Analytica Chimica Acta 661 (2010) 143–149

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Application of Fourier transform infrared spectroscopy and orthogonal projections to latent structures/partial least squares regression for estimation of procyanidins average degree of polymerisation

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ARTICLE INFO

Article history:
Received 2 June 2009
Received in revised form
17 December 2009
Accepted 18 December 2009
Available online 29 December 2009

Keywords:
Fourier transform infrared spectroscopy
Orthogonal projections to latent structures
Partial least squares regression-1
Grape seed
Flavan-3-ols
Graded procyanidin fractionation

ABSTRACT

Fourier transform infrared (FTIR) spectroscopy has being emphasised as a widespread technique in the quick assess of food components. In this work, procyanidins were extracted with methanol and acetone/water from the seeds of white and red grape varieties. A fractionation by graded methanol/chloroform precipitations allowed to obtain 26 samples that were characterised using thiolysis as pre-treatment followed by HPLC-UV and MS detection. The average degree of polymerisation (*DPn*) of the procyanidins in the samples ranged from 2 to 11 flavan-3-ol residues. FTIR spectroscopy within the wavenumbers region of 1800–700 cm^{-1} allowed to build a partial least squares (PLS1) regression model with 8 latent variables (LVs) for the estimation of the *DPn*, giving a RMSECV of 11.7%, with a R^2 of 0.91 and a RMSEP of 2.58. The application of orthogonal projection to latent structures (O-PLS1) clarifies the interpretation of the regression model vectors. Moreover, the O-PLS procedure has removed 88% of non-correlated variations with the *DPn*, allowing to relate the increase of the absorbance peaks at 1203 and 1099 cm^{-1} with the increase of the *DPn* due to the higher proportion of substitutions in the aromatic ring of the polymerised procyanidin molecules.

1. Introduction

Grape seeds are a rich source of flavan-3-ols. These structures are present in mixtures of monomers together with oligomeric and polymeric procyanidins, mainly composed by residues of (−)-epicatechin, (+)-catechin, and (−)-epicatechin-*O*-gallate (Fig. 1) [1,2]. In grape seed tissues, although the quantification of procyanidins has only been accurately performed for oligomers up until tetramers, they have been shown to represent more than 70% of the total flavan-3-ols [3]. Procyanidins with different degrees of polymerisation have different properties, namely, they confer different organoleptic properties to the food [4,5] and they have different absorption behaviour at the gastrointestinal tract level [6]. The estimation of the average degree of polymerisation (*DPn*) of procyanidins is thus a useful parameter to evaluate the type of procyanidins present in a sample. Because the majority of procyanidin-rich samples contain molecules with different degrees of polymerisation [7], fractionation steps can be used to improve their homogeneity.

For the calculation of the *DPn*, the polymers need to be submitted to an acid-catalysed degradation in the presence of nucleophilic agents, such as thiolysis that promote the formation of distinct monomers corresponding to the terminal and extension units of the polymer [1,8]. These compounds are then usually characterised by reversed-phase HPLC with UV detection at 280 nm [9–12]. More recently, advanced techniques based on mass spectrometry analysis have become very effective in qualitative analysis. These highly sensitive methods are able to characterise procyanidins in complex matrices, allowing the direct identification of the molecules with different degrees of polymerisation [13–16]. Anyway, the analyses using HPLC-UV are both time consuming and expensive and the advanced techniques such as HPLC–MS require highly specialised equipments. Therefore, versatile, cheap, and rapid analyses are desirable and of great interest for its use in routine and generalised analyses.

Infrared spectroscopy has been a very useful tool for rapid evaluation of the procyanidin composition of a given sample [17,18]. Nevertheless, the signals obtained for potential information are complex. Chemometric methods allow the extraction of useful information from the large amount and complex data generated. Among the vast field of chemometrics, the multivariate regression methods have been widely used to provide a better insight into the

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doi:10.1016/j.aca.2009.12.028

