Article

Effect of Chitosan on the Removal of Different Types of Tannins from Red Wines

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Abstract: (1) Background: most premium red wines are rich in tannins but the effect of chitosan on these macromolecules is unknown. In this work, the effect of a treatment with 0.5 g/L of chitosan on red wines, W, enriched with condensed tannins, CT, ellagitannins, ET, and gallotannins, GT, was evaluated. In addition, to understand if the effect of C was stable during wine evolution, treated wines underwent an oxidative stress simulating an exposure to 18 mg/L of oxygen. (2) Methods: parameters describing the reactivity of phenolic compounds were determined: iron reactive phenolics, BSA reactive tannins, vanillin reactive tannins, and the saliva precipitation index. Individual anthocyanins, total and polymeric pigments and chromatic characteristics were evaluated to determine the influence of each treatment on colour parameters. (3) Results: a decrease in BSA reactive tannins after the addition of C was detected for all wines and the effect persisted after oxidation. W and CT wines previously treated with C and oxidized showed a significant decrease in the reactivity towards salivary proteins. C caused a lower formation of polymeric pigments in all wines. (4) Conclusion: these results suggest a possible use of C to treat wines very rich in condensed tannins and excessively astringent.

Keywords: chitosan; tannins; protein reactivity; salivary proteins; red wine; astringency; gallotannins; condensed tannins; ellagitannins

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1. Introduction

Sustainability is progressively growing in importance in the wine industry [1,2]. In recent years various industries in oenological products have increased production of new commercial, eco-friendly products, among these, one of the most interesting with characteristics of biocompatibility, biodegradability, and low toxicity is the chitosan C, admitted as a food additive from 2009, (EC) No 606/2009 [3].

Chitosan is a natural polysaccharide derived from chitin with polycationic character and a solubility variable in acidic media due to its deacetylation degree and molecular weight [4]. The use of C has been authorized in must and wine for microbial stabilization or metal and protein removal. The admitted range for the addition of chitosan is 10–500 g/hL according to the aim (Commission regulation (EU)) [5].

Previous studies reported several beneficial effects due to chitosan additions in wine, such as antimicrobial [6,7] and antioxidant activity due to the direct radical scavenging mechanism [8] or indirectly, via metal chelation, which blocks the generation of radical species [9–11]. Chitosan is also of great relevance because it is effective in controlling *B. bruxellensis* during winemaking [12].

Given all these positive properties, C can be used on finished red wines to preserve them from microbial and oxidative spoilage. However, the possible effect on compounds

responsible for wine sensory quality should also be considered. Recently, Castro Marín and colleagues [13] showed that a post-fermentative treatment with 0.5 g/L of C on a Sangiovese wine slightly affected aroma compounds and did not severely impair the quality parameters of the final wines. However, in this same work authors also observed that ellagic acid, a tannic molecule released in wines after contact with wood, was adsorbed onto chitosan and it reduced this content by 40%. In a previous study, we also detected a decrease in BSA reactive tannins after a treatment of an Aglianico wine with C [14].

Tannins are very important for red wine quality as they are responsible for their high dry extract and longevity but also for the sensory attributes linked to their ability to precipitate salivary proteins during tasting, causing an astringent sensation. This sensory attribute is typical of red wine but, if present at high intensity, it results in negative consumer reactions. Because most of premium red wines are rich in tannins and often are previously aged in barrels or in contact with tannins extracted from grapes and wood, it is interesting to determine if C could influence their content and reactivity towards proteins.

During winemaking and storage, the addition of commercial tannins is definitively a practice often used to improve the wine quality. Tannins of grape origin, as well as of wood origin can be used [15]. Wood tannins are the hydrolysable tannins, gallotannins, and ellagitannins, which are extracted from different botanical species [16]. Ellagitannins originate from oak and chestnut wood [17], while gallotannins mainly originate from galla and tara nuts [18]. Grape tannins are condensed flavanols mainly present in berry skins, seeds, and stalks [15]. One of the effects of the addition of tannins is the variation in the pigments and chromatic characteristics of wine, but important is also the effect on sensory attributes such as the astringency and astringency subqualities [19]. This is of particular importance especially considering that red wines rich in tannins change their chromatic characteristics and astringency during aging and exposition to controlled moderate amounts of oxygen [20–22].

Taking into consideration all these aspects, it is worth investigating the possible effect of C on tannins (condensed and hydrolysable) in addition to their reactivity towards salivary proteins, one of the mechanisms at the base of the sensation of astringency of red wines [23,24]. Apart from the evaluation of the reactivity of tannins towards BSA, one of the most objective methods to evaluate their possible astringency is the evaluation of their reactivity towards proteins by means of the determination of the saliva precipitation index (SPI), an index pointed out by means of SDS-page analysis of salivary proteins before and after the binding with wine in lab conditions that simulate the phenomenon occurring during wine tasting and aging [23,24].

The aim of this work is to determine the influence of a treatment with C on red wines with condensed tannins, ellagitannins, and gallotannins previously added. The effect is evaluated before and after exposure of red wines to an oxidative stress aimed at simulating wine aging.

2. Materials and Methods

2.1. Experimental Plan

The experimental plan was performed on an Aglianico red wine produced in South Italy named W. The main oenological parameters of wines (free sulphur dioxide, total sulphur dioxide, residual sugar, malic acid, and lactic acid) were determined using a Y15 BioSystems analyser (BioSystem, Barcelona (Spain) for Sinatech, Montegranaro (FM), Italy). Alcohol, titratable acidity, volatile acidity, and pH were measured according to the OIV Compendium of International Methods of Wine and Must Analysis [25]. The main parameters were alcohol 14.88 $\pm$ 0.33% v/V, titratable acidity 5.23 $\pm$ 0.03 g/L of tartaric acid, pH 3.12 $\pm$ 0.01, volatile acidity 0.16 $\pm$ 0.03 g/L of acetic acid, malic acid 0.90 $\pm$ 0.01 g/L, and lactic acid 0.09 $\pm$ 0.01 g/L.

Wine was divided in 16 aliquots of 500 mL each to have 4 sets of samples, the first set of samples was prepared to evaluate the effect of tannins addition, the second set to evaluate the effect of C addition, and the third and fourth sets to evaluate the effect of forced oxidation.

2.2. Tannins and C Addition

The first set of 4 samples contained: the untreated wine W and samples treated by adding 400 mg /L of three different types of oenological tannins: Oligomeric Tannins (VR Grape Laffort Oenologie, Bordeaux, France) named CT, Gallotannins (Galalcool Laffort Oenologie, France) named GT, and ellagitannins (Quertanin Laffort Oenologie, Bordeaux France) named ET. Thus, the first four wines were obtained: W, CT, ET, and GT. The chemical characterisation of the commercial tannins formulations was previously reported [19]. To obtain the second set of 4 samples, wines prepared as for W, CT, ET, and GT were successively treated with 500 mg/L of C (Sigma-Aldrich CAS: 9012-764) to have the four experimental samples W-C, CT-C, ET-C, and GT-C. Seven days after the treatment, the first and the second sets of wines were centrifuged and filtered (0.45 micron) and then analysed.

2.3. Forced Oxidation Tests

The oxidative response of the different wines was assessed by applying an accelerated oxidation test recently published by Coppola et al. [26]. Wines prepared as previously described for the preparation of the first and second sets of wines (W, CT, ET, GT, and W-C, CT-C, ET-C, and GT-C) were treated by adding 19 mg/L of H_2O_2 (equivalent to 18 mg/L O_2) (30% Fluka, Sigma-Aldrich Chemie GmbH Steinheim, France). The forced oxidation tests were performed at 18 °C, considering a 1:1 stoichiometry of oxygen to hydrogen peroxide. Two other sets of samples were obtained: oxidized wine named Wo, oxidized wine treated with condensed tannins named CTo, oxidized wine treated with ellagitannins named ETo, oxidized wine treated with gallotannins named GTo, oxidized wine treated with chitosan named W-Co, oxidized wine treated with condensed tannins and chitosan named CT-Co, oxidized wine treated with ellagitannins and chitosan named ET-Co, and oxidized wine treated with gallotannins and chitosan named GT-Co.

