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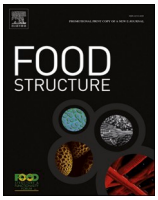

Diverse interaction of commercial enological tannins with the protein fraction of saliva. Association with astringency

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ABSTRACT

Commercial enological tannins (CETs) are wine additives that are mostly aimed at modulating astringency. This study aims at comparing the effects of eleven CETs (supplied by 4 companies) extracted either from grapes (n = 2) or oak wood (n = 9) on the salivary protein fraction and on the astringency they provoke. Phenolic compounds were characterized by spectrophotometry and HPLC methods. Interaction of CETs with the salivary protein was assessed by diffusion on cellulose membranes and precipitation assays. Astringency was measured by a trained sensory panel. The study showed major differences among CETs in both phenolic composition, strength of the interaction with the salivary protein fraction and ability to provoke astringency. Those differences were observed even among products that are marketed under common designations. Altogether, functional characterization of CETs is recommended as a preliminary necessary step to decide on which CET could be more appropriate to modulate wine style.

1. Introduction

Commercial enological tannins (CETs) are wine additives authorized by the OIV (Organization Internationale de la Vigne et du Vin). Those products are obtained from a diversity of plant sources mostly comprising proanthocyanidin-rich extracts from grape skins or seeds and/or hydrolyzable tannin-rich extracts from oak wood (Versari, du Toit, & Parpinello, 2013). A number of reports support a highly diverse list of uses they have in winemaking. Thus, CETs would provide antioxidant protection to components of must and wines, enhance their aging, act as fining agents, promote expression and stabilize wine color, improve wine structure and contribute with a variety of important beneficial biological effects (Baker & Ross, 2014; Hartzfeld, Forkner, Hunter, & Hagerman, 2002; Vignault et al., 2019; Zanchi et al., 2007). CETs would also contribute to modulate astringency, a complex sensation generally thought to be produced by the interaction of red wine tannins with the protein fraction of saliva to form tannin-protein complexes (Laghi et al., 2010; Obreque-Slier, López-Solís, Peña-Neira, & Zamora-Marín, 2010; Prinz & Lucas, 2000; Sanz, Martínez, & Moreno, 2008; Zanchi, Poulain, Konarev, Tribet, & Svergun, 2008). Among salivary proteins, proline-rich proteins, mucins, α-amylases and histatins have been pointed as high affinity targets of both condensed and hydrolyzable tannins (Obreque-Slier, López-Solís et al., 2010; Terrier, Poncet, & Cheynier, 2009; Zanchi et al., 2008). However, tannin-protein interactions are also highly dependent on the concentration, size and molecular structure of the polyphenol (Obreque-Slier, Peña-Neira, & López-Solís, 2010). Accordingly, Bacon and Rhodes (2000) have reported that tannic acid (a gallotannin-rich oak wood extract) would display a high affinity for salivary proteins quite likely due to its high content of covalently bonded gallic acid units. Likewise, Baxter, Lilley, Haslam, and Williamson (1997), have provided solid evidence that the larger and more complex polyphenols interact more strongly with a synthetic proline-rich polypeptide fragment. In previous studies we have shown that, on a mass basis, a sensory panel perceived a proanthocyanidin-rich CET as more astringent than a hydrolyzable tannin-rich CET in close association with the dissimilar abilities of both types of tannins to precipitate gelatin (Obreque-Slier, López-Solís et al., 2010). However, despite the widespread use of CETs in winemaking not much else has been reported in connection with the physicochemical nature of CETs (hydrolyzable, condensed or mixtures) or with their abilities to interact with the salivary protein fraction and, thence, with astringency. This study aims at assessing and comparing the effects of

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CETs extracted either from grapes or oak wood on the salivary protein fraction and on the astringency they provoke.

2. Materials and methods

2.1. Materials

Eleven commercial enological tannins (CETs) were purchased from various distributor companies of enological supplies (Table 1). Standards for gallic acid (G-7384), (+)-catechin (C-1251), (-)-epicatechin (E-1753), ellagic acid (E-2250), Coomassie blue R-250 and bovine serum albumin (BSA) were purchased from Sigma Chemical Company (Saint Louis, Missouri, USA). Cellulose membranes (Whatman 1) were purchased from Whatman Ltd., Maidstone, U.K. Solvents used for cellulose membrane processing were purchased from Merck, Santiago, Chile. High-performance liquid chromatography (HPLC)-grade acetic acid and acetonitrile were purchased from Merck, Darmstadt, Germany. The HPLC system (Agilent Technologies 1200 series) consisted of a G1311A quaternary pump, a G1315B photodiode-array detector, an ALS G1329A autosampler and reversed-phase Nova Pack C18 columns (4 μm, 3.9 mm ID * 300 mm) (Waters Corporation, Milford, MA, USA). Spectrophotometric measurements were performed on an Unicam Helios-Gamma 2000UV–Vis equipment.

2.2. Standard BSA solution

BSA was dissolved in water at 40 °C using mechanical agitation for 20 min. The resulting solution was centrifuged at 3000 rpm for 5 min. Absorbance of the supernatant at 280 nm was adjusted to 0.7 to represent 1 mg/mL. Such protein in solution was used as a model in the diffusion and precipitation tests for CET-protein interaction.