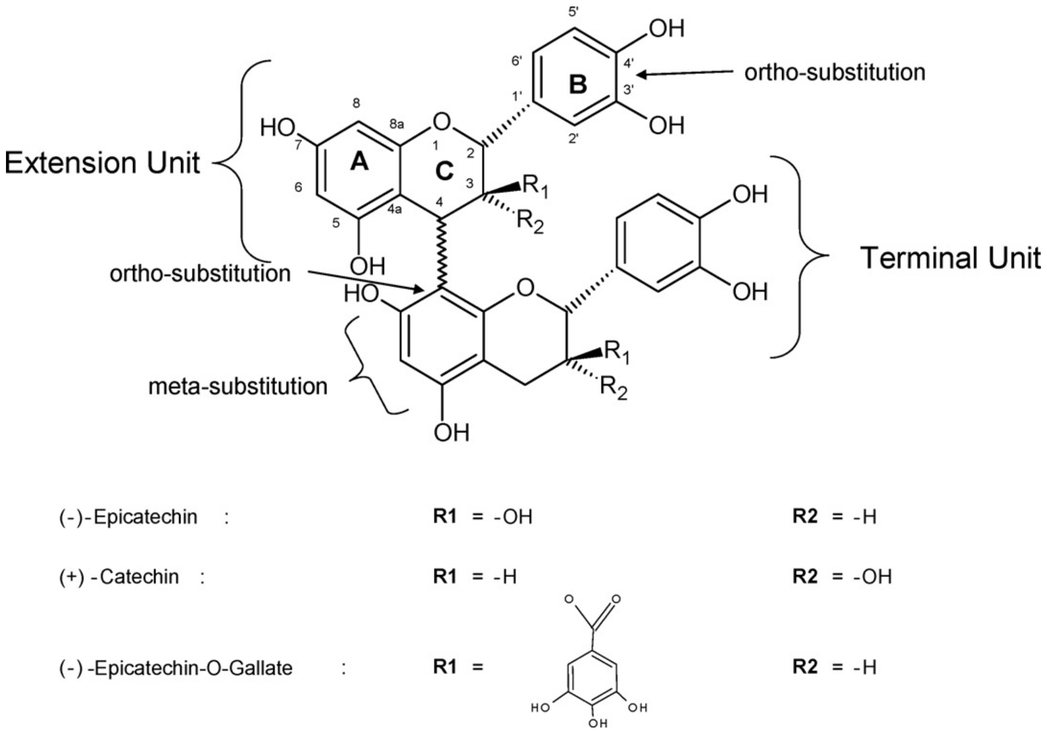

Fig. 1. Structure of procyanidin dimer containing a C4–C8 interflavanic linkage.

systems and to build calibration and prediction models. Partial least squares (PLS) regression is one of the most used models [19–22]. The PLS algorithm is based on a bilinear model, where the information contained in the **X** data matrix is projected over a small number of latent variables known as PLS components. The **Y** data matrix is actively used for estimating the latent variables and ensure that the first components of these are the most relevant for predicting the **Y** dependent variables. The interpretation of the relationships between the **X** and **Y** data is simplified to the relationships between the smaller number of PLS components [23,24].

The method of multivariate regression by PLS has been extensively used in chemometrics, where a wide field of applications has been found. This has been proposed and implemented in the routine analysis of a large number of parameters on wine industry [17,25,26]. Also, it has been used for the identification and characterisation of polysaccharides and proteins and for their quantification [27–29]. The estimation of the procyanidins *DPn* in the range of 2–6 residues by using PLS algorithm was recently attempted in dried red wine samples previously purified by C18-solid phase extraction [30].

In this work, regression models based on the FTIR spectral region between 1800 and 700 cm^{-1} using PLS1 and O-PLS1 were assayed in grape seeds freeze-dried procyanidin extracts. Identification of procyanidins molecular features related to the absorbance characteristics in the mid infrared region were established and correlated with procyanidins *DPn*.

2. Experimental

2.1. Materials

Methanol, ethyl acetate, *n*-hexane, and acetone, from Sigma–Aldrich Co. (St. Louis, MO, USA), were of analytical grade. Water, from Merck (Darmstadt, Germany), acetic acid and acetonitrile, from Sigma–Aldrich Co. (St. Louis, MO, USA), were of chromatographic grade quality. (+)-Catechin, (−)-epicatechin, (−)-epicatechin-*O*-gallate, procyanidin B2, and benzyl mercaptan, were purchased from Fluka Sigma–Aldrich Co. (St. Louis, MO, USA). Other reagents were of analytical grade or higher available purity.

2.2. Plant material

Seeds were obtained from grapes (*Vitis vinifera* L.) of the white variety ‘Chardonnay’ at technological maturity, in Bairrada Appellation, from an experimental vineyard (Estação Vitivinícola da Bairrada, Anadia, Portugal), during transfer of the musts for fermentation. A mixture of red grape varieties ‘Touriga Nacional’, ‘Touriga Francesa’, and ‘Tinta Roriz’, were also obtained from the wine producers of Adega Cooperativa de Pinhel (Pinhel, Portugal). The remaining pulp and skins were separated from grape seeds by decantation and sieving (pore size <2.8 mm diameter). The seeds were then submitted to several wash cycles with water (200 g L^{-1}) under gentle stirring with a magnetic bar at 4 °C for a minimum of 3 days, with two water exchanges a day, until a minimum turbidity was constant, assuring that no remaining adherent tissues were present. The purified seeds were then washed with ethanol, air dried at room temperature, and stored at 4 °C until further analysis.

2.3. Data set preparation and characterisation

2.3.1. Procyanidin crude extracts (PCE)

Seeds were immersed into liquid nitrogen, milled in a domestic coffee mill and sieved (pore size <0.75 mm diameter). The extraction methodology was adapted from Guyot et al. [12], as described by Cardoso et al. [31]. Seed powder was extracted three times with *n*-hexane to remove the lipids. It was then treated three times with methanol containing 5% acetic acid to extract the phenolic compounds. The methanol extracts were filtered through a G3 sintered glass filter, combined, concentrated under vacuum at 40 °C, with several additions of water to assure the complete removal of methanol and acetic acid, frozen, and freeze-dried, to give the methanol procyanidin crude extracts (PCE). The residue resultant from the methanolic treatment was extracted three times with acetone/water solution containing 5% acetic acid, whereas the acetone