After fifteen days, the samples were centrifuged and filtered (0.45 micron) and then analysed. All the samples were prepared in duplicate.

2.4. High-Performance Liquid Chromatography Determination of Acetaldehyde

Acetaldehyde was determined by HPLC analysis as described by Han et al. [27].

The derivatization of samples was performed by adding 20 μL of freshly prepared 1120 mg/L SO_2 solution to an aliquot of wine sample (100 μL). The samples were then acidified with 20 μL of 25% sulfuric acid (Carlo Erba reagent 96%), and then 140 μL of 2 g/L 2,4-dinitrophenylhydrazine reagent (Aldrich chemistry) were added.

After mixing, the solution was allowed to react for 15 min at 65 °C and then promptly cooled to room temperature. Analysis of carbonyl hydrazones was conducted by an HPLC (HPLC Shimadzu LC10 ADVP apparatus (Shimadzu Italy, Milan)), consisting of a SCL-10AVP system controller, two LC-10ADVP pumps, a SPD-M 10 AVP detector, and an injection system full Rheodyne model 7725 (Rheodyne, Cotati, CA) equipped with a 50 μL loop. A Waters Spherisorb column (250x4.6 mm, 4 mm particles diameter) was used for separation. The chromatographic conditions were: sample injection volume, 15 mL; flow rate, 0.75 mL/min; column temperature, 35 °C; mobile phase solvents, (A) 0.5% formic acid (Sigma-Aldrich 95%) in water milli-Q (Sigma-Aldrich) and (B) acetonitrile (Sigma-Aldrich 99.9%); and gradient elution protocol, 35% B to 60% B (t = 8 min), 60% B to 90% B (t = 13 min), 90% B to 95% B (t = 15 min, 2-min hold), 95% B to 35% B (t = 17 min, 4-min hold), total run time, 21 min. Eluted peaks were compared with derivatized acetaldehyde standard.

All analyses were conducted through two experimental replicas and two analytical replicas. Data are the means of four values.

2.5. High-Performance Liquid Chromatography (HPLC) Analyses of Monomeric and Polymeric Phenolics

HPLC analysis was performed for the separation and quantification of monomeric and polymeric phenolics according to [28].

A HPLC Shimadzu LC10 ADVP apparatus (Shimadzu Italy, Milan, Italy) equipped with a SCL-10AVP system controller, two LC-10ADVP pumps, a SPD-M 10 AVP detector, and an injection system full Rheodyne model 7725 (Rheodyne, Cotati, CA, USA) was used.

An Agilent PLRP-S 100- Å reversed phase polystyrenedivinyl benzene column (4.6 × 150 mm, 3 μm particle size) protected with a guard cartridge with the same packing material (PLRP-S, 5 × 3 mm) kept at 35 °C was used as the stationary phases.

The HPLC solvents were: solvent A: 1.5% *v*/*v* ortho-phosphoric acid (EMP Chemicals, Gibbstown, NJ, USA) and solvent B consisting of 80% acetonitrile (HPLC grade, Honeywell, Muskegon, MI, USA) with 20% of solvent A.

The following gradient was established: 0-time conditions, B 6%; 73 min, B 31%; 78 min, B 62%, staying constant until 86 min; and 90 min, B 6%. This zero-time solvent mixture was followed by a 15 min equilibrium period prior to injecting the next sample. The flow rate of the mobile phase was 1 mL/min.

Twenty μL of wine or calibration standards were injected onto the column. All the samples were filtered through 0.20 μm Microliter PTFE membrane filters (DWK Life Sciences, Wheaton, Milville, NJ 08332 USA) into dark glass vials and immediately injected into the HPLC system. Detection was carried out by monitoring the absorbance signals at 520 nm for monomeric anthocyanins and polymeric anthocyanins. Eluted peaks were compared with malvidin-3-monoglucoside (Extrasynthese, Lyon, France) on the basis of peak area.

All analyses were conducted through two experimental replicas and two analytical replicas. Data are the means of four values.

2.6. Spectrophotometric Analyses

The chromatic characteristics and spectrophotometric measures were determined using a spectrophotometer (Jenway 7305 Spectrophotometer). Colour intensity (Abs 420 nm + Abs 520 nm + Abs 620 nm) and hue (Abs 420 nm/Abs 520 nm) were evaluated according to the Glories methods [29].

The CIELAB parameters (L∗, a∗, b∗) were determined by using NomaSense Color P100 Equipped with a 0.5 cm cell, the colour differences were calculated as the Euclidean distance between two points in the 3D space defined by L∗, a∗, and b∗, as reported by the Commission Internationale de L'Eclariage (CIE).

Total anthocyanins, short polymeric pigments (SPP), large polymeric pigments (LPP), and bovine serum albumin (Sigma-Aldrich SRL, Milano, Italy) reactive tannins (BSA reactive tannins) were determined by the Harbertson–Adams assay [30].

Vanillin reactive flavans (VRF) were determined as reported in [31].

All analyses were conducted through two experimental replicas and two analytical replicas.

2.7. Saliva Precipitation Index

For the binding assay, 50 μL of saliva was mixed with 25 μL of diluted wine (1:4).

Human saliva used for binding reactions was obtained by mixing resting saliva samples from different individuals. Saliva collection was performed between 10 and 11 a.m. Subjects were asked to follow teeth cleaning instructions in the early morning and not to consume any food and beverage for 2 h before saliva collection. Saliva was collected from six non-smoking volunteers (three males and three females) by expectorating saliva into a pre-weighted ice-cooled tube for 5 min. The resulting mix was centrifuged at 10,000× *g* for 10 min to remove any insoluble material and the supernatant was used for analysis.

Binding assays were performed in Eppendorfs maintained at 37 °C for 5 min. The mixture was then centrifuged for 10 min at 10,000 g. The electrophoretic analysis was performed on the resulting supernatant, representing the salivary proteins that remained in solution after the precipitation by wine tannins. Two binding assays were performed on the two wine replicates.

Saliva samples (before and after the binding assay) mixed with an equal volume of 2xelectrophoresis sample buffer (0.125 M Tris–HCl, 4% SDS; 20% v/v glycerol, 0.2 M DTT, 0.02% bromophenol blue, pH 6.8) and heated at 95 °C for 5 min were analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE) according to the method of [32] using 14% acrylamide resolving gels. The stacking gel was 4% acrylamide (Bio-Rad). Electrophoresis was performed on a Bio-Rad MiniProtean Cell electrophoresis apparatus (Bio-Rad, Milan, Italy) and a PowerPac 1000 Bio-Rad power supply set a 150 V for the stacking and resolving gels. The gels were fixed with a mixture of ethanol, acetic acid, and deionized water (40:10:50) for 1 h. After washing in water for 5 min, the gels were stained with Coomassie Brilliant Blue R250 staining solution (Bio-Rad, Milan, Italy). The destain step was performed by incubation in the destain solution Coomassie Blue R250 (Bio-Rad, Milan, Italy). Molecular weights were estimated by comparison with the migration rates of Precision Plus Protein All Blue protein standards (Bio-Rad, Milan, Italy 3). Densitometric tracing of minigels was performed with a Biorad GS800 Densitometer. The SPI was calculated by the percentage decrease in the bands of saliva before and after the binding assay and expressed in g/L of gallic acid equivalent (GAE), as previously reported [33,34].

