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## **Feasibility study on the use of UV-VIS and FT-IR spectroscopies for the classification and quality control of food-grade tannins**

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### **Abstract**

In this study, fifty botanical extracts commonly referred as ‘food-grade tannins’ and currently allowed in foods and beverages as coloring agents, emulsifiers, stabilizers (E181) were analyzed for their botanical origin, content in bioactive compounds and extent of the polymeric fraction. Selected spectroscopy approaches, including UV-visible (UV-VIS) and mid-infrared (MIR), were tested as fast tools for discrimination and classification.

The MIR region of the electromagnetic spectrum enabled the discrimination between condensed and hydrolyzable structures by means of the wavelengths 1800-1500  $\text{cm}^{-1}$  (hydrolyzable) and 1300-900  $\text{cm}^{-1}$  (condensed). A more detailed discrimination between tannins was achieved by mean of UV-VIS spectra and PCA analysis: the range 190-320 nm enabled a clustering of tannins based on their botanical source, whereas the range 190-450 nm discriminated oak blends from other ellagic- and gallotannins. Moreover, the quantitative prediction of the tannin content was achieved by PLS regression of the UV spectral data ( $R^2 > 0.79$ ) using the Adams-Harbertson colorimetric assay as a reference method. Spectroscopic methods provided both qualitative and quantitative compositional information able to disclosure tannins based on their origin, therefore representing a valuable tool for rapid food quality control.

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### **Introduction**

Tannins are naturally occurring bioactive compounds, produced as secondary metabolites by plants. According to their botanical origin, tannins are classified on a structural basis as follow: (i) hydrolysable tannins, including ellagitannins (lactones of ellagic acid) and gallotannins (natural polymers of gallic acid esters); (ii) condensed tannins (procyanidins) composed of flavanol monomeric units,

mainly catechins, and a variable degree of polymerization. Besides their multiple functional properties, a further advantage of tannins is the large availability of their natural sources, and the increasing interest in the recovery of agro-wastes to produce ‘green’ byproducts. Tannins find application in different industry settings, and their use in the food industry is encouraged due to their bioactivity and bioavailability (Versari et al., 2013).

Several commercial formulations are currently used as enological additives, flavoring agents, dietary supplements, soliciting a detailed information on their chemical composition to predict their technological and nutraceutical impacts (Ricci et al., 2016). The development of rapid analytical techniques for effective discrimination between tannins derived from different botanical sources could further enhance and tailor industrial applications of tannins, enabling an effective monitoring along the supply chain.

In this context, UV-VIS and FT-IR spectroscopies, being simple and sensitive techniques, are suitable analytical approaches for quality control. Spectroscopic techniques are fast and versatile, with minimum samples manipulation (Ricci et al., 2015).

The aim of this work is to identify spectral patterns for discrimination and classification of botanical extracts by screening fifty food-grade commercial tannins from different botanical sources. Spectral raw data were processed using statistical multivariate analysis: Principal Component – Clustering Analysis (PCA-CA) and Partial Least Square (PLS) to evaluate the authenticity and the effective content in bioactive tannins in a fast, reliable and non-destructive way.

## Materials and methods

### Commercial extracts

Fifty water-soluble commercial food-grade powder tannins from grape seed and skin, quebracho, green tea, American oak, European oak, chestnut, gallnut, and related blended formulations were analyzed as provided by suppliers (Enologica Vason, Verona, Italy; HTS enologia, Marsala, Italy; Laffort, Bordeaux Cedex, France; AEB Group, Brescia, Italy; Enobiotech S.r.l., Montebello Vicentino, Italy). Samples were analyzed in solid-state by means of FT-IR spectroscopy and dissolved in pure distilled water for UV-VIS analysis.

