THE USE OF GROUP CONTRIBUTION METHOD FOR PREDICTING THE SOLUBILITY OF SEED POLYPHENOLS OF VITIS VINIFERA L. WITHIN A WIDE POLARITY RANGE IN SOLVENT MIXTURES

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ABSTRACT
Polyphenols recovery from the seeds of red-grape (Vitis vinifera L.) in different ethanol-water mixtures at different temperatures and times of extraction was studied. The extracts were screened for total polyphenols content using the modified Folin-Ciocalteu spectrophotometric method. Total polyphenols extracted varied sensibly over the range of 0.7-24 mg g⁻¹ depending on operating conditions. A recovery maximum was obtained using 50 % ethanol, ratio of solvent-to-solid of 7.5 ml g⁻¹, extraction time of 60 min and heating at 65°C. The variation in solubility produced by binary ethanol-water mixtures was studied against Hansen solubility parameters to estimate the effect of changing polarity in solvent mixtures.

Keywords: extraction, grape seeds, polyphenols, solubility parameter, solubility prediction.

INTRODUCTION
Many bioingredients derived from plant sources are high-value-added chemicals with expanding applications in food, cosmetics, and pharmaceutical sectors. To meet an increasing demand for shorter time and reduced cost in developing their manufacturing processes, a systematic procedure is needed for generating extraction process alternatives that transform the harvested plants into desired products. Many factors affect the extraction and need to be optimized for maximum recoveries - particles size, type of the solvents, pH of the extraction medium, time and temperature of extraction, number of extractions, and degradation of compounds during extraction. Among these factors, the type of solvent is the most important because it can affect the amount, the type and the biological activity of compound extracted.

Although many different approaches are available to assess solute-solvent interactions, there has been a strong tendency to use Hildebrand and Hansen solubility parameters (HSP) as a valuable practical aid in selecting solvent [1]. Hildebrand solubility parameter indicates the strength of the intermolecular forces (cohesive energy $E_{coh}$) which in turn is calculated via the enthalpy of vaporization $\Delta H_{vap}$ and the molar volume $V_m$

$$\delta_H^2 = \frac{E_{coh}}{V_m} = \frac{\Delta H_{vap} - RT}{V_m}$$  \hspace{1cm} (1)

The Hildebrand parameter works well for low molecular mass non-polar solvents. However, this simplistic parameter cannot adequately describe solubility behavior when polar and hydrogen bonding solvents are included in the system.

Solubility parameters proposed by Hansen addresses this problem by using three parameters to describe solubility behavior. The total cohesive energy density is approximated by the sum of the energy densities required to overcome atomic dispersion London
forces ($\delta_i^2$), forces between permanent dipoles of adjacent molecules (polar interaction) ($\delta_p^2$), and to break hydrogen-bonds (exchange of electrons, proton donor/acceptor) between molecules ($\delta_h^2$):

$$\delta_i^2 = \delta_d^2 + \delta_p^2 + \delta_h^2$$  \hspace{1cm} (2)

where the total solubility parameter $\delta_i$ should be identical to $\delta_i^r$. The mutual solubility between a solute $i$ and a solvent $j$ is quantified by the following parameter:

$$\Delta\delta_{ij} = \sqrt{(\delta_d^i - \delta_d^j)^2 + (\delta_p^i - \delta_p^j)^2 + (\delta_h^i - \delta_h^j)^2}$$  \hspace{1cm} (3)

The parameters follow the rule that the smaller $\Delta\delta_{ij}$ is, the greater the affinity between solute and liquid, also known as “like dissolve like”. Recently, HSP approach has been extended to interpret the solubility of compounded materials such as high polymer materials, drugs and bioingredients in plants in various solvents [2].