Table 1
Summary of the technical data sheets provided by the supplying companies of the eleven CETs in the study.

CET	Key	Supplier	Origin	Description
Premium Uva	T1	Enológica Vason	Grape skins	Condensed tannin (catechin)
Premium Vinacciolo	T2	Enológica Vason	Grape seeds	Condensed tannin (catechin)
Ambrosia. French complex	T3	Tonelería Nacional	Oak	Aqueous extract from toasted oak chips [*sic*]
Ambrosia. American complex	T4	Tonelería Nacional	Oak	Aqueous extract from toasted oak chips [*sic*]
Premium Limousin	T5	Enológica Vason	Oak	Hydrolyzable ellagitannins and gallotannins from French oak
Premium Whiskey Lattone	T6	Enológica Vason	Oak	Hydrolyzable ellagitannins and gallotannins from American oak
QuerPlus. Natural oakextract	T7	Laffort	Oak	Ellagitannin extract from oak staves
Trŭ/Tan. Innovative oak tannins. Vb	T8	Oaksolutions	Oak	Mixture of gallotannins and ellagitannins from toasted French oak
Trŭ/Tan. Innovative oak tannins. Fi	T9	Oak solutions	Oak	Mixture of gallotannins and ellagitannins from toasted French oak
Trŭ/Tan. Innovative oak tannins. FF	T10	Oaksolutions	Oak	Mixture of gallotannins and ellagitannins from toasted French oak
Trŭ/Tan Innovative oak tannins. Rf	T11	Oaksolutions	Oak	Mixture of gallotannins and ellagitannins from toasted French oak

2.3. Saliva collection

A 24-year-old male volunteer, with no history of tobacco, alcohol or medicine consumption, no evidence of disease and normal salivary flow (over 1 mL/min) was included as a healthy volunteer under the terms of a signed informed consent. The Ethics Committee of the Faculty of Medicine, University of Chile, approved the study protocol, which was in accordance with the Declaration of Helsinki. Saliva was collected passively (no stimulation). Immediately after collection, saliva was centrifuged at 750 g for 3 min and the supernatant was kept at 4 °C until just before using (Obreque-Slier, Peña-Neira et al., 2010).

2.4. Chemical characterization of CETs

The commercial enological tannins were dissolved (5 g/L) in a hydroalcoholic solution (20 % v/v of ethanol, 0.5 % w/v of tartaric acid adjusted to pH 3.5) at 20 °C with mechanical stirring for 20 min. Total phenols were determined by measuring absorbance at 280 nm (Glories, 1984). Total tannin content was determined by the methyl cellulose procedure (Mercurio, Dambergs, Herderich, & Smith, 2007). For characterization of phenolic compounds, a 50-mL aliquot of each CET was extracted successively with ethyl ether (3 × 20 mL) and ethyl acetate (3 × 20 mL). The combined total extract was evaporated to dryness at 30 °C, dissolved in 2 mL of 1:1 (v/v) methanol/water and filtered through 0.45 μm pore-size membranes. Aliquots of 20 μL were subjected to HPLC fractionation. The mobile phase (constant flow rate of 1 mL/min) was produced by mixing solution A (98:2 (v/v) water/acetic acid) with solution B (78:20:2 (v/v/v) water/acetonitrile/acetic acid). The gradient profile was 100-20 % A between 0 and 55 min, 20-10 % A from 55 to 57 min, and 10-0 % A from 57 to 90 min. Detection was performed by UV absorptiometry at various wavelengths in the range from 210 to 360 nm, with an acquisition speed of 1 s^{-1}. Identification of compounds corresponding to individual HPLC peaks was performed by comparing their UV absorption spectra to those of pure standards (Cadahía, Muñoz, Fernández de Simón, & García-Vallejo, 2001; Obreque-Slier, Peña-Neira, López-Solís, Ramírez-Escudero, & Zamora-Marín, 2009; Santos-Buelga, García-Viguera, & Tomás-Barberán, 2003). Both fractionation and composition analysis were performed in three independent experiments.

2.5. CET–salivary protein interactions: Diffusion and precipitation assays

Salivary protein-CET interactions were assessed using both a protein diffusion assay and a precipitation assay (Obreque-Slier, Peña-Neira et al., 2010; Obreque-Slier, Mateluna, Peña-Neira, & López-Solís, 2010). Thus, 200 μL aliquots of each of the CET dilutions (concentration range 0.4−5 g/L) were thoroughly mixed for 15 s with 200 μL of saliva or standard BSA solution and allowed to stand for 5 min. In the diffusion assay, 20 μL aliquots of each mix were dotted on a cellulose membrane and allowed to dry spontaneously at room temperature. The membrane was fixed, rinsed and stained for proteins with Coomassie blue R-250 as described elsewhere (López, Castillo, Traipe, & López, 2007; Obreque-Slier, López-Solís et al., 2010, 2010b, 2010c). After exhaustive washing in 7 % acetic acid, the membrane was rinsed once in distilled water and dried under a heat lamp. A digital image of the blue spots on the membrane was obtained using an Epson 4855 scanner. In the precipitation assay, the series of CET dilutions-saliva mixtures in Eppendorf tubes were allowed to stand for 5 min and then were centrifuged at 750 *g* for 5 min. Aliquots of 20 μL from each of the supernatants were dotted on a cellulose membrane. The membrane was processed for protein staining as indicated above. The blue–stained area of protein distribution corresponding to each of the extract dilution–saliva mixtures was quantified by Image *J* 1.45 software (See Supplementary Materials). Each of the assays was performed at least three times.

