AAPS PharmSciTech, Vol. 9, No. 2, June 2008 (© 2008)
DOI: 10.1208/s12249-008-9100-6

Research Article

Supercritical Extraction of Carotenoids from *Rosa canina* L. Hips and their Formulation with β-Cyclodextrin

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Received 15 February 2008; accepted 23 April 2008; published online 5 June 2008

Abstract. The purpose of this research was to preliminary assess the suitability of a new method for the preparation of a solid formulation in form of powder composed by β-cyclodextrin and the supercritical extract of *Rosa canina* hips. The method implies the extraction of carotenoids, in particular β-carotene, from freeze dried fruits of *R. canina* with supercritical CO_2 at 70 °C and 300 bar, in the presence of varying quantity of ethanol as entrainer. The obtained supercritical solution is then expanded at ambient conditions into an aqueous solution of β-cyclodextrin to favour the interaction between β-cyclodextrin and the lipophilic components of the extract. β-carotene solubility (mole fraction) in supercritical CO_2 or in supercritical CO_2/ethanol mixtures were in the order of $1\ 10^{-7}$. The β-carotene extracted from *R. canina* fruits (nearly 10 μg/g of dry matrix), interacts almost quantitatively with β-cyclodextrin affording a solid phase, which presents a low apparent solubility in water. Finally the interaction with β-cyclodextrin results in a higher concentration of the β-carotene *trans*- form relative to the *cis*- form in the extracted product when collected in an aqueous solution of β-cyclodextrin with respect to the extract in *n*-hexane.

KEY WORDS: β-carotene; β-cyclodextrin; interaction compound; *Rosa canina*; supercritical CO_2.

INTRODUCTION

In recent years great deal of interest has been devoted to the extraction of active components from natural sources, aiming at satisfying the increasing request of natural products not only for therapeutic use but also as preventing and protecting agents.

Among the large number of active substances in the focus, carotenoids have received particular attention in the last decade (1).

Carotenoids are used in the food industry for their nutritional and antioxidants properties; in particular, it was reported that they are able to reduce the risks connected to the action of free radicals and other oxidant chemicals (2,3).

β-carotene is a carotenoid of relatively high molecular weight, constituted by eight isoprene units, cyclized at each end, easily degradable by light, heat and air (4).

Numerous reports suggested that β-carotene possesses biological properties implying protection against cardiovascular disorders, arteriosclerosis, visual disorders as well as pathologies correlated with the age and cancer (5). In particular many retrospective clinical studies suggested that a suitable consume of β-carotene can be important to reduce the risk of pulmonary carcinoma, particularly in smokers (6).

Different species of Rosa are known in European Pharmacopoeias, in which the fruits and/or the pseudo-fruits of *Rosa canina* L., are considered as drugs. This part of the plant is known to contain Vitamin C, carotenoids, mainly lycopene and carotene and xantophylls (7–10), while only traces of flavonoids and phenols have been detected (7). It has been also suggested that these fruits could constitute, together with orange, mango and red pepper one of the main vegetal sources of natural carotenoid pigments (8–10) and particular attention has been devoted to the extraction of carotenoids from this natural source (11).

Typical extraction processes for carotenoids from natural matrices implies the use of organic solvent or relatively high temperatures.

Processes based on supercritical fluids (SFE) are an environment-friendly alternative to traditional solvent extraction techniques (TSE) (12,13). TSE often requires long extraction time and consumes large amount of organic solvents that may remain as residues in the final extract. Overall the most discernible features that differentiate the SFE from TSE are the high compressibility and diffusivity of the supercritical fluid.

The most widely used supercritical fluids (SFs) is carbon dioxide (SC CO_2) because of the low critical temperature (31.18 °C) and pressure (7.4 MPa), inexpensiveness, non-toxicity, non-flammability, recyclability and environmental benignity.

Several papers were published on the use of SC CO_2 to extract β-carotene from vegetables (13–18), while some works deal with the SC extraction from *R. canina* L. (19–23).