2.8. Statistic Analysis

These analyses were performed using XLSTAT (software Addinsoft, 2017.1). The effect of treatments were evaluated by the analysis of the variance (ANOVA) using the Tukey method for the significant differences procedure ($p < 0.05$). All data are means of four values (2 experimental replicates $\times$ 2 analytical replicates).

3. Results and Discussion

The study of the effect of C on wines containing different oenological tannins is divided in two parts: the first aimed at evaluating the effect of tannins and C addition on phenolic compounds, pigments, and chromatic characteristics of treated wines; the second focussed on understanding if changes detected just after the addition of C also persisted after an oxidation treatment aimed at simulating wine evolution.

3.1. The Effect of Chitosan on Wines Rich in Tannins of Different Origin

The effect of tannins addition and C on main phenolic parameters of wines is reported in Figure 1. As expected, the addition of tannins increased the concentration of total phenols and BSA reactive tannins compared with the control wine. The highest significant increase in total phenolics was detected only for GT. As the method we used to determine total phenols evaluated their reactivity towards iron [30], it is expected that gallotannins, molecules that form water-insoluble complexes with iron [35], gave a higher absorbance after the reaction of GT with the ferric chloride used in this assay. Similar results were showed by [36] in a study in which several enological tannins were assessed for their abilities to precipitate BSA protein and to react with ferric chloride.

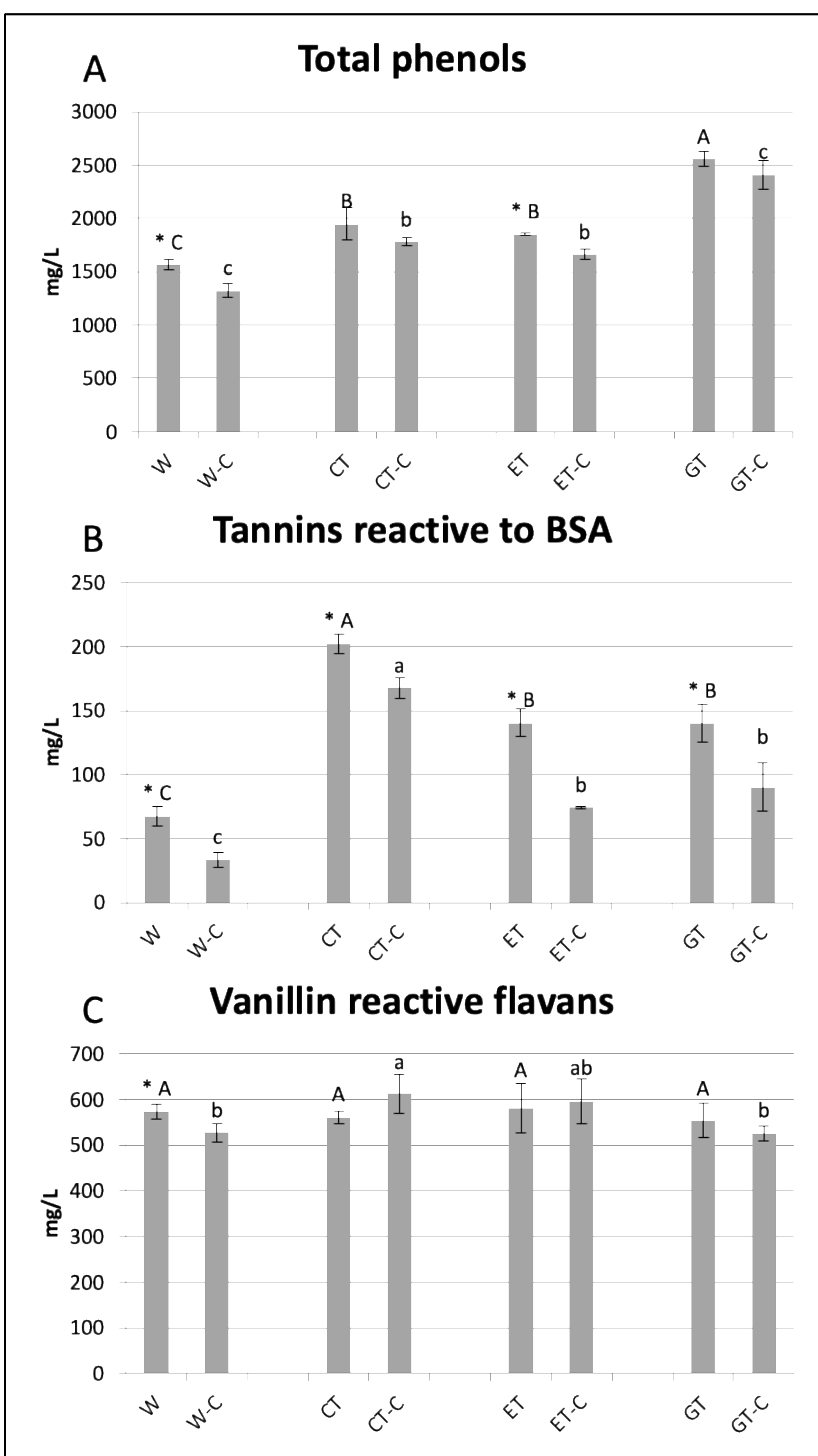

Figure 1. Fining effect on total phenols (**A**), tannins reactive to BSA (**B**), and vanillin reactive flavans (**C**). Different letters indicate a statistically significant difference among treated wines, according to Tukey's (HSD) analysis ($p < 0.05$). The effect of tannin addition is expressed with a capital letter on wines W, CT, ET, and GT (A, B, C), and the effect of chitosan addition is expressed with a lowercase letter on wines W-C, CT-C, ET-C, and GT-C (a, b, c). The asterisk (*) indicates a statistical difference between each kind of wine before and after the treatment with chitosan. All data are expressed as means ± standard deviation.

In agreement with a previous study [37], the CT sample showed the greatest increase in BSA reactive tannins compared with the treated wines. The increase is due to the molecular structure of condensed tannins which mainly are constituted of proanthocyanidins with a polymerization degree and composition in monomers highly reactive towards BSA [36]. No effect of enological tannins was detected for vanillin reactive flavans, VRF, probably because vanillin is mainly reactive towards low molecular weight proanthocyanidins [38].

Concerning the effect of C addition, a slight decrease in total phenols in W and ET (−15.68% and −10.03%, respectively) was determined. According to Bassi et al. (2000) [39], C does not directly interact with phenolics and the decrease in phenolics compounds is more ascribable to a sedimentation effect [40]. In agreement with recent results [14], a decrease in BSA reactive tannins after the addition of C was detected for all wines. A slight VRF removal was instead detected only for W. In a previous study on white wines, Spagna et al. [41] detected a decrease in VRF after a treatment with C of a Sauvignon Blanc in which the levels of VRF were largely accountable for the presence of monomer catechins. In red wines, the amount of catechins is low compared with the structures with a higher polymerization degree and this could justify the absence of an effect of the treatment with C on the reactivity of wines towards vanillin, especially in wines treated with commercial tannins. A possible competition for the adsorption on C of different tannic structures is not ruled out. If this is the case, the adsorption of phenolic structures highly reactive towards BSA is favoured.

As reported in Table 1, after the addition of tannins, a decrease in individual anthocyanins analysed by HPLC was detected and the decrease was higher for ET. Simultaneously, the polymeric pigments increased in all experimental samples, probably due to the involvement of native anthocyanins in the formation of new pigments at higher polymerization degree, as already observed in previous experiments [42].

Table 1. Effect of tannins and C on total anthocyanin, polymeric pigments, and acetaldehyde determined by HPLC methods.