2.6. Sensory evaluation

Each CET solution (0.7 g/L) was assessed for astringency by a 14–member trained sensory panel (7 men, 7 women; age range 24–56 years old). CET solutions (15 mL) at 20 °C (± 1 °C) in black cups were presented at random to the panel members, who were asked to describe the intensity of the perceived astringency on a 0–15 score scale. The extracts were evaluated twice in two independent sessions separated by a 48–h interval. A water solution of 0.1 % pectin was used for mouth rinsing between consecutive samples (Medel-Marabolí, Romero, Obreque-Slier, Contreras, & Peña-Neira, 2017).

2.7. Statistical analysis

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

3. Results

3.1. General information and phenolic characterization of the CETs in the study

General features of eleven CETs marketed by 4 supplier companies are shown in Table 1. According to technical data sheets provided by the corresponding companies, two CETs were obtained from skin or seed grapes and are described as condensed tannin-rich products (T1 and T2, respectively) while the other nine CETs are hydrolyzable tannin-rich products obtained from oak wood (T3 through T11). Phenolic characterization of the eleven CETs in our study showed that T8, T9, T10 and T11 displayed the highest apparent contents of total phenols (avg 1041.1 mg/g) and total tannins (average 2123.2 mg/g), that is, about 3 times the corresponding contents in the rest of the CETs (Fig. 1). Certainly, those figures have a nominal character mostly derived from the highly different molar absorptivities at 280 nm of gallic acid and epicatechin (around 3-fold) used for reference in the UV absorptiometric assays for total phenols and total tannins, respectively, together with the diverse contribution of both proanthocyanidins and hydrolysable tannins to the overall composition of each CET. In that regard, all those four CETs (T8 through T11) also displayed the highest relative contents of gallic acid (avg 4.1 mg/g), ellagic acid (avg 5.0 mg/g), gallotannins (avg 205.5 mg/g) and proanthocyanidin gallate (avg 69.0 mg/g), that is, values several-fold the ones in the rest of the CETs in the study (Fig. 2). Particularly, the relative content of gallotannins in the whole group of CETs in the study was distributed unevenly over a several hundred-fold range (Fig. 2). By contrast, T4 showed the lowest content of total phenols and T5 and T6 showed the lowest contents of total tannins (Fig. 1). On the other hand, the procyanidins (+)-catechin and (-)epicatechin were detected largely in the CETs T1 and T2. All rest of the CETs, excepting T7, showed basically no presence of these procyanidins.

3.2. Sensory evaluation of the CETs in the study

As shown in Fig. 1, a trained sensory panel perceived T2, T8, T10 and T11 as the most astringent CETs in the study (scores in the range 9.8–9.9) whereas T3 through T7 obtained the lowest scores for astringency intensity (range 4.8–6.7). T1, T8 and T9 obtained intermediate scores (range 8.5–8.9).

3.3. Physicochemical interaction of a CET with BSA: diffusion and precipitation assays

Coomassie blue R-250 staining reveals the distribution of BSA when an aliquot of the protein solution is placed on a horizontally positioned cellulose membrane (Fig. 3). Thus, a 20 μL aliquot of BSA in aqueous solution (0.5 mg/mL) that is placed sharply on a point of the cellulose membrane undergoes a radial and homogeneous diffusion to produce a