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In principle, the low critical temperature of CO_2 may be beneficial for thermally labile substances such as carotenoids. However, the extent of degradation of β-carotene through oxidation or isomerization during SFE is a matter of debate in the relevant literature. Chang and Randolph (24), reported that β-carotene was oxidized by CO_2, producing epoxide compounds. On the contrary, Jay *et al.* (25,26) did not find any evidence of β-carotene oxidation using CO_2 as solvent, while Cocero *et al.* (27) affirmed that this kind reaction is not even possible from chemical point of view.

From an applicative point of view, and in particular when the formulation of the extracts is concerned, the most important issues that have to be addressed are the aggregation and degradation of the solid material obtained after gas expansion.

In the case of β-carotene the availability of a stable and free-flowing powder would be desirable to implement the extract quality as well as to make easy further manufacturing operations.

β-cyclodextrins (CDs) are used in the pharmaceutical field for several applications mainly related to their ability to interact with other molecules, usually hydrophobic in nature, forming inclusion compounds or complexes. CDs have been proposed extensively for increasing aqueous solubility, stability and bioavailability (28–30) of drugs as well as for converting liquid drugs into powders (31).

Lancrajan *et al.* (32) have shown that the β-cyclodextrin can be considered a very good carrier for carotenoids. In this respect, Kaiser *et al.* (33) investigated the possibility of an in-line inclusion of chamomile CO_2 extracts in β-cyclodextrin during the extraction process, by placing predefined amount of solid β-cyclodextrin in the separation vessel of the extractor.

In the present work a method to prepare a solid formulation in form of powder composed by β-cyclodextrin and the supercritical extract of *R. canina* hips was investigated.

The method implies the extraction of carotenoids, in particular β-carotene, from freeze dried fruits of *R. canina* with SC CO_2 at 70 °C and 300 bar, in the presence of varying quantity of ethanol as entrainer. The obtained supercritical solution is then expanded at ambient conditions into an aqueous solution of β-cyclodextrin to favour the interaction between β-cyclodextrin and the lipophilic components of the extract.

MATERIALS AND METHODS

Phase Solubility Studies

Accurately weighed amounts of β-carotene (Sigma-Aldrich Inc. St. Louis, MO, USA purity ≥95%), in excess with respect to saturation, were added under nitrogen to 1 mL of distilled water or aqueous solutions of β-cyclodextrin with concentration ranging from 2.5 and 16.8 mg/mL and magnetically stirred in Eppendorf™ vials at 25±0.5 °C for 24 h. After centrifugation the supernatant was submitted to spectrophotometric UV analysis (V570, Jasco, Tokyo, J) at the wavelength 452 nm in order to quantify the amount of dissolved β-carotene.

The concentration of the β-carotene was calculated in comparison to a β-carotene standard solution.

CO_2–Ethanol Phase Behaviour

The CO_2–ethanol phase diagram was calculated using the PE-2000 software (Technische Universität Hamburg–Harburg, Germany).

The program was developed to model phase equilibria with a variety of equations of state. In the present study, the binary system CO_2–ethanol was calculated with Peng–Robinson Equation of State using quadratic mixing rules (34) in order to draw the binary mixture critical curve (pressure *vs.* temperature).

The binary interaction coefficients (k_{ij}), critical temperature (T_c), pressure (p_c), and the acentric factor (w) for the pure components were taken from the literature (35).

Determination of the Drug Content in the Natural Matrix

The total concentration of carotenoids in freeze-dried, seeds-free *R. canina* hips (200 mg) was determined by performing three consecutive macerations (lasting 24 h each) with *n*-hexane (25 mL). Each extraction was replicated three times.

The solutions obtained from each maceration were analysed separately by HPLC (see below) in order to quantify the carotenoids content and to verify the complete exhaustion of the matrix.

High Performance Liquid Chromatography (HPLC)

Carotenoids in the extracted samples were identified according to Hodisan *et al.* (7) using a isocratic HPLC system (LC-10 ATvp, Shimadzu, Tokyo Japan) equipped with a Spherisorb® column (5 μm, 4.6×250 mm, ODS1, Waters, Milford, USA) and a DAD detector (SPD-M10Avp, Shimadzu, Tokyo Japan) set at 451 nm. The volume of the injected samples was 20 μL.

As mobile phase an acetonitrile–methanol–ethyl acetate (79:11:10) mixture containing 1% triethylamine at 1.5 mL/min flow rate of was used.

