

Solubility of Purified Lutein Diesters Obtained from *Tagetes erecta* in Supercritical CO₂ and the Effect of Solvent Modifiers

Sandra Naranjo-Modad,[†] Agustín López-Munguía,[‡] Gérard Vilarem,[§] Antoine Gaset,[§] and Eduardo Bárzana^{*,†}

Departamento de Alimentos y Biotecnología, Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, 04510 México, D.F., Mexico; Instituto de Biotecnología, UNAM, Av. Universidad 2001, Col. Chamilpa, C.P. 62210, Cuernavaca, Morelos, Mexico; and Laboratoire de Chimie Agro-Industrielle, École Nationale Supérieure de Chimie de Toulouse, Institut National Polytechnique, 118 route de Narbonne, 31077 Toulouse Cedex 4, France

Purified lutein diesters deposited on commercial nonporous glass beads were solubilized in supercritical CO₂ in a computerized batch extractor, and their solubilities were compared to their solubilities in hexane. Densities of 0.7, 0.8, and 0.9 g/mL were evaluated without modifiers. Both pressure and temperature increased solubility, although temperatures >50 °C promoted carotenoid loss as determined by mass balance. Solubility was enhanced by the use of modifiers and was related to their log *P*. Chloroform (log *P* = 2) increased 2.8 times the amount of solubilized lutein diesters compared to pure CO₂ at the same extraction conditions (0.9 g/mL and 40 °C) to yield 65% of the amount extracted with hexane. Supercritical CO₂ extraction of lutein diesters could represent a cleaner technology as compared to the current industrial use of hexane with important ecological and health-related implications.

Keywords: *Supercritical CO₂; carotenoids; lutein; Tagetes erecta; modifiers; log P*

INTRODUCTION

Carotenoids are one of the most used natural food colorants due to their innocuous effect, wide availability, and presence in nature in various forms. Within these, xanthophylls or oxycarotenoids comprise a group that occurs naturally in the free or esterified form (Davies, 1976). Marigold (*Tagetes erecta*) is considered of commercial importance because it is a rich source of xanthophylls present in the form of lutein diesters and has been used for years in poultry feed to confer an acceptable color to skin and egg yolk.

Industrial extraction is performed with hexane from the milled dried flower, followed by solvent evaporation to obtain a lutein-rich oleoresin. This process is of commercial importance in Mexico.

Supercritical CO₂ extraction (SCCE) has attracted attention as an alternative to most hexane extraction processes, on the basis of its lack of residual toxicity and low environmental impact. Previous studies on extraction of carotenoids with supercritical CO₂ have focused mostly on β -carotene from carrots (Barth et al., 1995), sweet potatoes (Spanos et al., 1993), and leaf protein concentrates (Favati et al., 1988). In these studies, the reported optimum conditions depend on the maximum pressure delivered by the extraction equipment used. No systematic studies have been published on the use of modifiers for the supercritical extraction of carotenes or esters of xanthophylls, although it is

known that ethanol enhances the solubility of α - and β -carotene (Barth et al., 1995).

Our research is aimed to assess supercritical CO₂ as an alternative to traditional hexane extraction for the production of marigold oleoresin. Given that marigold meal contains a complex mixture of lipid soluble materials, it is essential to establish as a first step the capacity of supercritical CO₂ to solubilize pure lutein diesters and to evaluate the effect of different modifiers on the solubilization process.

MATERIALS AND METHODS

Sample Preparation. Lutein diesters were purified to 92% from oleoresin obtained by hexane extraction, according to the method of Philip and Berry (1976). The purity was measured using a standard method for the determination of carotenoids (AOAC, 1992). Purified diesters were added directly into the extraction cell or deposited on the surface of nonporous glass beads prior to CO₂ solubilization to increase the area of contact. For this deposition, 50 mg of purified lutein diesters was dissolved in 10 mL of hexane and 10 g of clean glass beads (mean diameter = 1.06 mm, Sigma Chemical Co., St. Louis, MO) was added to the preparation. After thorough mixing, hexane was evaporated using a gentle nitrogen stream. To measure the load of lutein diesters deposited on the beads, a specific amount of colored beads was weighed and extracted with hexane in a flask. An aliquot of hexane was diluted, and the absorbance at 474 nm was compared to a standard curve of the purified lutein diesters supplied by Bioquimex-Reka, Querétaro, Qro, Mexico.

