

Effect of enological tannin addition on astringency subqualities and phenolic content of red wines

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Abstract

Four Italian red wine varieties (Sangiovese, Montepulciano, Barbera, Nero d'Avola) were used to investigate the effect of enological tannins on astringency characteristics and phenolic content of red wines. Wines were treated with three tannins of different origins (G = grape, E = oak, and P = exotic wood) at two concentrations (10, 20 g/hl) and aged for 1 year. Wines were evaluated for astringency and for 16 subqualities using check-all-that-apply questions. In addition, polymeric pigments, tannins, flavans, total anthocyanins, and color parameters were analyzed. Enological tannin promoted color stability by pigmented polymers formation. Astringency intensity was not enhanced, even better an improvement of mouthfeel sensations was achieved with wood-derived tannins. Multivariate analysis revealed a great influence of the variety on astringency and phenolic characteristics of wines. Therefore, the initial phenolic composition of wine seems to be the main driver of the evolution of wine during aging.

Practical applications

Tannin addition is an enological practice widely widespread because of many economical benefits. The use of enological tannins during aging can contribute to color stabilization and to an improvement of astringency subqualities of wines. Training on astringency subqualities with touch standards coupled with the check-all-that-apply questions can provide an interesting way to reveal the different aspects of red wine astringency. Despite high astringency and high phenolic content, a wine may present desirable subqualities which can improve wine experience. Finally, a tailored use of enological tannins depends on wine variety.

1 | INTRODUCTION

In winemaking the use of enological tannins is officially authorized by the Organisation Internationale de la Vigne et du Vin (OIV) for musts and wines clarification (OIV, 2012). They can be of two typologies: condensed and hydrolyzable tannins, if they derive from grape seeds and skins (*Vitis vinifera*), exotic wood (e.g., quebracho: *Schinopsis balansae*) or nutgalls (of *Quercus* and *tara*), wood rich in tannin, such as chestnut (*Castanea sativa*), and oak (*Quercus* sp.) (Codex Cœnologique International; OIV, 2012). Enological tannins were also suggested for their ability to contrast wine oxidation and stabilize color (Ribereau-Gayon, Glories, Maujean, & Dubourdieu, 2006). In fact, thanks to their chemical nature, tannins are reactive molecules able to interact with metals, phenolic compounds, and macromolecules in wine. Once added into wine, tannins can participate to several reactions contributing differently to the stabilization of red wine color (Pérez-Lamela, García-Falcón, Simal-Gándara, & Orriols-Fernández, 2007) by forming

polymeric pigments with anthocyanins (Castañeda-Ovando, de Lourdes Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009; Liao, Cai, & Haslam, 1992), to the enhancement of the antioxidant activity (Baiano, Terracone, Gambacorta, & La Notte, 2009; Neves, Spranger, Zhao, Leandro, & Sun, 2010), and to the increase (Bautista-Ortín, Fernández-Fernández, López-Roca, & Gómez-Plaza, 2007) or not (Rinaldi, Gambuti, Moine-Ledoux, & Moio, 2010) of the astringency perception.

Oral astringency is a tactile sensation evoked in mouth by plant polyphenols-derived products, such as red wine. It has been generally described in sensory terms as a combination of three sensations of drying (lack of lubrication or moistness resulting in friction between oral surfaces), puckering (drawing or tightening sensation felt in the mouth, lips, and/or cheeks), and roughing (un-smooth texture in the oral cavity marked by inequalities, ridges, and/or projections felt when oral surfaces come in contact with one another) (ASTM, 2004; Lawless, Corrigan, & Lee, 1994). Bate-Smith (1954) first speculated that astringent

sensations were caused by the increase in friction between the mucosal surfaces which resulted from reduction in lubrication as salivary proteins were bound by astringent compounds. The soluble complexes and precipitates formed by polyphenols/salivary proteins binding stimulate and activate mechanoreceptors (MRs) hold in mouth. MRs are nerve endings that function like those of the skin, except that they have smaller receptive fields and lower activation thresholds (Trulsson & Essick, 1997). They are selectively sensitive to different *stimulus* properties, such as particle size and/or mouth movements, and project such information to the central nervous system (Chen & Engelen, 2012). Recently, the activation of G-coupled proteins seems to be involved in the perception of astringency, activating signal transduction pattern as that of taste recognition (Schöbel et al., 2014).

The activation of trigeminal nerve and chorda tympani could explain astringency as a multi perceptual phenomenon. In addition, side tastes as bitterness, sourness, and sweetness are able to highly modulate the overall astringency (Fleming, Ziegler, & Hayes, 2016). The sensitivity of MRs to astringents as well as basic tastes may elucidate the complexity of red wine astringency, which has been described by 33 different subqualities (Gawel, Iland, & Francis, 2001). Among these “hard,” “green,” and “rich” have been associated with bitterness, acidity, and high flavor concentration, respectively (King, Cliff, & Hall, 2003), “harsh,” “abrasive,” and “drying” have been found to define astringency as a negative sensation, while the “complex” and “mouthcoat” qualities have been associated to a positive impact during tasting (Gawel et al., 2001).

Tannins are the main responsible for the qualitative aspects of astringency as well for the intensity of the sensation. Grape skin and seed tannins are felt astringent as the degree of polymerization and galloylation increased. The latter seems to be responsible for the coarse perception (Vidal et al., 2003) which seems to be decreased by increasing the degree of B-ring trihydroxylation (given by epigallocatechin content) on tannin molecule. On the contrary, a recent work found that the hydroxylation of B-ring seems to decrease velvety astringency and increase the perception of puckering and drying astringency (Gonzalo-Diago, Dizi, & Fernández-Zurbano, 2013). Oak wood tannins were mainly associated to smooth and mouth-drying sensations at low concentrations (Stark et al., 2010).