β-carotene was quantified in comparison with a β-carotene (Sigma-Aldrich Inc. St. Louis MO, USA) solution of known concentration. Other carotenoids were identified in comparison to literature data (7).

The method was validated for linearity (R^2=0.999), limit of detection (6.2 ng/mL), limit of quantification (200 ng/mL) and relative standard deviation (< 2%).

Solubility of Crystalline β-Carotene in SC CO_2

The solubility measurements of pure β-carotene in supercritical CO_2 were performed by means of a laboratory scale apparatus (SPE-ED SFE, Applied Separation, USA), schematically represented in Fig. 1, operating under dynamic conditions at low flux according to the method described by Stassi *et al.* (36) and modified by Bettini *et al.* (37). Experiments were carried out at 70 °C and 300 bar (38).

The saturation cell (stainless steel column, internal volume 3 mL) was loaded, in a dark glove box under nitrogen atmosphere, with a 1:1 *v*/*v* mixture of β-carotene (450 mg) and glass beads (1 mm diameter), and placed in the thermostatic chamber.

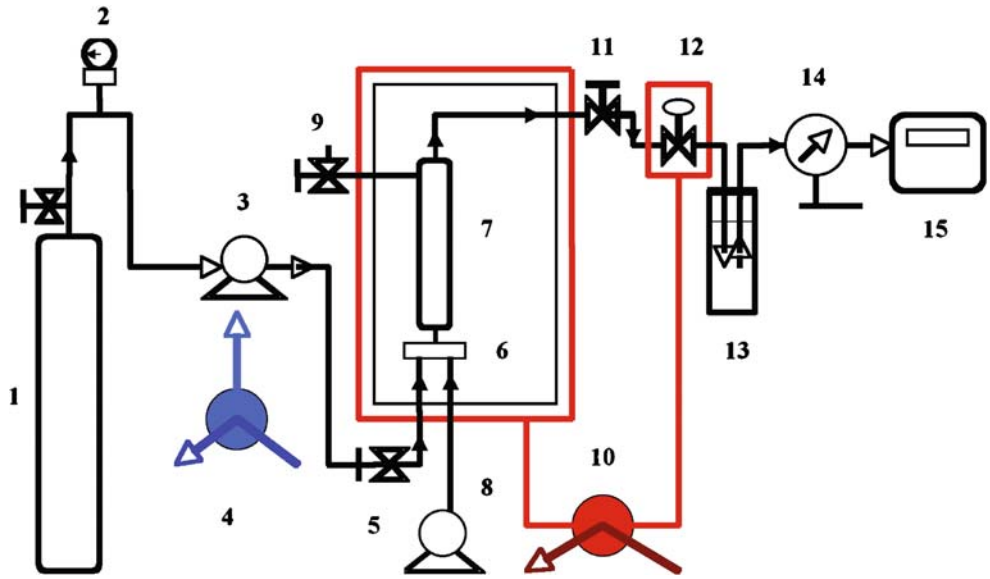

Fig. 1. Schematic representation of the apparatus used for the measurement of β-carotene solubility in SC CO_2 or SC CO_2–ethanol mixtures as well as for carotenoids extraction from lyophilized *R. canina* hips with SC CO_2 or SC–ethanol mixtures: CO_2 reservoir; *2* manometer; *3* CO_2 pump; *4* chiller; *5* inlet valve; *6* T connector; *6* inlet valve; *7* saturation/extraction column thermostatic chamber; *8* ethanol pump; *9* vent valve; *10* temperature controller; *11* outlet valve; *12* micrometric valve; *13* collecting flask; *14* flow-meter; *15* gas-meter

A pump operating at constant-pressure mode imposed the pressure, while a micrometric (expansion) valve allowed a manual tune of the flow rate, typically set around 5 mmol CO_2 min^{-1} (~ 0.2 g min^{-1}).

The system comprised a pump for HPLC that pumped ethanol (96% *v*/*v*) into the carbon dioxide stream through a "T" connector positioned before the saturation cell.

Beside pure CO_2, two CO_2/ethanol mixtures at different ethanol concentrations were used: 0.27 and 0.61 mole fraction, corresponding to 30 and 50% *v*/*v*.

