# Subcritical Water Extraction Kinetics of Polyphenols from Grape Skins and Defatted Grape Seeds: Experiment and Modeling

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## ABSTRACT

Polyphenols were extracted from skins and defatted Pinot Nero grape seeds by using subcritical water (SW) in a semi-continuous mode. Extractions were performed at a pressure of 10 MPa, at three different temperatures, namely 80, 100 and 120 °C, and with water flow rate of 2 mL/min. For both skins and defatted seeds, total polyphenol (TP) significantly increased with temperature: for skins from $44.3\pm0.4$ to $83.7\pm2.4$ mg/g, and for defatted seeds from $44.2\pm2.4$ to $123.9\pm0.7$ mg/g when the temperature increased from 80 to 120 °C. The extraction kinetics was simulated by two-site diffusion model. The adjustable parameters of the models were calculated by best fitting procedures with experimental data: they resulted in good agreement with literature values. The model fitted the experimental kinetics curves in a satisfactory way with root mean square error (RMSE) in the range of $10^{-2}$-$10^{-1}$.

**Keywords**: subcritical water, extraction, kinetics models, grape seeds, grape skins, polyphenols

## INTRODUCTION

Wine-making process generates substantial volume of solid by-products consisting of skins, stalks and seeds in different proportion. Researches in the past few decades have shown that the possibility of valorizing these by-products for the recovery of oil, phenolic compounds, and fibers are immense. Usually grape seeds are sold to the oil extraction industry and more recently they are asked for by food, cosmetic and pharmaceutical sectors for their use as a source of antioxidants [1]. The by-products are rich in polyphenols [2–6], compounds which contain aromatic rings with one or more hydroxyl groups. There are thousands of compounds identified under this category, the main classes including flavonoids, phenolic acids, tannins and stilbenes [7]. They exhibit wide range of bioactivities as antioxidants, antimicrobials, neuro-sedative, anti-inflammatory, anti-viral, anti-cancer, anti-ulcer, anti-carcinogenic, and anti-mutagenic [2–4].

Traditionally, polyphenols are extracted from natural products using organic solvents. However, these techniques require long extraction period and result in low yields of extract

[8]. To overcome these limitations, considerable amount of research is done in the extraction of plant constituents using non-conventional techniques like ultrasonic-assisted and microwave-assisted extraction [3,9,10]. Even though these techniques allow improving the extraction yield and reducing the extraction time, they still use conventional solvent and the urge for searching for an environmentally friendly solvent remains challenging.

Subcritical water extraction, also referred as pressurized or low polar water extraction, is an emerging technique for the extraction of both polar and non-polar compounds [11]. Subcritical water is defined as water at a temperature between its boiling and critical point where the pressure is regulated in such a way that water always remains in the liquid state. Lately the technique is getting much attention because water is readily available, non-flammable, non-toxic, low cost, and an environmentally acceptable solvent. Interesting reviews on the subcritical water extraction of natural products have been recently presented by several authors [11–14]. Under subcritical conditions, the dielectric constant of water can be tuned by changing the temperature which in turn changes its polarity. For instance under standard temperature and pressure (25 $^o$C and 101 kPa) water is a polar compound with dielectric constant of about 80 [13]; but, when the temperature is increased to about 200-350 $^o$C, the dielectric constant drops to around 20-30, which is similar to the range of the dielectric constant of conventional solvents like methanol, ethanol or acetone at room temperature.

In this work, subcritical water extraction of polyphenols from Pinot Nero grape skins and defatted seeds was investigated operating at constant pressure (10 MPa) and flow rate (2 mL/min), under the temperature range of 80-120 $^o$C.  The extraction kinetics was modeled and discussed.

## MATERIAL AND METHODS

Grape marc samples Pinot Noir, were obtained by winemakers in Northern Italy. At the winery, stalks were separated from the seeds and skins. The mixture of seeds and skins was taken to laboratory and stored at -20 °C before drying. The samples were dried at 55 °C for 48 h, and then the skins and seeds were separated by means of vibrating sieves and further cleaned manually and stored in dark under vacuum at ambient temperature. In order to perform the subcritical water extractions, the same equipment (Proras, Rome, Italy) previously utilized for defatting the grape seeds with supercritical $CO_2$ [1] was utilized with minor plant modifications. A nitrogen line was connected to the extractor to purge the system before extraction and to de-oxygenate the water utilized as solvent. The water was pumped to the extractor by means of HPLC pump (Gilson, Middleton, USA). For each test, 2 grams of substrate were utilized. The water/polyphenols extract was collected every 20 minutes during the 2 hours extraction time. The extract was concentrated in rotary evaporator (Heidolph, Schwabach, Germany) at a reduced pressure of 73 mbar, bath temperature of 40 °C and rotation speed of 30 rpm and stored at -20 $^o$C before analysis. The total polyphenol (TP) content was determined by a colorimetric method using the Folin-Ciocalteu assay resorting to the same procedure as previously reported [15]. TP was expressed as milligrams of gallic acid equivalent (GAE) per gram of dry substrate.

