EXTRACTION OF OIL ENRICHED IN a-TOCOPHEROL FROM GRAPE SEEDS (*VITIS VINIFERA*) USING SUPERCRITICAL CARBON DIOXIDE

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ABSTRACT

α-tocopherol is one of the most important natural anti-oxidizing agent. Obtaining an oil enriched with this molecule by means of a renowned clean process could be very important for the food industry that could use it for fortification.
In this work, milled grape seeds (*Vitis vinifera*), a residue obtained from the wine industry, were extracted with supercritical carbon dioxide (SC-CO_2) as solvent, to determinate the optimal extraction conditions to obtain an α-tocopherol enriched oil.
All the extractions were carried out at the pressure of 25 Mpa, based on earlier extraction of oil enriched in α–tocopherol from other seed matrices.
Both total oil and α-tocopherol extracted from milled grape seeds were determinated as a function of particle size ($0,85mm<d_1=1,4mm$, $0,425mm<d_2=0,85mm$, $0,3mm<d_3=0,425mm$), temperature (40, 80°C) and CO_2 to seed mass ratio (25, 60, 90).
The optimal extraction conditions were determined considering the maximum concentration of α-tocopherol in the extracted oil.

INTRODUCTION

In the last years a variety of processes involving extraction with Supercritical Carbon Dioxide have been developped in the food industry, as promissing alternatives to the current conventional processes (distillation, solvent extraction, ecc.). Actually, SC-CO_2 extraction offers many advantages, above all easy separation of the solvent from the extracted materials, leaving no residue of the solvent (which, anyway, is no toxic) in the processed materials and featuring a low extraction temperature, which minimizes thermal degradation of the products.
Currently grape seeds, a residue of the wine industry, are industrially extracted with hexane, to obtain an oil which has many nutritional advantages for human consumption, owing to its high level of unsaturated fatty acids [1].
The objective of this study was to obtain a grape seed oil enriched in α-tocopherol, which is the isomer of tocopherols, with the highest vitamin E activity.
The chemical structure of α-tocopherol is shown in Figure 1.

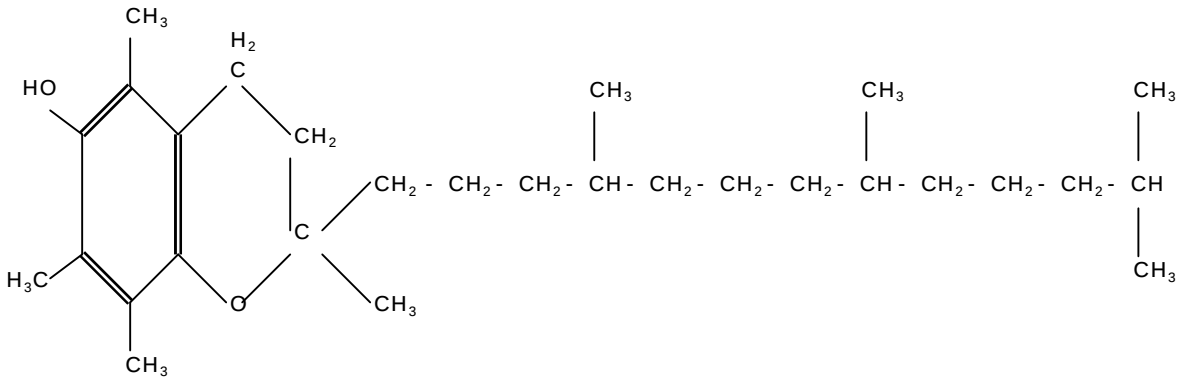

Figure 1 : Chemical structure of α-tocopherol

Supercritical Carbon Dioxide extration has recently been applied to extract an oil enriched in α-tocopherols from other natural matrices, such as palm leaves [2], soybean flakes, rice bran and wheat germ [3], olive tree leaves [4].
In these works the maximum α-tocopherol concentration in the extracted oil was obtained at 25-30 MPa, while, with a further increase of pressure, the concentration decreased because of competitive extraction of other matrix coupounds, due to their higher solubility in SC-CO_2. Therefore, in all experiments of this work, the pressure was kept at 25 MPa.
The extractions were carried out at a temperature of 40°C and 80°C based on earlier results [3], [4].
Total oil yield and α-tocopherol concentration in the extracted oil were determined for three different CO_2 to seed mass ratios: 25, 60 and 95.