### ATR-FTIR spectroscopy

ATR-FTIR analysis was carried out using a diamond ATR Smart Orbit™ accessory (Thermo Optec, Waltham, MA USA), equipped with a DGTS detector and a KBr window for measuring in the medium infrared (MIR) region; the incident beam had a 45° geometry. The spectral range selected was 4000–520 cm<sup>-1</sup>; spectra were averaged over 128 scans and the resolution was 4 cm<sup>-1</sup>. A Savitzky-Golay (9 pts)

smoothing filter was used, and spectra were normalized over their maximum absorbance.

### UV-VIS spectroscopy

UV-VIS spectra were collected in the range 190-600 nm using a Cary 60 UV-VIS Spectrophotometer (Agilent Technologies, Santa Clara, CA, US), equipped with a pulsed xenon lamp. A Savitzky-Golay (5 pts) smoothing filter was used, and spectra were normalized over their maximum absorbance.

The Adams-Harbertson colorimetric assay (Harbertson et al., 2002) was used as a reference method to quantify the effective tannin content in the extracts, dissolving tannin powders in pure distilled water (1 g/L of lyophilized powder) prior to analysis.

### Multivariate analysis

Principal Component Analysis (PCA) and Cluster Analysis (CA) (Unscrambler 9.7, Oslo, Norway) were used as unsupervised methods to disclose the hidden structure of botanical sources based on their spectral fingerprints.

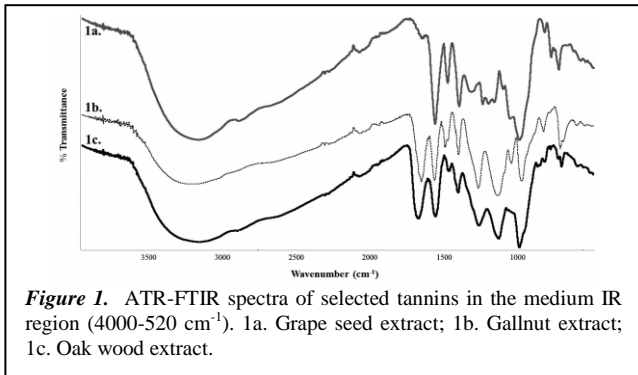
PLS regression (Unscrambler 9.7, Oslo, Norway) was used to model the relationship between the effective amount of tannins in commercial samples and the spectral profiles in selected UV-VIS regions. Multivariate analyses were performed using full-cross validation, i.e. the leave-one-out model, pre-processing x-variables as Standard Normal Variate (SNV).

## Results and Discussion

A detailed investigation of the molecular structure of up to fifty commercial food-grade tannins has been performed using the electronic and vibrational spectroscopies as valuable tools for the characterization of the botanical extracts on a quality basis.

The ATR-FTIR spectra allowed specific band profiles to be identified, arising from specific functional groups and hydroxylation patterns. In more detail, the hydrolyzable tannins showed intense absorption in the carbonyl stretch region, with a typical peak around 1700 cm<sup>-1</sup>. Two major single bands were also related to hydrolyzable structures, being the C-O single bond stretching of the carboxyl group. The main spectral feature for condensed tannins was constituted by the intense 1600 cm<sup>-1</sup> peak, related to aromatic ring breathing vibrations. Besides these specific vibrational modes, the whole 1400-900 cm<sup>-1</sup> was responsible for

samples discrimination, being sensitive to the number and position of hydroxyl substituents (Figure 1). The broad peak around 4000 and 2500  $\text{cm}^{-1}$  contained the stretching vibration for C-H and O-H groups of phenolic compounds, among other (Figure 1).



The multivariate approach applied to spectral data provided a simple PCA classification followed by cluster analysis. The model allowed to discriminate six clusters: among them, procyanidins were easily classified, with a further discrimination between condensed tannins from grape and procyanidins from other botanical sources (data not shown).

Oak wood extracts were classified in a cluster including both American and French *Quercus* species. Two gallnut extracts formed a distinguished cluster, with a large spatial distribution in the score plot area. The remaining clusters are more generally characterized by non-*Quercus* wood extracts (data not shown).

