

RAPID SCREENING METHOD TO ASSESS TANNIN ANTIOXIDANT ACTIVITY IN FOOD-GRADE BOTANICAL EXTRACT

A. SCHWERTNER PALMA^{a,b}, A. RICCI^c, G.P. PARPINELLO^{*a} and A. VERSARI^a

^aDepartment of Agricultural and Food Sciences, University of Bologna, Piazza Goidanich 60, 47521 Cesena (FC), Italy

^bCAPES foundation, Ministry of Education of Brazil, 70040-020 Brasilia (DF), Brazil

^{*}Corresponding author. giusi.parpinello@unibo.it

ABSTRACT

Fourier transform infrared (FTIR) spectroscopy measurements were used for the prediction of commercial tannins antioxidant capacity, i.e. DPPH, through Partial-least squares (PLS) regression. Plot of the leave-one-out full cross-validated PLS predicted scavenging activity values (DPPH %) with a good correlation ($r= 0.82$), proving FTIR succeeded to rapidly provide information on commercial tannins antioxidant capacity.

Keywords: chemometric, DPPH, FTIR, PLS, tannins

1. INTRODUCTION

Tannins are naturally occurring polyphenols produced by plants via secondary metabolic processes. Their ability to bind proteins, pigments, and complex metallic ions, together with their flavouring effect are the basis for their extensive use as additives in the food industry. Tannins are commercially available in water suspension liquids, which can be stabilized by the addition of (poly)saccharides, or as lyophilized powders, which can be either a single extract or a blend of two or more tannins, which can have one or more degree of purity (VERSARI *et al.* 2013).

Taking into account their nutritional and technological potentials, the main challenge is their analytical characterization, since suppliers seldom label their composition and the degree of purity. Manufacturers provide general information only regarding the source of the product and its use, whereas additional details such as their antioxidant activity – which makes tannins of great interest in the wine industry, due to a diminished addition of sulphur dioxide in this beverage – are often needed.

In this view, there is an ongoing interest to improve the measurement of the tannin antioxidant activity. It is well known that tannins bind proteins therefore the assays based on reaction catalysed by enzymes for generating radicals are inadequate because they use proteins sensible to oxidation to measure the antioxidant activity. These leads to the disability of tannins to be measured with, since there is the possibility that tannins can interact with the radical generator *per se*, or sensor, or even to scavenging the radicals themselves. It is important to notice that these both ways of measurements can be seen as antioxidant activity, but it may not be clear whether the tannins are acting or not (HAGERMAN *et al.*, 1998; RIEDL *et al.*, 2002; MILLER *et al.*, 1993; CAO *et al.*, 1993).

Currently there are several antioxidant assays available: ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) cation radical (ABTS•+) scavenging ability), DPPH (2,2-diphenyl-1-picrylhydrazyl radical, DPPH• scavenging capacity), FRAP (ferric reducing antioxidant power), electrochemistry (HAGERMAN *et al.*, 1998; BOUCHET *et al.*, 1998; MUCCILLI *et al.*, 2017; OSZMIANSKI *et al.*, 2007), each of them presenting both advantages and limitations. For example, the ABTS assay needs the time of incubation to be optimized depending on each and every substrate (WALKER and EVERETTE, 2009). Concerning electrochemical analysis, it is necessary to have trained professionals as an expensive apparatus.

Conversely, the application of Fourier transforms infrared spectroscopy (FTIR) analysis in oenology has had a considerable boost in recent times, due to its fastness, reliability and versatility of use. Analytical devices based on the vibrational spectroscopy method are widespread in analytical laboratories of oenological companies, providing the most important quality parameters and supporting oenologists in different process stages. Vibrational spectroscopy enables to disclose the molecular composition of unknown samples, also including complex matrices, and to highlight interactions involved in the molecular arrangements; this latter is especially useful to determine the occurrence of intermolecular interactions.