MATERIALS AND METHODS

Materials
As solvent, CO_2 N45 (99,995 % of purity), manufacturated by Air Liquide, was used.
Milled grape seeds were supplied by Oleificio Medio Piave (Fontanelle, Treviso - Italy). The samples were sieved to obtain three size fractions: $0{,}85mm<d_1=1{,}4mm$, $0{,}425mm<d_2=0{,}85mm$, $0{,}3mm<d_3=0{,}425mm$.
Hexane RG (Reagent Grade), 2-propanol RG, dichloromethane RG, potassium hydroxide pellets and anhydrous sodium sulphate were supplied by Ashland Italia, S. Giuliano Milanese (MI).
Hexane HPLC grade and methanol RG were supplied by Baker, Deventer, Holland. Prior to use, HPLC hexane was filtered with Millipore Type HA 0,45 μm filters, then mixed with the required amount of 2-propanol.
95% α-tocopherol and L-ascorbic acid were supplied by Sigma-Aldrich Chemie, Steinheim, Germany.
Standard α-tocopherol solutions were prepared before analysis by weighing the needed amount of α-tocopherol in a volumetric flask and diluting with RG hexane.
L-ascorbic acid solutions in acqueous methanol were prepared following the instructions reported by Krukowsky [5].

Chromatographic analysis

The HPLC apparatus consisted of the following parts: a Perkin Elmer series 200 binary pump and a Perkin Elmer 785A UV/VIS detector. The loop injector had a 20 μL volume.
The detector was linked to a PC via a Perkin Elmer NCI 900 electronic interface.
Turbochrom Navigator program (Copyright Perkin Elmer) was used to record and elaborate chromatographic data.
Chromatographic column was a Varian Lichrosorb Si 60-5, 150 x 4,6 mm, 0,5 μm particles. The column was protected by a 10 mm guard column with the same characteristics.
Direct phase HPLC analysis of tocopherols and carotenes is reported by Ye [6], and was followed with some differences.
The specimen was eluted using a mixture of hexane containing 0,45 % in volume of 2-propanol. Flow was kept at 1,00 mL/min. UV-VIS detector was fixed at 290 nm.
Each analysis was followed by a coinjection of the specimen with a standard solution of α-tocopherol, to confirm the assignation of the peak. In these conditions, the retention time of α-tocopherol was found to be about 6 minutes.
Quantitation of α-tocopherol was made with a reference curve, prepared in the expected analytical concentration range of 1-10 ppm.
Five solutions of different concentration in the given range were injected, and the peak area values were interpolated using the Origin 6.1 program (Copyright Origin Lab).

Saponification

The saponification of specimens was performed following the instructions reported by Krukovsky [5], with some modifications.
About 100 mg of the extracts were transferred in a tube and weighed. 1 mL each of methanol, 3% ascorbic acid solution and 12 M KOH were added in this sequence. The mixture was then heated with reflux for ten minutes. The mixture was transferred in a 25 mL separator funnel and extracted three times with dichloromethane. Organic extracts were then washed three times with water, vacuum dried, transferred with hexane in a volumetric flask, and diluted to volume.

Apparatus and Procedure

The equipment used for supercritical extractions was Dionex 703-SFE.
The flow diagram extraction equipment is shown in Figure 2: after filtering, the CO_2 was compressed by a high pressure pump, then the compressed fluid was passed through the extraction cell (8 or 32 ml). The Dionex extractor allows the simultaneous extraction from up to 8 cells arranged parallelly and horizontally in the oven and connected between the manifolt and restrictors: only one cell a time was used in the present work. The extract was accumulated in collection vials.
The experimental procedure started by heating the oven to the desired temperature and feeding the cell with 3 g of milled grape seeds evenly distributed in inert glass beads. Then, after the desired temperature was reached, the cell was placed in the oven cavity and connected to the manifold and restrictors and the SC-CO_2 extraction was started.
After the desired CO_2 to seed mass ratio was reached, the extraction process was stopped, the extract recovered, and the cell was cleaned and loaded with new grape seeds for the next run.