	Total Native Anthocyanins (mg/L)		Polymeric Pigments (mg/L)		Acetaldehyde (mg/L)	
W	1330.18 ± 20.44	* A	137.30 ± 9.39	C	11.74 ± 0.10	AB
W-C	1231.73 ± 13.07	a	136.74 ± 24.49	c	11.81 ± 0.23	a
CT	1235.17 ± 27.14	B	204.92 ± 34.33	B	11.75 ± 0.07	B
CT-C	1137.24 ± 74.98	a	207.01 ± 18.26	b	11.83 ± 0.05	a
ET	1173.72 ± 44.15	C	216.44 ± 2.40	* B	11.90 ± 0.09	AB
ET-C	1147.02 ± 29.01	a	199.27 ± 3.60	b	11.76 ± 0.06	a
GT	1185.37 ± 16.59	* BC	264.72 ± 6.49	* A	11.92 ± 0.02	A
GT-C	1132.69 ± 25.32	a	246.25 ± 4.40	a	11.88 ± 0.04	a

Different letters indicate a statistically significant difference among treated wines, according to Tukey's (HSD) analysis ($p < 0.05$). The effect of tannins addition is expressed with a capital letter on wines W, CT, ET, and GT before the addition of C (A, B, C), and the effect of chitosan addition on wines W, CT, ET, and GT is expressed with a lowercase letter on wines W-C, CT-C, ET-C, and GT-C (a, b, c). The asterisk (*) indicates a statistical difference between each kind of wine before and after the treatment with chitosan. All data are expressed as means ± standard deviation.

Concerning C, in agreement with literature [14,43] the effect on pigments was limited but significant. The removal of total monomeric anthocyanins was significant for W and GT and those of polymeric pigments for ET and GT. However, the loss was never higher than 7.40%. Looking at the individual anthocyanins (Table 2), only in W the addition of C determined a loss of almost all anthocyanins suggesting that in wines enriched with enological tannins, a possible competition for the adsorption on C occurred. The effect of tannins and C on acetaldehyde content of wines was not significant.

Table 2. Effect of tannins and C on native anthocyanins concentrations (mg/L).

	Delphinidin 3-Glucoside (mg/L)		Cyanidin 3-Glucoside (mg/L)		Petunidin 3-Glucoside (mg/L)		Peonidin 3-Glucoside (mg/L)		Malvidin 3-Glucoside (mg/L)		Malvidin 3-(6II-Acetyl)-Glucoside (mg/L)		Malvidin 3-(6II-Coumaroyl)-Glucoside (mg/L)	
W	248.92 ± 14.36	* A	10.97 ± 0.76	AB	174.10 ± 4.40	* A	91.77 ± 0.64	B	609.42 ± 16.31	* A	150.20 ± 2.50	* A	44.80 ± 1.48	AB
W-C	204.16 ± 6.36	a	9.96 ± 0.54	b	162.20 ± 4.06	a	89.67 ± 3.78	a	577.55 ± 8.49	a	144.79 ± 1.41	a	43.40 ± 1.85	a
CT	242.62 ± 20.99	AB	7.85 ± 1.08	C	169.50 ± 4.96	* AB	98.58 ± 3.27	* A	520.92 ± 17.89	B	148.40 ± 3.00	A	47.28 ± 2.03	A
CT-C	214.95 ± 24.50	a	9.80 ± 0.83	b	154.95 ± 4.54	ab	92.38 ± 1.22	a	470.10 ± 31.68	c	140.01 ± 6.77	a	55.04 ± 17.57	a
ET	205.24 ± 22.95	BC	9.48 ± 1.14	BC	155.15 ± 9.65	C	90.33 ± 2.78	B	549.18 ± 6.10	* B	126.45 ± 14.41	B	37.89 ± 1.10	C
ET-C	201.81 ± 22.04	a	10.05 ± 0.70	b	146.95 ± 4.12	b	88.39 ± 2.16	a	527.28 ± 5.00	ab	131.95 ± 8.51	ab	40.59 ± 3.61	a
GT	203.45 ± 4.72	* C	11.69 ± 0.21	A	158.82 ± 3.18	* BC	93.90 ± 1.59	AB	544.34 ± 26.09	B	129.31 ± 7.75	B	43.87 ± 0.99	B
GT-C	194.60 ± 3.22	a	12.68 ± 1.07	a	152.31 ± 1.29	b	90.03 ± 4.73	a	516.52 ± 26.71	bc	124.36 ± 6.41	b	42.19 ± 2.51	a

Different letters indicate a statistically significant difference among treated wines, according to Tukey's (HSD) analysis ($p < 0.05$). The effect of tannins addition is expressed with a capital letter on wines W, CT, ET, and GT (A, B, C), and the effect of chitosan addition is expressed with a lowercase letter on wines W-C, CT-C, ET-C, and GT-C (a, b, c). The asterisk (*) indicates a statistical difference between each kind of wine before and after the treatment with chitosan. All data are expressed as means ± standard deviation.

The effect detected for monomeric and polymeric anthocyanins is evident also for total anthocyanins and for main chromatic characteristics (Table 3). In agreement with literature [43,44], colour intensity and tonality slightly increased after the addition of tannins (Table 3). However, for all wines the addition of chitosan determined a slight decrease in CI and, for CT and ET, also in hue. Because CT and ET showed a lower content of polymeric pigments compared with GT, it is likely that, in wine treated with condensed tannins and ellagitannins, anthocyanins are more involved in copigmentation reactions influencing colour intensity [45].

Table 3. Effect of tannins and C on total anthocyanins, colour intensity, and tonality (evaluated by using spectrophotometric methods).

	Total Anthocyanins (mg/L)		Colour Intensity (420 nm + 520 nm + 620 nm abs units)		Tonality (420 nm/520 nm)	
W	301.32 ± 2.77	* AB	4.11 ± 0.10	* B	0.54 ± 0.01	B
W-C	288.90 ± 3.00	a	3.83 ± 0.07	c	0.53 ± 0.00	a
CT	308.01 ± 4.38	* A	4.38 ± 0.27	* A	0.61 ± 0.05	* A
CT-C	292.13 ± 1.91	a	3.96 ± 0.03	ab	0.56 ± 0.00	a
ET	307.79 ± 5.20	* A	4.25 ± 0.03	* AB	0.57 ± 0.00	*AB
ET-C	289.63 ± 2.37	a	4.02 ± 0.17	a	0.53 ± 0.02	a
GT	298.01 ± 1.56	* B	4.19 ± 0.08	* B	0.53 ± 0.00	* B
GT-C	285.96 ± 4.37	a	3.87 ± 0.06	bc	0.53 ± 0.00	a

Different letters indicate a statistically significant difference among treated wines according to Tukey's (HSD) analysis ($p < 0.05$). The effect of tannins addition is expressed with a capital letter on wines W, CT, ET, and GT (A, B), and the effect of chitosan addition is expressed with a lowercase letter on wines W-C, CT-C, ET-C, and GT-C (a, b, c). The asterisk (*) indicates a statistical difference between each kind of wine before and after the treatment with chitosan. All data are expressed as means ± standard deviation.

A parameter useful to understand the effect of a treatment on human visual perception was given by CieLAB analysis and it is the ΔE. The ΔE value is used to express the overall colour difference between a sample and the control. According to Mokrzycki et al. (1999) [46], two samples show a visible difference if ΔE calculated between them is higher than 1.5. In this study, we performed the calculation of ΔE with the aim to understand if the effect of C addition was significant (Table 4).

Table 4. Effect of C on ΔE calculated between samples treated with C and related controls.

	ΔE	
W-WC	1.99 ± 0.59	b
CT-CT C	2.73 ± 0.73	b
ET-ET C	4.37 ± 0.54	a
GT-GT C	2.81 ± 0.89	b

W-WC: the ΔE value related to the overall colour difference between W and WC; CT-CT C: the ΔE value related to the overall colour difference between CT and CT C; ET-ET C: the ΔE value related to the overall colour difference between ET and ET C; GT-GT C: the ΔE value related to the overall colour difference between GT and GT C. Different letters indicate a statistically significant difference among treated wines according to Tukey's (HSD) analysis ($p < 0.05$). The effect of tannins or chitosan addition is expressed with letters (a, b). All data are expressed as means ± standard deviation.

