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Phenolic Characterization of Commercial Enological Tannins

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Abstract Enological tannins are widely used in winemaking process to improve different characteristics of wines. A wide spectrum of enological tannins is now available on the market; however, the tannins' chemical nature and botanical origin are not always clearly defined in the commercial products. The aim of this work was the chemical characterization of ten commercial plant-derived tanning agents of enological use. Enological tannins were analyzed by spectrophotometry (total phenols, total tannins and gelatin index) and High Performance Liquid Chromatography (HPLC-DAD) (low molecular weight phenolic compounds). In general it was possible observe important differences in the concentration of total phenols, total tannins and gelatin index values among the commercial products studied. By using HPLC-DAD it was possible classified different types of tannins (mainly hydrolyzable (gallotannins and ellagitannins), condensed or proanthocyanidic tannins and blends of these groups). Clear differences were evident between the study results and the information on the type of tannin indicated on some of the commercial tannin labels. These discrepancies could lead to technological problems in the winery industry because of the different aims that guide the use of different types of these enological products.

Keywords Commercial tannins • enological tannins • grapes tannins • oak wood tannins

Abbreviations

HPLC-DAD	High Performance Liquid Chromatography
GAE	Gallic acid equivalents

ET Enological tannins

Introduction

Vegetable tannins are natural polyphenols ubiquitously distributed in plants, frequently occurring in a variety of foods such as vegetables, fruits, seeds, and plant-derived beverages [1]. Proanthocyanidinic or condensed tannins are naturally found in red wines, but another group of tannins, known as hydrolyzable tannins (gallotannin and ellagitannin), can also be present depending on the conditions employed during winemaking and ageing [2]. The traditional source of hydrolyzable tannins is the oak barrels where the wine is kept during the ageing process. Recently, however, a new source of hydrolyzable tannins has emerged, via the direct addition of enological tannins (ETs) or oak chips to the wine [3]. The use of ETs in winemaking is a longstanding technological practice, and these tannins are usually natural substances of plant origin, obtained from several botanical species. These products are used to ensure wine palate balance and complexity. In addition, the use of enological tannins as fining agents for white wines has been authorized by the International Organization of Vine and Wine (OIV), due to the their ability to precipitate proteins. However, enological tannins also serve other applications. For example, they can be used to inhibit lacasse in Botrytis-infected grapes, and in the particular case of proanthocyanidinic tannins, they can contribute to stabilize the color of red wine [4]. For these reasons, grape ETs are being used more frequently; consequently, their market share is increasing significantly every year, even though they are more expensive than other conventional ETs (such as ellagitannins, gallotannins or Quebracho tannins). Sources for commercial tannins, from high to low prices, are arranged in the following order: skins > seeds > stems ≥

Quebracho tannins ≥ other vegetal origins [5]. A wide spectrum of enological tannins is now available on the market, classified mainly according to the enological properties. However, the tannins' chemical nature is not always clearly defined, and it is not always possible to know their botanical origin [2]. From an economical and technological point of view, it is important to know the differences among commercial tannins and to verify the information presented by suppliers. For that reason, the aim of this work was the chemical characterization of ten commercial tannins sold for enological use.

Materials and methods

Materials

Ten enological commercial tannins were purchased from the Chilean market from different companies that distribute enological products. Standards for gallic acid (G-7384), protocatechuic acid (P-5630), protocatechuic aldehyde (E-24859), quercetin (Q-0125), miricetin (M-6760), kaempherol (420325), (+)-catechin (C-1251), (-)-epicatechin (E-1753), and (-)-epicatechin-gallate (E-3893) were purchased from Sigma Chemical Company (Saint Louis, Missouri, USA). Cyanidin chloride was purchased from Extrasynthese (Genay, France). HPLC-grade acetic acid and acetonitrile were purchased from Merck (Darmstadt, Germany). Other solvents and reactive pro-analysis degrees were purchased from Oxiquim-Chile. The chromatographic system had a photodiode-array detector model G1315B, pump model Quat G1311A, autosampler model ALS G1329A (Agilent Technologies 1200), and Reversed-phase Nova Pack C_{18} (4 µm, 3.9 mm ID x 300 mm) (Waters Corporation, Milford, Massachusetts, USA). Absorbance measurements were conducted with an UNICAM UV–Vis (model Helios-Gamma 2000) spectrophotometer.

Preparation of tannin solutions

The commercial tannins (3 g/L) were dissolved in a hydroalcoholic solution (10% v/v of ethanol and 0.5% w/v of tartaric acid) at 20ºC for 20 min of agitation.