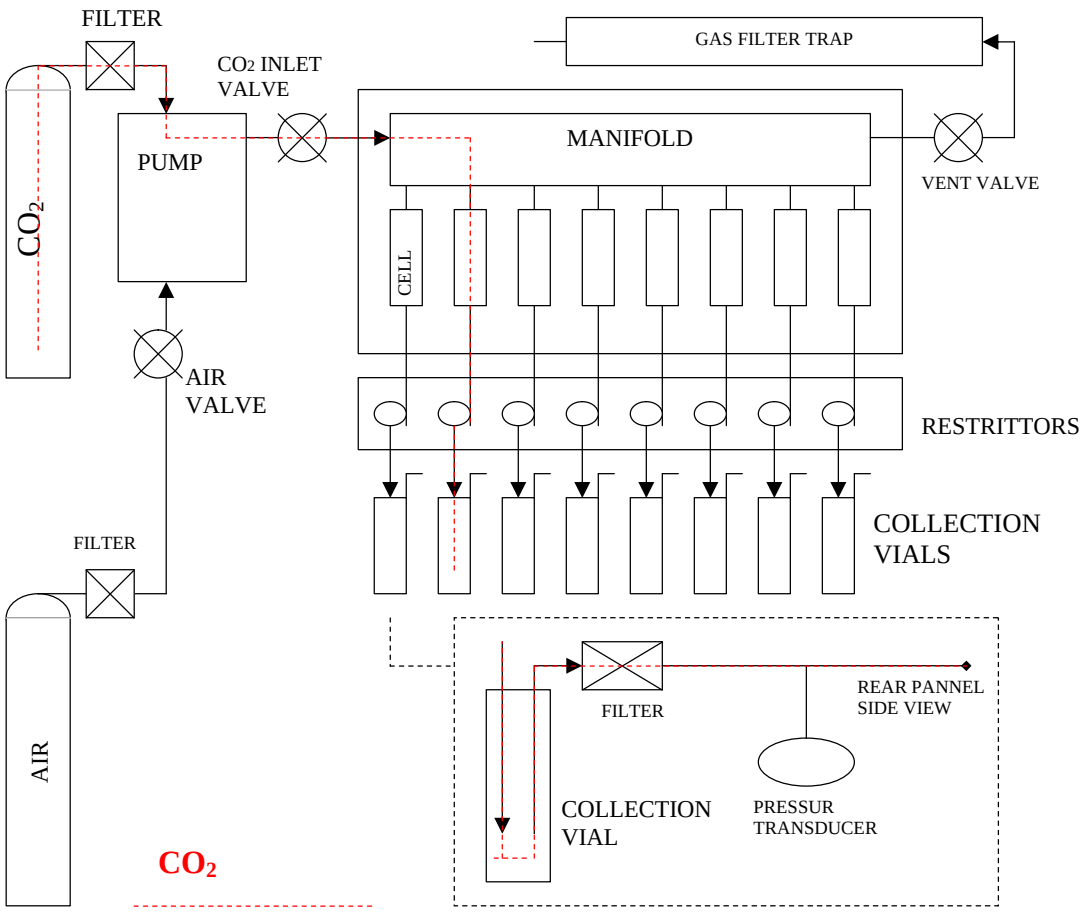

Figure 2 : Schematic diagram of Dionex 703-SFE

RESULTS AND DISCUSSION

Oil yields at temperature extraction of 40 and 80°C are shown respectively in Figures 3 and 4.

α-tocopherol extracted from 3 g of grape seeds is shown in Table 1.

α-tocopherol concentration in the extracted oil is shown in Table 2 and in Figure 5.

The results of the present study indicate that the total oil yield obtained with SC-CO_2 is always lower than that obtained by conventional extraction with n-hexane (14% (w/w) of the seed charge [1]) (see Figures 3 and 4).