All wines treated with C showed a ΔE higher than 1.5 when compared with the respective untreated sample and the higher difference was observed for ET. This suggests a possible well visible impact of C especially on wines rich in ellagitannins.

3.2. Forced Oxidation Trial

Several studies showed the antioxidant activities of tannins [47] and chitosan, but no study addresses the effect of both treatments on the main phenolic parameters of red wines after an exposure at controlled amounts of oxygen. To obtain this information, all experimental wines underwent a controlled oxidative stress [26].

A significant variation in the reactivity of tannins after oxidation was detected (Figure 2). For all wines, the BSA reactive tannins increased (Figure 2B) and for W-Co, CT-Co, ET-Co, and GT-Co a decrease in VRF occurred (Figure 2C). Data on total phenols did not follow

a clear trend, which can be justified considering all factors influencing the formation of coloured complexes among phenolic structures and iron chloride used for this assay [36]. Concerning the treatment with C, data highlighted that, after oxidation, the effect of this treatment on BSA reactive tannins and VRF is still evident but less enhanced.

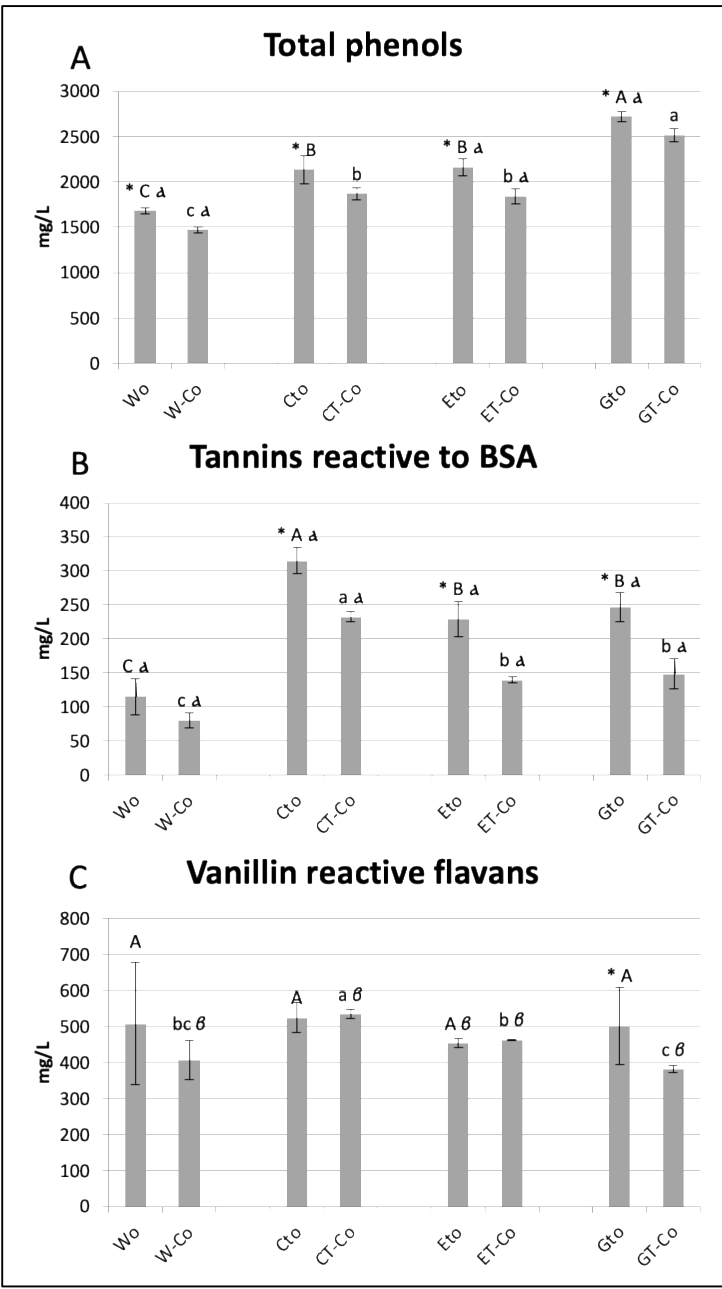

Figure 2. Oxidation effect on total phenols (**A**), tannins reactive to BSA (**B**), and vanillin reactive flavans (**C**) of treated wines. Different letters indicate a statistically significant difference among treated wines according to Tukey's (HSD) analysis ($p < 0.05$). The effect of tannins addition is expressed with a capital letter on wines Wo, CTo, ETo, and GTo (A, B, C), and the effect of chitosan addition is expressed with a lowercase letter on wines W-Co, CT-Co, ET-Co, and GT-Co (a, b, c). The asterisk (*) indicates a statistical difference between each kind of wine before and after the treatment with chitosan. The Greek symbol (α) is used if the value is significantly higher than the non-oxidized sample, and the symbol (β) is used if the value is significantly lower than the non-oxidized sample. All data are expressed as means ± standard deviation.

As expected, after oxidation total individual anthocyanins dramatically decreased while polymeric pigments and acetaldehyde increased (Table 5). The highest increase in polymeric pigments was detected for CT as already observed in a comparative experiment performed on red wines added with the same kind of tannins [37]. The effect of C on the content of polymeric pigments was higher than those observed before oxidation (between 10.02% and 19.70%). This is not surprising because, as observed for BSA reactive tannins, in this case it is also possible that the removal of molecules with a molecular size ranging from trimer to octamer, showed to be more reactive to BSA, is favoured.

Table 5. Oxidation effect on acetaldehyde, total anthocyanins, native anthocyanins, and polymeric pigments of treated wines.

	Total Native Anthocyanins (mg/L)		Polymeric Pigments (mg/L)		Acetaldehyde (mg/L)	
Wo	58.10 ± 4.02	A β	367.88 ± 13.67	* C α	66.10 ± 0.30	* A α
W-Co	46.77 ± 6.66	a β	331.01 ± 5.39	b α	62.76 ± 0.34	a α
CTo	50.62 ± 7.99	A β	507.34 ± 12.28	* A α	64.27 ± 0.13	* C α
CT-Co	46.39 ± 2.27	ab β	418.53 ± 15.84	a α	60.74 ± 0.13	b α
ETo	49.74 ± 0.23	* A β	330.24 ± 2.02	* D α	64.69 ± 0.30	* C α
ET-Co	38.78 ± 0.67	b β	265.16 ± 2.18	c α	58.47 ± 0.57	d α
GTo	46.33 ± 6.67	* A β	416.83 ± 4.08	* B α	65.42 ± 0.15	* B α
GT-Co	41.33 ± 1.18	ab β	337.17 ± 8.56	b α	59.71 ± 0.12	c α

Different letters indicate a statistically significant difference among treated wines according to Tukey's (HSD) analysis ($p < 0.05$). The effect of tannins addition is expressed with a capital letter on wines Wo, CTo, ETo, and GTo (A, B, C,D). The effect of chitosan addition is expressed with a lowercase letter on wines W-Co, CT-Co, ET-Co, and GT-Co (a, b, c, d). The asterisk (*) indicates a statistical difference between each kind of wine before and after the treatment with chitosan. The Greek symbol (α) is used if the value is significantly higher than the non-oxidized sample, and the symbol (β) is used if the value is significantly lower than the non-oxidized sample. All data are expressed as means ± standard deviation.