## MODELING

The SW extraction kinetics of TP was modeled by the so called "two-site kinetic model". The literature reports that this model has been applied to the SW extraction of essential oil from savory [16] and *Z. Multiflora* [17], an anti-cancer (*damnacanthal*) from roots of Morinda [18]

and polycyclic aromatic hydrocarbons from contaminated soils [19]. The model is an extension of the "one site kinetic model", mostly referred as Crank's [20] hot ball diffusion model which is based on Fick's second law of diffusion and exploits the similarities with the diffusion of heat in a spherical hot ball cooling down in a uniform medium. It assumes that initially the solute is uniformly distributed in the solid matrix, which contains small quantities of extractable materials so that the extraction is not limited by solubility and the solute concentration in the solvent is close to zero. The two site diffusion model overcomes this shortcoming by considering a fast and slow extraction periods relevant to two different fractions of solute. The desorption rate of fast extracted fraction of the polyphenols, $F$, is given by first-order rate constant $k_1$, and that of slowly released fraction $(1 - F)$ is given by first-order rate constant $k_2$ [16]. Thus, the extraction profile is given by Eq. (1).

$$C/C_o = 1 - [Fe^{-k_1 t}] - [(1 - F)e^{-k_2 t}] \qquad (1)$$

Where $C$ is the TP concentration in the fluid phase and $C_o$ is the initial TP concentration in the matrix. A more explicit form of Eq. (1) is given by Sovová [21] for extraction of solutes under the assumption of mixed flow condition with existence of solute-matrix interaction. In this case the first-order rate constants $k_1$ and $k_2$ represent lumped parameters and are expressed by Eq.s (2) and (3).

$$k_1 = \frac{q'K_m}{1 + \varepsilon q'/\gamma k_f a_0} \qquad (2)$$

$$k_2 = \frac{1}{\lambda R/5D_e + \varepsilon/K_m \gamma k_f a_0} \qquad (3)$$

Where $q'$ is specific flow rate, $K_m$ is mass partition coefficient, $\varepsilon$ is bed void fraction, $\gamma$ is solvent-to-solid mass ratio in the extractor, $k_f$ is mass transfer coefficient in the fluid, $a_0$ is specific surface area, $\lambda$ is characteristic particle dimension (volume-to-surface ratio), R is particle radius, and $D_e$ is effective diffusion coefficient.

In order to reduce the number of adjustable parameters in the model, the value of $F$ is determined according to Reverchon & Marrone [22], which assumes that the particle surface is completely covered with free solute and the thickness of this layer is equal to the radius of solute bearing cell. Fiori et al., [23] found a better agreement between experimental data and model predictions by doubling the thickness of this layer under what is called "double shell hypothesis". If the solute is uniformly distributed in the solid, $F$ is given by Eq. (4).

$$F = 2\frac{\pi d_p^2(d_c/2)}{\pi d_p^3/6} = 6\frac{d_c}{d_p} \qquad (4)$$

Where $d_p$ is diameter of the particle and $d_c$ is solute bearing cell diameter which is assumed equal to 20μm. Accordingly, the value of $F = 0.24$ is taken for all investigated conditions.


**RESULTS AND DISCUSSION**

The extraction yield of TP for both grape skins and defatted seeds at different temperature is presented in Table 1. All the data points represent an average of at least two repeated experiments, each analyzed for TP in triplicate.

