SEPARATION OF ANTIOXIDANT COMPONENTS FROM WHITE GRAPE SKIN AND SEEDS USING COMPRESSED CARBON DIOXIDE.

Catarina M.M. Duarte,* Ana A. Matias, Ana V. M. Nunes, M. Rosário Bronze, Helena I. Motta Veiga, M. Nunes da Ponte.

Instituto de Biologia Experimental e Tecnológica, Aptd. 12 – 2780 Oeiras, Portugal.

Email: cduarte@itqb.unl.pt Fax: 351-21-4421161

The possibility of extracting antioxidant components with supercritical carbon dioxide from a hydroalcoholic mixture was investigated. High-pressure phase equilibrium behaviour on quaternary systems (CO_2 + ethanol + water + antioxidant compound) was investigated at 40ºC and between 15 and 19 MPa. The separation factors for mixtures of different compositions – 60%(v/v) water + 40% (v/v) ethanol and 90 %(v/v) water + 10%(v/v) ethanol were determined and compared. The viability of scCO_2 extraction process is discussed for Quercetin-3-glucoside. Different combinations of pressures and solvent ratios were studied. The separation factors are determined and discussed.

INTRODUCTION

Since the last decade there is a considerable interest in the food industry and in preventive medicine in the development of "natural antioxidants" from plant material.[1] Grapes, wines and grape byproducts contain large amounts of phenolic compounds, mostly flavonoids, at high concentrations.[2] Previously, several studies have reported grape's phenolic composition, which, comprises flavonols, flavan-3-ols and hydroxycinnamic acids. The polyphenolic compounds present in grapes pass to wine more or less depending on the characteristics of the wine making process, however a high proportion remains in the wine production residues or wastes.[3]

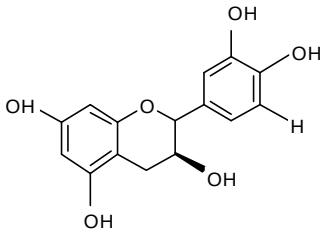
The work presented is part of a major project designed to study the viability of an alternative clean process for the extraction of antioxidant compounds from botanical source matter.

Supercritical carbon dioxide is perceived as a non-toxic solvent. If it is to be used only in a second step of a separation process, the first extraction should use one of the few other solvents that are also considered suitable for contact with products for human consumption. Hydroalcoholic mixtures are in this category and they are currently used to extract many different substances from plants. Moreover, phase equilibrium in the ternary system water + ethanol + carbon dioxide has been extensively studied.[4]

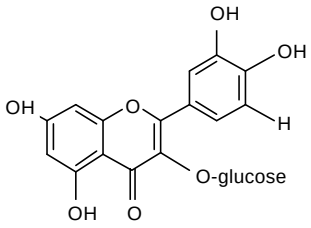
In this work, several extractions using dried solid mixtures of white grape skin and seeds, as the matrix feed, were performed using different water-ethanol compositions. Furthermore the possibility of extracting the antioxidant compounds from the hydroalcoholic mixtures using supercritical fluid extraction was studied. This possibility is attracting considerable interest in

what concerns the reuse of the grape byproducts and therefore the valorization of a residue, minimizing its environmental impact.

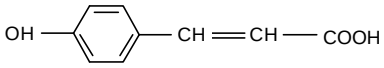
Catechin, Quercetin-3-glucoside and Coumaric acid are representative of three different phenolic groups and therefore were used as model compounds is this study.



Catechin



Quercetin-3-glucoside



Coumaric acid

The design of the supercritical extraction process requires a detailed knowledge of the vapour-liquid equilibrium compositions of the quaternary systems CO_2 + ethanol + water + antioxidant compound. Therefore high-pressure phase equilibrium on the quaternary systems for Catechin, Coumaric acid and Quercetin-3-glucoside was studied.

The viability of the extraction process is discussed for Quercetin-3-glucoside. Different combinations of pressures and solvent ratios were studied. The separation factors are determined and compared.

MATERIALS AND METHODS

Materials

The substances used in this work were: Residues (grape seeds and skin) of white wine production from *J. Maria da Fonseca, Portugal*, 96% (+)-Catechin [154-23-4] CAS, 90% Quercetin-3-glucoside [776-86-3] CAS and 98% p-Coumaric acid [501-98-4] CAS from Fluka; 99,8% ethanol from Riedel-de Häen [64-17-5] CAS, 99.998 mol% carbon dioxide from Air Liquide. All chemicals were used without further purification.

VLE Measurements

Vapour-liquid equilibrium measurements were performed using the static analytical method schematically presented in Fig. 1. The equilibrium cell is a stainless steel cylinder with an internal capacity of approximately 40 cm^3. This cell is immersed in a thermostated water bath and has magnetic internal stirring. Operating the CO_2-compressor, the desired pressure is brought into the cell. The pressure is monitored in a Digital Gauge Pressure instrument.