Spectrophotometric characterization of enological tannins

Total phenols were determined by a direct readout of sample absorbance at 280 nm [6] and expressed as gallic acid equivalents (GAE). Quantification was based on the standard curve of 50, 100, 150, 200, 250, 300, 350, 400, and 500 mg/L of gallic acid prepared at the same time and conditions. Tannin concentration was measured according to the method of Ribereau-Gayon and Stonestreet [7]. Briefly, two tubes with 4 ml of previously diluted (1:50) sample, 2 ml of distilled water, and 6 ml of HCl (12 N) were prepared and hermetically sealed. One tube was heated to 100°C in a water bath, and the other was maintained at room temperature. After 30 min, 1 ml of ethanol (95%) was added to both tubes. After stirring, absorbance at 550 nm was measured. Results were expressed as mg cyanidin equivalent/g (cyanidin chloride was used as standard to prepare the calibration curve). Although this is a specific method to measure proanthocyanidinic tannins, this analysis was applied to all the samples in order to determine if products with hydrolyzable tannins (gallotannins and ellagitannins) contain proanthocyanidinic tannins too. The gelatin index of the different tannin solutions was measured using the methodology described by Glories [6]. Briefly, either 5 ml of distilled water or 5 ml of gelatin solution (70 g/L) was added to two Erlenmeyer flasks containing 50 ml of tannin solution. After three days, the samples were centrifuged at 11700 g for 10 min (Sorval RC5C). The supernatants were assayed to determine the tannin concentration [7], and the results were expressed as astringency intensity and as a percentage. Astringency intensity was calculated as the difference between the total solution tannin concentration and the concentration after gelatin precipitation. The percentage was calculated by relating this astringency intensity to the total tannin concentration [6].

Characterization of enological tannins by HPLC–DAD

A 50-ml solution of each enological tannin was extracted first with ethyl ether (3 x 20 ml) and then with ethyl acetate (3 x 20 ml). The total extract was evaporated until dryness at 30ºC. The residue was collected in 2 ml of methanol/H_2O (50:50; v/v), filtered with a 0.45-µm membrane, and 20 µL was injected into the HPLC. Two mobile phases were employed for elution: A [water-acetic acid (98:2), v/v)] and B [water-acetonitrile-acetic acid (78:20:2, v/v/v)]. The gradient profile was 0-55 min, 100-20% A; 55-57 min, 20-10% A; 57-90 min, 10-0% A. Detection was performed by scanning from 210 nm to 600 nm with an acquisition speed of 1 sec [8, 9]. Identification of specific compounds was carried out by comparing their spectra to standard spectra. Gallotannins and proanthocyanidins which have no available standards were identified by comparison of their spectra with those previously reported [10, 11]. Quantitative determinations were accomplished using the external standard method with commercial standards. Gallotannins were quantified with the gallic acid curve and proanthocyanidins with the curve of the (+)-catechin. The calibration curves were calculated by injection of different volumes of standard solutions under the same conditions used for the samples analyzed over the range of concentrations observed. All the phenolic composition analyses were performed in triplicate.

Statistical analysis

Minitab Release software version 13.32 and Tukey's t-test were applied to compare the averages of properties with a 95% confidence interval.

Results

Supplier information

Table 1 shows the technical information presented in labels and catalogs for the ten enological tannins purchased from the Chilean market (chemical composition, botanical origin, and commercial value). Taking into consideration the information provided by the suppliers with regards to the chemical composition of the enological tannins, the products were classified into three groups: "100% proanthocyanidinic" (products T_1 and T_2), "100% hydrolyzable" (products T_3, T_4, and T_5), and "mixture of proanthocyanidinic and hydrolyzables" (products T_6, T_7, T_8, T_9, and T_{10}). The ten enological tannins encompassed a diverse array of botanical origins, with three tannins originating exclusively from oak wood, two from grapes/oak wood, and the other five from grape pomace, vegetable, oak gall-nut, Quebracho wood, and vegetable/oak mixes. The price for the ten enological tannins also varied widely, with the differences in cost up to 695%. Products T_1, T_3, and T_9 had the highest prices, fluctuating between US$ 1375.5 and US$ 119.5 per kilo. The price was not related with the botanical origin of the commercial products.

Spectrophotometric characterization

Table 2 shows the content of total phenols, total tannins, and gelatin index of extracts from the products studied. Total phenol concentration ranged from 260.1 to 1036.8 mg GAE/g of enological tannin, with an average value of 437 mg GAE/g. Within this range, product T_6

had the lowest concentration and product T_5 the highest concentration of total phenols. With regard to the total tannin content, products T_1, T_2, T_3, T_5, T_7, and T_{10} had concentrations up to 14.1 mg cyanidin equivalent/g, and products T_4, T_6, and T_9 had an average of 23 mg cyanidin equivalent/g. Product T_8 had the highest concentration of total tannins, with more than 72.8 mg cyanidin equivalent/g. For the gelatin index values, products T_3 and T_{10} had the lowest percentage (under 9%), whereas products T_4, T_5, T_7, and T_9 had over 48%.

Identification and quantification of phenolic compounds in extracts of enological tannins for HPLC-DAD