Therefore, this study aimed to develop a fast analytical approach suitable for screening antioxidant activity of tannins, which can provide reliable information in the wine and food industry to tailor at best the use of commercial tannins.

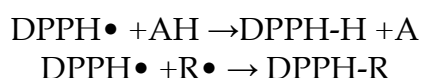
2. MATERIALS AND METHODS

2.1. Samples

Thirty commercial tannins were provided by suppliers as powder and dissolved in model wine (pH 3.6, 12% EtOH) in a concentration of 1 g L⁻¹.

2.2. DPPH scavenging activity

The amount of 'bioactive tannins' was calculated with the method of scavenging the DPPH radical (BRAND-WILLIAMS, 1995), which is based on ability of the sample to act as antioxidant agent within the methanolic solution of the free radical 2, 2 diphenyl-1 picryl-hydrazyl (DPPH), which shows a maximum of absorption at 517 nm. Once the antioxidant is added to the solution, absorption diminishes. The chemical reactions that take place are as follows:



DPPH radical reagent was prepared in methanol (25 mg L⁻¹). Tannin solutions (100 µL) were mixed with 2.9 mL DPPH radical solution (NIXDORF and HERMOSÍN-GUTIÉRREZ, 2010) and after 60 min, absorbance were measured at 517 nm against methanol. The results are given considering the solution of 2.9 mL of radical and 100 µL as control (100%), and to tannins sample it was considered their ability to neutralize the DPPH radical, in relation to control, according to the following equation:

$$\% \text{ Inhibition} = [(\text{Abs}_{\text{DPPH}} - \text{Abs}_{\text{tannin}}) / \text{Abs}_{\text{DPPH}}] \times 100$$

2.3. FTIR Analysis - Spectra acquisition

Fourier-Transform Mid-Infrared (FT-MIR) spectral analysis was performed using a Tensor 27 spectrometer (Bruker Optics) equipped with a horizontal attenuated total reflectance (ATR) zinc selenide (ZnSe) crystal (HATR, PIKE Technologies, Madison, US). A remote-control thermosetting device was used to keep samples (1 mL) at 40 ±1°C for the whole duration of measurements. Spectra, with a spectral resolution of 4 cm⁻¹ and 64 scans averaged for each spectrum, were recorded in duplicates from 4000 to 700 cm⁻¹ for each tannin. The same number of scans was used for background subtraction. Prior to data analysis each spectrum was corrected for the variation in effective path-length using the ATR correction option available in the Spectrum One 5.3.1 software (Perkin-Elmer, Waltham, MA). The spectra were exported in ASCII format to the statistical software for statistical analysis.

2.4. Chemometrics and data analysis

Partial Least Square regression (PLS) (Unscrambler 9.7, Camo, Oslo, Norway) was used to model the relationships between the amount of bioactive tannins in commercial samples and the selected wavelengths of FTIR spectra. Multivariate analyses were performed using full cross-validation, i.e. the leave-one-out model, and with x-variables pre-processed as 'Standard Normal Variate' (SNV).

3. RESULTS AND DISCUSSION

The tannins were coded by manufacturers (same letter), with info on their chemical classification, if available (Table 1).

Table 1. Code, chemical classification and DPPH scavenging activity (%) of commercial tannins.