The pressure of the saturation cell was raised up to 300 bar, the outlet valve being closed. Then, the outlet valve was opened in order to allow the CO_2 (99.99%, Sapio, Piacenza, Italy) to flow through the saturation cell. The run was stopped when nearly 0.95 moles of CO_2 had passed through the cell.

Upon expansion through the micrometric valve, the dissolved drug conveyed by the CO_2 stream was collected in a 50 mL amber glass flask containing either ethanol or an aqueous solution of β-cyclodextrin (1.5% *w*/*v*).

To avoid the risk of missing portions of the solute, at the end of each measurement all valves and connection tubings were carefully washed with 1.15 mol of CO_2/ethanol mixture (0.6 ethanol mole fraction).

The total amount of β-carotene collected either in *n*-hexane or β-cyclodextrin solution was determined by HPLC.

In the case where the supercritical stream was collected the aqueous β-cyclodextrin solution, at the end of the experiment the solution was stores in a refrigerator at 4 °C for 12 h in order to allow the separation of the solid β-carotene/β-cyclodextrin interaction compound. Thereafter, the vessel was centrifuged at 5,000 rpm for 10 min and the solid phase was dried under vacuum at 35 °C for 24 h. The amount of β-carotene in the dried solid (10 mg) was determined by performing three consecutive extractions each one with 10 mL *n*-hexane under magnetic stirring for 30 min. The solutions obtained from each extraction were analysed separately by HPLC.

Extraction of Carotenoids from *R. canina* L. hips

The extraction of carotenoids from freeze dried, seeds-free *R. canina* hips were carried out with the same equipment and the same procedure used for the solubility measurements of pure β-carotene; the only difference in the case of freeze dried powder (200 mg) was that some specimens were also macerated for 24 h in alcohol or *n*-hexane before performing the extraction process.

The extracted carotenoids were collected either in *n*-hexane or in an aqueous solution of β-cyclodextrin. In the latter case, after separation of the solid as above reported the presence of carotenoids and the amount of β-carotene in the dried solid (10 mg) was determined by performing three consecutive extractions each one with 10 mL *n*-hexane under magnetic stirring for 30 min. The solutions obtained from each extraction were analysed separately by HPLC.

Each measurement was performed at least in triplicate.

All operations implying β-carotene handling were conducted under dry nitrogen atmosphere, solid and liquid samples were collected and maintained in amber glass vials and all samples were stored at 4 °C for a time lower than 24 h before analysis.

RESULTS AND DISCUSSION

Phase Solubility Studies

Phase solubility measurements on β-carotene in β-cyclodextrin solutions (Fig. 2) indicated that the apparent solubility of β-carotene increased linearly with β-cyclodextrin concentration (linear regression coefficient R^2=0.933), giving

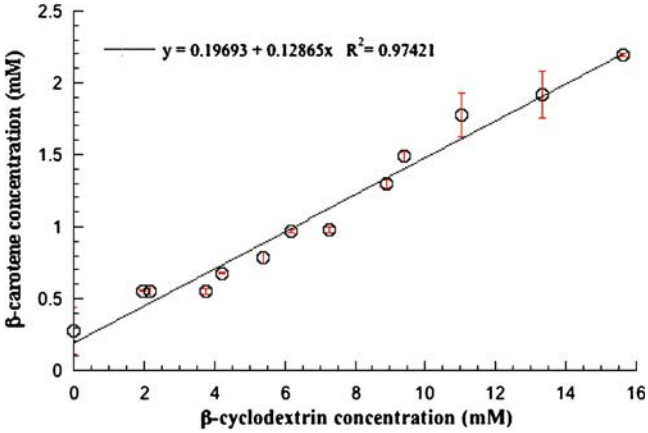

Fig. 2. β-carotene solubility as a function of β-cyclodextrin concentration in aqueous solutions at 25 °C

rise to a typical A_L diagram according to the Higuchi and Connors classification (39).

The apparent solubility of β-carotene increased from 0.32 mM up to 2.22 mM.