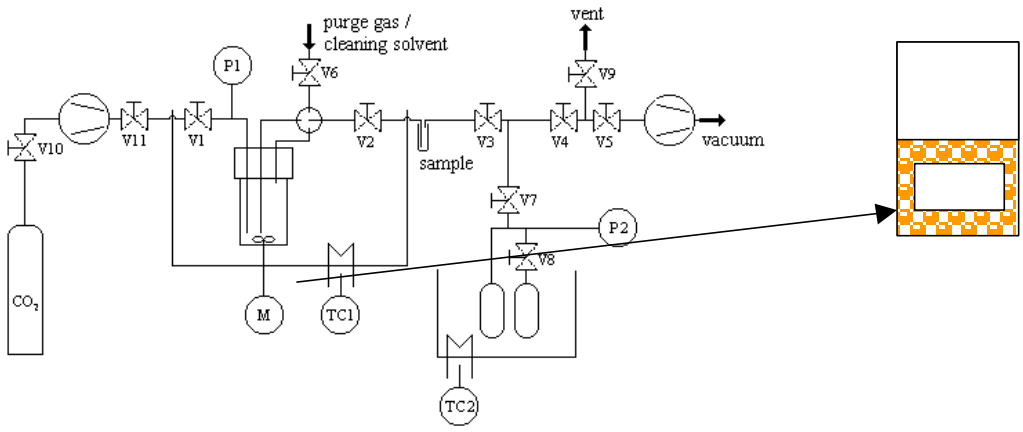


Fig. 1- Experimental vapour-liquid equilibrium apparatus.

The required time to reach equilibrium varied from 45 min to 1 hour. After that, a sample from the liquid (bottom) phase and the gas (top) phase was taken through a six port sampling valve. These samples were collected by quick depressurization and expansion into small glass traps, connected to large steel bottles of calibrated volume in a thermostated water bath. Measurement of pressure in these volumes, after the expansion, allowed the calculation of the amount of CO_2 present in the sample. To ensure that all solute was recovered in the trap some cleaning solvent (ethanol) was injected through the lines.

The samples collected are diluted in ethanol to a convenient volume.The samples were analysed by UV spectrophotometry (in the case of a single phenolic compound) or HPLC-UV (for the samples containing multiple phenolic compounds together). All the compounds absorb in the region of ultraviolet. Calibration was obtained via use of standard samples between 10^{-6} and 10^{-5} M.

RESULTS

Preliminary hydroalcoholic extractions of dried solid mixtures of white grape skin and seeds, as the matrix feed, were performed using different water-ethanol ratios.
Fig. 2 shows the chromatograms of the extracts obtained with 60%(v/v) water and 90%(v/v) water. Quercetin-3-glucoside, Catechin and Coumaric acid were all identified in the resultant hydroalcoholic extracts.

The design of the supercritical extraction process to carry out as a secondary stage separation requires a detailed knowledge of the vapour-liquid equilibrium compositions of the quaternary systems CO_2 + ethanol + water + antioxidant compound.

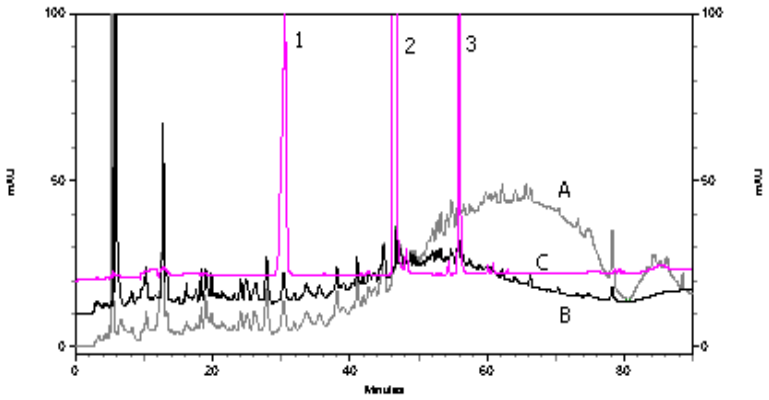


Fig.2 – Resulting HPLC chromatogram of white grape skin and seeds extracts. A-extract obtained with 90%(v/v) water; B-extract obtained with 60%(v/v) water; C-pure components: 1-Catechin, 2-Coumaric acid, 3- Quercetin-3-glucoside.

Vapour-liquid equilibrium experiments were performed using two hydroalcoholic mixtures of different water:ethanol composition (60%(v/v) water and 90%(v/v) water), in order to study the effect of the water content in the selectivity of the extraction process. The phenolic compound (Catechin, Quercetin-3-glucoside or Coumaric acid) was added to these mixtures until a mass ratio of 5×10^{-5} was obtained. These concentrations are of the order of magnitude of those obtained in the hydroalcoholic extracts from wine production residues or wastes.