Objects of this study were red-grape (Vitis vinifera L.) seeds that contain large amount of natural polyphenols (vegetable tannins). During the last years the interest in these compounds has increased due to their strong antioxidant activity [3, 4]. The seeds are a part the richest in polyphenols but a part in a grape solid cluster where polyphenols are the most difficult to extract. The seeds contain lipid, protein, carbohydrates, and 5-8% polyphenols, concentrated in the seed coat. The amount and types of polyphenols present in a particular grape seeds may vary and is greatly influenced by the extraction process, as well as the source, variety and storage of seeds used. From the quantitative point of view, the procyanidins are the major group of polyphenols accounting for 60 to 70% of their total content. Procyanidins are derivatives of the flavan-3-01 class of flavonoids. The major compounds that were identified as preferentially soluble in water-ethanol mixture include catechin $C_{15}H_{14}O_{6}$ and its epimer epicatechin; gallicatechin and epigallocatechin (EGC) $C_{15}H_{14}O_{6}$, that contain an additional phenolic hydroxyl group; and gallic acid esters such as epigallocatechin gallate (EGCG) $C_{22}H_{18}O_{13}$ and epicatechin gallate (ECG) $C_{22}H_{18}O_{13}$ [5]. These monomer units polymerize to give dimers (such as proanthocyanidin PAC B1 $C_{31}H_{26}O_{14}$), trimers, oligomers and condensed polymers linked most commonly by acid-labile 4-8 bonds (Fig. 1).

The term “proanthocyanidins” was defined because these colorless compounds release colored anthocyanidins by cleavage of the interflavan C-C bond on heating in acidic medium. Typically grape polyphenols are presented

Fig. 1. Structures of major polyphenols identified in grape seed extract.
essentially in polymeric forms (60-80 %), followed by oligomeric forms (15-30 %) while monomer flavan-3-ols represent only a small proportion (less than 10 %) [5].

The aim of the present study was to evaluate the effect of changing polarity of the solvent on the polyphenols recovery in a red-grape seeds/ethanol-water system. The experimental results were compared to the calculated solubility of polyphenols in grape seeds by group contribution method in order to validate the use of HSP values for selecting a preferential solvent with a considerable solution capacity.

**EXPERIMENTAL**

**Solid-liquid extraction**

Dried (moisture content of 6-8 %) red-grape seeds originate from Moldova were used during experiments. Grape seeds extracts were prepared using distilled water and ethanol-water mixtures as solvents (ethanol concentration of 20, 40, 50, 60, 80 and 95 %). The experiments were performed in a conventional batch-type stirred vessel at angular velocity of n > 5 s⁻¹, which eliminates the external mass transfer resistance. Non-crushed seeds samples (3 g) were extracted with the appropriate solvent volume (22.5 ml) at temperature of 25, 45 and 65°C and time of extraction up to 1 h. After extraction, dry extracts were obtained by evaporating the solvent and the total extractive yields were determined by mass differences. Results are expressed as a mass fraction (mg total extractive yield /g seed).

**Determination of the total polyphenols content**

Total polyphenols content of the extracts was determined by the Folin-Ciocalteu spectrophotometric method [6] with the following modifications. Grape seed extract (0.5 - 3 ml) was placed in a volumetric flask of 50.00 ml and was diluted with distilled water (approximately 25 ml); Folin-Ciocalteu phenol reagent (2.5 ml) and sodium carbonate solution (10 ml of 2 % m/v) were added and then the sample was diluted with water to 50.00 ml. The sample was shaken thoroughly to homogenize, and after resting time of 30 minutes the blue color formed was measured at 750 nm using a SPECOL 11 spectrophotometer.

Because of a relatively short linear part of the calibration curve (the range of 2.10⁻⁶ - 7.10⁻⁶ mol dm⁻¹ tannins) absorbance values read can be between 0.210 to 0.340. The measurement was carried out by using an blank sample (mixture of all reagents except for the extract). Results are calculated by the following analytical function:

\[
m_l = \frac{C_i (M_r x 10^3) (50.00 x 10^{-3}) V_{ext}}{V_i} = 0.8506 x 10^5 \frac{C_i V_{ext}}{V_i} (mg)
\]  

where \( m_l \) - mass of the total tannins (mg), \( C_i \) (mol l⁻¹) - concentration of the total tannins accounted from the calibration curve, \( M_r \) - molecular mass of tannins (g mol⁻¹), \( V_{ext} \) - total volume of extract obtained (ml), and \( V_i \) - volume of extract analyzed (varied from 0.5 to 3 ml). The mixture was additionally diluted when the values of absorbance fell out of the calibration curve range assigned, and then the dilution volumes were taken into account in equation (4).