Although the data reported by Szente *et al.* (40) would suggest a β-carotene/β-cyclodextrin weight ratio = 10 corresponding to a 1:4–1:5 mole ratio, the assumption of a 1:1 complex formation seems to be more realistic (41). In this case the $K_{1:1}$ stability or complex formation constant can be calculated from the slope and the intercept, S_0, of the linear regression of the data reported in Fig. 2 as (39):

$$K_{1:1} = \frac{slope}{S_0(1 - slope)} \quad (1)$$

The obtained $K_{1:1}$ was equal to 749 M^{-1} which indicates the formation of a stable complex in solution.

Solubility of Crystalline β-Carotene in SC–CO_2–Ethanol

The solubility of the crystalline β-carotene in supercritical CO_2 was determined at 300 bar and 70 °C in the presence of various amount of ethanol as entrainer.

These conditions were adopted taking into consideration literature reports which indicate that the highest β-carotene solubility in pure SC CO_2 as well as yield of solubilization/extraction are obtained in the 60–80 °C range and above 250 bar (11,18,38,42).

On the other hand it is well known that the use of a co-solvent or an entrainer often results in an increase of the solubilization of the active component and a enhancement of the CO_2 extraction power (43). According to Sovova *et al.* (44) the increase of β-carotene solubility is proportional to square root of the entrainer concentration; in this respect ethanol, resulted more efficient than vegetable oil.

In particular, Sanal *et al.* (43) indicated 69 °C, 311 bar and 27.4% *v/v* ethanol concentration as optimal conditions for extracting β-carotene from apricot pomace. Therefore, these experimental conditions were taken as the *golden standard* for the present work.

However, the use of a co-solvent implies the preliminary study of the CO_2-cosolvent phase equilibria in order to draw the binary mixture critical curve, namely the separation line between sub-critical and supercritical region in the p/T plane where both components are represented. This information is required in order to set-up the experimental conditions for a suitable supercritical extraction.

It is known that the binary system CO_2–ethanol exhibits a complete miscibility, meaning that both components are miscible in all ratios. This type of mixture shows a Type I fluid phase behaviour according to classification for binary mixtures proposed by Van Konynenburg and Scott (45).

Binary mixtures exhibiting Type I fluid phase behaviour only show a continuous vapour–liquid critical line linking the critical points of the two components, above which the fluids are completely miscible in the supercritical phase. Unfortunately, it is not possible to calculate the critical point of a mixture by determining the phase equilibrium from the behaviour of the pure compounds alone, because binary systems cannot usually be described as the simple sum of the pure components. For this reason, the pressure (p) *vs.* composition (χ, mole fraction) diagram of the system CO_2–ethanol had to be calculated at different temperatures.

In the p, χ diagram, the pressure is plotted against the mole fraction of the mixture at a constant temperature. As an example in Fig. 3, the calculated "phase envelope" of CO_2–ethanol at 70 °C is drawn.

Considering a mixture of CO_2–ethanol with composition χ at different pressures and constant temperature, at low pressures the mixture only shows a homogeneous vapour phase. By raising the pressure the mixture exhibits a dew point when the first droplet condenses. With further pressure increase, the mixture shows the coexistence of two phases (vapour and liquid) until the bubble point is reached and only an infinitively small bubble remains. The critical point of the mixture is the point where the dew point line meets the bubble point line. Above the critical point all mixtures are supercritical.

From data in Fig. 3, it can be noticed that at 70 °C and above 120 bar the system CO_2–ethanol is in the supercritical condition. Similarly by means of PE program the critical points of CO_2–ethanol system at different compositions were

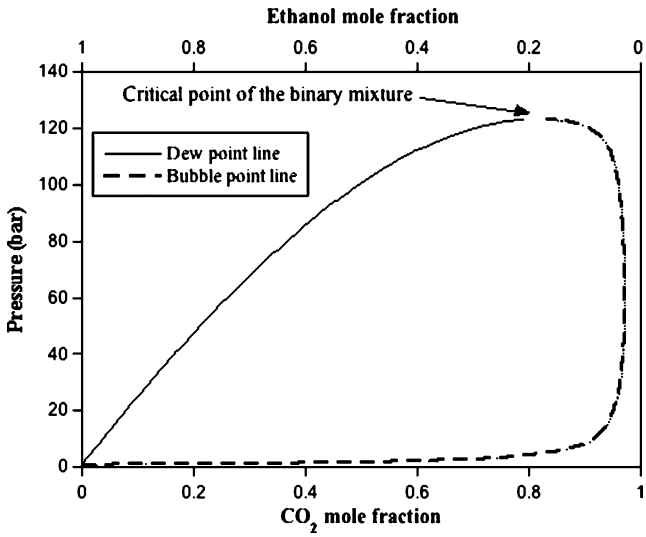

Fig. 3. Pressure *vs.* composition diagram for CO_2–ethanol binary mixtures at 70 °C