**Table 1:** Extraction yield of TP for Pinot Nero grape skins and defatted seeds

| | Matrix | |
|---|---|---|
| Temp.($^o$C) | Skins TP (mgGAE/g) | Defatted seeds TP (mgGAE/g) |
| 80 | 44.3±0.4 | 44.2±2.4 |
| 100 | 66.3±4.2 | 101.6±1.6 |
| 120 | 83.7±2.4 | 123.9±0.7 |

The TP yield from defatted grape seeds is higher than that from skins. Even though there is not direct comparison of yield of TP from defatted grape seeds and skins in the literature using subcritical water (to the best of our knowledge), Casazza et al. [3] made a comparison between un-defatted grape seeds and skins of Pinot Nero extracted by different non-conventional techniques. They found out that TP in seeds is one order of magnitude higher than that in skins and the yields of TP increased up to 390% simply by changing extraction technique. Aliakbarian et al. [4] performed subcritical water extraction of grape pomace and found a yield of 30.80±3.38 mgGAE/g at operation conditions of 140 $^o$C and 11.6 MPa when the flow rate was 1-2 mL/min. Bucic´-Kojic´ et al. [24] reported a TP yield from grape seeds of 130 mgGAE/g when extracting at a temperature of 80 oC. In fact, wide ranges of TP yield from wine industry by-products are reported in the literature due to the several factors which influence the total yield, such as the extraction temperature, time, technique, solvent type, and cultivars. This preliminary finding shows, particularly for defatted seeds the TP are comparable to the result reported by Bucic´-Kojic´ et al. [24] for non-defatted seeds extracted using conventional technique in batch operation, which hints the need of further rigorous investigation of the technique.

The extraction kinetics of both grape skins and defatted seeds were modeled with the two site desorption model. The results are presented in Figure 1 and 2 respectively.
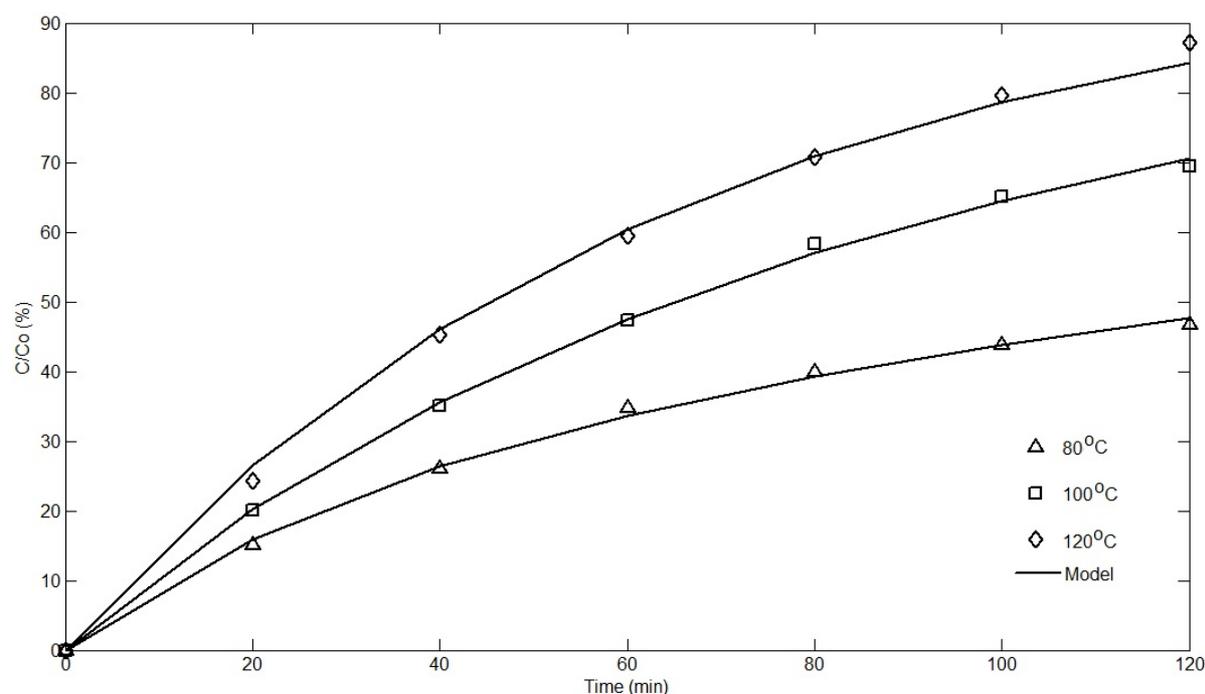