Each determination was performed in triplicate. Results are expressed as tannic acid equivalent (mg total tannins/g seed). Folin-Ciocalteu reagent and tannic acid (\( M_r = 1701.2 \) g mol⁻¹) used as standard were purchased from Sigma-Aldrich (Germany).

**RESULTS AND DISCUSSION**

**Effect of ethanol-water ratio**

Fig. 2 presents results concerning the yields of extraction for different ethanol-water ratio at 60°C after 30 min of extraction. Total polyphenols content varied widely from 0.7 to 13.1 mg g⁻¹, and the variations in the total extractive yield over the range of 1.4 - 22.5 mg g⁻¹ correlated with the trend in the polyphenols content.

In addition, the colours of the extracts were complicatedly varied from colourless to yellow to brown-red with the ethanol-water mixtures suggesting selective extraction of a particular group of tannins. Among the solvents tested the highest levels of polyphenols in the extract were achieved using 50 % ethanol, followed by 60 % ethanol > water > 40 % ethanol = 20 % ethanol > 80 % ethanol > 95 % ethanol, respectively.

**Effect of the temperature and the time**

The effect of temperature on the extraction was studied with a heating time up to 60 min (Fig. 3). The integral part of compounds extractable in 50 % aqueous ethanol cannot be removed at ambient temperature. The
Table 1. Number of structural groups for the major polyphenols in grape seed extract.

<table>
<thead>
<tr>
<th>Group</th>
<th>$F_d$ J$^{1/2}$cm$^{3/2}$mol$^{-1}$</th>
<th>$F_e$ J$^{1/2}$cm$^{3/2}$mol$^{-1}$</th>
<th>$E_h$ J mol$^{-1}$</th>
<th>Catechin</th>
<th>ECG</th>
<th>EGC</th>
<th>EGCG</th>
<th>PAC</th>
<th>B1</th>
</tr>
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<tr>
<td>- CH$_2$ -</td>
<td>270</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt; CH -</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>= CH -</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>= C &lt;</td>
<td>70</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>12</td>
<td>8</td>
<td>13</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>- O -</td>
<td>210</td>
<td>250000</td>
<td>20000</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Ring</td>
<td>190</td>
<td>160000</td>
<td>3000</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Group contribution technique was used for the calculation of $F_d$ (dispersion group molar attraction), $F_p$ (polar group molar attraction) and $E_h$ (H-bonding) contribution for major polyphenols in grape seed extract (Table 1).

The numerical values for the solubility parameters were summarized in Table 2. The different phenolic compounds are distinguished by minor variations on their structures (Fig. 1), and consequently the calculated values of $\delta_d$ (24.76 ± 0.56 MPa$^{1/2}$) are closely related, classifying the polyphenolic mixture as polar fraction. The results confirmed experimental results previously reported by other authors that acetonitrile ($\delta_d$ = 24.8 MPa$^{1/2}$) and 75% acetone-water mixture ($\delta_d$ = 26.78 MPa$^{1/2}$) were the best extraction solvents both for catechin and oligomeric and polymeric procyanidins from grape seeds [4, 5].

The solubility parameter theory uses the so-called geometric mean of interaction in two pure liquids to estimate the interaction between unlike molecules in their mixtures. According to this principle the calculated HSP values for ethanol-water mixtures have intermediary values within the large polarity range of ethanol ($\delta_d$ = 26.2 MPa$^{1/2}$) and water ($\delta_d$ = 48.00 MPa$^{1/2}$).