Supercritical CO₂ Extraction. An analytical scale extractor (HP 7680T) was used for solubility studies. The operating temperature, pressure, time, and flow were set and controlled by a computer interface. A sample of 1.0 mg of purified diesters or 200–300 mg of beads containing 900–1100 μ g of lutein diesters was placed into a 7 mL extraction cell, and the supercritical fluid was pumped into the cell. Conditions were

* Author to whom correspondence should be addressed [telephone (52-5) 622-53-10; fax (52-5) 622-53-45; e-mail ebg@servidor.unam.mx].

[†] Departamento de Alimentos y Biotecnología.

[‡] Instituto de Biotecnología.

[§] Laboratoire de Chimie Agro-Industrielle.

Table 1. Modifier Percentages, log *P* Values, and Critical Variables of Their Mixture with Supercritical CO₂

modifier	% vol	% mol fraction	log <i>P</i> ^a	critical variables ^b (atm/K)
methanol	5	5.8	-0.76	98.5/328.5
acetonitrile	6	5.4	-0.33	105.1/334.4
ethanol	7	5.7	-0.24	104.1/331.6
acetone	9	5.9	-0.23	97.4/332.0
isopropanol	9	5.7	0.20	107.7/335.3
methylene chloride	7	5.2	0.60	90.3/325.0
ethyl acetate	11	5.6	0.66	103.0/336.2
<i>n</i> -butanol	11	5.9	0.80	122.9/339.6
chloroform	9	5.5	2.0	98.0/330.7
carbon tetrachloride	11	5.1	2.8	100.9/332.7
hexane	14	5.5	3.5	95.4/333.8

^a log *P* is the logarithm of the partition coefficient of the solvent in the two-phase system octanol–water. Its is calculated according to the method of Rekker (1977). ^b Critical variables were calculated with software as described previously (Solórzano-Zavala et al., 1996).

as follows: flow, 4 mL/min; total CO₂ volume, 7 mL; densities, 0.7, 0.8, or 0.9 g/mL; temperatures, 40, 45, 50, or 55 °C. Holding times were varied from 0 to 60 min at intervals of 10 min to determine equilibrium conditions. Once the holding time had concluded, the supercritical phase was swept to a nozzle where CO₂ was expanded to atmospheric pressure. The nozzle was connected to a stainless steel trap held at 40 °C, where the extract precipitated as fine powder. The powder was then washed into vials with hexane and, after dilution, the absorbance was read at 474 nm and compared to the standard curve previously described. The carotenoids remaining in the extraction cell were quantitatively washed with hexane and measured in the same way to close the mass balance of the process. Extraction yields are reported as percent of the hexane extraction of the colored glass beads (which was fixed as 100% recovery).

Supercritical CO₂ Extraction with Modifiers. On the basis of results obtained at different operating conditions, a density of 0.9 g/mL and a temperature of 40 °C were chosen as the experimental conditions for assessing the effect of modifiers added to the supercritical fluid for the solubilization of lutein diesters on glass beads. Modifiers were chosen in terms of their polarity referred to by their log *P* values. An HPLC pump (HP 1050), connected immediately after the liquid CO₂ supply, ensured mixing of CO₂ and modifier prior to the high-pressure pump and extraction cell. The nozzle temperature was fixed for evaporation of the modifier prior to the trap, to avoid extract carry-over through the waste port. From previous runs, the holding time was set to 45 min to reach equilibrium conditions, and triplicates were run for each modifier. Extracted lutein diesters were measured as described above.