Figures 1, 2, 3, and 4 show four representative chromatograms of extracts from enological tannins of different botanical origins (grape pomace, vegetable, oak wood, and oak gall-nut). Each UV spectrum of peaks in the chromatograms was compared with the UV spectral information of standard compounds and also with previously published work from other authors [11, 12]. The compounds identified in the extracts of enological tannins were: gallic acid, several ellagitannins, protocatechuic acid, flavonol glycosides, protocatechuic aldehyde, several gallotannins, vanillic acid, ellagic acid and proanthocyanidins (Figure 5). After identifying the peaks in each chromatogram of the tannin extracts, the amounts of non-flavonoid and flavonoid phenolic compounds in the samples were quantified. Among the non-flavonoid phenolic compounds (Table 3), gallic acid was the only one present in all the extracts. Gallic acid was notable in products T_4, T_7, and T_8, each of which had statistically significant differences in comparison to other extracts. Likewise, ellagic acid was present in 6 out of the 10 extracts, with product T_3 having the highest concentration. The presence of gallotannins was clearly higher in product T_4, as its concentration was almost 15 times higher than the next highest sample, product T_8. Finally, ellagitannins were detectable in 5 out of the 10 products, with product T_3 being the most enriched in this substance. In case of the flavonoid compounds (Table 4), two families of this group of substances were detected in the extracts of enological tannins: flavonol glycosides and proanthocyanidins. These compounds appeared in all the extracts except for product T_4 (ellagitannin). Flavonol concentration ranged between 2.8 to 9.9 mg/g, with product T_6 having the highest values and significantly higher concentrations compared to the other nine products. In contrast, the proanthocyanidins were the most abundant flavonoids found in the samples studied, with concentrations between 9.8 and 146.8 mg/g. For this substance, products T_6 and T_{10} had the highest concentrations, with statistically significant differences relative to the other eight extracts. The extracts with the lowest concentration of proanthocyanidins were products T_3 and T_8, with concentrations of 9.8 and 16.1 mg/g of enological tannin, respectively.

Discussion

Spectrophotometric characterization

Commercial enological tannins are used in the wine industry for a multitude of reasons: to stabilize the color of red wines, to ensure palate balance and complexity in wines, to inhibit lacasse in botrytis-infected fruit, and to serve as fining agents to reduce protein concentrations [2, 4, 5, 13, 14]. Importantly, the nature of the tannin (hydrolyzable or proanthocyanidin) determines its the effects on the wine [3, 15]. Other researchers have demonstrated that the average concentration of total phenols in skins and seeds of grapes ranges between 5-15 and 10-30 mg GAE/g, respectively [9, 15, 16, 17]. However, in this study, the lowest concentration of total phenols was found in product T_6, with 260.1 mg GAE/g of enological tannin, a value that is already much higher than what is naturally found in grapes. Similarly, 80% of the enological tannins studied averaged 368.3 mg GAE/g, and product T_5 had an exceptionally high concentration, 1036.8 mg GAE/g. In the case of total tannins, the skins and seeds of grapes have an average concentration between 5-20 mg and 10-60 mg cyanidin equivalent/g, respectively [9, 18]. In this study, the concentration of total tannins ranged widely among the ten samples studied, in some cases showing a nearly 70-fold difference, as seen for products T_3 and T_8 (1.2 and 72.8 mg cyanidin equivalent/g, respectively). Generally, however, with the exception of product T_8, 70% of the enological tannins used in this study had a concentration over 15 mg cyanidin equivalent/g of enological tannin, which is comparable to the ranges found in grape seeds and skins. There was no relationship between the content of total phenols and content of total tannins, since a high content of total tannins did not necessarily indicate a high concentration of total phenols. For example, product T_6 had the second-highest amount of total tannins but a lower concentration of total phenols when compared to the other samples, and product T_5 had a high content of total phenols but a total tannin concentration below the average. The products T_7 and T_3 also had lower values for total tannins but had values of total phenols closer to average. Although are tree products that according with the providers information are 100% hydrolyzable tannins (products T_3, T_4 and T_5), in two of them were found proanthocyanidins (products T_3 and T_5). These results are in agreement with those reported by Sarneckis et al. [19], whom studying the content of total condensed tannins in enological tannins from oak wood and three commercial gallotannins, found higher concentrations of condensed tannins in these products, inclusive compare with those observed in grape seed extracts. The gelatin index is still one of the most widely used analytical methods for estimating astringency in red wine [20]. The higher the index (over 50%), the more astringent or "rougher" the wine will seem [6]. In 3 out of the 10 products, the value of the gelatin index was over 50%, and the others presented very low percentage values (for example, products T_3 and T_{10} had less than 9%). Tannins that have a higher value for the gelatin index generally also have a higher affinity for proteins. It is important to take this characteristic into account prior to application, as it may lead to undesirable side effects in the final product, although normally the standard industry practice in enological tannin use consider the preparation of a range of tannin additions to wine on a small scale and taste these prior to making large scale additions.

HPLC-DAD characterization

Tables 3 and 4 summarize the great variety of phenolic compounds present in the extracts of enological tannins. The variation in the phenolic composition among the samples studied is related to the raw materials used in the extraction of these enological tannins.

Grape enological tannin

The grapes of *Vitis vinifera* consist mainly of compounds from both the non-flavonoids (benzoic and hydroxycinnamics acids) and flavonoids (anthocyanins, flavanols, and flovonols) families [9, 21]. During the winemaking process, these polyphenols are partially extracted into the must. In the case of red wines, the crushed grapes are kept in contact with the juice during fermentation for several days in order to enrich the juice with these compounds [22]. However, the extraction is far from complete – the estimated yield for anthocyanins is 30–40% [9] and, consequently, the skins and seeds, which represent 50% of the remaining weight of solid waste resulting from the pressed grapes (otherwise known as the grape pomace), constitute a very abundant source of these phenolic compounds (proanthocyanidinic tannin) [22]. In agreement with the information given by the product T_1 supplier, the origin of this product was from the solid residues of the grapes (seeds and skins) after fermentation (grape pomace). In agreement with the HPLC-DAD analysis, product T_1 had a high concentration of proanthocyanidins and a void concentration of gallotannins and ellagitannins. However, it is important to note that this product also had low concentrations of ellagic acid, which implies that oak wood (e.g., chips or slabs) was used in the elaboration process of the wine when the tannin was being extracted from the pomace to produce this product, resulting in the presence of this compound. This analysis confirmed the origin of this sample.