The addition of tannins from grape or wood into wine can differently modulate the sensory perception of wine, and in particular the effect on wine astringency depends on many factors such as tannin typology, timing (Parker et al., 2007), dose (Harbertson, Parpinello, Heymann, & Downey, 2012), and grape variety (Versari, Toit, & Parpinello, 2012). Until now no studies on the evaluation of the subqualities of wines treated with enological tannins have been conducted.

In the present work, we evaluated the effect of enological tannin addition on astringency (intensity and subqualities), phenolic content, and color parameters of different Italian monovarietal wines (Sangiovese [SG], Montepulciano [MA], Barbera [BA], Nero d'Avola [NV]) after an aging period of 1 year with three tannins (from grape, oak, exotic wood) at two concentrations (10, 20 g/hl). Moreover, the impact of the variety on subqualities and phenolic characteristics of wine was assessed, and some correlations between astringency subqualities and polyphenols were found.

2 | MATERIALS AND METHODS

2.1 | Reagents

Solvents of High Performance Liquid Chromatography (HPLC) grade were purchased from Merck Millipore (Darmstadt, Germany). Caffeine was purchased from ACEF (Piacenza, Italy). Tannic acid was purchased by Extrasynthèse (Lyon, France). Enological tannins for sensory training as well for the experiment (E from oak, P from exotic wood, and G from grape) were all provided by Laffort (Bordeaux, France). L(+)-tartaric acid, bovine serum albumin (BSA) and vanillin from SIGMA Life Science.

2.2 | Wines samples

SG, BA, MA, and NV wines were obtained from wineries located in Toscana, Piemonte, Abruzzo, and Sicilia regions, respectively. Wines of 2015 vintage, after 9 months from the harvest, were analyzed according to the OIV (2007). The physical-chemical composition of each wine was the following: SG alcohol content (% vol/vol) 13.2, pH 3.4, titratable acidity (g/L tartaric acid) 5.9; BA alcohol content (% vol/vol) 13.4, pH 3.4, titratable acidity (g/L tartaric acid) 6.6; MA alcohol content (% vol/vol) 12.5, pH 3.6, titratable acidity (g/L tartaric acid) 5.4; NV alcohol content (% vol/vol) 13.5, pH 3.2, total acidity (g/L tartaric acid) 6.2. For all wines the residual sugars were <1 g/L, and free SO₂ <20 mg/L. This represents the starting point of the aging period ($t = 0$), when SG, BA, MA, and NV were treated with G, P, E tannins at 10 g/hl (G1-, P1-, E1-) and 20 g/hl (G2-, P2-, E2-). Wines were homogenized by stirring, bottled under N₂, and stopped with screw cap, to avoid oxygen entry. Bottles (375 ml) were stored at about 18°C for 12 months ($t = 12$). C-SG, C-BA, C-MA, and C-NV represented the wines with no tannins added (control wines) at $t = 12$. Treatments were made in duplicate. After $t = 12$, wines were filtered with a filter paper of general use (Albet 400, weight 80 [g/m²]) under vacuum to remove sediments, and used for sensory and phenolic analyses.

2.3 | Sensory analysis

2.3.1 | Training

A jury composed of students and researchers of the Division of Sciences of Vine and Wine, Department of Agriculture, University of Naples Federico II, in Avellino (Italy), was trained for the evaluation of astringency and mouthfeel sensations. At first, basic tastes and mouthfeel solutions were presented to jury to select the participants able to recognize sweetness (sucrose 10.0 g/L), acidity (tartaric acid 1.0 g/L), bitterness (caffeine 1.0 g/L), and astringency (tannic acid 2.0 g/L) in water. Over 18 participants, 13 passed the selection. Then, such solutions were proposed at lower concentrations in water, white, and red wine. Successively, panelist was asked to individuate the different stimuli (acid, sweet, sour, bitter, and astringent) in binary mixtures in white wine. For rating tests, scaling solutions of sucrose, tartaric acid, caffeine, tannic acid were presented in water and white wine.

In the following sessions, the training focused on astringency. Panelists have been introduced first to the theory of astringency and then,

TABLE 1 Description of the astringency subqualities terms listed in the check-all-that-apply questions and touch-standards used during training

Subquality term	Description	Grouping	Touch-standard
Silk	Tactile sensation like silk	Surface smoothness	Silk
Velvet	Tactile sensation like velvet	Surface smoothness	Velvet
Dry	Feeling of lack of lubrication in mouth	Drying	–
Corduroy	Sensation of a light wrinkling of the soft palate that can be felt by tongue movements	Surface smoothness	Corduroy
Adhesive	The feeling that mouth surfaces are sticking, yet can be pulled away from each other with slight pressure	Dynamic	Double-sided scotch
Hard	Combined effect of astringency and bitterness	Harsh	–
Aggressive	Excessive astringency of strong roughing nature	Harsh	Sand paper 600 grade
Soft	A light and finely textured astringency	Complex	Fur
Mouthcoat	Like a coating film that adheres to mouth surfaces	Complex	Suede
Rich	High flavor concentration with balanced astringency	Complex	–
Green	Combined effect of excess of acidity and astringency	Unripe	–
Grainy	Sensation of micro-particles in mouth	Particulate	Sand paper 1000 grade
Satin	A smooth and sliding astringency	Surface smoothness	Satin
Pucker	A reflex action of mouth surfaces being brought together and released in attempt to lubricate mouth surfaces	Dynamic	Burlap
Full-body	Sensation of high viscosity	Complex	–
Persistent	An overall sensation (flavor, tactile, taste) which lasts over time	Complex	–