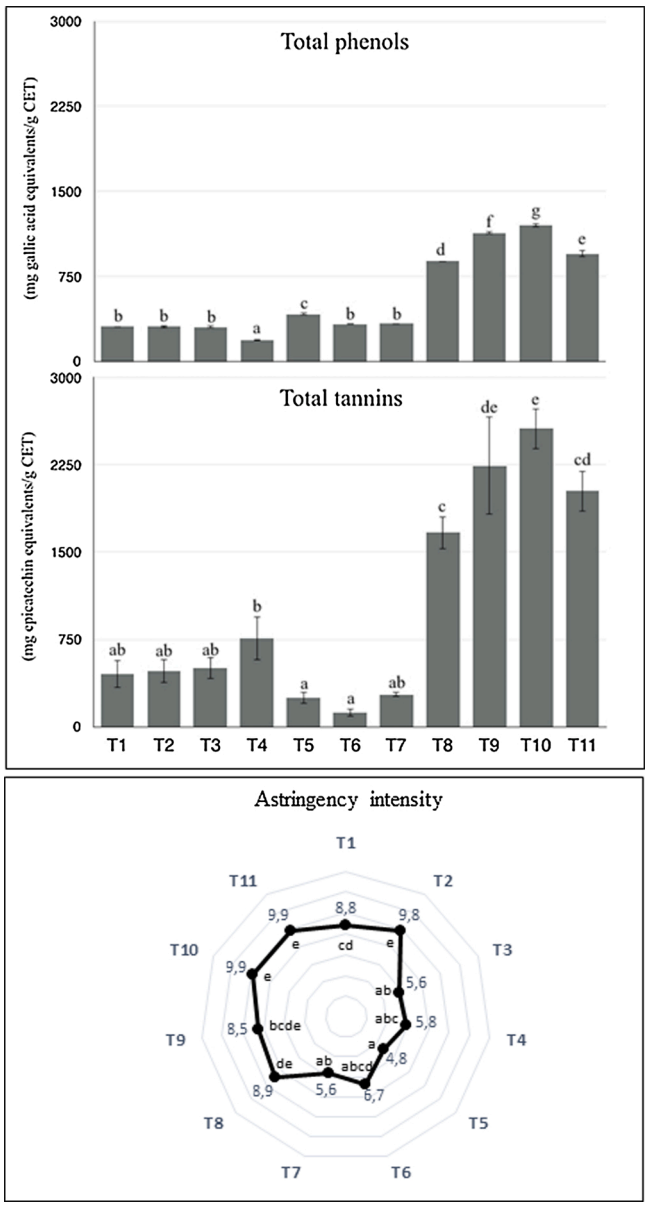

Fig. 1. Total phenolic and tannin contents in the commercial enological products and astringency intensity evoked by the CETs in the study. For comparison purposes between all CETs in the study, in the panels of the figure the total contents of both groups of phenolics are expressed on the basis of a single common standard (epicatechin or gallic acid). Nominal contents (ordinate axis) of both groups of polyphenols in at least some CETs are clearly overestimated quite likely due to a ratio close to 3.0 between the molar absorptivities at 280 nm of gallic acid to epicatechin and the expectedly diverse contributions of gallic acid-rich, ellagic- acid rich and proanthocyanidin-rich polyphenols in the composition of different CETs.

diffusion circle (Fig. 3A). In presence of 10 % ethanol at pH 3.5 (control condition), protein staining becomes somewhat nonhomogeneous, that is, a minor fraction remains close to the spotting site (non diffusible fraction or NDF) whereas the rest of the protein diffuses freely (diffusible fraction or DF) (Fig. 3B). Then, growing amounts of the CET T1 were mixed with BSA in an equivalent hydroalcoholic medium and protein diffusion on the cellulose membrane was assayed (Fig. 3C-L). In this diffusion assay, addition of 0.4 g/L of T1 to the standard aliquot of BSA provoked a marked decrease in the area of protein diffusion, that is, in the area of the DF fraction (Fig. 3C), whereas additions of TI at concentrations of 0.8 g/L or over provoked the full disappearance of that

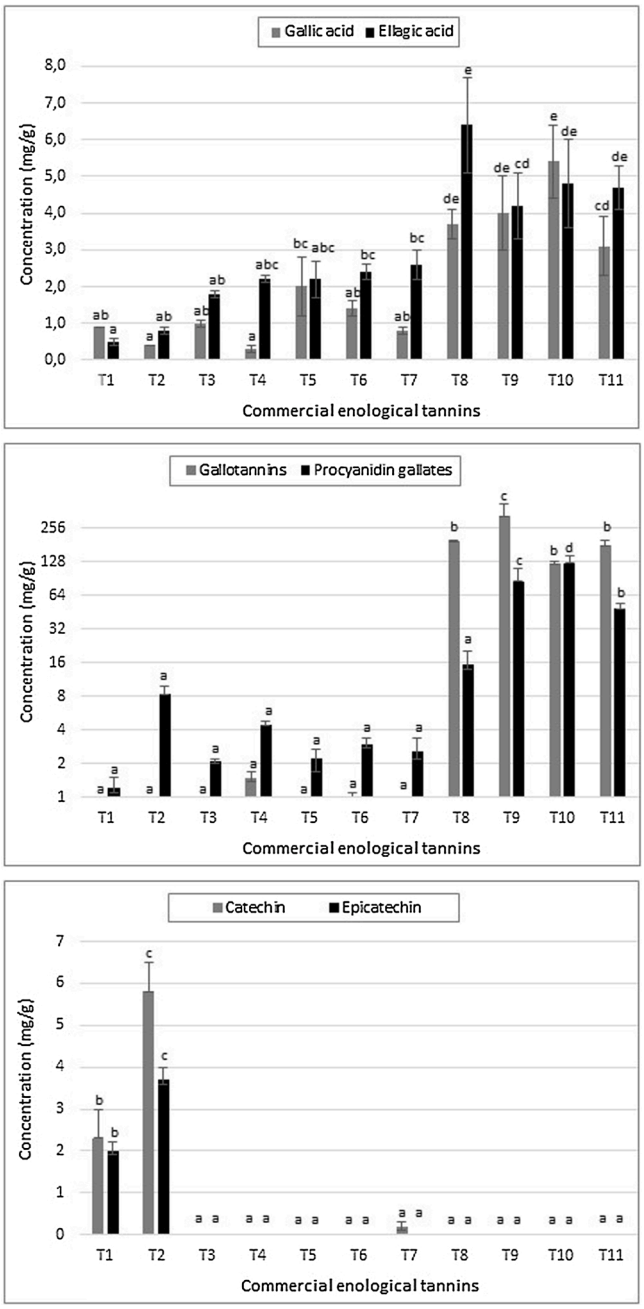

Fig. 2. Low molecular weight phenol contents in the commercial enological products of the study.

fraction (Fig. 3D). This drastic antidiffusive effect of T1 on BSA was observed even on the NDF fraction (BSA-T1 complexes) at concentrations of 2.8 g/L of T1, or over (Fig. 3I-L). When the BSA-T1 mixtures were centrifuged just before placing an aliquot of the supernatant on the cellulose membrane (precipitation assay) (Fig. 3M-W), the DF was drastically reduced up to disappear by the addition of 0.8 g/L of T1, or over (Fig. 3O-W). Higher amounts of T1 (4 g/L or over) resulted in full disappearance of BSA from the supernatant, that is, its precipitation (Fig. 3K-W).

