



## Experimental and theoretical binding affinity between polyvinylpyrrolidone and selected phenolic compounds from food matrices



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### ABSTRACT

Polyvinylpyrrolidone (PVPP) is a fining agent, widely used in winemaking and brewing, whose mode of action in removing phenolic compounds has not been fully characterised. The aim of this study was to evaluate the experimental and theoretical binding affinity of PVPP towards six phenolic compounds representing different types of phenolic species. The interaction between PVPP and phenolics was evaluated in model solutions, where hydroxyl groups, hydrophobic bonding and steric hindrance were characterised. The results of the study indicated that PVPP exhibits high affinity for quercetin and catechin, moderate affinity for epicatechin, gallic acid and lower affinity for 4-methylcatechol and caffeic acid. The affinity has a direct correlation with the hydroxylation degree of each compound. The results show that the affinity of PVPP towards phenols is related with frontier orbitals. This work demonstrates a direct correlation between the experimental affinity and the interaction energy calculations obtained through computational chemistry methods.

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## 1. Introduction

Phenolic compounds are important secondary metabolites that are ubiquitous to several fresh and processed food products (Balasundram, Sundram, & Samman, 2006; Cheyner, 2012). In fermented alcoholic beverages, such as wines, they contribute with sensorial characteristics that are critical to the quality of the finished product, such as colour, astringency and bitterness (Baiano et al., 2014; Marquez, Serratos, & Merida, 2014; Sun, Liang, Bin, Li, & Duan, 2007). Moreover, phenolic compounds have been proven to be effective radical scavengers that upon regular consumption have been linked with health benefits such as anti-inflammatory, antimutagenic, and anticancer effects (Dong et al., 2011; Fernandez, Oliva, Barba, & Camara, 2005; Gollucke et al., 2013; Nunes et al., 2013; Sergent, Piront, Meurice, Toussaint, & Schneider, 2010). In wines, the presence of large amounts of these

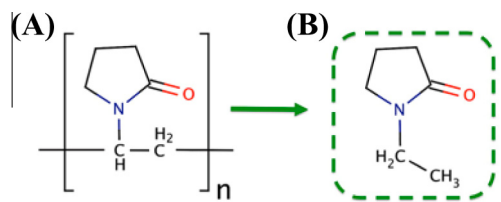
phenolic compounds may relate with sensorial or cosmetic problems (Lorrain et al., 2013; Villamor & Ross, 2013) which, in some instances, are solved by reducing their concentration using fining agents such as polyvinylpyrrolidone, PVPP (Caceres-Mella et al., 2013; Mcmurrough, Madigan, & Smyth, 1995; Sen et al., 2012).

The PVPP polymer (Fig. 1) was introduced commercially as an adsorbent for beer phenolics in 1961 (Caceres-Mella et al., 2013; Mcmurrough et al., 1995). Since then, it has been widely used as an agent for prolonging the stability of beers against haze formation (McMurrrough, Kelly, Byrne, & O'Brien, 1992), as well as for the modulation of the concentration of phenolics in wines (Magalhães et al., 2010; Mcmurrough et al., 1995).

Although few studies have applied computational chemistry methodologies to estimate the molecular interactions between PVPP and target molecules of industrial interest (Laborde et al., 2006; Le Bourvellec & Renard, 2012; Panarin, Kalninsk, & Pestov, 2001), none of them have correlated their results with experimental data, particularly regarding to phenolic compounds. Therefore, the aim of this study was to evaluate the experimental affinity between phenolic compounds such as quercetin, catechin, epicatechin, 4-methylcatechol, gallic acid and caffeic acid towards PVPP

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**Fig. 1.** (A) Chemical structure of PVPP –  $(C_6H_9NO)_n = (111.1)_n$ . (B) Monomeric unit used to calculate the interaction energy.

polymer (adsorption test in model solutions evaluated by HPLC–DAD), and correlate these results with information obtained through the structure-based molecular characterisation using semi-empirical and quantum mechanical studies (Gontijo et al., 2012; Louli, Ragoussis, & Magoulas, 2004; Stewart, 2007; Yilmaz & Toledo, 2006).

## 2. Materials and methods

### 2.1. Reagents

Quercetin hydrate  $\geq 95\%$  (Sigma), (–)-epicatechin (Sigma), 4-methylcatechol  $\geq 95\%$  (Aldrich), gallic acid monohydrate  $\geq 98\%$  (Aldrich), caffeic acid (Sigma), (+)-catechin hydrate  $\geq 98\%$  (Sigma), ethanol HPLC grade  $\geq 99.8\%$  (Merck), acetonitrile HPLC grade  $\geq 99.5\%$  (Sigma) and polyvinylpyrrolidone powder, average Mw  $\sim 40,000$  (Sigma). All solutions were prepared using MilliQ water.

### 2.2. Adsorption of phenolic compounds by PVPP in model solutions

Model solutions containing a mixture of phenolic compounds were prepared in (a) 12% v/v ethanol solution at pH 3.5 (a mixture commonly used to model wine samples), and (b) water at pH 6.5 adjusted with formic acid. The concentration of phenolic compound was adjusted to 0.16 mM for each of the following phenolic species (gallic acid, catechin, caffeic acid, epicatechin, 4-methylcatechol and quercetin) affording a 2.25 mg mL<sup>-1</sup> of phenolics. Considering that quercetin was insoluble, or slightly soluble in pure water, this phenol was only evaluated in the acidified 12% ethanol solution.