were familiarized with astringency rating. They were asked to evaluate the overall astringency of water solutions spiked with five different enological tannins at different concentrations (from 0.1 to 5.0 g/L) on a 9-point scale (named: absent, very weak, weak, weak moderate, moderate, moderate strong, strong, very strong, extremely strong). Each sample was evaluated within 5 min. Astringency was expressed as the maximum of intensity perceived. The same tannins were assessed in white wine for intensity rating tests at concentrations from 0.1 to 1.5 g/L. Analysis of variance (ANOVA) on astringency was made considering “sample” as a fixed source of variation, “session” and “panelist” as random effects, and the two-way interactions. Performance of the trained panel was considered adequate since interactions of panelist * session, panelist * sample, and sample * session were not significant ($p > .05$).

A discussion on the perception of subqualities according to the mouthfeel wheel was made after tasting (Gawel et al., 2001; King et al., 2003). The most familiarized terms were selected among 33 astringency definitions. Only terms cited by more than 20% judges of the panel were considered (Campo, Ballester, Langlois, Dacremont, & Valentin, 2010) and were introduced in the check-all-that-apply (CATA) questions (Ares et al., 2015). CATA question is a form of multiple choice question where a list of 16 subqualities terms (sensations of touch, taste, and flavor) is presented and respondents tick the options that they consider to be applicable to the wine. In order to deepen insight the subqualities of astringency, the CATA method was coupled with the use of touch standards as described by different authors (De Miglio & Pickering 2008; Oberholster, Francis, Iland, & Waters, 2009). The novelty consisted into place the standards in a closed

box to avoid the involvement of sight and to enhance the tactile sensation felt by MRs receptors put in fingers (McCabe, Rolls, Bilderbeck, & Mcglone, 2008; Merabet et al., 2004). Training for astringency subqualities was made by evaluating six commercial red wines spiked with 0.2 to 0.5 g/L of five enological tannins, by using CATA question as described in Table 1. To assess the reproducibility of trained assessors when using CATA questions for evaluating the subqualities of wines, the global reproducibility index, proposed by Jaeger et al. (2013), was calculated as follows:

$$Rli = 1/J \sum_{j=1}^{j=J} \left(\frac{ter_{id\ ij}}{ter} \right)$$

where $ter_{id\ ij}$ is the number of terms used by assessor i identically in both repetitions for sample j , ter is the total number of terms of the CATA question, n is the number of samples, and Rli is the global reproducibility index of assessor i , which ranges from 0 (lack of reproducibility) to 1 (perfect reproducibility). The Rli was 0.89, suggesting that on average, trained panelists used reliably by ticking or not ticking 89% of the CATA terms to describe wine.

2.4 | Sample evaluation

Sensory evaluation of wines at $t = 12$ months was made in duplicate. In each session, two tasting evaluations of four unknown samples were performed. They were presented in balanced random order at room temperature ($18^{\circ}\text{C} \pm 2^{\circ}\text{C}$) in black tulip-shaped glasses coded with three-digit random numbers. The assessors were

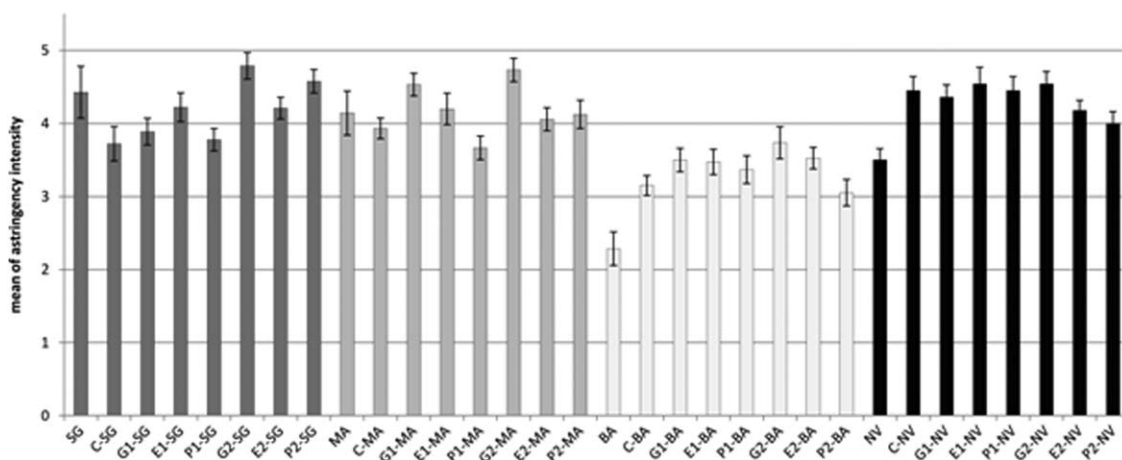


FIGURE 1 Mean of the astringency intensity for Sangiovese, Montepulciano, Barbera, Nero d'Avola wines before (SG, MA, BA, NV) and after 12 months of aging, with tannins at 10 g/hl (G1-E1-P1) and 20 g/hl (G2-E2-P2), and with no tannins added (C-SG, C-MA, C-BA, C-NV). Error bars are calculated as $s/(n)^{1/2}$, where (s) is the standard deviation and (n) is the number of panelists

instructed to pour the whole sample in their mouth, hold it for 8 sec, expectorate and rate the perceived overall astringency using a 9-point scale. Judges waited for 4 min before to rinse with mineral water (Sorgesana, pH \approx 7) for 10 sec twice, and then waited at least 30 sec before the next sample. Each sample was evaluated within 5 min. At the same moment, judges were presented with a CATA question with the 16 terms shown in Table 1. The evaluation procedure was the same as the training sessions.