3.4. Physicochemical interaction of CETs with the protein fraction of saliva: diffusion assay

Fig. 4 shows the effect of each one of the CETs in the study on the diffusion of the protein fraction of saliva on cellulose membranes. At variance of BSA, the protein component of saliva diffuses biphasically on a cellulose membrane, that is, in the absence of any additional compound in the medium both a non diffusible fraction and a diffusible fraction of salivary protein can be readily identified (Fig. 4, first column of spots on the left). When growing amounts of CETs were mixed with saliva just before placing aliquots of the mixtures on the cellulose membrane, significant and quantitatively different anti diffusive effects were put in evidence. In effect, 1:1 (v/v) mixtures of saliva with 0.4 g/L of T11 or with 0.8 g/L of either T1, T8, T9 or T10 displayed a reduced diffusion of the DF of saliva whereas 1.2 g/L of T9 or T10 provoked full disappearance of that fraction (Fig. 4, Supplementary Material Table S1). Such maximal effect was produced by concentrations as high as 1.6 g/L of T8 and T11 or 2.0 g/L, or over, of T1, T2 and T5. In the same regard, concentrations over 2 g/L of T1, T2 or T5 were necessary to produce full loss of the DF of saliva. Likewise, concentrations higher than 4 g/L of T3, T6 or T7 were necessary to produce either full (T3) or almost full (T6, T7) disappearance of the DF of saliva. By the same token, growing amounts of T4 all over the range of concentrations in the study did not result in full disappearance of the DF of saliva (Fig. 4, Supplementary Material Table S1). On the other hand, addition of growing amounts of CETs resulted in a progressive decrease in the area of the NDF of saliva, excepting for T4 (Fig. 4, Supplementary Material Table S2). Those decreases were statistically significant following the mix with 0.4 and 0.8 g/L of T10 and T11, respectively, or with 1.2 g/L of T5, T8 and T9. For T1, T3 and T7, such effect was observed after mixing saliva with concentrations of 4 g/L or over (Supplementary Material Table S2).

3.5. Physicochemical interaction of CETs with the protein fraction of saliva: precipitation assay

Centrifugation of each one of the CET/saliva mixtures just before spotting the cellulose membrane with 20 μL aliquots of the corresponding supernatants put in evidence marked differences among CETs to provoke full precipitation of the salivary protein (Fig. 5). In effect, T8, T9, T10 and T11 provoked practically full precipitation at concentrations of 2.8 g/L whereas, at the other end, T4 was mostly unable to produce salivary protein precipitation at any of its concentrations in the study. Thus, the order of precipitant power in this assay was determined as T8=T9=T10=T11 > T1 > T2 > T3=T5=T6=T7 > T4 (Fig. 5, Supplementary Material Table S3 and Table S4). Complementary, the precipitation assay also showed the occurrence of less diffusible non precipitating CET-salivary protein complexes at CET concentrations usually somewhat higher than those responsible of producing full precipitation of the salivary protein. For instance, full loss of the DF of the salivary protein occurring in the supernatants of the CET-saliva mixtures was observed at CET concentrations around 1.6–2.4 g/L of both T1, T8, T9, T10 and T11 and around 2.8–4.0 g/L of both T2, T3, T5, T6 and T7. Such effect was only marginal in the case of T4 (Fig. 5, Supplementary Material Table S3).

4. Discussion

Commercial enological tannins (CETs) are widely used in wine industry. They have proved useful to modulate a number of wine properties, particularly astringency. Currently, distributor companies of enological supplies provide a gamma of CETs (Chira & Teissedre, 2013) comprising a high content of polyphenols from various plant species and geographical origins (Obreque-Slier et al., 2009). However, it is a common observation that not much information about the physicochemical composition of these products or about their functional recommendations as astringency modulators is included in the corresponding technical data sheets. Considering that astringency has been closely associated with the ability of tannins to interact with saliva, in this study we compared 11 CETs (named T1 through T11, for convenience) from four different supplier companies as to their abilities to