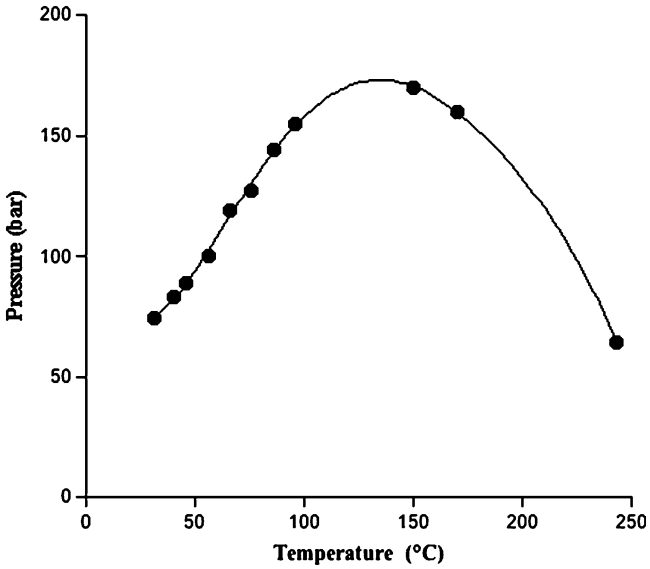

Fig. 4. Critical line of the CO_2–ethanol mixture

calculated to draw the vapour–liquid critical line (Fig. 4). From the data reported in Fig. 4, it can be noted that the point 300 bar and 70 °C is located well above the supercritical curve of the binary, thus it can be affirmed that the system would definitely be in the supercritical condition even taking into account the presence of a small amount of a third component (the solute).

The solubility data of β-carotene measured at 300 bar and 70 °C are reported in Table I.

The solubility of crystalline β-carotene at the studied conditions resulted in the order of 1.12×10^{-7} mole fraction (Table I, column 2). This value is about 20 times lower than those reported by Johanessen and Brunner (38) in similar conditions. However, Saldaña *et al.* (3) already underscored the large discrepancy among β-carotene solubility data reported in literature where largely scattered data can be found. This might be attributed to differences in purity of β-carotene and/or CO_2 used as well as to the different experimental set-up and techniques adopted. In the present work, measurements were carefully carried out by using highly pure compound and validated methods (36).

The introduction of ethanol as co-solvent resulted in a significant increase in β-carotene solubility that augmented about two- and four-folds with 0.27 and 0.61 ethanol mole fraction respectively. The highest figure corresponded to an amount of β-carotene around half mg per g of crystalline material submitted to dissolution (Table I, column 3).

Not statistically different data were obtained when the dissolved β-carotene was collected in β-cyclodextrin aqueous solution of (Table I, column 4) instead of *n*-hexane (Table I, column 2). In this case (β-cyclodextrin solution) part of the drug was in solution (Table I column 7), likely owing to the above reported high stability constant of the complex, and part was collected as a solid powder (β-carotene+β-cyclodextrin) after cooling at 4 °C for 12 h (Table I, column 6). It should be underlined that the pure β-cyclodextrin, at the concentration used in these experiments (1.5% *w/v*) is soluble in water also at 4 °C. Data reported in column 8 refer to the content by weight of the solid fraction recovered: the addition of 27% (mol/mol) ethanol to the extracting solvent did not significantly improve the amount of β-carotene in this powder with respect to the powder obtained with pure CO_2. On the contrary, when the amount of ethanol became preponderant (0.61 mole fraction) a nearly ten-folds increase was observed.

