HIGH-PRESSURE SOLUBILITY DATA OF SYSTEM ETHANOL (1) + QUERCETIN (2) + CO$_2$ (3).

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Grape seeds extracts have shown a broad range of pharmacological activities including, among others, antiulcer and antioxidant properties. These properties are mainly attributed to phenolic compounds. To date, the most abundant phenolic compounds isolated from grape seed are catechins and their family as quercetin.

In the extraction of quercetin using supercritical CO$_2$ it is necessary the use of a cosolvent for his polar nature. This work use different mixtures ethanol-CO$_2$, ranging 5% to 30% the ethanol contained to optimise the amount of quercetin extracted. The solubility of quercetin was measured at 313.15 K and pressures ranging 80 to 120 bar.

In order to correlate the obtained results, two equations of state (EOS) were successfully used (Peng-Robinson (PR) and Soave-Redlich-Kwong (SRK)), showing PR the better correlation with experimental data.

INTRODUCTION

Extraction processes with supercritical fluids have been widely studied and used at laboratory, pilot, and industrial scales in the past decades. The food and pharmaceutical industries have rapidly taken advantage of the possibilities of carbon dioxide as a non-toxic, environmentally safe, cheap and selective extraction solvent [1][2]. Carbon dioxide has the advantage compared to liquid solvents that its selectivity or solvent power is adjustable and can be set to values ranging from gas to liquid like. Carbon dioxide has a low critical temperature (304.1 K) and moderate critical pressure (71.9 bar), thus being an ideal solvent for compounds that may suffer thermal degradation [3]. Furthermore, CO$_2$ processing creates a medium without oxygen (high pressure avoid the oxygen entrance), where oxidation reactions can be reduced, which is of great interest when antioxidant compounds are to be extracted.

Grape seeds extracts have shown a broad range of pharmacological activities including, among others antiulcer [4], antioxidative and protective properties [5],[6]. In most cases, the activity is related to the antioxidant properties of the compounds present in the extracts and is mainly attributed to phenolic compounds. Much of the interest in phenolic compounds from grape seeds stems their antioxidant properties [7] and their ability to serve as free radical scanvergers [8]. To date, the most abundant phenolic compounds isolated from grape seed are catechins and their polymers [9],[10]. Complex phenols possess distinct properties from one other, thus, their isolation is of high interest.

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The solubility of a solute in a supercritical fluid (SCF) is one of the most important thermophysical property that must be determined and modelled for an efficient design of an extraction process based on supercritical solvents. The most recient literature review on experimental methods and systems investigated, regarding solubilities, is given by Dohrn and Brunner [11]. Berna et al. [12-14] have provide solubility data of resveratrol, catechin and epicatechin in CO_2 – ethanol mixtures. Coutsikos et al. [15] reported the solubility of some simple phenols, Chen and Tsai [16] of methoxybenzoic acids, Uchiyama et al. [17] of flavone and 3-hydroflavone, and Choi et al. [18] of p-coumaric acid isomers. There is not available in literature solubility data of quercetin in supercritical CO_2 or CO_2 – ethanol mixtures.

This work has as objective the determination of solubilities of quercetin in a mixture ethanol + CO_2. This is a previous step for the study of the viability of supercritical extraction of phenolics (mainly catechin, epicatechin and quercetin) from skin grape in order to increase its presence in wines or pharmacological use. Also, this information is necessary to optimise operating conditions of the extraction.

MATERIALS AND METHODS

Quercetin (98 mass %, GC grade) was purchased from Aldrich Chemical Co. Inc. Ethanol absolute (99.8 mass %, GC grade) supplied by Prolabo S.A. was used as modifier and solvent to collect the extract. The reagents were used without further purification after chromatography failed to show any significant impurities. High-purity CO_2 (more than 99.9 vol % purity, SFC grade) was used as received. Silica (more than 99.8 mass %) extra pure (from Sigma Chemical Co. Inc.) was utilized as support of products. The properties of the pure components are listed in Table 1.

Table 1. Critical constants and acentric factor of pure components.

substance	T_c (K)	P_c (bar)	w
CO_2	304.2[a]	73.7[a]	0.225[a]
ethanol	513.9[a]	61.4[a]	0.644[a]
quercetin	978.6[b]	57.7[b]	1.363[c]

[a]Daubert and Danner [19] [b]Estimated by the Constantinou and Gani method [20]. [c]Estimated by the Han and Peng method [21].

The solubilities were measured with an SFX 3560 extractor with two syringe pumps (Model 260D) manufactured by ISCO (Lincoln, NE, USA). With the proper plumbing, two pump system can deliver modified supercritical fluid or a continuous flow of supercritical fluid. The cylinder capacity of the pump is 266 mL and the maximum pressure 510 bar. The temperature can range from 313.2 K to 423.2 K, and supercritical fluid flow rate between 0.5 and 5 mL/min. This equipment was described in a previous paper [12].