It is interesting to observe that the additions of C positively impacted the final concentrations of acetaldehyde in all treated wine. The best effect was observed for the combination of GT and C. In a previous study [11], the authors showed that C operates against oxidation by means of a direct radical scavenging mechanism, however, it can also act indirectly, via metal chelation [48], which would block the generation of radical species following the Fenton reaction [9,10,49]. However, it should also be considered that part of acetaldehyde may have been involved in the formation of ethyl-bridged anthocyanins and flavanols [50,51].

Concerning total individual anthocyanins, the presence of C did not influence the final concentration in wines, except for the ET-C and GT-C samples, while the effect on each individual anthocyanins was significant in few cases: delphinidin 3-glucoside in GT-C, petunidin 3-glucoside in W-C, malvidin 3-glucoside in ET-C, and malvidin 3-(6II-acetyl)-glucoside in W-C and ET-C (Table 6).

Table 6. Oxidation effect on native anthocyanins.

	Delphinidin 3-Glucoside (mg/L)		Cyanidin 3-Glucoside (mg/L)	Petunidin 3-Glucoside (mg/L)		Peonidin 3-Glucoside (mg/L)	Malvidin 3-Glucoside (mg/L)		Malvidin 3-(6II-Acetyl)-Glucoside (mg/L)		Malvidin 3-(6II-Coumaroyl)-Glucoside (mg/L)
Wo	5.88 ± 0.92	AB β	ND	6.20 ± 0.91	* A β	ND	30.79 ± 1.08	A β	10.92 ± 1.94	* A β	ND
W-Co	4.76 ± 1.02	ab β	ND	4.13 ± 0.69	a β	ND	26.24 ± 6.66	a β	7.40 ± 0.34	a β	ND
CTo	5.61 ± 0.78	A β	ND	4.45 ± 0.75	A β	ND	26.71 ± 5.41	A β	9.18 ± 1.37	A β	ND
CT-Co	5.40 ± 1.02	a β	ND	4.91 ± 0.83	a β	ND	24.67 ± 2.52	a β	6.91 ± 1.55	ab β	ND
ETo	4.26 ± 0.41	BC β	ND	4.55 ± 0.77	A β	ND	27.30 ± 0.12	* A β	10.66 ± 0.38	* A β	ND
ET-Co	3.16 ± 0.93	b β	ND	3.71 ± 0.67	a β	ND	22.44 ± 0.05	a β	5.48 ± 0.02	b β	ND
GTo	3.64 ± 0.44	* C β	ND	4.41 ± 0.86	A β	ND	26.85 ± 3.27	A β	7.75 ± 1.41	A β	ND
GT-Co	2.99 ± 0.27	b β	ND	3.97 ± 0.17	a β	ND	24.59 ± 0.54	a β	6.78 ± 0.63	ab β	ND

Different letters indicate a statistically significant difference among treated wines according to Tukey's (HSD) analysis ($p < 0.05$). The effect of tannins addition is expressed with a capital letter on wines Wo, CTo, ETo, and GTo (A, B, C). The effect of chitosan addition is expressed with a lowercase letter on wines W-Co, CT-Co, ET-Co, and GT-Co (a, b). The asterisk (*) indicates a statistical difference between each kind of wine before and after the treatment with chitosan. The Greek symbol (β) is used if the value is significantly lower than the non-oxidized sample. All data are expressed as means ± standard deviation.

The changes in total anthocyanins and chromatic characteristics after oxidation were as expected: a decrease in total anthocyanins and colour intensity and an increase in tonality (Table 7). Significant data points were those on ΔE between wines treated and untreated with C: for all experimental wines after oxidation the effect of C was well visible (ΔE > 3) (Table 8) and the trend was: ET > GT > CT and W. The great effect of C on ellagitannins agrees with previous results [43] and it deserves future investigation.

Table 7. Oxidation effect on total anthocyanins, colour intensity, and tonality of treated wines.

	Total Anthocyanins (mg/L)		Colour Intensity (420 nm + 520 nm + 620 nm)		Tonality (420 nm/520 nm)	
Wo	114.49 ± 2.11	* C β	3.01 ± 0.16	* A β	0.73 ± 0.01	* B α
W-Co	104.63 ± 0.56	b β	2.62 ± 0.13	ab β	0.75 ± 0.00	a α
CTo	123.16 ± 2.04	* A β	2.99 ± 0.21	A β	0.74 ± 0.00	AB α
CT-Co	110.74 ± 1.74	a β	2.64 ± 0.17	a β	0.74 ± 0.00	a α
ETo	119.56 ± 1.28	* AB β	2.83 ± 0.12	* A β	0.73 ± 0.01	* B α
ET-Co	94.26 ± 1.67	c β	2.27 ± 0.20	b β	0.77 ± 0.01	a α
GTo	118.09 ± 0.88	* BC β	2.91 ± 0.11	* A β	0.79 ± 0.06	A α
GT-Co	95.81 ± 2.08	c β	2.64 ± 0.15	a β	0.78 ± 0.00	a α

Different letters indicate a statistically significant difference among treated wines according to Tukey's (HSD) analysis ($p < 0.05$). The effect of tannins addition is expressed with a capital letter on wines Wo, CTo, ETo, and GTo (A, B, C), and the effect of chitosan addition is expressed with a lowercase letter on wines W-Co, CT-Co, ET-Co, and GT-Co (a, b, c). The asterisk (*) indicates a statistical difference between each kind of wine before and after the treatment with chitosan. The Greek symbol (α) is used if the value is significantly higher than the non-oxidized sample, and the symbol (β) is used if the value is significantly lower than the non-oxidized sample. All data are expressed as means ± standard deviation.

Table 8. Oxidation effect on ΔE.

	ΔE	
Wo-WCo	2.79 ± 0.50	c
CTo-CT Co	3.49 ± 0.38	c
ETo-ET Co	7.64 ± 0.11	a
GTo-GT Co	5.09 ± 0.18	b

Different letters indicate a statistically significant difference among treated wines according to Tukey's (HSD) analysis ($p < 0.05$). The effect of tannins or chitosan addition is expressed by a lowercase letter (a, b, c). All data are expressed as means ± standard deviation.

3.3. Impact of C and Oxidative Stress on the Reactivity of Wines towards Saliva

The SPI (saliva precipitation index) was performed to evaluate the reactivity of the wines towards salivary proteins, representing an indirect method to measure wine astringency [34] before and after the treatment with C. According to the ANOVA analysis ($p = 0.005$) that considered the effect of C on SPI, the C highly influenced the precipitation of salivary proteins, resulting in a possible decrease in the astringency in control wine and wine treated with condensed tannins (Figure 3).

This result was also observed in a previous work in which the treatments with C at different phases of winemaking significantly reduced the precipitation of polymeric tannins reactive to BSA [14]. The most astringent wine was GTo, confirming that the addition of gallotannins can influence the astringency negatively [33]. Even with the addition of CTo tannin (condensed tannin), the wine may result more reactive towards salivary proteins and likely astringent. However, the chitosan treatment decreased the SPI values in a significant way, and the percentage decrease was higher in CT-Co (−23%) than in the other treated wines. The chitosan probably showed a higher affinity for condensed tannins, allowing more precipitation of the formed complexes with salivary proteins [41]. The control wine Wo did not differ from the wine added with ET tannin (ellagitannin), and after the treatment W-Co and ET-Co were less astringent, similar also to CT-Co.