Oak wood enological tannin

Oak wood is often used during the aging of wine in order to enrich it with substances that will take part in the evolution of its flavor. The phenolic compounds found in oak wood are phenolic acids (gallic and ellagic), gallotannins, ellagitannins, and some flavanoles and cumarins [23-26]. The suppliers indicated that the active ingredients were 100% of hydrolyzable tannins from oak wood for product T_3, and a mixture of oak wood hydrolyzable tannins and proanthocyanidinic tannins for products T_6 and T_7. However, in the case of product T_3, there is no agreement between the commercial information and the results obtained, because it was possible observe proanthocyanidinic tannins in the samples analyzed. In contrast, the supplier information for the composition of products T6 and T7 was confirmed by HPLC-DAD analysis. Lastly, products T8 and T9 were characterized by the supplier as a mixture of proanthocyanidinic and hydrolyzable tannins. Their origins from grape/oak were also confirmed by HPLC-DAD analysis.

Vegetable enological tannin

Vegetable tannins is a very ambiguous historical term that refers to compounds used in the tanning of hides. However, this term is used nowadays for different enological tannins providers to refer to their products. For several authors, vegetable tannins are natural compounds distributed ubiquitously in plants and are present in a great variety of foods, such as vegetables, fruits, seeds, and plant-derived beverages. Without a doubt, the term "vegetable" tannin is a concept that encompasses a wide range of origins, which may in turn include a large selection of compounds including proanthocyanidinic and hydrolyzable tannins [13, 27, 28]. Tannin T_2 is described by the supplier as consisting of 100% proanthocyanidinic tannin. These data were completely validated by HPLC-DAD analysis, which showed that this product has the third highest concentration of proanthocyanidins among the samples tested. In contrast, the origin of product T_{10} is defined by the supplier as vegetable/oak, composed of 70% proanthocyanidinic tannins and 30% hydrolyzable tannins. However, as shown in Table 3, hydrolyzable tannins and related compounds only comprise a low percentage of the total content. In fact, it is worthwhile to note that product T_{10} actually has the second-highest value of proanthocyanidins among the samples studied.

Quebracho enological tannin

Two species of Quebracho tree, *Schinopsis balansae* and *Schinopsis lorentzii*, are grown in South America, particularly in northern Argentina and eastern Paraguay. These species are of interest given the high concentration of phenolics that accumulate in their wood [29]. Quebracho phenolics have been classified and determined to be rich in gallic acid or catechin derivatives, and this group of tannins is an excellent source of proanthocyanidinic tannins (profisitinidins) [29] and hydrolyzable compounds (gallic, protocatechuic, and caffeic acids) [30]. In this study, product T_5 (originating from Quebracho tree wood) had only 3.5 mg of gallic acid/g, a concentration that was below the average (4.1 mg/g) observed among the samples studied. This product also had a proanthocyanidin concentration of 56.9 mg/g that also correlated with the large amount of profisitinidins found in this type of tannin, as shown by previous work [5]. For product T_4, originating from gall-nut oak, the supplier states in the commercial catalog that it consists of 100% hydrolyzable tannins. This tannin is unique among all the commercial tannins studied in this work because the supplier information was absolutely consistent with the results obtained by HPLC-DAD analysis.

Conclusions

In this study, ten enological tannins were characterized by classification into three groups: enological products composed of proanthocyanidins, hydrolyzable, and the mixture of both types of tannins. Within each group, tannin composition varied greatly, mainly defined by the botanical origin of each commercial product. However, in some cases, information delivered by the suppliers with regard to the botanical origin and the chemical composition of these products proved inconsistent as compared to the analysis carried out in this work.

The differences found between the supplier's technical information and the real chemical composition of these commercial tannins must be kept in mind. Since these products are used for different technological aims (e.g., color stabilization, to ensure body, etc.), different classes of tannins (proanthocyanidin and hydrolyzable) are not necessarily the same and may have differing effects on the wine. It would be better if tannin manufacturers gave more accurate information about the origin and chemical composition of their products, in order for the user to select and use the proper tannin with the correct chemical composition to fulfill the desired function, and at the most reasonable commercial price.