From the experiments it was also realized that α-tocopherol concentration in the oil extracted with SC-CO_2 (Table2) increases with temperature, due to the higher solubility of that molecule in SC-CO_2 at 80°C rather than at 40°C [7].

The results also indicate that α-tocopherol concentration in the extracted oil (Table 2) decreases with increasing particle size, because α-tocopherol extraction is retarded by the diffusion in the solid phase [8].

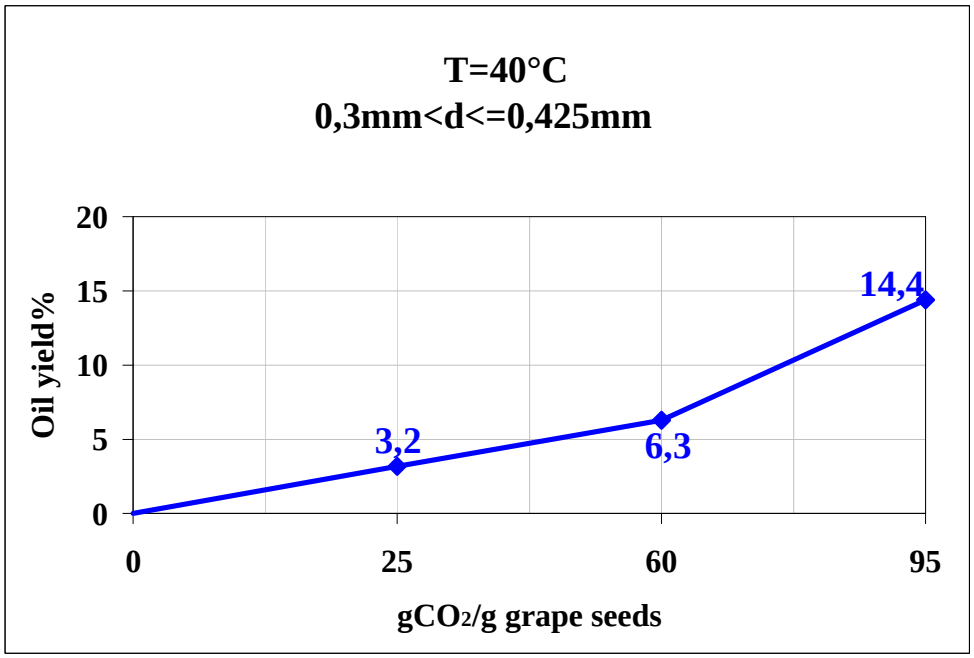

Figure 3 : Oil yield of SC-CO_2 extraction at 25 MPa, 40°C and particle size d=d_3