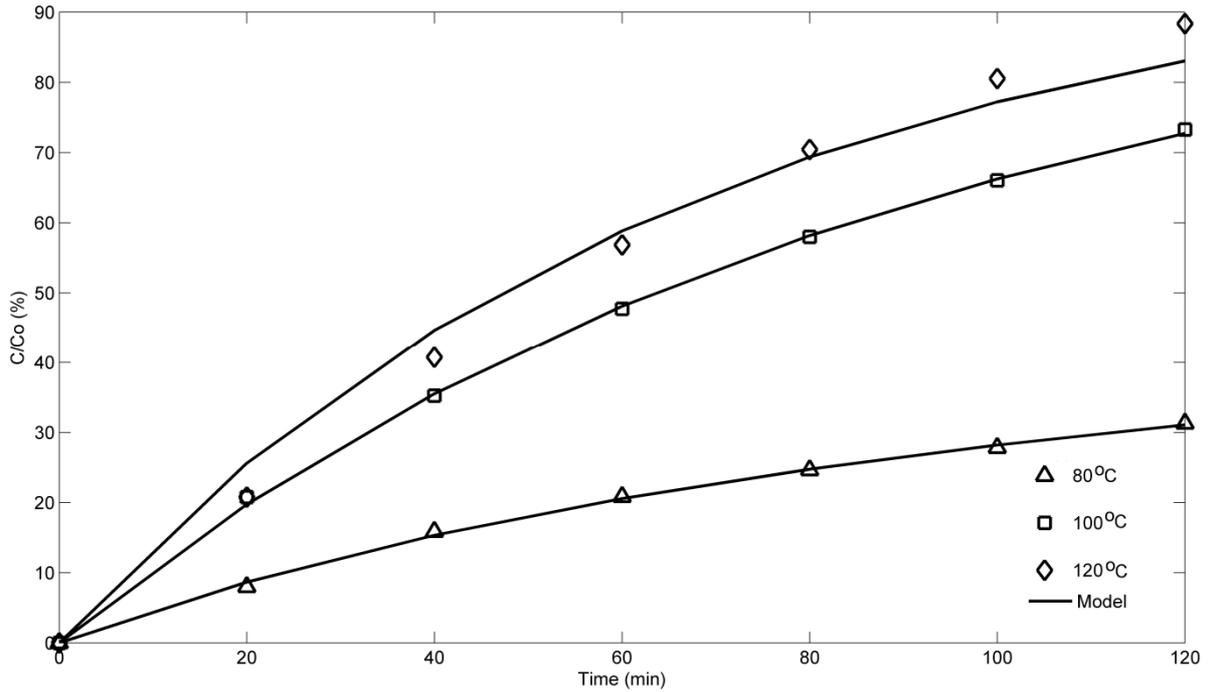**Figure 1**: SW extraction kinetics of Pinot Nero grape skins

**Figure 2**: SW extraction kinetics of Pinot Nero grape defatted seeds

As shown in the Figure 1 and 2, in both cases the rate of extraction significantly increases with the increase in temperature (and at constant pressure), which is in line with the dielectric constant of water. It is widely reported that the solubility of organic compounds in subcritical water depend on several factors like chain length, type and position of side group, molecular weight, position of hydrogen bonding etc. Increase in temperature results in reduction of hydrogen bonding strength in water which makes the water to behave more like non polar compound which in turn increases the solubility of organic compounds [13]. The polyphenols contains wide range of compounds, therefore each compound has its own preferential optimum solubility within SW and the optimum yield depends on proper selection of operating conditions.

**Table 2**: Model adjustable parameters for SW extraction of grape skins and defatted seeds

| Temp.($^{o}$C) | Matrix | | | | | |
|---|---|---|---|---|---|---|
| | Skins | | | Defatted seeds | | |
| | $k_1$(min$^{-1}$) | $k_2$(min$^{-1}$) | RMSE*10$^{-2}$ | $k_1$(min$^{-1}$) | $k_2$(min$^{-1}$) | RMSE*10$^{-2}$ |
| 80 | 0.0154 | 0.0032 | 1.92 | 0.0146 | 0.0012 | 0.99 |
| 100 | 0.0216 | 0.0084 | 1.92 | 0.0148 | 0.0099 | 1.28 |
| 120 | 0.0317 | 0.0154 | 3.91 | 0.0168 | 0.0148 | 9.11 |

The model adjustable parameters are presented in Table 2 along with the deviation of model prediction from experimental data. As can be observed, there are clear trends for the first order rate constant $k_1$ and $k_2$ for both the skins and defatted seeds and the values are higher for the skins. Since the experiments were conducted at constant specific flow rate and bed void fraction, the external mass transfer coefficients are largely expected to be similar within specific category of experiments. Besides the characteristic particle dimensions were similar for all the tests, therefore the variation of the parameters $k_1$ can be explained in terms of the mass partition coefficient of the solute (which is defined as the ratio of equilibrium

concentration of the solute in the fluid phase at the particle surface to the solute concentration in the solid phase). As the temperature increases, the polarity of SW decreases, the solute solubility increases which results in the increase of partition coefficients. It can also be seen from Eq.s (2) and (3) that the first order rate constants are directly proportional to the partition coefficient. So, with increase in temperature the solute partition coefficient will increase and hence desorption rate constants also increase.