![Fig. 2. Effect of different solvents on extractive yields.](image-url)
comparative analysis shown dispersion values ($\delta_d = 13.4 \pm 0.2$ MPa$^{1/2}$) for polyphenols similar to that of single-used ethanol ($\delta_d = 12.6$ MPa$^{1/2}$) or water ($\delta_d = 12.2$ MPa$^{1/2}$). However, water contains significantly higher values for $\delta_p (22.8$ MPa$^{1/2}$) and $\delta_h (40.8$ MPa$^{1/2}$) compared to the moderate-to-low polar component $\delta_p (4.0$-$0.7$ MPa$^{1/2}$) and relatively high H-bonding compound $\delta_h (20.5 \pm 0.6$ MPa$^{1/2}$) for the polyphenols. Thus, the high value of calculated in polyphenols/water system that increased with the number of units of polymerization (Table 2) confirmed that water alone cannot dissolve these polyphenols. Theoretically, the addition of ethanol ($\delta_p = 11.2$, $\delta_d = 20.0$ MPa$^{1/2}$) diminished the values of and improved the mutual solubility in polyphenols/ethanol system at room temperature. However, the position of the minimum shifts to a single-used ethanol and then HSP concept was unable to predict the recovery maximum at 50% ethanol as found experimentally (Fig. 2).

The discrepancy can be due to some limitations for predicting solubility in the case of aqueous mixtures. As ethanol is added to water, the entropy of mixing increases and the structure of the solvent mixture became less ordered, favoring the interaction of the solute with the solvent mixture. Also, since entropy is also affected by the number of elements in the system (more elements-more disorders) smaller and more linear molecules diffuse more rapidly than larger more bulky ones. Beside it, Hansen solubility parameters do not include kinetic effects on diffusion rates. Generally increasing temperature increases the solubility and diffusion of the compounds to be extracted, decreases the viscosity of solvent and thus enhanced extraction efficiency. The difference in the recovery profiles in ethanol-water mixtures at room temperature and at 65°C suggested that diffusivity rather than the solubility had a major effect on the extraction performance at higher temperature.

**CONCLUSIONS**

The extraction of natural polyphenols can be effectively accomplished from the non-crushed grapes seeds at temperature up to 65°C in 50% ethanol-water mixture. Although ethanol is classified as a GRAS (Generally-Recognized-As-Safe) solvent, its utilization must be strictly accounted as preferential solvent for the production of grape seed extracts.

**Table 2. Calculated HSP parameters for major polyphenols in ethanol-water mixture.**

<table>
<thead>
<tr>
<th></th>
<th>$V_m$ cm$^3$/mol</th>
<th>$\delta_d$ MPa$^{1/2}$</th>
<th>$\delta_p$ MPa$^{1/2}$</th>
<th>$\delta_h$ MPa$^{1/2}$</th>
<th>$\delta_i$ MPa$^{1/2}$</th>
<th>$\Delta\delta_{i,j}$ water</th>
<th>$\Delta\delta_{i,j}$ C$_2$H$_5$OH</th>
<th>$\Delta\delta_{i,j}$ 50% C$_2$H$_5$OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>266</td>
<td>13.7</td>
<td>4.5</td>
<td>19.7</td>
<td>24.4</td>
<td>27.79</td>
<td>7.06</td>
<td>16.53</td>
</tr>
<tr>
<td>ECG</td>
<td>385</td>
<td>13.8</td>
<td>3.8</td>
<td>19.7</td>
<td>24.4</td>
<td>28.27</td>
<td>7.78</td>
<td>17.09</td>
</tr>
<tr>
<td>EGC</td>
<td>273</td>
<td>13.6</td>
<td>4.7</td>
<td>21.2</td>
<td>25.6</td>
<td>26.53</td>
<td>6.91</td>
<td>15.42</td>
</tr>
<tr>
<td>EGCG</td>
<td>392</td>
<td>13.8</td>
<td>4.0</td>
<td>20.8</td>
<td>25.3</td>
<td>27.37</td>
<td>7.63</td>
<td>16.28</td>
</tr>
<tr>
<td>PCA B1</td>
<td>518</td>
<td>13.4</td>
<td>3.3</td>
<td>19.9</td>
<td>24.2</td>
<td>28.46</td>
<td>8.15</td>
<td>17.34</td>
</tr>
</tbody>
</table>
The used Hansen solubility parameters to reproduce the experimental solubility profile was found to be good in predicting single-used solvents behavior and to have some shortcomings in case of non-ideal behavior of mixture, interference of competitive solutes in multi-component systems, and diffusion-dominated processes. However, although the calculated HSP values are sometimes only crude estimations of the relevant forces and interactions, their shortcomings do not eliminate their usefulness completely as a heuristic rules for selecting a preferential solvent.

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REFERENCES