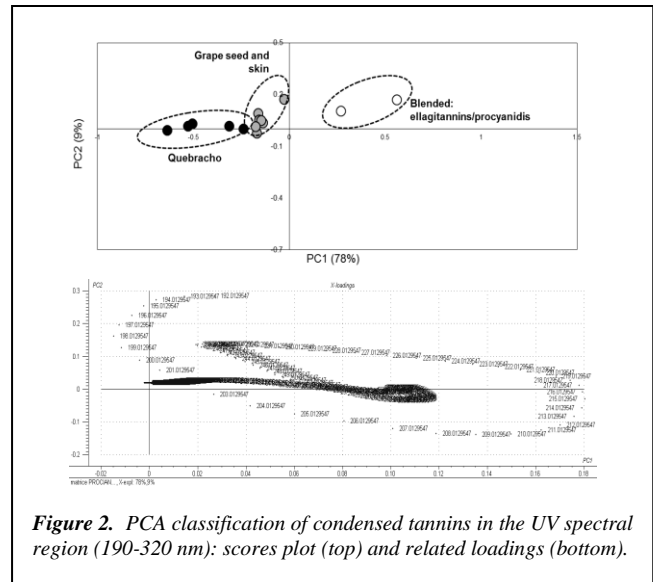
UV-VIS spectra were recorded in water solution: organic solvents were avoided to limit interferences in the far-UV region.)

PCA analysis was performed over selected spectral ranges for a preliminary screening. In the range 190-320 nm it was possible to discriminate the hydrolyzable tannins from condensed. Principal Component 1 (PC1 78%) was mainly responsible for this classification.

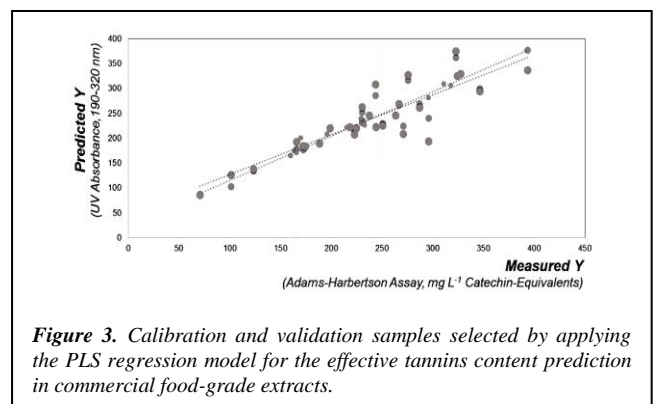
Spectral profiles were further investigated selecting the specific subclasses (condensed, hydrolyzable), and including some blended formulations in both groups of samples. The model enabled to classify procyanidins according to their botanical origin (spectral range 190-320 nm, Figure 2).

When the model has applied to the spectra of hydrolyzable tannins in the range 190-450 nm, PC2 (21%) was responsible for the clustering of American oak, European oak, and chestnut (data not shown).

UV spectral profiles were also used to build a model for the prediction of the effective tannin content in the extracts, which is usually related to the botanical sources, to the purity of the extracts and/or to the



effectiveness of the extraction procedures. Tannins were screened using the Adams-Harbertson colorimetric assay, showing variable degrees of purity; results were then correlated to the UV spectra in the spectral range 190-320 nm using a multivariate regression model ( $R^2 > 0.79$ , Figure 3). The model showed to get weaker at higher tannins concentrations; this might be caused by a lower accuracy of the Adams-Harbertson assay in increasing concentration levels, and this hypothesis needs for further experiments to be investigated. Despite the preliminary results showed potentialities of the forecasting model, a further sampling is required to increase representability and improve robustness.



## Conclusions

UV-VIS and FT-MIR spectroscopies provided both qualitative and quantitative compositional information able to disclose tannins based on their origin, therefore representing a valuable tool for rapid food quality control. The UV region was mainly responsible for tannins classification and showed potentialities for the quantitation of effective polymerized fraction in the extract.

## Acknowledgements

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