2.5 | Spectrophotometric analyses

All determinations were performed using a Spectrophotometer Shimadzu UV-1800 model. Wine colorant intensity (CI) and hue were analyzed by Glories (1984) method. Flavans reactive to vanillin were determined according to Di Stefano and Guidoni (1989). Anthocyanins, long polymeric pigments (LPP), short polymeric pigments (SPP), were determined by Harbertson, Picciotto, and Adams (2003). Analyses were made in duplicate on each treatment.

2.6 | Data analysis

As one-way ANOVA analysis, Fisher's least significant differences procedure were used to discriminate among the means of the variables for phenolic analyses when true the assumption of variance homogeneity. Astringency intensity was evaluated by Duncan test. Differences of $p < .05$ were considered significant. Multifactorial ANOVA with second-order interactions was used to evaluate the relationships between variety, concentration, and tannin typology. Correspondence analysis (CA) was performed on the contingency table containing the average citation frequency of terms. The average score of astringency subqualities grouped for tannin typology was projected as illustrative variable in the CA map. Elaborations were carried out by means of XLSTAT software (Addinsoft, XLSTAT 2017). Multiple factor analysis (MFA) was performed on phenolic analyses, astringency intensity, and subqualities with R, using FactoMineR (Lê, Josse, & Husson, 2008) package.

3 | RESULTS

3.1 | Sensory evaluation of astringency

One of the main sensory characteristics of red wine is astringency, which can be defined as drying, puckering, and roughing of the oral cavity after the exposure to tannin-rich wines. SG, BA, MA, and NV wines were treated with three tannins of different origin (G = from grape, E = from oak, and P = from exotic wood) at two concentrations (10 g/hl = 1; 20 g/hl = 2) and aged for 1 year in bottle ($t = 12$). Wines at $t = 0$ and after 1 year were evaluated for the astringency sensation as the mean of the maximum intensity perceived by a trained jury, and results are shown in Figure 1.

After aging, astringency in control wines did not change for SG and MA, while for BA and NV increased respect to time zero. Treated wines were not different from their controls, except SG-P1 and MA-P1, which were considered less astringent (Duncan test $p < .05$). In addition, during wine tasting judges were asked to indicate the astringency subqualities felt in mouth according to CATA methodology. Citation frequencies (%Cf) of the 16 subqualities included in CATA question (Table 1) were organized in a contingency table on which was applied the CA to better visualize the relationship between astringency subqualities and samples categorized for tannin typology (Figure 2).

The first and second dimensions of the CA explained the 83.84% of total variance, and allowed a clear separation between the controls (C) and the treatment with G, from P and E. In the first dimension ($F1 = 60.97\%$), terms such as *green* (acid and astringent), *aggressive*, and *pucker* were mainly associated with no tannin addition (C), and *adhesive* and *grainy* with grape-derived tannin (G). Terms as *mouthcoat*, *full-body*, *persistent* were located at negative values of the first dimension, and were mainly associated with oak-derived tannin (E). Similarly, the *velvet*, *soft*, and *satin* terms were associated with the exotic wood-derived tannin (P).

3.2 | Phenolic content of wines

Before tannin addition ($t = 0$) wines differed for the main phenolic classes (Table 2), in particular SG had the lowest content of total

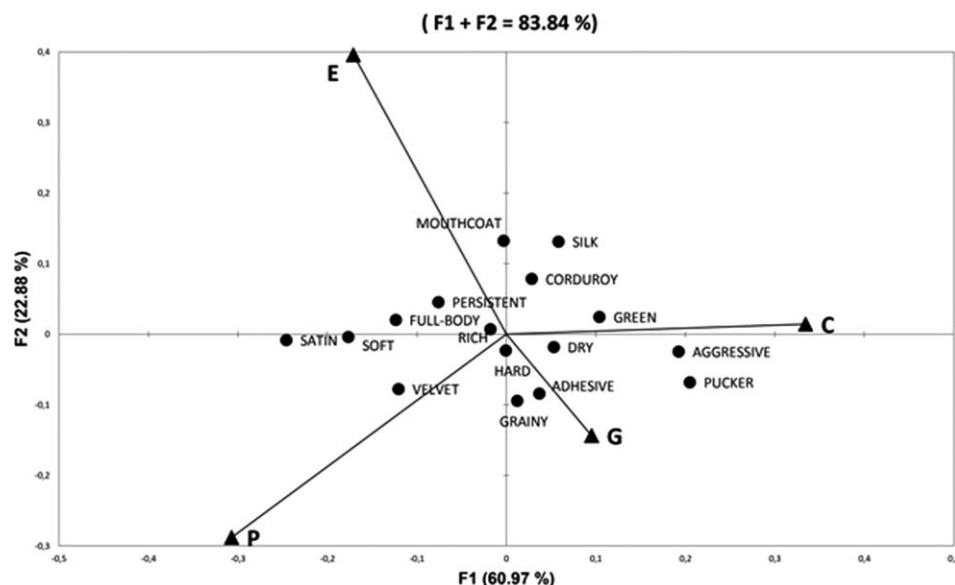


FIGURE 2 Correspondence analysis performed on the mean of citation frequencies of the astringency subqualities included in the check-all-that-apply question and grouped for tannin typology