## CONCLUSION

Subcritical water extraction of polyphenols from grape skins and defatted grape seeds were conducted in semi-continuous extractor. Relatively high yields of TP were obtained in this work for both skins and seeds. The kinetics of extraction was modeled by the two site kinetic model; remarkable agreement between the model and experimental data was observed with root mean square error in the range of $10^{-2}$-$10^{-1}$. The model adjustable parameters were also in satisfactory agreement with the value reported elsewhere in the literature.

## REFERENCES

[1]     L. Fiori, J. Supercrit. Fluids. 43 (**2007**) , p.43.

[2]     M. Palma, L.T. Taylor, J. Chromatogr. A. 849 (**1999**) , p.117.

[3]     A. a. Casazza, B. Aliakbarian, S. Mantegna, G. Cravotto, P. Perego, J. Food Eng. 100 (**2010**) , p.50.

[4]     B. Aliakbarian, A. Fathi, P. Perego, F. Dehghani, J. Supercrit. Fluids. 65 (**2012**), p. 18.

[5]     S. Bail, G. Stuebiger, S. Krist, H. Unterweger, G. Buchbauer, Food Chem. 108 (**2008**) , p.1122.

[6]     L. Casas, C. Mantell, M. Rodríguez, E.J.M.D. La Ossa, a. Roldán, I. De Ory, et al., J. Food Eng. 96 (**2010**), p. 304.

[7]     I. Ignat, I. Volf, V.I. Popa, Food Chem. 126 (**2011**) , p.1821.

[8]     P.P. Singh, M.D. a. Saldaña, Subcritical water extraction of phenolic compounds from potato peel, Food Res. Int. 44 (**2011**), p. 2452.

[9]     H. Bagherian, F. Zokaee Ashtiani, A. Fouladitajar, M. Mohtashamy, Process Intensif. 50 (**2011**), p. 1237.

[10]     M.F. Barrera Vázquez, L.R. Comini, R.E. Martini, S.C. Núñez Montoya, S. Bottini, J.L. Cabrera, Ultrason. Sonochem. 21 (**2014**) , p.478.

[11]     L. Ramos, E.M. Kristenson, U. a T. Brinkman, J. Chromatogr. A. 975 (**2002**), p. 3.

[12]     E.S. Ong, J.S.H. Cheong, D. Goh, J. Chromatogr. A. 1112 (**2006**) , p.92.

[13]     A.G. Carr, R. Mammucari, N.R. Foster, Chem. Eng. J. 172 (**2011**) , p.1.

[14]     M. Herrero, a Cifuentes, E. Ibanez,  Food Chem. 98 (**2006**) , p.136.

[15]     L. Fiori, D. de Faveri, a. a. Casazza, P. Perego, CyTA - J. Food. 7 (**2009**), p. 163.

[16]     A. Kubátová, B. Jansen, J.-F. Vaudoisot, S.B. Hawthorne,  J. Chromatogr. A. 975 (**2002**), p.175.

[17]     M. Khajenoori, a. H. Asl, F. Hormozi, Chinese J. Chem. Eng. 17 (**2009**), p. 359.

[18]     T. Anekpankul, M. Goto, M. Sasaki, P. Pavasant, a Shotipruk,  Sep. Purif. Technol. 55 (**2007**), p.343.

[19]     M.N. Islam, Y.-T. Jo, S.-K. Jung, J.-H. Park, J. Ind. Eng. Chem. 19 (**2013**), p. 129.

[20]     J. Crank, Clarendon Press, Oxford, **1975**.

[21]     H. Sovová, J. Supercrit. Fluids. 66 (**2012**), p. 73.

[22]     E. Reverchon, C. Marrone, J. Supercrit. Fluids. 19 (**2001**) , p.161.

[23]     L. Fiori, D. Basso, P. Costa, J. Supercrit. Fluids. 48 (**2009**) , p.131.

[24]     A. Bucić-Kojić, H. Sovová, M. Planinić, S. Tomas, Food Chem. 136 (**2013**), p. 1136.