The extractor measured the amount of supercritical CO_2 and modifier ethanol used during the extraction. The flow rate of supercritical fluid used for all experiments was 2 mL/min. It was checked that this is a suitable flow to achieve the equilibrium with saturation of supercritical phase in a reasonable time. Ethanol was used to trap the quercetin extracted.

After that, the extract was transferred to 10 mL calibrated flasks and diluted with ethanol. In some cases, a higher dilution could be necessary to achieve a concentration level adequate for UV spectrometry determination.

For sample preparation, first 0.5 g of quercetin was dissolved in 25 mL of ethanol. Second, 2 mL of solution was added to diatomaceous earth (1 g) previously weighed in the sample cartridge. Finally, the spiked solid was allowed to heat (60 ºC) for at least 2 h in order to evaporate ethanol and precipitate quercetin over the surface of solid particles. This procedure is necessary in order to avoid preferential ways during extraction and the formation of masses of particles.

A 8453 Hewlett-Packard diode array UV_VIS spectrophotometer was used for the determinations of quercetin in the extract, after calibration with gravimetrically prepared standard solutions.

Solubility is obtained as the slope of the lineal representation at different extraction times (5 values at least), of quercetin extracted vs. solvent (carbon dioxide + ethanol), in a molar basis.

RESULTS AND DISCUSSION

The solubilization of quercetin using CO_2 supercritical was tested. At extreme conditions of pressure and temperature (400 bar and 100ºC), it only extracts traces of product ($< 10^{-6}$ mole fraction), in accordance with the results obtained for other authors [12], [22-24], for compounds of the same family. That could be due to the polar nature of quercetin and polyphenols in general. So, it is necessary to use a cosolvent. Ethanol is appropriate because it is a polar solvent and it is permitted in food industry for its harmless. Also, it is easily removed of the extract by evaporation at relatively low temperatures.

The solubility of quercetin in a supercritical phase were measured at 313 K, pressures ranging 80 to 120 bar and for different mixtures ethanol-CO_2. These results are shown in Figure 1.

This work use different mixtures of ethanol-CO_2, ranging 5% to 30% of ethanol, in order to optimise the amount of quercetin extracted with the minimal consumption of cosolvent. So, the extract is cleaner and the cost of solvent and separation is smaller than using bigger cosolvent percentages.

The extraction work temperature (313.15 K) is the minimal temperature allowed for the equipment. This temperature has been selected in order to avoid the opposed effect of temperature on solubility [2]. Furthermore, the main advantage of supercritical extraction it is being an ideal extraction method for compounds that may suffer thermal degradation [3].

The inferior limit of 80 bar was chosen because it was near of CO_2 critical pressure, so the behaviour of system at conditions near the critical point of system was studied. It was not possible to measure the solubility of quercetin at pressures over of 110 bar with this equipment because, at these conditions, the entire product contained in the sample cartridge was extracted. We can measure it just to 120 bar only using 5% of ethanol.

Table 2 Parameters and deviations between experimental and calculated values of system ethanol (1) + quercetin (2) + CO_2 (3) at 313.15 K.

model	k_{12}	k_{13}	k_{23}	h_{12}	h_{13}	h_{23}	ARD
PR EOS	0,030	0,082	0,186	-0,018	-0,021	-0,135	0.059
SRK EOS	0,020	0,012	0,154	-0,014	-0,028	-0,015	0.231

ARD = (1/N) Σ | y_{ex}— y_{cal} | / y_{ex}

In order to correlate the obtained results, two equations of state were successfully used: Peng-Robinson [25] (PR EOS) and Soave-Redlich-Kwong [26] (SRK EOS). The parameters of equations of state were determined using the solver function of Microsoft Excel. These parameters and the average relative deviation (ARD) are shown in Table 2. The parameters estimation was performed considering all the experimental data by minimising the following objective function:

$$OF = \sum \left(\frac{ABS(y_{exp} - y_{calc})}{y_{exp}} \right) \tag{1}$$

where y is the solubility of solute (quercetin) in the supercritical phase.