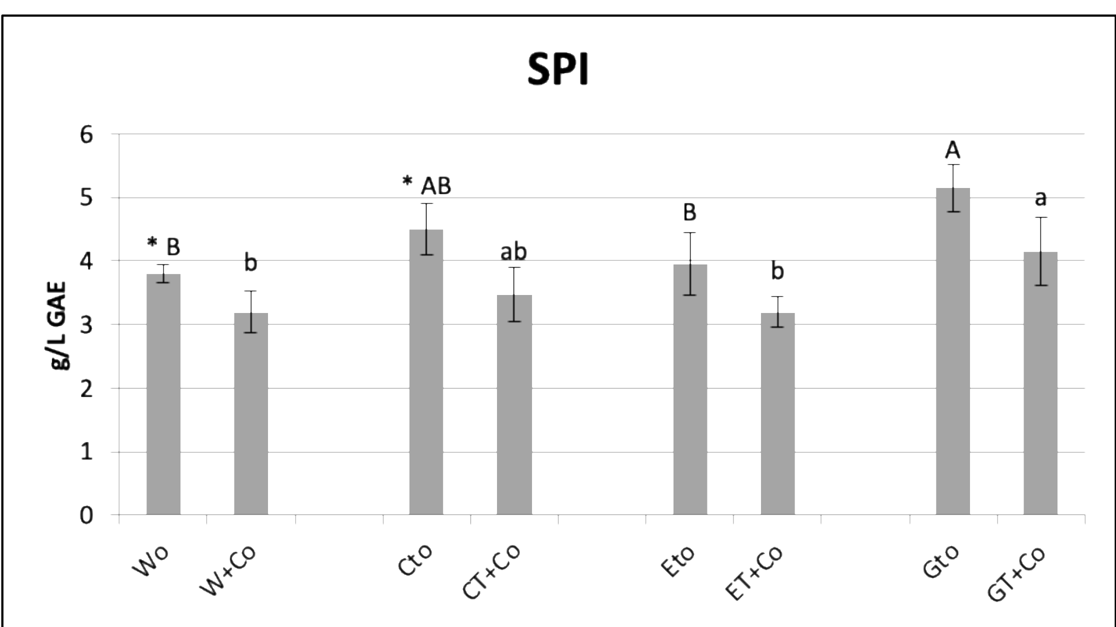

Figure 3. Oxidation effect on the SPI (saliva precipitation index) of the treated wines. Different letters indicate a statistically significant difference among treated wines according to Tukey's (HSD) analysis ($p < 0.05$). The effect of tannins addition is expressed with a capital letter on wines Wo, CTo, ETo, and GTo (A, B), and the effect of chitosan addition is expressed with a lowercase letter on wines W-Co, CT-Co, ET-Co, and GT-Co (a, b). The asterisk (*) indicates a statistical difference between each kind of wine before and after the treatment with chitosan. All data are expressed as means ± standard deviation.

4. Conclusions

Data obtained in this study showed a significant effect of C on both red wine and tannin-enriched wine. The greatest effect was detected for ET and CT. After oxidation, the treatment with C of wines added with condensed tannins determined the higher formation of polymeric pigments while a great variation in chromatic characteristics was detected for control wines and wines added with CT. For all wines, the treatment with C determined a lower production of acetaldehyde after oxidative stress. The removal of tannins, due to the absorption on C before oxidation, and the lower production of acetaldehyde, due to the antioxidant effect of C, caused a lower formation of polymeric pigments in all wines.

This is the first study in which an effect of C on the reactivity towards salivary proteins of wines was detected. As a decrease of SPI was detected, these results suggest a possible use of C to treat wines very rich in tannins that could be not well accepted by consumers for their excessive astringency. Mechanisms involved in the removal of tannins of different origin in red wines should be better elucidated in future studies.

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References

1. Borsellino, V.; Migliore, G.; D'Acquisto, M.; Di Franco, C.P.; Asciuto, A.; Schimmenti, E. Green' wine through a responsible and efficient production: A case study of a sustainable Sicilian wine producer. *Agric. Agric. Sci. Procedia* **2016**, *8*, 186–192. [CrossRef]
2. Chiusano, L.; Cerutti, A.K.; Cravero, M.C.; Bruun, S.; Gerbi, V. An industrial ecology approach to solve wine surpluses problem: The case study of an Italian winery. *J. Clean. Prod.* **2015**, *91*, 56–63. [CrossRef]
3. Luo, Y.; Wang, Q. Recent advances of chitosan and its derivatives for novel applications in food science. *J. Food Process. Beverages* **2013**, *1*, 1–13.
4. Friedman, M.; Juneja, V.K. Review of antimicrobial and antioxidative activities of chitosans in food. *J. Food Prot.* **2010**, *73*, 1737–1761. [CrossRef]
5. European Union (EU). Commission regulation (EU) 53/2011 of 21 January 2011. *Off. J. Eur. Union*. 2011, pp. L19/1–L19/6. Available online: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:019:0001:0006:EN:PDF (accessed on 5 December 2021).
6. Brasselet, C.; Pierre, G.; Dubessay, P.; Dols-Lafargue, M.; Coulon, J.; Maupeu, J.; Delattre, C. Modification of chitosan for the generation of functional derivatives. *Appl. Sci.* **2019**, *9*, 1321. [CrossRef]
7. Kong, M.; Chen, X.G.; Xing, K.; Park, H.J. Antimicrobial properties of chitosan and mode of action: A state of the art review. *Int. J. Food Microbiol.* **2010**, *144*, 51–63. [CrossRef] [PubMed]
8. Park, P.; Je, J.; Kim, S. Free radical scavenging activities of differently deacetylated chitosans using an ESR SPECTROMETER. *Carbohydr. Polym.* **2004**, *55*, 17–22. [CrossRef]
9. Guibal, E. Interactions of metal ions with chitosan-based sorbents: A review. *Sep. Purif. Technol.* **2004**, *38*, 43–74. [CrossRef]
10. Schreiber, S.B.; Bozell, J.J.; Hayes, D.G.; Zivanovic, S. Introduction of primary antioxidant activity to chitosan for application as a multifunctional food packaging material. *Food Hydrocoll.* **2013**, *33*, 207–214. [CrossRef]
11. Castro Marín, A.; Culcasi, M.; Cassien, M.; Stocker, P.; Thétiot-Laurent, S.; Robillard, B.; Pietri, S. Chitosan as an antioxidant alternative to sulphites in oenology: EPR investigation of inhibitory mechanisms. *Food Chem.* **2019**, *285*, 67–76. [CrossRef]
12. Taillandier, P.; Joannis-Cassan, C.; Jentzer, J.B.; Gautier, S.; Sieczkowski, N.; Granes, D.; Brandam, C. Effect of a fungal chitosan preparation on Brettanomyces bruxellensis, a wine contaminant. *J. Appl. Microbiol.* **2015**, *118*, 123–131. [CrossRef]
13. Castro Marín, A.; Colangelo, D.; Lambri, M.; Riponi, C.; Chinnici, F. Relevance and perspectives of the use of chitosan in winemaking: A review. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 3450–3464. [CrossRef]
14. Picariello, L.; Rinaldi, A.; Blaiotta, G.; Moio, L.; Pirozzi, P.; Gambuti, A. Effectiveness of chitosan as an alternative to sulfites in red wine production. *Eur. Food Res. Technol.* **2020**, *246*, 1795–1804. [CrossRef]
15. Versari, A.; Du Toit, W.; Parpinello, G.P. Oenological tannins: A review. *Aust. J. Grape Wine Res.* **2013**, *19*, 1–10. [CrossRef]
16. Fernández de Simón, B.; Cadahía, E.; Conde, E.; García-Vallejo, M.C. Low molecular weight phenolic compounds in Spanish oak woods. *J. Agric. Food Chem.* **1996**, *44*, 1507–1511. [CrossRef]
17. Jourdes, M.; Pouységu, L.; Quideau, S.; Mattivi, F.; Truchado, P.; Tomás Barberán, F.A. Hydrolyzable tannins: Gallotannins, ellagitannins, and ellagic acid. *Handb. Anal. Act. Compd. Funct. Foods* **2012**, 435–460.
18. Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. *Handbook of Enology, Volume 2: The Chemistry of Wine Stabilization and Treatments*; John Wiley & Sons: Hoboken, NJ, USA, 2006.
19. Picariello, L.; Rinaldi, A.; Forino, M.; Errichiello, F.; Moio, L.; Gambuti, A. Effect of Different Enological Tannins on Oxygen Consumption, Phenolic Compounds, Color and Astringency Evolution of Aglianico Wine. *Molecules* **2020**, *25*, 4607. [CrossRef]
20. Singleton, V.L.; Trousdale, E.K. Anthocyanin-tannin interactions explaining differences in polymeric phenols between white and red wines. *Am. J. Enol. Vitic.* **1992**, *43*, 63–70.
21. Gambuti, A.; Picariello, L.; Rinaldi, A.; Moio, L. Evolution of Sangiovese wines with varied tannin and anthocyanin ratios during oxidative aging. *Front. Chem.* **2018**, *6*, 63. [CrossRef] [PubMed]
22. Rinaldi, A.; Picariello, L.; Soares, S.; Brandão, E.; de Freitas, V.; Moio, L.; Gambuti, A. Effect of oxidation on color parameters, tannins, and sensory characteristics of Sangiovese wines. *Eur. Food Res. Technol.* **2021**, *247*, 2977–2991. [CrossRef]
23. Gambuti, A.; Rinaldi, A.; Pessina, R.; Moio, L. Evaluation of aglianico grape skin and seed polyphenol astringency by SDS–PAGE electrophoresis of salivary proteins after the binding reaction. *Food Chem.* **2006**, *97*, 614–620. [CrossRef]
24. Gambuti, A.; Picariello, L.; Rinaldi, A.; Ugliano, M.; Moio, L. Impact of 5-year bottle aging under controlled oxygen exposure on sulfur dioxide and phenolic composition of tannin-rich red wines. *OENO One* **2020**, *54*, 623–636. [CrossRef]
25. Office International de la Vigne et du Vin. *Compendium of International Methods of Wine and Must Analysis*; Office International de la Vigne et du Vin: Paris, France, 2019.
26. Coppola, F.; Picariello, L.; Forino, M.; Moio, L.; Gambuti, A. Comparison of three accelerated oxidation tests applied to red wines with different chemical composition. *Molecules* **2021**, *26*, 815. [CrossRef]
27. Han, G.; Wang, H.; Webb, M.R.; Waterhouse, A.L. A rapid, one step preparation for measuring selected free plus SO2-bound wine carbonyls by HPLC-DAD/MS. *Talanta* **2015**, *134*, 596–602. [CrossRef] [PubMed]
28. Waterhouse, A.L.; Price, S.F.; McCord, J.D. Reversed-phase high-performance liquid chromatography methods for analysis of wine polyphenols. *Method Enzymol.* **1999**, *299*, 113–121.
29. Glories, Y. La couleur des vins rouges: 2e. Partie: Mesure, origine et interpretation. *Connaiss. De La Vigne Et Du Vin* **1984**, *18*, 253–271. [CrossRef]