Code	Chemical classification	DPPH scavenging activity (%) (mean±SD)
A1	Not available	25.7±0.2
A2	Condensed	44.3±0.7
A3	Hydrolysable / condensed	29.1±0.6
A4	Condensed	18.7±0.3
A5	Hydrolysable / condensed	26.4±0.01
A6	Hydrolysable / condensed	49.6±2.0
A7	Hydrolysable	19.3±0.2
A8	Hydrolysable	25.0±0.3
A9	Hydrolysable / condensed	40.8±0.9
A10	Not available	66.9±2.0
A11	Hydrolysable	34.7±1.6
A12	Hydrolysable stabilized with natural polysaccharides	27.8±0.7
A13	Hydrolysable / condensed	41.9±0.2
A14	Hydrolysable	36.0±0.4
B1	Hydrolysable	42.8±0.4
B2	Hydrolysable	59.4±0.5
B3	Condensed	30.7±0.6
B4	Hydrolysable	38.3±0.8
B5	Hydrolysable	37.9±0.01
B6	Hydrolysable	29.6±0.7
C1	Hydrolysable	28.8±0.7
C2	Hydrolysable / condensed	29.8±0.4
C3	Hydrolysable	34.6±0.1
C4	Condensed	24.2±1.2
C5	Condensed	25.4±0.4
C6	Condensed	32.5±0.3
C7	Condensed	22.0±0.8
D1	Hydrolysable	21.3±2.7
D2	Hydrolysable	36.9±0.2
D3	Hydrolysable	29.3±2.6
D4	Hydrolysable	26.2±0.4

DPPH values ranged between 18.7-49.6%, which can be considered a representative interval suitable for PLS modelling of FTIR spectra. Although the entire IR spectra was acquired, after a preliminary attempt using the whole IR signal (4000 to 700 cm⁻¹), a specific region 1750-900 cm⁻¹ – which is well known as ‘fingerprint’ for polyphenolic compounds – was considered for the PLS modelling, using 6 latent variables explaining up to 77% total X-variance, and 61% total Y-variance. The selection of spectral regions was consistent with

info from the literature (JENSEN *et al.* 2008; RICCI *et al.* 2015), which identified two IR regions, 1485-1425 and 1060-995 cm^{-1} , as mostly important for tannin quantification.

The fast-molecular screening provided by MIR spectroscopy showed satisfactory correlation with the effective DPPH scavenging activity of commercial tannins ($r=0.817$; slope 0.82) with the root mean square error of full cross-validation (RMSECV) of 6.6 (Fig. 1). This result is consistent with previous finding (VERSARI *et al.*, 2010), therefore confirmed the reliability of FTIR analysis combined with PLS regression as a fast screening method for antioxidant prediction in foodstuffs.

Moreover, it is also known that FTIR associated with chemometric can be useful for the classification of tannin, and fraud disclosure (GRASEL *et al.*, 2016a; GRASEL and FERRAO, 2016b).

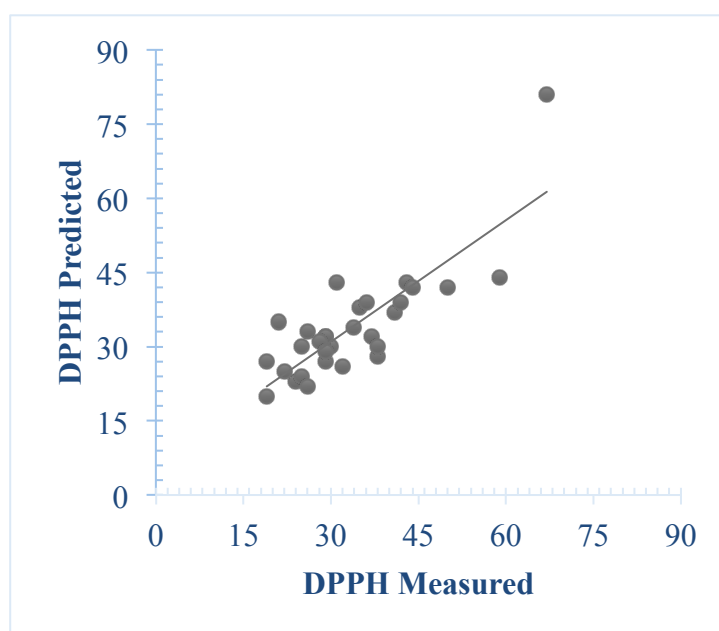


Figure 1. Prediction of antioxidant activity (DPPH) of commercial tannins using FTIR and Full Cross PLS Validation.