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References

1. Haslam E (2007) Phytochem 68: 2713-272

2. Luz-Sanz M, Martínez-Castro I, Moreno-Arribas M (2008) Food Chem 111: 778-783

3. Zamora F (2003) Elaboración y crianza del vino tinto: aspectos científicos y prácticos. Madrid Vicente, España

4. Obradovic D, Schulz M, Oatey M (2005) Aust New Zealand Grapegrow Winemaker 493:52-54.

5. Vivas N, Nonier MF, Vivas de Gaulejac N, Absalon C, Bertrand A, Mirabel M (2004) Anal Chim Acta 513: 247-256.

6. Glories Y (1984) Connaiss Vigne Vin 18: 253-271

7. Ribereau-Gayon P, Stonestreet E (1966) Chem anal 48: 188-192

8. Peña-Neira A, Hernández T, García-Vallejo C, Estrella-Suárez JA (2000) Eur Food Res Technol 210: 445-448

9. Peña–Neira A, Dueñas M, Duarte A, Hernández T, Estrella I, Loyola E (2004) Vitis 2004. 43: 51-57.

10. Cadahía E, Conde E, Fernández de Simon B, García-Vallejo MC (1997) Int J Biol Chem Phys Technol Wood 51, 2: 125-129

11. Santos-Buelga G, García-Viguera C, Tomás-Barberan A (2003) On-line identification of flavonoids by hplc coupled to diode array detection. In: methods in polyphenol analysis. Cambridge: Royal Society of Canada

12. Cadahía EL, Muñoz B, Fernández de Simón, García-Vallejo M (2001) J Agric Food Chem 49: 1790 -1798.

13. Zywicki B, Reemtsma T, Jekel M (2002) J Chromatogr 970: 191-200

14. Bautista-Ortín AB, Fernández-Fernández JI, López-Roca JM, Gómez-Plaza E (2007) J Food Compos Anal 20: 546-552

15. Canals R, Llaudy M, Valls J, Canals J, Zamora F (2005) J Agric Food Chem 53: 4019-4025

16. Pérez-Magariño S, González-San José ML (2006) Food Chem 96, 197–208.

17. Yilmaza Y, Romeo T (2006) J Food Compos Anal 19: 41–48.

18. Canals R, Llaudy M, Valls J, Canals J, Zamora F (2005) J Agric Food Chem 53: 4019-4025.

19. Sarneckis CJ, Dambergs RG, Jones P, Mercurio M, Herderich MJ, Smith P (2006) Aust J Grape Wine Res 12:39-49.

20. Llaudy M, Canals R, Canals J, Rozes N, Arola L, Zamora F (2004) J Agric Food Chem 52: 742-746

21. Kennedy J, Jones GP (2001) J Agric Food Chem 49: 1740 -1746

22. Luque-Rodríguez JM, Luque de Castro MD, Pérez-Juan P (2007) Bioresour Technol 98: 2705–2713.

23. Matejícek O, Mikes O, Klejdus B, Sterbová D, Kubán V (2005) Food Chem 90: 791-800

24. Cadahía E, Muñoz L, Fernandez de Simón B, García-Vallejo M (2001) J Agric Food Chem 49: 1790 -1798

25. De Simon BF, Cadahía E, Conde E, García-Vallejo MC (1996) J Agric Food Chem 44: 1507–1511

26. Chatonnet P, Boidron JN, Dubourdieu D, Pons M (1994) J Int Sci Vigne Vin 28: 337-357

27. Cadahía E, Conde E, García Vallejo M, Fernández de Simón B (1996) Chromatogr 42: 95-100

28. Cadahía E, Conde E, García Vallejo M, Fernández de Simón B (1999) Phytochem Anal 8: 78-83

29. Rautio P, Bergvall UA, Karonen M, Salminen J (2006) Biochem Syst Ecolog 35: 257-262

29. Lopez-Fluza J, Omil F, Mendez R (2003) Water Sci Technol 48: 157–163

30. Marín-Martinez R, Veloz-García R, Veloz-Rodríguez R, Guzmán-Maldonado S, Loarca-Pina G, Cardador-Martinez A, Guevara-Olvera L, Miranda-López R, Torres-Pacheco I, Pérez Pérez C, Herrera-Hernández G, Villaseñor-Ortega F, González-Chavira M, Guevara-Gonzalez R (2008) Biosour Technol 100:434-439

TABLES

Table 1. Technical information for the ten enological tannins as given by the commercial suppliers.

Tannin	US$/kg	Botanic Origin	Chemical composition given by supplier
T1	1375.5	Grapes Pomace	100% condensed
T2	4.7	Vegetable	100% condensed
T3	222.7	Oak Wood	100% hydrolysable
T4	35.2	Gall nut Oak Wood	100% hydrolysable
T5	1.9	Quebracho Wood	100% hydrolysable
T6	33.6	Oak Wood	Mix condensed and hydrolysable (ellagic)
T7	18.9	Oak Wood	Mix condensed and hydrolysable (ellagic)
T8	27.8	Grapes/Oak Wood	Mix condensed and hydrolysable (ellagic and gallic)
T9	119.5	Grapes/Oak Wood	Mix 50% condensed and 50% hydrolysable (ellagic)
T10	39.0	Vegetable/Oak Wood	Mix 70% condensed and 30% hydrolysable

