INNOVATIVE METHOD FOR THE DIRECT MEASUREMENT OF TANNINS IN RED WINES

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Article extracted from the presentation held during Enoforum Web Conference (23-25 February 2021)

Introduction

The term ‘tannins’ refers to polyphenolic compounds with variable degree of polymerizations, which are biosynthesized by plants as protective secondary metabolites. Red grape is a renowned source of condensed tannins (more specifically referred as ‘proanthocyanidins’), and their balanced occurrence in musts and wines is deemed as a quality index: along with polyphenolic compounds, tannins exhibit enhanced technological properties being involved in wine and musts clarification, protection against oxidation, color stabilization mechanisms, and contributing to the mouthfeel and sensory attributes of wines, among others (Versari et al., 2013). Tannins in musts/wines are both derived from grapes and added as exogenous extracts, the latter in compliance with the current OIV Regulation (Resolutions OIV-OENO 567C-2018; OIV-OENO 613-2019).

Tannins reactivity and efficiency mostly correlate to their effective content in musts and wines. The most recent scientific advances report a key technological role for these compounds; in particular, a proper dosage may contribute to enhance the wine properties and stability over time, reversely, their overdosage may produce unbalanced wine with serious implications in the organoleptic properties and physical-chemical stability (Vignault et al., 2019; Jeremic et al., 2020).

The valorization of tannins in oenology (both endogenous and from exogenous sources) have elicited increasing interest, and in recent years efforts have been made to increase knowledge about reaction mechanisms underlying their technological properties; nevertheless, there is a need to fill the gap for their fast and effective monitoring along the oenological supply chain. The quantification of tannins along the production process provides guidance to the oenologist in decision support strategies, enhancing wine quality and shelf-life durability. On the other hand, information of effective polymerized fraction of polyphenols requires fractionation processes and cannot prescind from laboratory analyses.

In this work, an innovative analytical method has been proposed for fast and reliable in-line analysis of tannins in wines; the proposed method advantages from the selective reactivity of tannins in a mixture containing proteinaceous matter (i.e. gelatin), under pH 3.5, resulting in the formation of white cloudiness. The modification of the optical properties of the wine/gelatin mixture is selectively associated to the tannins content, and it is detected with high accuracy by an innovative optical prototype, namely a Spectral-Sensitive Pulsed Photometer (SSPP) which has been previously described in the literature (Ragni et al., 2016). The SSPP operate with a wavelength-sensitive pulsed electromagnetic source, with maximum emission around 850 nm, and with a detector whose maximum sensitivity is at around 890 nm, eliminating potential interferences from wine color. The extent of turbidity provoked by the procyanidin-protein reaction is measured combining different intensity and spectral emission of a light source (tungsten lamp) with the photodiode wavelength sensitivity. Preliminary results have advanced the technology to the monitoring of tannins in commercial red wine, obtaining non-linear correlations with tannin dosage obtained by the Adams-Harbertson colorimetric assay (Ricci et al., 2020). Main advantages involve the high selectivity of the reaction responsible for turbidity formation, and simple, cost-effective technology of the optical device; results have boosted the improvement of the readiness level of the technology for its application under relevant oenological conditions and to support the enologist in decisional strategies.

Materials and Methods

Several experiments have been performed using both standard solutions of commercial tannins dissolved in model wine solution and commercial red wines, belonging to different varieties and from different geographical origin. In a previous experiment, which is discussed herein, the wines assayed showed a broad selection of tannins (ranging 10 to 2700 mg/L) as previously determined using the Adams-Harbertson colorimetric assay (Harbertson et al., 2002). A saturated solution of gelatin has been prepared by dissolving the lyophilized powder in a model wine solution (12% ethanol, pH 3.5). Wines have been directly mixed with the gelatin solution (1:1 v/v) in a glass cuvette suitable for optical determinations, and instantly inserted in the measuring chamber of the optical device for direct measurement.

The prototype optical device (Figure 1) consists of a reaction chamber (glass cuvette with 1 cm optical path), an optical system which has been previously described in the literature (Ragni et al., 2016), and a software interface to measure and process the optical signal.
The optical source (tungsten lamp) operated in a continuous wavelength range, with a maximum of emission around 850 nm. Every determination was averaged over 5 subsequent measurements, confirming the stability of the optical response for the whole measurement cycle. Every sample was measured against a blank (red wine/model wine solution, 1:1 v/v), to confirm that the wine color did not interfere with the optical determination. Measures were assessed in duplicates, showing satisfactory inter-day and intra-day reproducibility.

Results

The results of the optical measurements were processed to investigate the specific portion of the signal vs time curve which showed the higher correlation with the tannins content of wines. Best fitting was obtained using a non-linear correlation function; Figure 2 reports the correlation curve for a selection of the red wines object of this study.