TABLE 2 The phenolic content of wines at $t = 0$ (SG, MA, BA, NV), and after 12 months of aging

	$t = 0$ SG	Control C-SG	10 g/hl			20 g/hl		
			G1-SG	E1-SG	P1-SG	G2-SG	E2-SG	P2-SG
Total anthocyanins	151.16	68.07	74.19	71.42	69.13	82.84	90.32	81.62
SD	28.76	3.69	5.67	9.53	7.48	2.97	3.95	1.52
CI	7.06	5.66	5.77	5.72	5.49	6.54	6.99	6.45
SD	0.11	0.15	0.26	0.17	0.32	0.38	0.46	0.22
Hue	0.50	0.92	0.90	0.92	0.91	0.96	0.96	0.96
SD	0.01	0.00	0.02	0.02	0.04	0.01	0.02	0.03
SPP	0.94	1.11	1.03	1.12	1.21	1.37	1.01	1.26
SD	0.11	0.06	0.10	0.12	0.11	0.12	0.10	0.12
LPP	0.34	1.21	1.30	1.35	1.24	1.45	1.86	1.47
SD	0.17	0.07	0.10	0.19	0.14	0.08	0.17	0.16
BSA-p tannins	158.40	294.47	315.93	351.85	328.66	378.94	338.77	319.28
SD	27.28	14.54	18.00	21.39	19.17	13.84	13.77	16.07
Flavans	941.64	553.56	567.71	520.57	457.72	540.21	493.86	596.77
SD	69.01	17.66	11.58	32.19	92.59	37.44	44.91	36.65
	$t = 0$ MA	Control C-MA	10 g/hl			20 g/hl		
			G1-MA	E1-MA	P1-MA	G2-MA	E2-MA	P2-MA
Total anthocyanins	323.19	169.73	180.27	175.18	181.86	191.54	188.11	182.11
SD	48.14	2.49	14.37	11.37	8.87	5.78	7.45	8.25
CI	9.69	8.38	9.02	8.90	8.92	9.40	9.94	10.05
SD	0.07	0.31	0.16	0.30	0.30	0.98	0.57	0.21
Hue	0.75	0.84	0.83	0.83	0.84	0.86	0.86	0.87

(Continues)

TABLE 2 (Continued)

	t = 0 MA	Control C-MA	10 g/hl			20 g/hl		
			G1-MA	E1-MA	P1-MA	G2-MA	E2-MA	P2-MA
SD	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SPP	2.04	2.06	2.39	2.04	2.09	2.53	2.43	2.72
SD	0.14	0.28	0.49	0.16	0.16	0.10	0.42	0.19
LPP	0.86	1.28	1.51	1.61	1.53	1.78	1.70	1.69
SD	0.08	0.14	0.11	0.09	0.11	0.15	0.22	0.18
BSA-p tannins	203.90	317.49	317.69	271.16	234.17	222.63	221.84	173.32
SD	14.41	58.24	20.09	18.25	24.96	33.13	42.93	19.24
Flavans	777.29	629.77	790.81	771.95	838.73	875.65	776.67	891.36
SD	2.96	44.62	79.30	25.06	84.36	73.44	74.91	28.50
	t = 0 BA	Control C-BA	10 g/hl			20 g/hl		
			G1-BA	E1-BA	P1-BA	G2-BA	E2-BA	P2-BA
Total anthocyanins	242.78	159.44	151.10	169.71	159.17	162.79	155.39	156.00
SD	37.98	10.13	12.98	8.84	6.04	9.87	9.41	4.81
CI	8.22	9.51	9.10	8.83	8.74	9.10	8.82	8.62
SD	0.22	1.71	0.99	0.65	0.91	1.20	0.81	0.78
Hue	0.73	0.79	0.80	0.78	0.78	0.79	0.78	0.78
SD	0.01	0.03	0.02	0.00	0.01	0.01	0.03	0.01
SPP	1.37	2.59	2.55	2.28	2.36	2.36	2.52	2.51
SD	0.11	0.11	0.20	0.19	0.17	0.10	0.08	0.16
LPP	0.56	1.09	1.27	1.61	1.45	1.74	1.65	1.33
SD	0.12	0.07	0.06	0.08	0.02	0.03	0.13	0.16
BSA-p tannins	0.00	39.84	62.19	77.86	57.66	91.54	77.94	51.61
SD	0.00	4.53	7.02	2.18	6.35	4.22	21.49	7.73
Flavans	490.72	359.53	380.74	309.25	309.25	382.31	268.40	366.60
SD	35.68	36.78	38.00	43.73	74.82	34.65	32.61	35.12
	t = 0 NV	Control C-NV	10 g/hl			20 g/hl		
			G1-NV	E1-NV	P1-NV	G2-NV	E2-NV	P2-NV
Total anthocyanins	330.32	213.60	220.10	201.35	207.11	201.47	214.34	214.22
SD	58.79	2.64	5.69	6.47	3.59	4.87	2.13	1.44
CI	10.04	12.66	13.07	12.78	12.49	13.13	12.93	13.27
SD	0.22	0.57	0.35	0.10	0.39	0.27	0.42	0.50
Hue	0.64	0.70	0.71	0.70	0.70	0.70	0.69	0.71
SD	0.00	0.01	0.02	0.01	0.00	0.01	0.03	0.02
SPP	1.82	3.06	2.80	3.62	3.25	3.56	3.16	3.04
SD	0.04	0.19	0.19	0.10	0.21	0.10	0.22	0.11
LPP	0.79	2.59	2.54	2.53	2.58	2.33	2.55	2.48
SD	0.08	0.13	0.08	0.17	0.12	0.27	0.08	0.16
BSA-p tannins	194.04	287.86	314.11	248.49	276.33	279.11	341.95	284.28
SD	27.61	25.03	30.22	37.95	37.31	13.89	23.54	26.84