Table 2. Chemical composition of ten enological tannins

Tannin	Total phenols[&]	Total tannins[#]	Gelatin index[†]
T1	368.3 ± 16 e*	3.5 ± 1.5 a	29.3 ± 1.7 b
T2	438.8 ± 8.8 g	14.1 ± 2.0 c	37.7 ± 3.9 c
T3	330.2 ± 12 c	1.2 ± 1.5 a	2.5 ± 1.2 a
T4	308.9 ± 16 b	23.1 ± 2.2 d	73.7 ± 4.2 e
T5	1036.8 ± 9.3 i	9.0 ± 2.9 b	67.2 ± 2.3 e
T6	260.1 ± 14 a	23.7 ± 5.6 d	36.5 ± 3.0 c
T7	351.9 ± 16 d	1.2 ± 1.4 a	52.7 ± 1.1 d
T8	363.0 ± 16 de	72.8 ± 2.8 e	32.2 ± 0.7 bc
T9	391.9 ± 5.8 f	21.1 ± 3.6 d	48.2 ± 0.4 d
T10	520.0 ± 2.2 h	12.0 ± 1.5 c	8.61 ± 1.2 a

* Different letters in the same column indicate a statistically significant difference between treatments in agreement with the Tukey test ($p > 0.05$).

[&] mg equivalent gallic acid g^{-1} tannin

[#] mg equivalent cyanidin g^{-1} tannin

[†] expressed as percentage

Table 3. Concentration of non-flavonoid phenolic compounds (mg g^{-1} tannin) in ten enological tannin extracts.

Tannin	Gallic acid	Ellagic acid	Gallotannins	Ellagitannins
T1	1.9 ± 0.2 ab*	0.4 ± 0.0 a	ND a	ND a
T2	0.6 ± 0.1 a	ND a	ND a	ND a
T3	2.4 ± 0.2 ab	5.5 ± 2.9 b	0.5 ± 0.1 a	2.4 ± 0.5 b
T4	9.9 ± 1.3 d	ND a	533.6 ± 240.6 b	ND a
T5	3.5 ± 0.8 b	ND a	ND a	ND a
T6	2.7 ± 0.2 ab	1.4 ± 0.1 a	ND a	1.0 ± 0.4 a
T7	6.7 ± 1.3 c	1.7 ± 0.7 a	2.8 ± 1.0 a	0.6 ± 0.6 a
T8	7.0 ± 1.8 c	2.7 ± 1.3 ab	36.8 ± 11.6 a	0.9 ± 0.9 a
T9	3.0 ± 0.4 ab	2.0 ± 0.7 a	ND a	0.2 ± 0.2 a
T10	3.5 ± 0.3 b	2.2 ± 0.4 a	0.6 ± 0.7 a	ND a

* Different letters in the same column indicate a statistically significant difference between treatments in agreement with the Tukey test ($p > 0.05$). ND “not detected”.

Table 4. Concentration of flavonoid phenolic compounds (mg g^{-1} tannin) in the ten enological tannin extracts.

I.D.	Flavonol glycosides	Flavan-3-ols
T1	3.5 ± 0.2 ab*	74.0 ± 14.5 ab
T2	3.9 ± 0.6 a	79.9 ± 4.9 ab
T3	2.8 ± 0.0 ab	9.8 ± 2.3 a
T4	ND a	ND a
T5	7.8 ± 1.7 cd	56.9 ± 1.8 ab
T6	9.9 ± 1.0 d	146.8 ± 98.4 b
T7	5.0 ± 1.1 bc	50.6 ± 10.2 ab
T8	8.1 ± 3.1 cd	16.1 ± 11.3 a
T9	4.6 ± 0.9 bc	47.2 ± 4.6 ab
T10	6.1 ± 0.2 bc	139.0 ± 81.0 b

- Different letters in the same column indicate a statistically significant difference between treatments in agreement with the Tukey test ($p > 0.05$). ND “not detected”.

Figure Captions

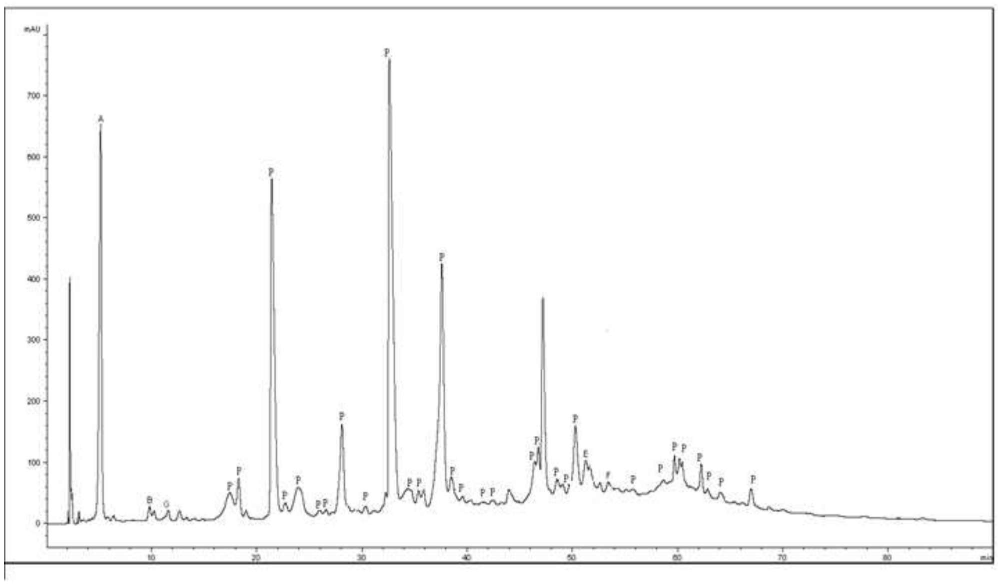
Figure 1. HPLC-DAD chromatogram of grape pomace enological tannin (T_1). The compounds identified were: (A) gallic acid, (B) protocatechuic acid, (C) protocatechuic aldehyde, (D) vanillic acid, (Ea) ellagic acid, (F) flavonol glycosides, (G) gallotannins, (P) proanthocyanidins and (E) ellagitannins.