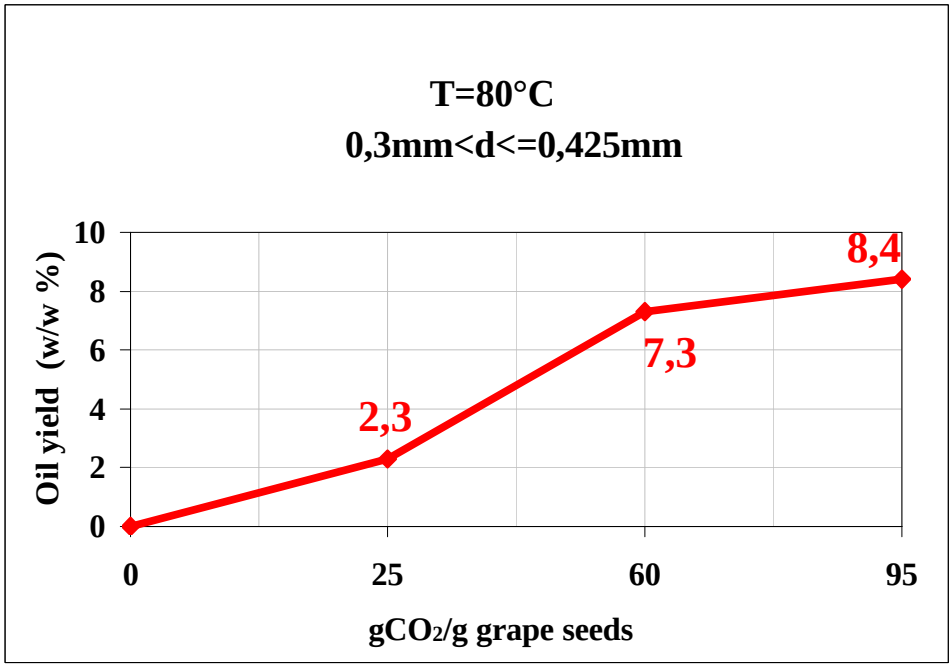

Figure 4 : Oil yield of SC-CO_2 extraction at 25 MPa, 80°C and particle size d=d_3

	mg					
	d_1	d_2	d_3	d_1	d_2	d_3
25 ***$gCO_2/gSeed$***	**3,6 ± 0,6**	**9,2 ± 0,7**	**15 ± 1**	**4,1 ± 0,5**	**6,5 ± 0,6**	**19 ± 1**
60 ***$gCO_2/gSeed$***	**4,4 ± 0,5**	**8,5 ± 0,7**	**19 ± 1**	**7,1 ± 0,7**	**21 ± 1**	**18 ± 1**
95 ***$gCO_2/gSeed$***			**19 ± 1**			**24 ± 1**
	40°C			**80°C**		

Table 1 : α-tocopherol extracted from 3 g of grape seeds using SC-CO_2

	(ppm)					
	d_1	d_2	d_3	d_1	d_2	d_3
25 ***$gCO_2/gSeed$***	**45 ± 7**	**106 ± 8**	**153 ±10**	**67 ± 9**	**99 ± 10**	**265 ± 17**
60 ***$gCO_2/gSeed$***	**36 ± 4**	**49 ± 4**	**102 ± 6**	**44 ± 4**	**106 ± 8**	**105 ± 6**
95 ***$gCO_2/gSeed$***			**44 ± 4**			**95 ± 6**
	40°C			**80°C**		