(Continues)

TABLE 2 (Continued)

	t = 0 NV	Control C-NV	10 g/hl			20 g/hl		
			G1-NV	E1-NV	P1-NV	G2-NV	E2-NV	P2-NV
Flavans	1,059.47	799.45	808.88	757.03	881.94	836.37	785.31	806.52
SD	57.68	38.44	12.96	32.15	42.19	51.34	26.79	26.83

Note. Values are expressed as the means \pm standard deviations (SD) over four replications. Anthocyanins are expressed as mg/L of malvidin-3-glucoside equivalent. CI is the sum of 420, 520, 620 Abs. Hue is the 420/520 Abs ratio. LPP and SPP are expressed as 520 Abs. Vanillin reactive flavans are expressed as mg/L. BSA-p tannins are expressed in mg/L of catechin equivalent. BA = Barbera; BSA-p tannins = BSA-precipitable tannins; CI = colorant intensity; MA = Montepulciano; NV = Nero d'Avola; SG = Sangiovese.

anthocyanins, BA did not presented polymerized tannins precipitable by BSA protein (BSA-p tannins), NV had a high content of flavans, and MA a medium content of anthocyanins. Total anthocyanins, polymeric pigments (SPP-LPP), BSA-p tannins, flavans, and color parameters of treated wines and controls (the wines at $t = 12$ without tannins) were shown in Table 2.

After 12 months, total anthocyanins drastically decreased in wines, due to the natural degradation of these compounds during aging. The treatment of SG wine with the higher concentration of oak-derived tannin (E2-SG) determined a lower decrease of anthocyanins respect to control wine C-SG ($p < .05$). The CI of SG and MA wines decreased during aging, except in wines treated with tannins at 20 g/hl. The hue (Abs 420/520 nm) increased after 1 year, especially in SG, but did not differ in between wines. The short (SPP) and long (LPP) polymeric pigments represent the compounds resistant to SO_2 bleaching, and provide the stability of wine color. After 1 year, the SPP were significantly higher in G2-MA, P2-MA, and E1-NV, G2-NV than respective controls. Addition of enological tannins in BA promoted the formation of LPP, which in SG and MA was obtained only at 20 g/hl. The BSA-p tannins increased during the aging period, especially in BA. On the contrary, in MA the content of BSA-p tannins decreased as the concentration of enological tannins increased, probably for the incorporation in LPP or precipitation. In NV there were no significant differences.

In order to understand the effect of variety (VARIETY), concentration (CONC), and tannin typology (TANNIN), as well the interactions

between them, a three-way ANOVA was performed, as shown by F ratio and Pr value in Table 3.

For all parameters the major influence was stated by the VARIETY. The factor CONC influenced color parameters (total anthocyanins, CI, hue, LPP, SPP), but not BSA-p tannins and flavans, which were influenced by TANNIN. The three-way ANOVA results revealed also that interactions between factors occurred, and in particular CONC*VARIETY had a significant influence on anthocyanins, CI, hue, and SPP, while for LPP, the interaction between TANNIN*VARIETY was the most significant. The interaction between enological tannins concentration and variety (CONC*VARIETY) highly influenced the BSA-p tannins content of wines.

Excluding the VARIETY effect, a two-way ANOVA was performed on wines of the same variety. It asserted that for SG the higher concentration (20 g/hl versus 10 g/hl) had a great effect on anthocyanins, CI, hue, and LPP, and that oak-derived tannin (E) was the most encouraging LPP formation ($p = .01$). As SG at $t = 0$ showed a low anthocyanin content, enological tannins permitted to stabilize anthocyanins by forming pigmented polymers or co-pigments also thanks to the high content of co-factors as flavans. In a different way, two-way ANOVA results for BA stated that LPP ($p = .0006$), flavans ($p = .0055$), and BSA-p tannins ($p = .0001$) were mainly influenced by tannin typology. Independently from concentration the addition of tannins determined an increase of LPP, the major effect asserted by oak- and grape-derived tannin. In BA wine that at $t = 0$ was medium in anthocyanins,

TABLE 3 A three-way ANOVA results (F ratio and Pr value) on phenolic analysis for concentration (CONC), tannin typology (TANNIN), and variety (VARIETY) effects, as well their interactions