Figure 2. HPLC-DAD Chromatogram of vegetable enological tannin (T_2). The compounds identified were: (A) gallic acid, (B) protocatechuic acid, (C) protocatechuic aldehyde, (D) vanillic acid, (F) flavonol glycosides and (P) proanthocyanidins.

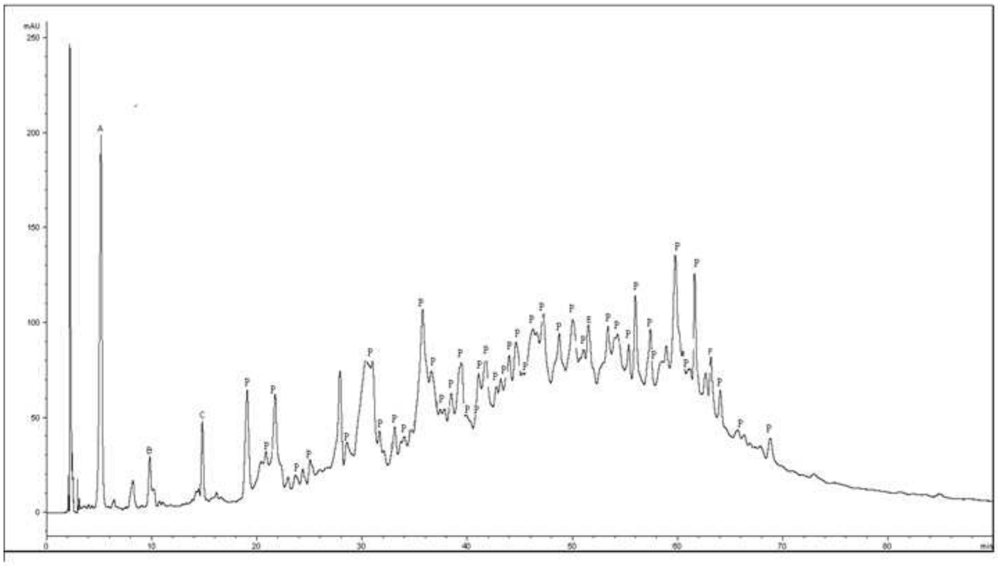
Figure 3. HPLC-DAD Chromatogram of oak wood and oak enological tannin (T_3). The compounds identified were: (A) gallic acid, (B) protocatechuic acid, (C) protocatechuic aldehyde, (D) vanillic acid, (E) ellagitannins, (Ea) ellagic acid, (F) flavonol glycosides, (G) gallotannins and (P) proanthocyanidins.

Figure 4. HPLC-DAD chromatogram oak gall-nut enological tannin (T_4). The compounds identified were: (A) gallic acid, and (G) gallotannins.

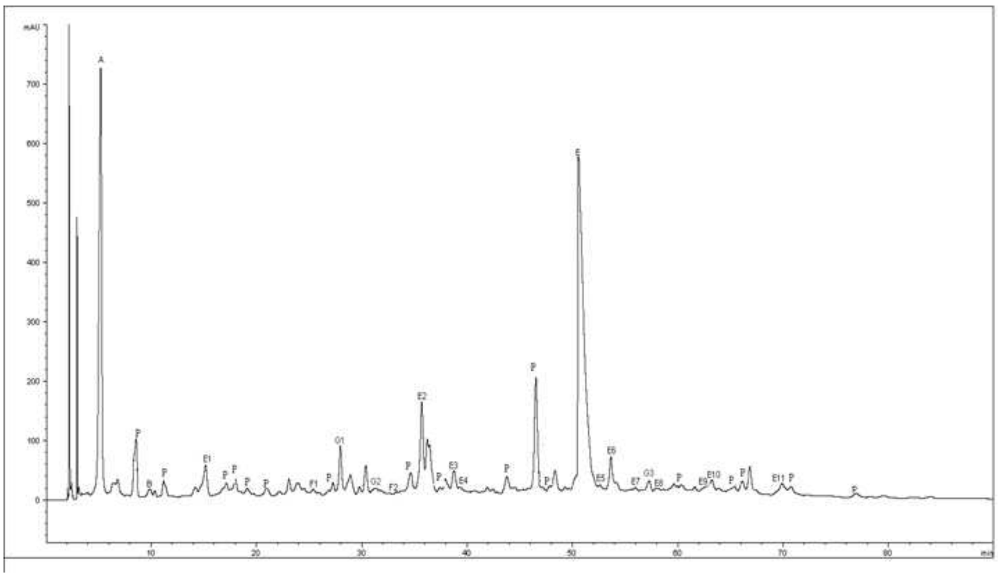
Figure 5. UV spectra of phenolic compounds detected and identified by HPLC-DAD in the ten enological tannin samples.