To have comparable results for the extraction with modifiers, equivalent molecular amounts of the different modifiers were used (i.e., mole fraction percentages rather than volume percentages). The computer software used permits only the setting of the percentage in volume of modifiers, so the corresponding mole fractions were adjusted as close to each other as possible. Pressure conditions were set to operate above the critical point of the mixture. Table 1 shows information regarding the modifiers used in this study.

RESULTS AND DISCUSSION

Effect of Pressure and Temperature. Direct supercritical CO₂ processing of purified lutein diesters resulted in low statistical reproducibility of extraction yield, due to variations and difficulties in handling a sample of small size within the extraction cell. Deposition of the diester on nonporous glass beads prior to supercritical fluid solubilization allowed a more uniform handling, which resulted in an improved reproducibility

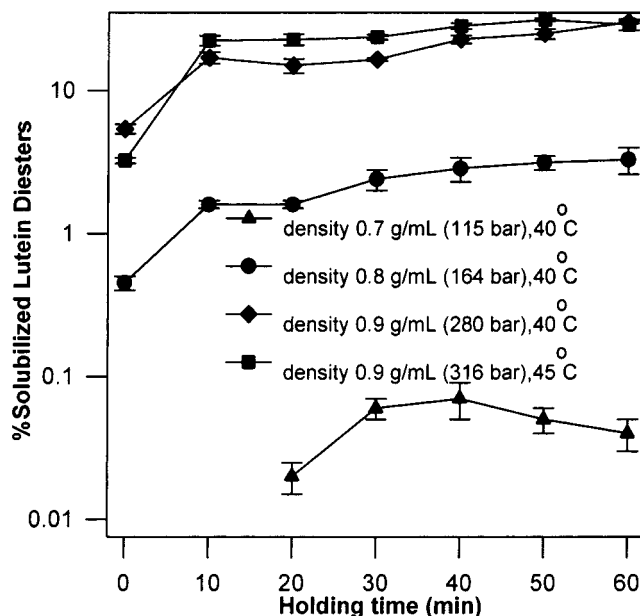


Figure 1. Solubility of lutein diesters in supercritical CO₂ as a function of extraction time and fluid density. Lutein diesters (100 µg) were deposited on glass beads in a 7 mL fixed cell volume. No color was extracted at 0.7 g/mL after 0 and 10 min of contact time.

(<10% standard deviation). This procedure was employed in all further experiments.

In Figure 1 the recovery of lutein diesters at three different densities and the corresponding conditions is presented. It may be observed that the yield increases dramatically in proportion to density, obtained by incrementing the pressure at constant temperature for the range studied. For all cases, solubilization of lutein diesters occurs mostly during the first 10 min followed by a slow increment as holding time progresses. This indicates that a portion of lutein diesters is adsorbed on the surface of the beads that is not easily removed by the CO₂ solvent. This is consistent with previous reports that describe the competition for sorption sites on solid matrices (or supports) between the solvent and the target analyte, in terms of the forces involved (Jeong and Chesney, 1999). It is then possible that some layers of the diester interact preferentially with the hydrophilic glass surface, in analogy with the tightly bound monolayer encountered in sorbed water. After 40 min, the increase in absorbance was marginal, so it was assumed that equilibrium conditions were reached. At a constant temperature of 40 °C the highest extraction yield reached $22.8 \pm 1.5\%$ at 280 bar and 45 min of holding time. The lowest density at which lutein diesters could be solubilized at 40 °C was 0.7 g/mL (115 bar), with <1% in yield. This threshold density is close to the 0.65 g/mL value at 37 °C obtained by Subra et al. (1994) for β -carotene, taking into consideration that the molecular weight of lutein diesters is on average 1031 g/mol, whereas that of β -carotene is 536.9 g/mol.