Generally both equations show similar deviations at different conditions of pressure and temperature, but PR EOS [25] shows the better correlation with experimental points.
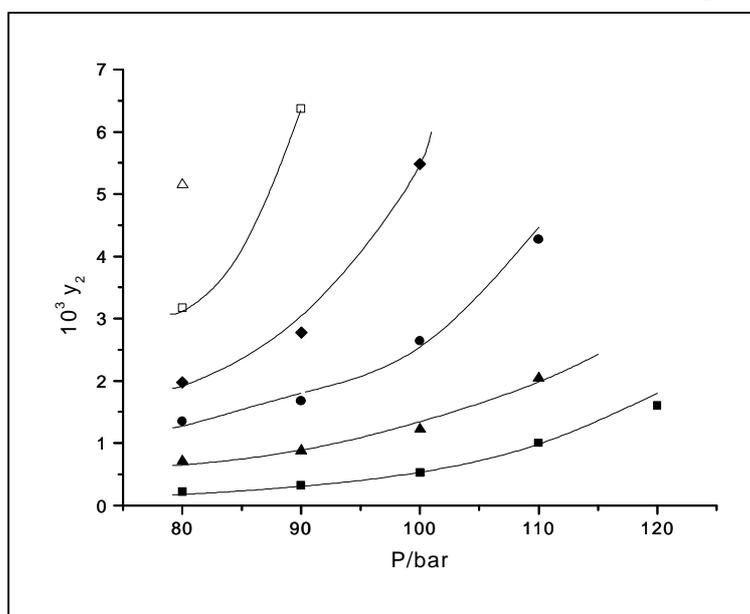

Figure 1. Solubility (10^3 y_2) of quercetin (2) in supercritical CO_2 (3) at 313.15 K at a % of modifier ethanol (1) of: (■) 5%, (σ) 10%, (•) 15%, (υ) 20 %, (□) 25 %, (△) 30 %. (——) Modeling by PR EOS [25].

At a constant temperature, raising the pressure increases the density of the supercritical fluid, that is, its solvating power becomes greater and more solute can be transferred to the supercritical phase. So, the solubility, as it was shown in Figure 1, rises with pressure. The extraction will be carried out at elevated pressure. On the other hand, the extraction pressure will be limited by operational costs and safety cautions.

The evolution of the solubility of quercetin vs. the modifier fraction shows an increase with the amount of ethanol employed. So, the optimal concentration of cosolvent will be fixed by separation costs. This behaviour was shown in Figure 1.

CONCLUSION

As conclusions, the present study reveals the influence of process conditions on the solubility of quercetin in supercritical CO_2. It was necessary the use of a cosolvent and ethanol was appropriate. The solubility increases with the amount of ethanol. At a constant temperature, raising the pressure increases the density of the supercritical fluid, that is, its solvating power becomes greater and more solute is transferred to the supercritical phase. The extraction pressure will be limited by operational costs and security cautions. Finally, PR EOS [25] was successfully applied to the ternary system quercetin-ethanol-CO_2.

REFERENCES

[1]   BRUNNER, G. Gas Extraction; An Introduction to Fundaments of Supercritical Fluids and the Application to Separation Processes; Baumgürtel, H., Franck, E.U., Grünbein, W., Eds.; *Springer, New York,* 1994.
[2]   MCHUGH, M.A.; KRUKONIS, V.J. Supercritical Fluid Extraction, Principles and Practice; *Butterworths-Heinemann: Stoneham, MA; Boston*, 1994.
[3]   PALMER, M.V.; TING, S.S.T. Applications for Supercritical Fluid Technology in Food Processing, *Food. Chem.* Vol.52, **1995**, 345-352.
[4]   SAITO, M.; HOSOYAMA, H.; ARIGA, T.; KATAOKA, S.; YAMAJI, N. Antiulcer Activity of Grape Seed Extract and Procyanidins. *J. Agric. Food Chem.* Vol. 46, **1998**, 1460-1464.
[5]   BAGCHI, D.; GARG A.; KROHN, R.L.; BACHI, M.; BACHI, D.J.; BALMOORI, J.; STOHS, S.J. Protective Effects of Grape Seed Proanthocyanidins and Selected Antioxidants against TPA-Induced Hepatic and Brain Lipid Peroxidation and DNA Fragmentation, and Peritoneal Macrophage Activation in Mice. *Gen. Pharmac.* Vol. 30, **1998**, 771-776.
[6]   MEYER, A.S.; YI, O.S.; PEARSON, D.A.; WATERHOUSE, A.L.; FRANKEL, E.N. Fruit Hydroxycinnamic Acids Inhibit Human Low-Density Lipoprotein Oxidation in Vitro. *J. Agric. Food Chem.* Vol. 46, **1998**, 1783-1787.
[7]   VINSON, J.A.; DABBAGH, Y.A.; SERRY, M.M.; JANG, J. Phenol Antioxidant Quantity and Quality in Foods: Vegetables. *J. Agric. Food Chem.* Vol. 43, **1995,** 2800-2802.
[8]   MAFFEI-FACINO, R.; CARINI, M.; ALDINI, G.; BOMBARDELLI, E.; MARAZZONI, P.; MORELLI, R. Free Radicals Scavenging Action and Anti-enzyme Activities of Procyanidines from Vitis Vinifera. A Mechanism for their Capillary Protective Action. *Arzneimittelforschung,* Vol. 44, **1994**, 592-601.