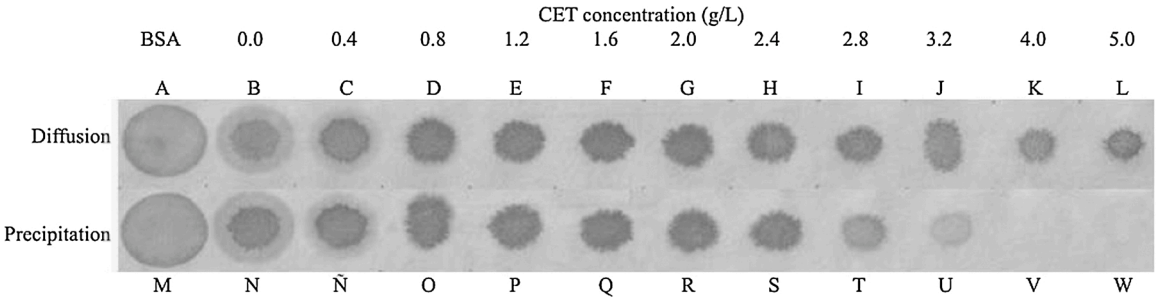

Fig. 3. Diffusion and precipitation assays for the interaction of T1 and BSA. Twenty μL aliquots from 1:1 (mL: mL) mixtures of an aqueous solution of BSA (1 mg/mL) with an array of T1 solutions (concentration range 0−5 g/L) in a hydroalcoholic medium (20 % ethanol, 0.5 % tartaric acid, pH 3.5) were dotted on a horizontally positioned cellulose membrane (diffusion assay). All mixtures were then centrifuged (750 g × 5 min) and 20 μL aliquots from each supernatant were also dotted on a cellulose membrane (precipitation assay). Membranes were processed for protein staining as described in Materials and Methods. Numbers on the top of the figure represent T1 concentration before mixing with BSA solution.

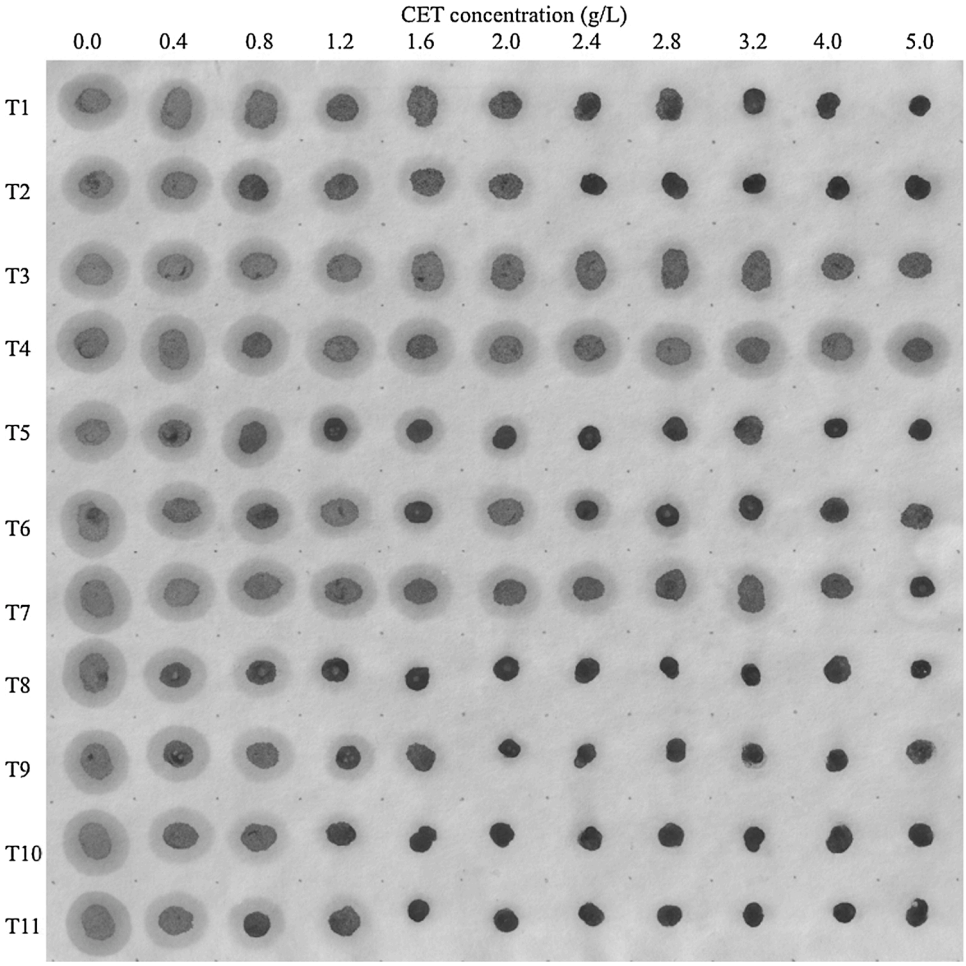

Fig. 4. Interaction of CETs with the protein fraction of saliva: diffusion assay. The assay was similar to the diffusion assay described in Fig. 2 except that here each one of the CETs in the study (T1 through T11) were mixed at a 1:1 (mL: mL) ratio with saliva. Numbers on the top of the figure represent CET concentrations before being mixed with saliva. Each row corresponds to the assay with the CET indicated on the left of the panel.