It was also of interest to evaluate the effect of temperature on solubilization at constant density reached by a consequent adjustment in pressure. Although temperatures <40 °C are usually recommended for extraction and manipulation of carotenoids (Davies, 1976), previous groups have extracted carotenoids with supercritical CO₂ at temperatures as high as 70 °C (Cygnarowicz et al., 1990). In these cases, thermal degradation of carotenoids has been recognized as an

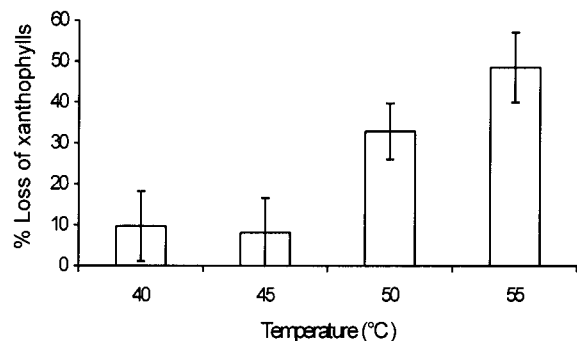


Figure 2. Color loss during supercritical CO₂ solubilization as a function of temperature at a constant fluid density (0.9 g/mL). Sample was prepared as indicated in Figure 1.

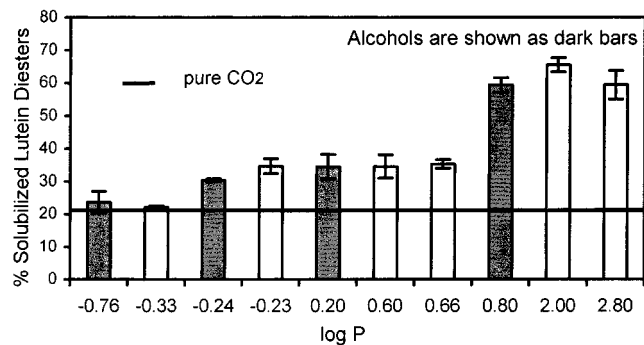


Figure 3. Effect of log P of modifiers in the solubilization of lutein diesters in supercritical CO₂. Fluid density was 0.9 g/mL at 40 °C. Sample was prepared as indicated in Figure 1.

undesirable phenomenon, but no precise measurements have been made of it. In principle, because solute vapor pressure increases with temperature, a compromise between solute solubilization and thermal degradation should result in an optimum temperature.

In our study, it was observed that as temperature increased at a constant fluid density, a larger quantity of pigments was solubilized, but product loss also increased in proportion to the temperature, as established by a mass balance. The results are shown in Figure 2. For instance, operation at 0.9 g/mL (364 bar) and 55 °C resulted in the solubilization of approximately half of the initial sample, but the other half was not recovered from the cell. At 40 and 45 °C the loss was reduced to ~10% of the initial pigment and was associated more to a deficit resulting from manipulation than to thermal degradation. According to these results, it was decided to conduct further experiments at 40 °C to minimize degradation losses of the colorant.

Effect of Modifier Addition. To increase the solubility of lutein diesters, a second strategy, based on a modification of the polarity of the CO₂ solvent through the addition of cosolvents, was explored. These results are shown in Figure 3, from which it may be observed that modifiers with log P > -0.24 increased lutein diester solubility compared to pure CO₂ at the same temperature–pressure combination, which is represented by a continuous line (23%). For the alcohol series (methanol, ethanol, isopropanol, and *n*-butanol), in-

creasing log P values resulted in higher extraction yields, reaching 60% for the case of CO₂ containing 5.9% of butanol, which is equivalent to a 2.4-fold increase in lutein diester solubility. No higher molecular weight alcohols were tested due to limitations in handling viscous fluids. For chloroform (log P = 2), a 2.6 increment was obtained (this is 65% of the amount extracted with hexane), whereas for carbon tetrachloride (log P = 2.8) the solubilization tended to level off. Hexane was also attempted (log P = 3.5), but the volume handled was too high and it was not possible to attain the mole percentage used for the rest of the modifiers (see Table 1).

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