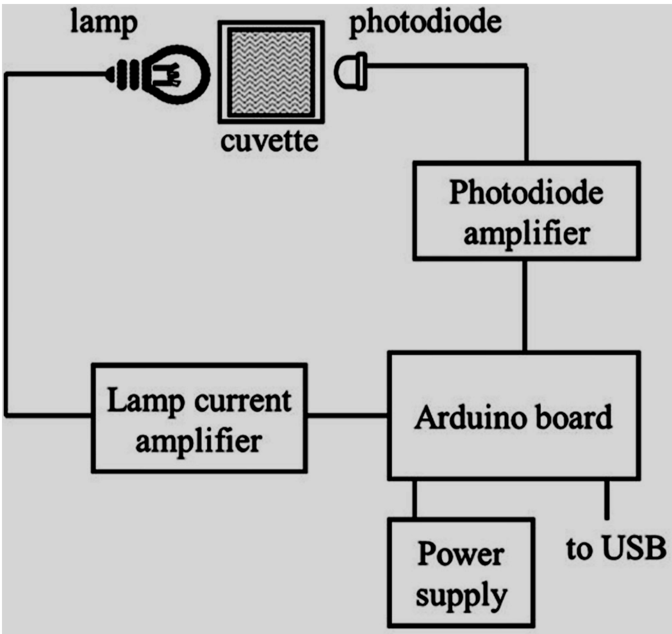

Figure 1. *Schematic representation of the optical device described in Ragni et al., 2016 as reported in Ricci et al., 2020.*

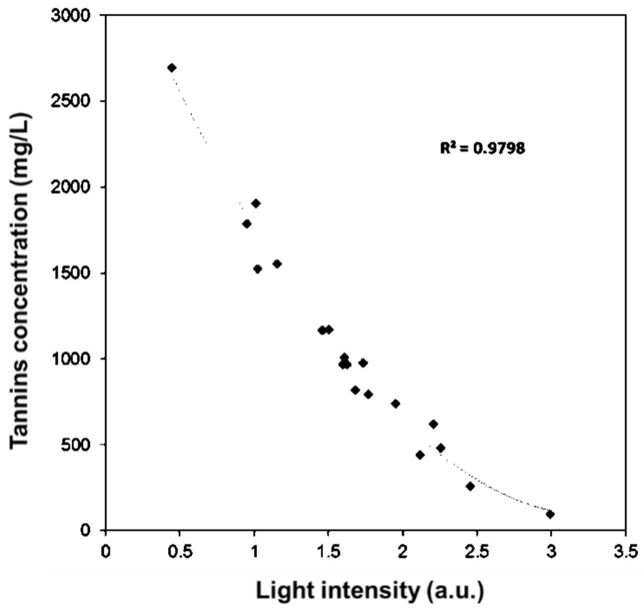

Figure 2. *Non-linear correlation between the tannin content in wines and the peak light intensity as measured following the mixture of wine with gelatin solution and development of turbidity.*

The development of the method and the assessment of optimal conditions for the measurements were supported by previous studies from the literature. It has been previously observed that the efficiency of complex formation is highly variable, depending on the protein structure and several physico-chemical factors (Hagerman, 2012; Canon et al., 2011). The protein source used in this experiment, gelatin, is industrially obtained from the elaboration of animal tissues, resulting in a rich proline ad hydroxyproline – like proteins mixture. These proteins exhibit high reactivity against tannins under controlled pH and polarity conditions; results from our experiment corroborates previous studies after Calderon et al. (1968), showing higher interaction under ethanol 12% and pH 3.5 condition, with a weaker complexation capacity when increasing the pH value up to 7.

The wine / gelatin solution volume ratio (v/v) also influenced the extent and the optical properties of turbidity in solution, as previously reported in the literature (Le Bourvellec and Renard, 2012); in more detail, a low tannin/protein ratio (observed in wines with low tannin content) encourage the interlinking between small and soluble complexes, with progressive clusters growing and precipitation. When the tannins/protein ratio was increased, the protein rapidly interact with tannin, through a surface mechanism of absorption, with formation of large aggregates and precipitation. In the present experiment the concentration of the gelatin and the relative volume ratio of the reagents (wine and gelatin solution) were kept constant, to appreciate the different kinetics of the turbidity onset during the optical measurement and correlate them with the effective tannin content in wines.

Conclusion

In this work an innovative method for the direct measurement of tannins in red wines was presented in the context of its development, validation, and possible technology implementations.
The method is geared toward the wine industry; the original idea and future developments aims at making this easy-to-use and cost-effective approach an efficient tool to support the work of the oenologist, giving the possibility to monitor a key technological parameter (tannin content) along the wine production chain.
The analytical method has been patented at the National (Italian) level (Ricci et al., 2021), and it is currently Patent pending at the International level (Ricci et al., 2020); future perspectives involve its exploitation in the oenological industry and the implementation of the technology readiness level.
The study is currently supported by ‘Proof of Concept’ development programs funded by the University of Bologna and the Ministry of Economic Development (MISE), Italy.

Summary

The present work introduces a fast, rapid, and reliable optical method to quantify the tannin content in red wines. Wine samples are directly mixed with a protein solution (gelatin) under controlled pH conditions, resulting in a modification of the optical properties of the mixture. The extent of turbidity arising from the tannin-protein interaction is directly measured with an optical device operating with a wavelength-sensitive pulsed electromagnetic source. Preliminary measures on a selection of commercial red wines showed a high predictive capability in tannins quantitation. The instrumental signal generated by the sample turbidity was modelled as a function of peak intensity, amplitude, and curvature, obtaining a non-linear correlation compared to a colorimetric tannin assay as reference method.
Results confirmed the suitability of the method for direct measurement of tannins, overcoming laborious lab protocols; further advantages consist of its affordability and the limited cost of the prototype used for the optical measurements. All these considered, the method can be easily exploited for quality control in the oenological industry, supporting strategies to produce quality wines.

Keywords: Tannin-gelatin reactivity, turbidity, spectral pulsed photometry, tannins quantitation, red wine quality

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