interact with the salivary fraction of saliva and to evoke astringency. Firstly, CETs were dissolved in a common hydroalcoholic solution and were characterized by spectrophotometry and HPLC-DAD analysis. Overall quantitative analysis of these products showed that the total contents of phenols and tannins are substantially higher than those reported for *Vitis vinifera* grape seeds and skins, which are in the range of 5−30 mg GAE/g and 5−60 mg EE/g, respectively (Canals, Llaudy, Valls, Canals, & Zamora, 2005; Obreque-Slier et al., 2013). Such high levels of both phenols and tannins have been previously observed in studies from other laboratories working with CETs other than those of the present study (Bautista-Ortín, Cano-Lechuga, Ruiz-García, & Gómez-Plaza, 2014; Obreque-Slier et al., 2009). On the other hand, the use of gallic acid or epicatechin as a single standard to express contents of either total phenols or total tannins in all CETs in the UV-absorptiometric assay at 280 nm resulted in nominal figures whose levels of underestimation or overestimation were strongly influenced by the about 3-fold ratio between the molar absorptivities of gallic acid to epicatechin (Lin & Harnly, 2012), and the expectedly diverse contributions of gallic

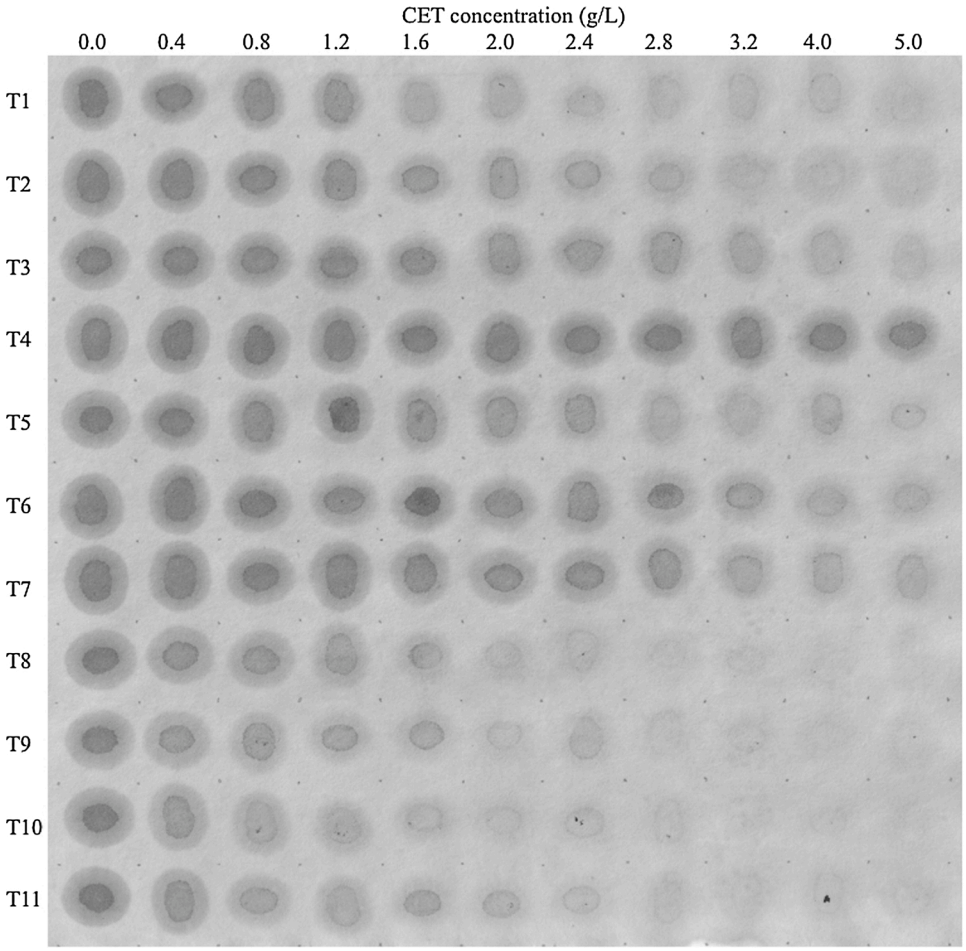

Fig. 5. Interaction of CETs with the protein fraction of saliva: precipitation assay. The assay complements the diffusion assay shown in Fig. 4, except that in this case 20 μL aliquots from the supernatants of each mixture were dotted. Both legends and numbers have the same meaning as in Fig. 4.

acid-rich, ellagic- acid rich and proanthocyanidin-rich polyphenols in the composition of different CETs. Thus, more realistic total contents of both types of polyphenols can be produced by correcting, as appropriate, the nominal contents as a function of the prevalent contents of either epicatechin (CET1 and CET2) or gallic/ellagic acids (CET3 through CET11). According to HPLC analysis, the grape-derived CETs in the study (T1 and T2) displayed the highest contents of monomeric (+)-catechin and (-)-epicatechin whereas the mixtures of gallotannins and ellagitannins from toasted French oak (T8-T11) showed the highest contents of gallic acid, ellagic acid, gallotannins and proanthocyanidin gallates. About a half of the CETs in the study (T3 through T7) showed no particular quantitatively prominent low molecular weight polyphenol. These observations lend full support to the grape-derived CETs as important sources of proanthocyanidin monomeric polyphenols and to some French oak-derived CETs as main sources of wood phenols. However, striking compositional and functional differences were observed among the products that are marketed under the common designation of oak-derived CETs. Thus, the content of gallic acid in T10 was about 20-times the one in T4 and the gallotannin content in T9 was about 300-times the one in T3. In addition, our compositional analysis revealed that even the oak-derived CETs from common origins (American oak or French oak) showed a wide group heterogeneity in the polyphenol content. Such wide diversity may be accounted for by local factors of production, such as the standard extraction methods or the raw materials used by every CET producing company. Thus, characterization of these enological products should be a necessity for the wine-making industry in order to orientate purchase decisions about CETs and to facilitate predictability of the effects of CETs on wine quality.