Fluid phase equilibrium experiments were carried out between 15 and 19 MPa, and 40 °C. Samples taken from both gas (CO_2-rich) and liquid (water + ethanol-rich) phases were analysed for the antioxidant compound and carbon dioxide content, as described in the Materials and Methods section. The compositions of both phases in water and ethanol were calculated from the measured amounts of carbon dioxide. Mass balances of the whole contents of the cell were performed, on the basis of the correlation of phase equilibrium data of several authors for the CO_2 + water + ethanol system.[4] It was assumed that the presence of the antioxidant compound, due to the very small concentrations in both phases, did not significantly affect the equilibrium ratios for the other components.

The experimental results for the studied mixtures, are given in terms of the mass of the phenolic compound per unit of mass of carbon dioxide and separation factors α, expressed as:

$$\alpha = \frac{(Wt_{\text{phenolic comp.}} / Wt_{\text{water+ethanol}})_{\text{gas}}}{(Wt_{\text{phenolic comp.}} / Wt_{\text{water+ethanol}})_{\text{liquid}}}$$

Table 1 summarises the solubility results obtained for Quercetin-3-glucoside (the highest value-product) in the two different initial liquid solutions (60%(v/v) water and 90%(v/v) water), at 40 °C. A clear distinction emerges between the two hydroalcoholic compositions. It can be seen that Quercetin-3-glucoside solubilises better in the gas phase when the carbon dioxide-rich phase is in contact with a water-rich liquid phase than when in contact with an ethanol-rich one.

Table 1
Phase equilibrium compositions on the quaternary system CO2 + ethanol + water + quercetin

T	P	Liquid Phase			Gaseous Phase			Solubility in CO_2
(ºC)	(MPa)	x_{water}	$x_{ethanol}$	x_{CO2}	y_{water}	$y_{ethanol}$	y_{CO2}	(x 10^{-7} g/g)
			wt. %			*wt. %*		
Quercetin in 60% water + 40% ethanol								
40	15.7	58	31	11	0.6	4.4	95	5.82
40	18.7	57.8	30.9	11.3	0.8	5.3	93.9	4.85
Quercetin in 90% water + 10% ethanol								
40	16.9	86.5	7.7	5.8	0.44	1.7	97.9	13.4

Solubility of quercetin, expressed in terms of mass of quercetin per mass of carbon dioxide in the gaseous phase.

In Figure 3, the separation factors for Quercetin-3-glucoside are plotted as a function of pressure for the two hydroalcoholic mixtures of different water:ethanol composition (60%(v/v) water and 90%(v/v) water), in order to study the effect of the water content in the selectivity of the extraction process.

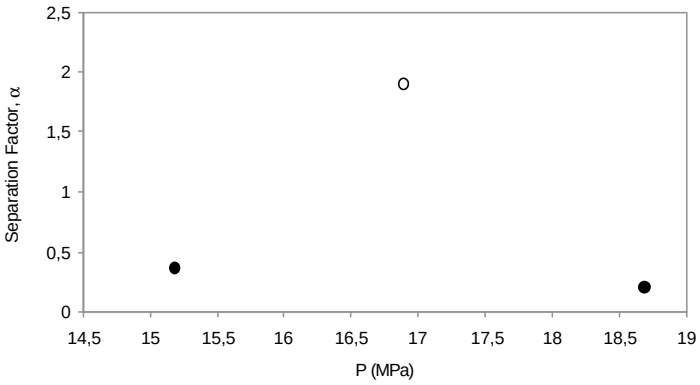


Fig.3-Separation factors of Quercetin-3-glucoside between the gaseous and liquid phases, as function of pressure. Results obtained for the mixture of 60%(v/v) water (•) and 90%(v/v) water (o), at 40ºC.

The separation factor did not show any special trend with pressure, between 15 MPa and 19 MPa. The effect of the water content in the separation factor of Quercetin-3-glucoside is obviously very large. The separation factor, obtained for the mixture of 90 % (v/v) water was six times higher than the value obtained for the mixture of 60 % (v/v) water.

CONCLUSION

First stage hydroalcoholic extractions of dried solid mixtures of white grape skin and seeds using different water-ethanol ratios showed the presence of Quercetin-3-glucoside, Catechin and Coumaric acid in the resultant extracts. The possibility of carry out supercritical CO_2 extraction as a secondary stage separation was investigated through vapour-liquid equilibrium experiments performed using two hydroalcoholic mixtures of different water:ethanol composition (60%(v/v) water and 90%(v/v) water). At 40ºC and between 15 and 19 MPa Quercetin-3-glucoside solubilised better in the gas phase when the carbon dioxide-rich phase were in contact with a water-rich liquid phase than when in contact with an ethanol-rich one.

The effect of the water content in the separation factor of Quercetin-3-glucoside is very relevant in opposite of the effect of pressure. It may be concluded from the experimental results that Quercetin-3-glucoside should be easily extracted from water-rich mixtures.
These preliminary results encouraged the possibility of extracting phenolic compounds with antioxidant activity from wine production residues using an alternative clean method involving supercritical fluid technology.

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