Drug Content in the Natural Matrix

The chromatographic conditions for the separation and identification of carotenoids in the chromatogram obtained from the total extract of *R. canina* fruits upon maceration with n-hexane were similar to those described by Hodisan *et al.* (7). Accordingly, four main peaks, out of five, were identified, corresponding to: lutein+zeaxanthin (R_t= 3.56 min); rubixanthin (R_t=6.12 min); β-carotene (R_t=7.58); and cis β-carotene (R_t=8.43). Furthermore, β-carotene was also identified and quantified in comparison to a standard solution of known concentration.

The total β-carotene extracted resulted 95±7.1 $\mu g\cdot g^{-1}$ of dried *R. canina* fruits. This datum was in good agreement with previous reports relevant to a validated extraction from the same matrix (10).

The percentage of β-carotene, with respect to the others carotenoids, was determined from the relative height of the relevant peaks and resulted 58% while in the Hodisan's work (7) this figure was significantly lower (20%). The β-carotene concentration relative to rubixanthin and lutein + zeaxanthin was 4.5 and 6.3 respectively. However, it should be underlined that the isocratic HPLC method adopted in the present work was not able to properly separate lycopene from other carotenoids. Since this pigment was found in significant

Table I. β-carotene Solubility Data in SC CO_2 and in SC Mixtures of CO_2 and Ethanol (70 °C 300 bar) Determined Upon Collection of the Gas Stream in n-hexane or in an Aqueous solution of β-cyclodextrin

	β-carotene Collected in *n*-hexane		β-carotene Collected in β-cyclodextrin Solution				
Ethanol Mole Fraction in SC Mixture	Solubility (mol fraction×10^7)	μg/g of Crystalline β-carotene	Solubility (mol fraction×10^7)	μg /g of Crystalline β-carotene	Fraction in Precipitated Powder	Fraction in Solution	μg/g Complex
0	1.12 (0.09)	125 (9.8)	1.08 (0.8)	120	0.31	0.69	233 (200)
0.27	1.98 (0.14)	220 (16.2)	2.00 (0.14)	222	0.24	0.76	260 (60)
0.61	4.05 (3.40)	449 (378)	7.30 (1.2)	807	0.80	0.20	2,280 (450)

Standard deviation in parenthesis

Table II. Data of β-carotene Extraction from Lyophilized Rosa Hips Powder in SC CO_2 and in SC Mixtures (70 °C 300 bar) of CO_2 and Ethanol Determined Upon Collection of the Gas Stream in n-hexane

Ethanol Mole Fraction in SC Mixture	μg/g of Lyophilized Powder	μg/g of β-carotene in the Lyophilized Powder	μg/ mol CO_2
0	8.0 (0.04)	84	1.70
0.27	10.5 (0.001)	110	2.24
0.61	7.0 (0.03)	73	1.30

Standard deviation in parenthesis

amount by different authors in *R. canina* hips (7,9,10), it was hypothesised that it might co-elute with β-carotene or rubixanthin, thus slightly increasing the β-carotene/other carotenoids ratio.

Supercritical Fluid Extraction (SFE)

Table II reports the amount of β-carotene extracted both per g of lyophilized *R. canina* fruit and per g of β-carotene in the *R. canina* fruit. When pure CO_2 was used as extracting solvent 8 μg of β-carotene per gram of starting material were obtained. This amount corresponded to 8.4% of the total β-carotene present in the powder submitted to extraction. It increased to 11% when 0.27 mole fraction of ethanol were used, while it remained practically unchanged at the highest entrainer concentration.

Carotenoids extraction was then carried out in the same conditions using 0.27 mole fraction ethanol as entrainer starting from a solid matrix macerated for 24 h *n*-hexane before performing the extraction process. The amount of β-carotene obtained (12 μg/g lyophilized powder) was slightly higher but not statistically different relative to data in Table II.

Illés *et al.* (19) reported data concerning the SFE of hiprose fruit using CO_2 or propane as extracting solvent at 35 and 55 °C and in the 100–400 bar range.