[9]   FULEKI, T.; DA SILVA, J.M.R. Catechin and Procyanidin Composition of Seeds from Grape Cultivars Grown in Ontario. *J. Agric. Food Chem.* Vol. 45, **1997**, 1156-1160.

[10]   ESCRIBANO-BAILON, T.; GUTIERREZ-FERNANDEZ, Y.; RIVAS-GONZALO, J.; SANTOS-BUELGA, C. Characterization of Procyadinins of Vitis Vinifera Variety Tinta del País Grape Seeds. *J. Agric. Food Chem.* Vol. 40, **1992**, 1794-1799.

[11]   DOHRN, R.; BRUNNER, G. High-Pressure Fluid-Phase Equilibria: Experimental Methods and Systems Investigated (1988-1993). *Fluid Phase Equilib.* ,Vol. 106, **1995**, 213-282.

[12]   BERNA, A.; CHÁFER, A.; MONTÓN, J.B. High Pressure Solubility Data of System Resveratrol (3) + Ethanol (2) + CO_2 (1). *J. Supercritical Fluids* , Vol. 19, **2001**, 133-139.

[13]   BERNA, A.; CHÁFER, A.; MONTÓN, J.B.; SUBIRATS, S. High Pressure Solubility Data of System Ethanol (1) + Catechin(2) + CO_2 (3). *J. Supercritical Fluids,* Vol. 20, **2001**, 157-162.

[14]   BERNA, A.; CHÁFER, A.; MONTÓN, J.B.; MUÑOZ, R. High Pressure Solubility Data of System Ethanol (1) + Epicatechin(2) + CO_2 (3). *J. Supercritical Fluids*, Vol. 24, **2002**, 103-109

[15]   COUTSIKOS, P.; MAGOULAS, K.; TASSIOS, D. Solubilities of Phenols in Supercritical Carbon Dioxide. *J. Chem. Eng. Data,* Vol. 40, **1995**, 953-958.

[16]   CHEN, J.-W.; TSAI, F.-N. Solubilities of Methoxybenzoic Acid Isomers in Supercritical Carbon Dioxide. *Fluid Phase Equilib.* Vol. 176, **1995**, 189-200.

[17]   UCHIYAMA, H.; MISHIMA, K.; OKA, S.; EZAWA, M.; IDE, M.; TAKAI, T.; PARK, P.W. Solubilities of Flavone and 3-Hydroxyflavone in Supercritical Carbon Dioxide. *J. Chem. Eng. Data.* Vol. 42, **1997**, 570-573.

[18]   CHOI, E. S.; WONG, M.J.; CHENG, I.W.; HUANG, Y.W.; LIN, Y. Solubilities of o-, m- and p-Coumaric Acids Isomers in Carbon Dioxide. *J. Chem. Eng. Data.* Vol. 43, **1998**, 6-8.

[19]   DAUBERT T.C.; DANNER, R.P. Physical and Thermodynamic Properties of Pure Chemicals. *Taylor & Francis, Washington*, 1997.

[20]   CONSTANTINOU, L.; GANI, R. New Group Contribution Method for the Estimating Propierties of Pure Compounds. *AIChE J.,* Vol. 40, **1994**, 1697-1710.

[21]   HAN, B.; PENG, D.Y. A Group-Contribution Correlation for Predicting the Acentric Factors of Organic Compounds. *Can. J. Chem. Eng.,* Vol. 71, **1993**, 332-334.

[22]   TENA, M.T.; RIOS, A.; VALCARCEL, M. Supercritical Fluid Extraction of t-Resveratrol and other Phenolics from a Spiked Solid. *Fresenius J. Anal. Chem.*, Vol. 361, **1998**, 143-148.

[23]   PALMA, M.; TAYLOR, L.T. Extraction of Polyphenolic Compounds from Grape Seeds with Near Critical Carbon Dioxide. *J. Chromatogr. A,* Vol. 849, **1999**, 117-124.

[24]   MURGA, R.; RUIZ, R.; BELTRÁN, S. J.; CABEZAS, L. Extraction of Natural Phenols and Tannins from Grape Seeds by Using Supercritical Mixtures of Carbon Dioxide and Alcohol. *J. Agric. Food Chem.* Vol. 48, **2000**, 3408-3412.

[25]   PENG, D.; ROBINSON, D.B. A New Two-Constant Equation of State. *Ind. Eng. Chem. Fundam.* Vol. 15, **1976**, 59-64.

[26]   SOAVE, G. Equilibrium Constants from a Modified Redlich-Kwong Equation of State. *Chem. Eng. Sci.* Vol. 27, **1972**, 1197.