4. CONCLUSIONS

The preliminary findings of this short communication suggest that FTIR spectroscopy is suitable as a rapid screening tool to provide information on antioxidant activity of commercial tannins. Further FTIR analysis on a larger number of samples is needed to improve the prediction model at best using an independent set of samples.

ACKNOWLEDGEMENTS

Author Aline Schwertner Palma acknowledge CAPES foundation for the scholarship (BEX 12943/13-4).

REFERENCES

- Bouchet N., Barrier L. and Fauconneau B. 1998. Radical scavenging activity and antioxidant properties of tannins from *Guiera senegalensis* (Combretaceae). *Phytother. Res.* 12:159.
- Brand-Williams W., Cuvelier M.E., Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.* 28:25.
- Cao G., Alessio H.M. and Cutler R.G. 1993. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radic. Biol. Med.* 14:303.
- Grasel F.S., Marcelo M.C.A. and Ferrao M.F. 2016a. A non-destructive, rapid and inexpensive methodology based on digital images for the classification of natural tannin extracts. *RSC Adv.* 6:32358.
- Grasel F.S. and Ferrao M.F. 2016b. A rapid and non-invasive method for the classification of natural tannin extracts by near-infrared spectroscopy and PLS-DA. *Anal. Methods* 8:644.
- Hagerman A.E., Riedl, K.M., Jones G.A., Sovik K.N., Ritchard N.T., Hartzfeld P.W. and Riechel T.L. J. 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Agric. Food Chem.* 46:1887.
- Jensen J. S., Egebo M. and Meyer A.S.J. 2008. Identification of spectral regions for the quantification of red wine tannins with Fourier transform mid-infrared spectroscopy. *Agric. Food Chem.* 56:3493.
- Miller N. J., Rice-Evans C., Davies M.J., Gopinathan V. and Milner A. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.* 84:407.
- Muccilli V., Cardullo N., Spatafora C., Cunsolo V. and Tringali C. 2017. α -Glucosidase inhibition and antioxidant activity of an oenological commercial tannin. Extraction, fractionation and analysis by HPLC/ESI-MS/MS and ¹H NMR. *Food Chem.* 215:50.
- Nixdorf S. L. and Hermosín-Gutiérrez I. 2010. Brazilian red wines made from the hybrid grape cultivar Isabel: phenolic composition and antioxidant capacity. *Anal. Chim. Acta* 659:208.
- Oszmianski J., Wojdylo A., Lamer-Zarawska E. and Swiader K. 2007. Antioxidant tannins from *Rosaceae* plant roots. *Food Chem.* 100:579.
- Ricci, A., Parpinello, G.P., Olejar, K.J., Kilmartin, P.A., and Versari, A. 2015. Attenuated Total Reflection Mid-Infrared (ATR-MIR) Spectroscopy and Chemometrics for the Identification and Classification of Commercial Tannins. *Appl. Spectrosc.*, 69:1243.
- Riedl K.M., Carando S., Alessio H.M., McCarthy M. and Hagerman A.E. 2002. Antioxidant activity of tannins and tannin-protein complexes: Assessment *in vitro* and *in vivo*. Ch. 14. In: "Free Radicals in Food". M.J. Morello, F. Shahidi, C.T. Ho (Ed.), p. 188. ACS Symposium Series, Washington, DC.
- Versari A., Parpinello G.P., Scazzina F. and Del Rio D. 2010. Prediction of total antioxidant capacity of red wine by Fourier transform infrared spectroscopy. *Food Control* 21:786.
- Versari A., Toit W. and Parpinello G.P. 2013. Oenological tannins: a review. *Aust. J. Grape Wine Res.* 19:1.
- Walker R.B. and Everette J.D.J. 2009. Comparative reaction rates of various antioxidants with ABTS radical cation. *J. Agric. Food Chem.* 57:1156.

Paper Received November 8, 2016 Accepted February 26, 2017