Functionally, tannins are well-known for their abilities to interact *in vitro* with diverse salivary proteins, such as various families of proline-rich and histidin-rich proteins, and through that mechanism to produce physiological responses such as astringency perception (Bennick, 2002; Ramos-Pineda et al., 2019; Brandão et al., 2020). One of the CETs in this study (T1) was shown to interact with a pure model protein (BSA) by assaying its ability to affect both BSA diffusion on a cellulose membrane as well as BSA solubility. Accordingly, T1 reduced both parameters in a concentration-dependent manner and beyond enhancing effects of both acidic pH and ethanol (Obreque-Slier, Peña-Neira et al., 2010; Obreque-Slier, Peña-Neira, & López-Solís, 2012). When both functional assays were performed by mixing each of the CETs in the study with saliva, a biochemically complex body fluid mostly comprising water, inorganic electrolytes and proteins (some of the latter ones displaying high affinity for tannins), marked differences among the various CETs were observed (Obreque-Slier, Peña-Neira et al., 2010; 2012a; Obreque-Slier, Peña-Neira, & López-Solís, 2012). Salivary proteins from healthy subjects display a biphasic distribution on cellulose membranes, that is, when aliquots of saliva are placed on absorbing cellulose membranes and free diffusion of saliva is allowed to occur, a non diffusible protein fraction becomes surrounded by highly diffusible salivary proteins (Obreque-Slier, Peña-Neira et al., 2010, 2012b). In the present study, saliva was taken from a single healthy subject under the same experimental conditions in order to control for its compositional invariability. Under these conditions, the CETs T8, T9, T10 and T11, at concentrations lower than those of the rest of the CETs (1.2 g/L), were able to fully suppress protein diffusion, that is, to provoke disappearance of the diffusible fraction of the salivary protein. Such full antidiffusive

effect was also observed with the CETs T3, T6 y T7 at concentrations higher than 4 g/L. Contrarily, even the maximal concentration of T4 in the assay (5 g/L) was unable to modify the biphasic distribution of the salivary protein. Interestingly, the precipitation assay was mostly confirmatory of these observations because all CETs displaying the highest antidiffusive effects (*i.e.* T8, T9, T10 and T11) were also those that at low concentrations provoked full precipitation of the salivary protein. These differential effects of CETs on the salivary protein may well be a consequence of their differences in chemical composition. In effect, the grape-derived CETs in the study (T1 and T2) display high contents of the procyanidins (+)-catechin and (-)-epicatechin, and low contents of non flavonoid compounds. In addition, T1 and T2 are significantly less interactive with the salivary protein in comparison with the oak-derived CETs T8, T9, T10 and T11. This observation contradicts reports from other laboratories pointing to grape-derived proanthocyanidins as the wine components responsible of interacting with human saliva (Bautista-Ortín et al., 2014; De Freitas & Mateus, 2001). Indeed, our present results support the view that the strength of the interactions of the non flavonoid phenolic compounds occurring in oak-derived CETs may be quantitatively similar to that of proanthocyanidins also occurring in some other CETs (Obreque-Slier, López-Solís et al., 2010). Interestingly, although the diffusion and precipitation assays also revealed that T4 showed the lowest affinity for the salivary protein, physicochemical characterization of its polyphenols failed to show any peculiarity that may be associated to such scanty or nil interaction between T4 and the salivary protein. Thus, that observation depicts the huge functional diversity that CETs of a common nominal origin may exhibit.

Last but not least, sensory assessment of CETs (0.7 g/L) showed that T1, T2, T8, T10 and T11 are the most astringent products in the study. At roughly similar concentrations (0.8 g/L) all these CETs proved to produce a significant restriction of diffusion of the salivary protein on cellulose membranes and, also, to reduce protein solubility. Thus, both findings would indicate that both flavonoid-rich (condensed-tannin-rich) CETs and non-flavonoid-rich (hydrolyzable-tannin-rich) CETs may be endowed with a high ability to interact and affect the protein fraction of saliva, thus supporting the view that the close association between tannin-protein interaction and astringency (Obreque-Slier, López-Solís et al., 2010) in some cases may be more dependent on the concentration of the phenolic compounds and its surrounding medium than on the own physicochemical nature of the polyphenol (Obreque-Slier, Peña-Neira et al., 2010, 2012a). Altogether, functional characterization of CETs, such as the ones used in this study, may be a useful, necessary or critical step to decide on which CET can be more appropriate to modulate or impact on wine style.

5. Conclusions

Marked quantitative and qualitative differences in both phenolic composition, ability to interact with the protein fraction of saliva and to provoke astringency were observed among a group of randomly selected CET that are distributed by various supplying companies. Those differences were observed even among products with similar nominal origins and compositions shown in the corresponding technical data sheets. Accordingly, functional characterization of CETs, such as the one conducted in this study, is suggested as a relevant step to decide which particular CET is most appropriate to shape the style of a single wine, particularly in reference to astringency modulation.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.foostr.2020.100163.

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