO_2, and $V = 82cm^3$ is the volume of cell and other components in the solvent loop, according to Araus el al. [4].

Caution is suggested in interpreting the lines in Fig. 3, because they represent average values without its corresponding deviations. At the pressure range of Table 3, (19.10–33.55) MPa, the predictions of solubility enhancements in Fig. 3 decreased from 2.1 at 333 K to approximately 1.5, with the three lines overlapping.

Eq. (3) can be rearranged into a complex function of y_2 and other system conditions (p, T, y_3) as a linear function of ρ_1. Fig. 4 describes

the collapse of the isotherms to a single line, which confirms the self-consistency of the experimental data, a kind of precision according to Méndez-Santiago and Teja [38], for experimental solute solubility in mixtures of CO_2 and cosolvents. In Fig. 4 were also included experimental results of Jay et al. [20] showing that the effect of temperature on the solubility of lutein in SC-CO_2 differs from this study. In fact, using Eq. (3) for $\rho_1 \geq 740\,kg\,m^{-3}$ and $y_2 = 0\,mol\,mol^{-1}$, predicted values that where in average 62% larger at 288 K, 42% larger at 308 K, and 16% smaller at 328 K than corresponding values experimentally measured by Jay et al. [20]. Ruen-ngam et al. [18] carried out the extraction of lutein from mixtures of ethanol treated *Chlorella vulgaris* with SC-CO_2. Authors estimated the solubility of lutein in pure and modified CO_2 from the initial slope of the extraction curve. Results showed differences of two orders of magnitude compared with solubility values measured by Jay et al. [20] and this contribution.

4. Conclusions

Free, 92% pure solid *trans*-lutein was isolated from marigold flower (*Tagetes erecta* L.). Isothermal solubility of lutein in pure CO_2, and mixtures of CO_2 with (0.0194–0.0252) $mol\,mol^{-1}$ of ethanol at (313, 323 and 333) K, was experimentally determined using an analytic-recirculation methodology. The molar fraction of lutein in the supercritical phase was $\leq 2.45{\cdot}10^{-6}$ $mol{\cdot}mol^{-1}$ at (333 K, 32.91 MPa) with estimated uncertainties $\leq \pm 0.28{\cdot}10^{-6}$ $mol{\cdot}mol^{-1}$ for pure CO_2; and, $\leq 4.02{\cdot}10^{-6}$ $mol{\cdot}mol^{-1}$ at (333 K, 32.91 MPa) with uncertainties $\leq \pm 0.42{\cdot}10^{-6}$ $mol{\cdot}mol^{-1}$ for the fluid mixture (CO_2 + ethanol). Experimental results were represented with a correlation of Méndez-Santiago and Teja [38], in order to estimate the molar fraction of lutein as a function of ethanol content, system temperature, and pure CO_2 density. The effect of ethanol on the solubility of solid lutein was assessed with the enhancement factor (or the ratio of the solubility of lutein in the mixture (CO_2 + ethanol) to pure CO_2) that decreased from 2.2 to 1.5 as the amount of CO_2 loaded into to the cell increased. Ethanol added might promote the affinity between CO_2 and lutein molecules by the association lutein-ethanol decreasing the polarity and increasing the local density near solute molecules.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.supflu.2018.08.012.

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