Table 2 : α-tocopherol concentration in the oil extracted using SC-CO_2

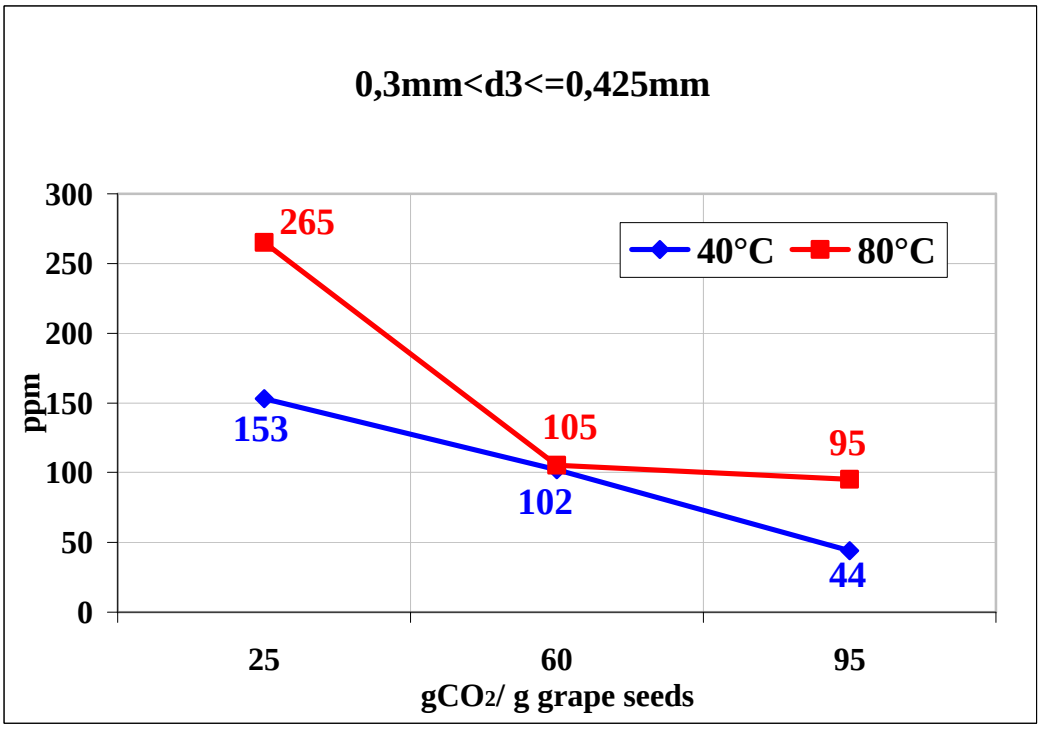

Figure 5 : α-tocopherol concentration in the oil extracted using SC-CO_2 (P=25 MPa, T=40, 80°C, d=d_3)

	*(mg a-t/g CO2 *100)*					
	d_1	d_2	d_3	d_1	d_2	d_3
25 ***gCO_2/gSeed***	**5 ± 1**	**12 ± 1**	**19 ± 1**	**5 ± 1**	**9 ± 1**	**24 ± 1**
60 ***gCO_2/gSeed***	**2 ± 0**	**5 ± 0**	**10 ± 1**	**4 ± 0**	**12 ± 1**	**10 ± 1**
95 ***gCO_2/gSeed***			**7 ± 1**			**8 ± 1**
	40°C			**80°C**		

Table 3 : Efficiency of the SC-CO_2 extraction

Finally it was observed that α-tocopherol concentration in the extracted oil (Table 2) is higher in the first step of the extraction process (with a CO_2 to seed mass ratio of 25), while decreases with further processing time: actually efficiency of SC-CO_2 is maximum in the first step (Table 3), probably due to the exhaustion of the α-tocopherol in the seed matrix.
Particularly for the extraction conditions set: T=80°C, particle size=d_3 and CO_2 to seed mass ratio = 25, α-tocopherol concentration in the oil extracted with SC-CO_2 is 265 ppm.

CONCLUSION

In this work SC-CO_2 was used for milled grape seeds extraction in order to determinate the optimal conditions to obtain an oil enriched in α-tocopherol.
The effect of particle size, temperature and CO_2 to seed mass ratio has been analyzed.
The experiments have demonstrated that SC-CO_2 extraction can be used to obtain an oil enriched in α-tocopherol: optimal extraction conditions are: P=25 Mpa, T=80°C, particle size=d_3 and CO_2 to seed mass ratio = 25.
With these extraction conditions the α-tocopherol concentration in the extracted oil is 265 ppm, while the total oil yield is 2,3 % (w/w) of the seed charge.
The extracted oil, enriched in α-tocopherol, which is the isomer of tocopherols, with the highest vitamin E activity, could be very important for food industry, which could use it as a fortifying agent.

This work is part of an experimental thesis on Chemical Engineering defended by Federico Spinoglio, ay University “La Sapienza” of Rome (Italy) – Department of Chemical Engineering.

REFERENCES

[1] GINO SECCHI, I nostri alimenti, Hoepli, XI, **1993**, p. 598
[2] BRUNNER, A.BIRTIGH, M. JOHANNSEN, J. Supercritic. Fluids, 8, **1995**, p.46
[3] J. W. KING, F. FAVATI, S. L. TAYLOR, Separation Sci. Technol., 31, **1996**, p.1843
[4] A. DE LUCAS, E. MARTINEZ DE LA OSSA, J. RINCON, M.A. BLANCO, I. Gracia, J. Supercritic. Fluids, 22, **2002**, p. 221
[5] KRUKOVSKY V.N., J. Agric. Food Chem., 12 (3), **1964**, p.289
[6] LIN YE, WILLIAM O. LANDEN, RONALD R. EITENMILLER, J. Agric. Food Chem., 48, **2000**, p.4003
[7] J. CHRASTIL, J. Phys. Chem., 86, **1982**, p.3016
[8] H. SOVOVA, Chem. Eng. Sci., 49, **1994**, p.409