According to the data reported by Illés *et al.* one can calculate an amount of the β-carotene extracted as 6.9 μg per mole of CO_2 used at 35 °C and 250 bar. This figure is higher with respect to the findings of the present work (Table II, column 4) even though the title of the solid starting material could be calculated as 0.72 μg/g. However, the same authors, contrary to what reported by other investigators (29) showed for the whole extraction process, the existence of a crossover pressure (between 35 and 55 °C isotherms) at ca. 320 bar. This means that below this pressure value the solubility of the extract components decreased with a temperature increase, suggesting that at 70 °C, (the temperature used in the present work) and 300 bar the extractability of hiprose fruit may be reduced.

Quite recently Saldaña *et al.* (3) have reported the extraction of β-carotene from dry carrots. These authors extracted ca. 80 μg/mol of CO_2 used from a matrix containing 1832 μg/g of dry matter.

As stated before for the large scatter of β-carotene solubility data, also in the case of β-carotene extraction the large difference observed among various literature data can be mainly ascribed to different experimental set-up, *i.e.* fluid-dynamics, heat exchange, solvent purity etc.

Nevertheless, the main goal of the present work was to select adequate extraction condition in order to get a proof of concept on the possibility to obtain a formulation in form of powder composed by β-cyclodextrin and the supercritical extract of *R. canina*.

Therefore, the dry matrix, previously macerated for 24 h *n*-hexane, was extracted at 70 °C, 300 bar in the presence of 0.37 mol fraction of ethanol, collecting the extract conveyed by the gas stream in an aqueous solution of β-cyclodextrin. The solid obtained upon filtration of the cooled suspension was analysed for determining the β-carotene content that resulted 10.6±1.25 μg/g of solid dry matrix namely a non significantly different amount with respect to that collected in *n*-hexane. Furthermore, contrary to what observed with crystalline β-carotene, in this case a negligible amount was found in the supernatant.

Therefore, these two points indicate that practically the total amount of extracted β-carotene interacted with the β-cyclodextrin and it could be recovered with this last upon filtration of the suspension obtained by cooling the solution at 4 °C. The difference observed between crystalline β-carotene and the extract may be likely ascribed to the effect of the others carotenoids on the stability and solubility of the complex.

Furthermore, the β-carotene concentration relative to lutein+zeaxanthin was 6.6, namely not significantly different to that obtained upon maceration with n-hexane, while a lower value (2.9 *vs.* 4.5) was obtained for the β-carotene/rubixanthin ratio indicating a higher rubixanthin concentration relative to β-carotene in the supercritical extract with respect to that obtained upon maceration in *n*-hexane. Again a possible effect of lycopene co-elution in HPLC analysis might play a role in this difference.

Finally, Saldaña *et al.* (3) indicated a significant isomerization of β-carotene from *trans*- to *cis*- form upon storage at 70 °C for 2 h. Therefore, the possible β-carotene isomerization from *trans*- to *cis*- form was investigated in the supercritical extract collected in a β-cyclodextrin solution with respect to that obtained by maceration in *n*-hexane. In both cases the ratio between the two forms was evaluated from the relative height of the relevant chromatographic peaks. This ratio resulted 16.2±0.3, 7.2±0.3 and 3.3±0.3 for pure β-carotene, the supercritical and the hexane extract respectively. The higher concentration of the *trans*- form in the β-cyclodextrin powder obtained from the supercritical extract relative to that of hexane extract can be explained both as a consequence of the higher selectivity of the solvent toward such form and/or the higher capability of cyclodextrin with respect to the hexane solution to stabilize the *trans*-β-carotene form.

CONCLUSIONS

Extraction of β-carotene and others carotenoids from a freeze dried *R. canina* L. hips using supercritical CO_2 containing 30% *v/v* ethanol as entrainer gives rise to a powdered product when the SC stream is collected in a β-cyclodextrin aqueous solution of subsequently cooled-down to 4 °C.

The extracted β-carotene interacts almost quantitatively with β-cyclodextrin affording a solid phase, which presents a very low apparent solubility in water.

Finally the interaction with β-cyclodextrin results in a higher concentration of the β-carotene *trans-* form relative to the *cis-* form in the extracted product when collected in an aqueous solution of β-cyclodextrin with respect to the extract in *n*-hexane.

ACKNOWLEDGEMENT

This work was partially supported by Italian Ministry of University and Research through its PRIN programme.

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