Phenolic analysis	CONC		TANNIN		VARIETY		CONC*TANNIN		CONC*VARIETY		TANNIN*VARIETY	
	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F
Total anthocyanins	8.265	0.005	0.442	0.644	1,085.781	<0.0001	0.584	0.560	4.052	0.009	0.779	0.588
CI	17.829	<0.0001	0.460	0.633	483.314	<0.0001	0.872	0.421	3.619	0.016	0.696	0.654
Hue	23.611	<0.0001	0.315	0.731	750.227	<0.0001	0.581	0.561	11.554	<0.0001	0.971	0.449
SPP	8.789	0.004	0.389	0.679	294.019	<0.0001	2.151	0.122	2.756	0.047	1.767	0.115
LPP	26.765	<0.0001	1.648	0.198	198.094	<0.0001	3.264	0.043	1.581	0.199	4.424	0.001
BSA-p tannins	1.118	0.293	11.020	<0.0001	398.104	<0.0001	1.552	0.217	12.579	<0.0001	1.291	0.270
Flavans	3.034	0.085	13.961	<0.0001	396.551	<0.0001	1.957	0.147	1.227	0.304	1.295	0.268

ANOVA = analysis of variance.

low in flavans, and with no polymerized tannins, the supplementation with exogenous tannins favored color stabilization. While for MA the concentration was the main driver on wine phenolic composition, in NV no significant effects were denoted. The NV being the richest in phenolic compounds was less influenced by tannin addition.

3.3 | Correlation between astringency characteristics and phenolic content of wines

In order to understand how the addition of enological tannins could affect overall wine characteristics a MFA was performed considering four groups of variables: one related to “colour” characteristics (anthocyanins, CI, hue, LPP, SPP), one to “astringency” and related phenolic classes (astringency intensity, BSA-p tannins, flavans), one to astringency “subqualities,” and one to the categorical variable “variety.”

In Figure 3a, on first dimension (Dim1) are positively projected the variables related to “colour” such as LPP, SPP, anthocyanins, CI, and to these were associated the subqualities *velvet*, *rich*, *mouthcoat*, *corduroy*, while the hue was projected at the negative values of Dim1. The “astringency” group (astringency intensity, BSA-p tannins, and flavans) was almost equally projected into the first and second dimensions (Dim2), and in particular the astringency intensity and BSA-p tannins mainly contributed to Dim2 (0.570 and 0.760, respectively), to which were associated the subqualities that classically define astringency as: *dry*, *pucker*, *adhesive*, *aggressive*. Flavans mainly contributed to Dim1 (0.792), probably for the involvement as co-factors in pigmented polymer formation. The “variety” group highly contributed to both dimensions (Dim1 = 0.985; Dim2 = 0.988). The “variety” highly influenced phenolic classes related to color and astringency characteristics.

In Figure 3b, the individual factor map revealed that wines grouped for variety. A clear distinction was observed between the NV, which is located in the positive traits of the “colour” group, and the SG highly characterized by the hue parameter. On the opposite of “astringency” group characteristics are located the BA wines, low in tannins and not astringent, also characterized by *satin* and *soft* subqualities. The MA wines, located at the center of the quadrants, showed intermediate characteristics.

4 | DISCUSSION

Italy represents a wide scenario of red wine varieties, so that same treatments on wines with different phenolic composition can lead to different results. Astringency depends on tannins typology, so that the addition of enological tannins may influence the sensory properties of red wines, and in particular the effect on astringency subqualities has not yet been studied. This study evaluated the effect of tannin addition on changes in astringency and subqualities of wines with different polyphenolic content typical of four Italian regions (SG [Toscana], MA [Abruzzo], BA [Piemonte], and NV [Sicilia]) after an aging period of 1 year.

It is known that during aging the astringency of red wines changes as a consequence of several reactions involving tannins, anthocyanins, and other components of wine matrix. Generally, it is believed that the

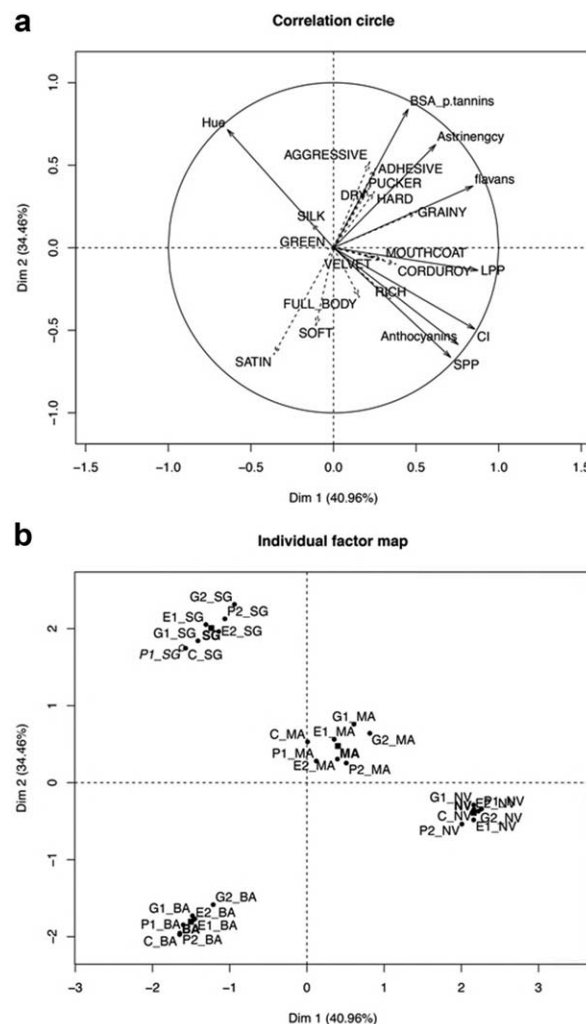


FIGURE 3 Representation of variables (a, correlation circle) and individuals (b, individual factor map) on the first two dimensions of the multiple factor analysis performed on phenolic analyses (Anthocyanins, CI, LPP, SPP, hue, BSA-p tannins, flavans), and astringency characteristics (intensity and subqualities) of Sangiovese, Montepulciano, Barbera, Nero d'Avola wine varieties (SG, MA, BA, NV) after aging