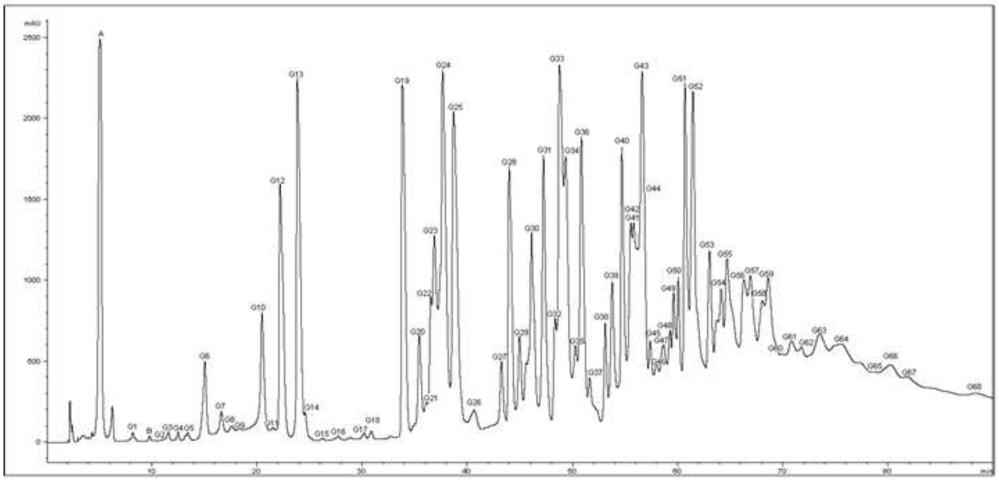
HPLC-DAD chromatogram of grape pomace enological tannin (T1). The compounds identified were: (A) gallic acid, (B) protocatechuic acid, (C) protocatechuic aldehyde, (D) vanillic acid, (Ea) ellagic acid, (F) flavonol glycosides, (G) gallotannins, (P) proanthocyanidins and (E) ellagitannins.
254x190mm (72 x 72 DPI)



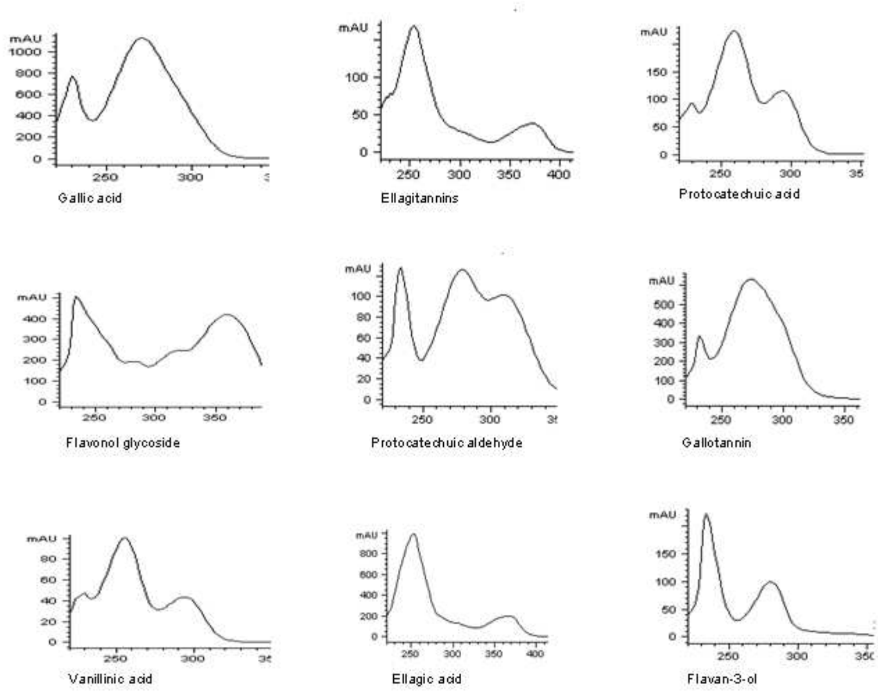
HPLC-DAD Chromatogram of vegetable enological tannin (T2). The compounds identified were: (A) gallic acid, (B) protocatechuic acid, (C) protocatechuic aldehyde, (D) vanillic acid, (F) flavonol glycosides and (P) proanthocyanidins.
254x190mm (72 x 72 DPI)



HPLC-DAD Chromatogram of oak wood and oak enological tannin (T3). The compounds identified were: (A) gallic acid, (B) protocatechuic acid, (C) protocatechuic aldehyde, (D) vanillic acid, (E) ellagitannins, (Ea) ellagic acid, (F) flavonol glycosides, (G) gallotannins and (P) proanthocyanidins.
254x190mm (72 x 72 DPI)



HPLC-DAD chromatogram oak gall-nut enological tannin (T4). The compounds identified were: (A) gallic acid, and (G) gallotannins.
254x190mm (72 x 72 DPI)



UV spectra of phenolic compounds detected and identified by HPLC-DAD in the ten enological tannin samples.
254x190mm (72 x 72 DPI)