An initial trial was performed to evaluate the time of interaction required between PVPP and phenolics in order to produce the maximum adsorption. The adsorption kinetics was evaluated at nine different times (0, 5, 10, 15, 20, 25, 30, 45 and 60 min after the addition of PVPP) at room temperature by measuring the solution's absorption at 280 nm (i.e., a simple and commonly used procedure to quantify the concentration of phenolics in solution). After identifying the time of maximum absorption, an HPLC–DAD method was tested (as detailed below) in order to assess the concentration of each individual phenol in solution. To evaluate the affinity of PVPP towards each selected phenolic compounds, 1.8 mg of PVPP (0.45 mg mL<sup>-1</sup>) was added to a 4 mL solution containing all phenols (2.25 mg mL<sup>-1</sup> of total phenols). The ratio by weight of the total phenolic compounds to PVPP was 5 (total phenolic compounds:PVPP, 5:1 w/w). The samples were incubated with stirring for 15 min at constant room temperature (25 °C) and then centrifuged at 10,000 rpm for 10 min. The concentration of phenolic compounds in the supernatant was analysed by HPLC–DAD (as further explained below). The quantification was conducted at 280 nm for catechin, 4-methylcatechol and epicatechin, for gallic acid at 271 nm, for caffeic acid at 323 nm, and for quercetin at 265 nm. Three independent measurements were made for each sample, and the results are presented as mean values with standard deviations.

The adsorption efficiency of each phenolic compound by PVPP was evaluated by determining the percentage decrease in the absorbance at each specific maximum absorbance wavelength using the following Eq. (1):

$$\text{Adsorption (\%)} = \frac{A_0 - A}{A_0} \times 100 \quad (1)$$

where  $A_0$  is the initial absorbance at specific wavelength and  $A$  is the final absorbance at the same wavelength (Table 1).

### 2.3. Chromatographic method and instrument employed

The HPLC system (Agilent ChemStation, 1200, USA) consisted of a low-pressure quaternary pump (model Agilent 1200), auto-sampler (model Agilent 1260 Infinity Autosampler), an in-line DG-model G1322A degasser and a model G1329B, and a photodiode array detector (model MD-1510 UV/visible multiwavelength detector). Separations were achieved on a LiChrospherRP-18 column of 250 mm (5  $\mu$ m) particle size. The chromatographic conditions were the following: Mobile phases were prepared with formic acid 4.5% and milliQ water (solution A) (filtered through a 0.45  $\mu$ m nylon filters), and acetonitrile, HPLC grade (solution B). The gradient was programmed as specified in Table S1 (Supplemental material) running 35 min. The injection volumes used were of 100  $\mu$ L, and the wavelengths selected for evaluation were at 265, 271, 280 and 323 nm. Column room temperature and flow rate were optimised for phenols separation, initial 1.2 mL min<sup>-1</sup> for 10 min, 1.3 mL min<sup>-1</sup> between 10 and 15 min, and 1.5 mL min<sup>-1</sup> between 15 and 35 min. Peak purity was checked to exclude any contribution from interfering peaks. The identification and quantification of phenolic compounds were performed by comparing their retention time against high purity standard. For this purpose, calibration curves were performed for each phenolic compound (catechin, epicatechin, gallic acid, caffeic acid, quercetin and 4-methylcatechol) in the range of 5–50 ppm.

### 2.4. Computational methods

To carry out the computational calculations a nanoinformatics strategy (Avila-Salas et al., 2012; Metropolis, Rosenbluth, Rosenbluth, Teller, & Teller, 1953) was used to calculate the interaction energy between pairs of molecules (Fan, Olafson, & Blanco, 1992; Gonzalez-Nilo, Urzua, Leiva, Gargallo, & Radic, 2003) using 1-methyl-2-pyrrolidone (monomeric unit of PVPP) with each phenol, as a way to estimate the molecular properties of PVPP. To obtain an accurate prediction of the interaction energies of these systems, it is important to use a representative sampling of the available conformations for each complex (molecule 1–molecule 2). For this reason, the algorithm used starts with a random sampling through Euler Angles between the pair of molecules under study (Fan et al., 1992; Metropolis et al., 1953). That strategy generates thousands of pairs of 1-methyl-2-pyrrolidone–phenol, allowing an exhaustive conformational sampling. Then, for each new pair conformation, a single energy point was calculated using the latter semi-empirical quantum mechanical methods, in this case, the Parameterization Method 7 (PM7) MOPAC, 2012. The advantage of using this new version of PM7 method is that allows the study of intermolecular interactions with a good accuracy and great speed. PM7 also includes empirical corrections for dispersion and hydrogen-bond interactions (Hostas, Rezac, & Hobza, 2013; Korth, 2010).

Semi-empirical calculations consider the valence shell electrons, and use empirical data (experimental information) to account for the energies of the inner shell electrons (Louli et al., 2004). The use of empirical information allows simplifying the quantum mechanical calculations. Although they are not as accurate as

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