formation of different structures between tannins and anthocyanins can lead to a decrease in astringency (Weber, Greve, Durner, Fischer, & Winterhalter, 2012), also promoted by enological tannins (Picariello, Gambuti, Petracca, Rinaldi, & Moio, 2018). After 1 year, the trained panel did not feel any differences in the perception of astringency intensity between wines treated or not with tannins (Figure 1). Notwithstanding, astringency intensity is not sufficient to fully characterize wine astringency. Thus, during wine tasting trained judges were asked to indicate the astringency subqualities felt in mouth according to CATA questions. CATA question consists of a list of subqualities from which the panelists have to select all the options they consider appropriate to that wine. A similar approach has been recently utilized for the characterization of the astringency subqualities of Tannat wine (Vidal et al., 2017). However, it is the first time that the astringency subqualities of wines aged with tannins have been evaluated.

Subqualities as *soft*, *silk*, *velvet*, *full-body*, *rich*, and *mouthcoat* highly characterized the wines aged with wood-derived tannins (E = oak and P = exotic wood) (Figure 2). Similarly, the term *mouthcoating* mostly characterized wines aged in oak barrel (Vidal et al., 2016). The term *rich*, related to wines with a balanced astringency and a high flavor concentration, was highly associated with *full-body*. In general, wines that are full-bodied are more intense in flavor and vice versa and were highly appreciated by consumers (Niimi, Danner, Li, Bossan, & Bastian, 2017). Soft-related textures (*silky*, *velvety*) have been showed to contribute positively to high quality wine's characterization (Vidal et al., 2017). The use of tannins E and P improved wines of positive and desirable subqualities of astringency. While the tannin from grape (G) enhanced the negative subqualities of astringency as *pucker*, *aggressive*, *adhesive*, and *dry*.

As regard color characteristics, addition of enological tannins enhanced the polymerization between anthocyanins and tannins or flavans to form colored species (LPP) more stable to SO₂ bleaching (Picariello, Gambuti, Picariello, & Moio, 2017) in BA, and at higher concentration in SG and MA. Enological tannins stabilize the color of wine with no polymerized tannins (BA), and with low and medium content of anthocyanins (SG, MA) thanks to the formation of anthocyanin-derived pigments (Chen et al., 2016; Garcia-Estevéz, Alcalde-Eon, Escribano-Bailon, & Puente, 2017; Picariello et al., 2018). In NV the LPP polymerization was achieved independently from tannin addition, meaning that the initial phenolic composition such as, in this case, the high content of anthocyanins respect to tannins (A/T ratio) may represent an important parameter for wine evolution. The different effect of tannins on wine color may depend on wine own characteristics as total phenolic content, A/T ratio, phenolic profile, and SO₂ level, and on the different reactions with wine matrix and other components in which tannins are involved.

In order to understand the impact of the variety on subqualities and phenolic content of wines, a MFA analysis was performed considering the color characteristics, the astringency-related parameters, the astringency subqualities, and the variety as categorical variable. Wines grouped for variety independently from tannin treatment (Figure 3). Each variety was associated to a specific group of variables: the NV was the richest in phenolic compounds and the highest in color parameters, the BA resulted the less tannic, and was felt as *soft* and *satiny*; the SG was highly characterized by hue, probably for the low content in acylated anthocyanins (Arapitsas, Perenzoni, Nicolini, & Mattivi, 2012), which can be responsible for color instability; and the MA was not only highly colored but also astringent.

Some relationships emerged between astringency subqualities and phenolic classes. Among these, anthocyanins are believed to contribute to the increase in the intensity of astringency-related sensory terms (Oberholster et al., 2009), while for others there were not relevant (Vidal et al., 2004). We found that anthocyanins and SPP were not correlated to the intensity of astringency, but conferred to wine a sensation of *corduroy*, that for us represented the sensation of a light wrinkling of the soft palate that can be felt by tongue movements, and a flavor richness (*rich*), probably for an enhanced effect on the volatility and subsequent sensory perception of aroma compounds (Lorrain

et al., 2013). Astringency intensity and BSA-p tannins are correlated with subqualities which classically define astringency: *dry*, *pucker*, *adhesive*, *aggressive*. Flavans are mainly related to *grainy*, which is the feeling in mouth of low molecular flavanols as particulate. Subqualities as *velvet* and *mouthcoat* are correlated with LPP formation which, while conferring color stability, contributed to the tactile sensation of velvety and to the suppleness in mouth.

5 | CONCLUSIONS

The effect of tannin addition on astringency subqualities and phenolic content was evaluated in different red wine varieties. Bottle aging is an usual practice for red wines before commercialization. After 1 year, the aging with enological tannins did not determine an increase in the intensity of wine astringency, even better an improvement of mouth-feel sensations was achieved with wood-derived tannins. In particular, *full-body*, *rich*, and *mouthcoat* subqualities were positively perceived. Enological tannin promoted color stability by pigmented polymer formation in varieties with a low content of anthocyanins and tannins. The variety had a great influence on phenolic content and subqualities of wines aged with enological tannins. So that studied wines preserved their varietal characteristics. In addition, the correlations between subqualities and polyphenolic characteristics of wine may help in understanding the sensory and phenolic evolution of wines during aging. A tailored choice of enological tannins for wine aging can be made considering the phenolic composition of wine variety.

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