30. Harbertson, J.F.; Picciotto, E.A.; Adams, D.O. Measurement of polymeric pigments in grape berry extract sand wines using a protein precipitation assay combined with bisulfite bleaching. *Am. J. Enol. Vitic.* **2003**, *54*, 301–306.
31. Gambuti, A.; Han, G.; Peterson, A.L.; Waterhouse, A.L. Sulfur dioxide and glutathione alter the outcome of microoxygenation. *Am. J. Enol. Vitic.* **2015**, *66*, 411–423. [CrossRef]
32. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–685. [CrossRef]
33. Rinaldi, A.; Gambuti, A.; Moine-Ledoux, V.; Moio, L. Evaluation of the astringency of commercial tannins by means of the SDS–PAGE-based method. *Food Chem.* **2010**, *122*, 951–956. [CrossRef]
34. Rinaldi, A.; Gambuti, A.; Moio, L. Application of the SPI (Saliva Precipitation Index) to the evaluation of red wine astringency. *Food Chem.* **2012**, *135*, 2498–2504. [CrossRef] [PubMed]
35. Haslam, E. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. *J. Nat. Prod.* **1996**, *59*, 205–215. [CrossRef] [PubMed]
36. Harbertson, J.F.; Parpinello, G.P.; Heymann, H.; Downey, M.O. Impact of exogenous tannin additions on wine chemistry and wine sensory character. *Food Chem.* **2012**, *131*, 999–1008. [CrossRef]
37. Picariello, L.; Gambuti, A.; Picariello, B.; Moio, L. Evolution of pigments, tannins and acetaldehyde during forced oxidation of red wine: Effect of tannins addition. *LWT* **2017**, *77*, 370–375. [CrossRef]
38. Vrhovsek, U.; Mattivi, F.; Waterhouse, A.L. Analysis of red wine phenolics: Comparison of HPLC and spectrophotometric methods. *Vitis* **2001**, *40*, 87–91.
39. Bassi, R.; Prasher, S.O.; Simpson, B.K. Removal of selected metal ions from aqueous solutions using chitosan flakes. *Sep. Sci. Technol.* **2000**, *35*, 547–560. [CrossRef]
40. Dıblan, S.; Özkan, M. Effects of various clarification treatments on anthocyanins, color, phenolics and antioxidant activity of red grape juice. *Food Chem.* **2021**, *352*, 129321. [CrossRef]
41. Spagna, G.; Pifferi, P.G.; Rangoni, C.; Mattivi, F.; Nicolini, G.; Palmonari, R. The stabilization of white wines by adsorption of phenolic compounds on chitin and chitosan. *Food Res. Int.* **1996**, *29*, 241–248. [CrossRef]
42. Picariello, L.; Gambuti, A.; Petracca, F.; Rinaldi, A.; Moio, L. Enological tannins affect acetaldehyde evolution, colour stability and tannin reactivity during forced oxidation of red wine. *Int. J. Food Sci. Technol.* **2018**, *53*, 228–236. [CrossRef]
43. Castro Marin, A.; Chinnici, F. Physico-Chemical Features of Sangiovese Wine as Affected by a Post-Fermentative Treatment with Chitosan. *Appl. Sci.* **2020**, *10*, 6877. [CrossRef]
44. Bautista-Ortín, A.B.; Martínez-Cutillas, A.; Ros-García, J.M.; López-Roca, J.M.; Gómez-Plaza, E. Improving colour extraction and stability in red wines: The use of maceration enzymes and enological tannins. *Int. J. Food Sci. Technol.* **2005**, *40*, 867–878. [CrossRef]
45. Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *Am. J. Enol. Vitic.* **2001**, *52*, 67–87.
46. Mokrzycki, W.S.; Tatol, M. Colour differenceΔ E-A survey. *Mach. Graph. Vis.* **2011**, *20*, 383–411.
47. Vignault, A.; González-Centeno, M.R.; Pascual, O.; Gombau, J.; Jourdes, M.; Moine, V.; Teissedre, P.L. Chemical characterization, antioxidant properties and oxygen consumption rate of 36 commercial oenological tannins in a model wine solution. *Food Chem.* **2018**, *268*, 210–219. [CrossRef] [PubMed]
48. Chinnici, F.; Natali, N.; Riponi, C. Efficacy of chitosan in inhibiting the oxidation of (+)-catechin in white wine model solutions. *J. Agric. Food Chem.* **2014**, *62*, 9868–9875. [CrossRef] [PubMed]
49. Waterhouse, A.L.; Laurie, V.F. Oxidation of wine phenolics: A critical evaluation and hypotheses. *Am. J. Enol. Vitic.* **2006**, *57*, 306–313.
50. Timberlake, C.F.; Bridle, P. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* **1976**, *27*, 97–105.
51. Atanasova, V.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Effect of oxygenation on polyphenol changes occurring in the course of winemaking. *Anal. Chim. Acta* **2002**, *458*, 15–27. [CrossRef]