



Microstructure and oenological potential of vine-shoot chips as affected by chips pre-treatments and addition strategy

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ABSTRACT

This work aims at evaluating the effect of vine-shoot chips pretreatment and their addition strategy on the final chemical composition of Aglianico and Nero di Troia wines. Primitivo vine-shoot chips were subjected to three pretreatments (toasted, boiled and toasted, steam-treated and toasted) and then added to the wines either during maceration or aging phases.

Microstructural analyses showed that toasting caused the formation and propagation of cracks in chips, increasing their surface area; while boiling and steam-treatment induced the collapsing of the chips inner structure, limiting wine-chip exchanges. Toasted chips in maceration reduced the titratable acidity, total phenolic and ethanol contents, flavonoids concentration and increased macro and microelement concentration of the wine. When vine-shoot chips were added during aging, S, P, K, Cl, Fe, quercetin, color intensity, vanillin-reactive flavans, antioxidant activity and anthocyanins reduced, and no effect of the chip pretreatment was observed.

1. Introduction

Nowadays, the concept of sustainability in waste management has been extended to many agri-food sectors including the wine production chain (Baiano, 2021). Some researchers have shown that wine consumers are generally interested in wines produced in environmentally friendly or sustainable conditions (Capitello & Sirieix, 2019). Indeed, the wine life cycle is not free from environmental problems, which include large amounts of organic and inorganic waste produced during the wine production process (Maicas & Mateo, 2020).

Wineries globally produce around 13 million tons of solid wastes and by-products (seeds, skins, stems, and grape marc) every year, and large amounts of wastewater during washing and clarification stages (Baiano, 2021). Among them, vine shoots, also called canes (Aliaño-González et al., 2020; Goufo et al., 2020), constitute the majority of viticulture wastes generated during vine annual pruning (Sánchez et al., 2002). They are usually left on site, directly used as organic fertilizer in

vineyards or burnt, causing significant environmental problems (Sánchez-Gómez et al., 2017). The amount of this waste generated every year is very high, considering that in 2022 the world vineyard surface area was estimated at 7.3 million hectares, with Italy representing the 9.9% of the world's vineyard area, positioned fourth in the world ranking, after Spain, France, and China (OIV, 2023). In particular, the Apulia region, situated in the south-east of Italy, covers a vineyard surface area of approximately 96,971 ha (15.4% of the national vineyard area) (ISTAT, 2020). Within this context, vine shoots valorization could have both a positive economic impact on the wine sector and an environmental benefit, reducing the life cycle impact of the wine production chain. Different approaches have been evaluated by researchers for the exploitation of vine pruning residues: biofuel production (Kovacs et al., 2022), extraction of bioactive compounds (Noviello et al., 2022), production of fertilizers and biostimulants (Sánchez-Gómez et al., 2017), preparation of activated carbon to remove from wine undesired compounds, like those responsible of browning (Calderón-Martín et al.,

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2022). In such a *circular economy* perspective, some researchers have proposed the use of toasted and untoasted vine-shoot chips as an alternative to common oak chips, during winemaking and aging processes, to modify the phenolic, volatile and mineral composition of wine (Cebrián-Tarancón et al., 2022; Cebrián-Tarancón et al., 2022, 2023; Cebrián-Tarancón, Sánchez-Gómez, Cabrita, et al., 2019; Cebrián-Tarancón, Sánchez-Gómez, Carot, et al., 2019; Fanzone et al., 2021; Noviello, Antonino, et al., 2024; Noviello et al., 2025), without cytotoxic effects (Cebrián-Tarancón et al., 2021).

The main components of vine shoots are cellulose and hemicellulose (68%), lignin (20%), carbohydrates, proteins, a small fraction of mineral elements, phenolic and volatile compounds (Sánchez-Gómez et al., 2017). However, the type of pretreatment can influence the type and concentration of the chemical compounds which can be extracted from vine-shoot chips (Noviello et al., 2025; Rajha et al., 2014). The toasting process of vine-shoot chips significantly changes shoots chemical composition, increasing the amount of extractable phenolic and volatile compounds. The best toasting conditions for vine-shoots chips were set at 180 °C for 45 min (Cebrián-Tarancón et al., 2018). High temperatures cause modifications in the physical structure of wood and induce the thermal degradation of wood biopolymers (such as lignin or polysaccharides) through hydro-thermolysis and pyrolysis reactions (Delgado de la Torre et al., 2015). It should be considered that vine-shoot chips can also contain relatively high amounts of starch (Lebon et al., 2005), whose granular structure (Buttrose, 1969), including amorphous fractions, can be more easily affected by physical pre-treatments compared to structural polysaccharides and lignin. These reactions also generate a great number of volatile compounds (such as volatile phenols, furanic compounds, aldehydes, and ketones) that will be transferred to wine during aging, improving their sensorial quality (Martínez-Gil et al., 2022). Therefore, investigating the effects of different thermal treatments on vine-shoots is essential to understand their oenological potentiality.

It has also been demonstrated that vine shoots are rich in some mineral elements such as K, Ca, Mg, Fe, Zn and Na, whose concentration depends on various factors such as the pedo-climatic conditions and the grape variety (Cebrián-Tarancón et al., 2021; Sánchez-Gómez et al., 2017). The elemental composition of wine is affected by many factors such as the soil characteristics, vineyard site, winemaking process, storage and ageing (Catarino et al., 2018; Grainger et al., 2021; Kaya et al., 2017; Shimizu et al., 2020), and it can have significant effects on wine quality, oxidative and tartaric stability, as well as on the consumers' health, as in the case of metals (Tariba, 2011). Research highlights that physical pre-treatments of wood and grape canes, such as boiling or steaming, could influence solute extractability, impact on subsequent thermal degradation reactions, induce starch granule swelling, therefore influencing their adsorption/desorption capacity and the pattern and amounts of released compounds (Andlauer et al., 2025; Jančíková & Jablonský, 2025).

Based on these premises, simple physical pretreatments could be considered as potential modulators of the oenological performances of vine-shoot chips, providing a tailored in-house tool to winemakers. The present study investigates, for the first time, the effect of vine-shoot chips pre-treatment on their microstructure and evaluates oenological performances of vine-shoot chips in two different application scenarios such as the maceration phase and aging. For this purpose, chips were produced from Primitivo vine shoots and added into two Apulian red wines: Aglianico and Nero di Troia.

2. Materials and methods

2.1. Vine shoots sampling and treatment

Vine shoots of Primitivo wine grape cultivar were winter pruned randomly from different areas of a commercial vineyard located in Corato (Puglia, Italy) (coordinates: 41° 05' 36" N, 16° 20' 24" E). About

20 kg of vine shoots were collected with a length of 40 cm and stored intact for 6 months at room temperature (18 ± 3 °C) in the dark (Cebrián et al., 2017), with a final humidity of 9.5 %, measured using a thermobalance (Ladwag Mac 110/NP, Radwag, Poland). Then, vine shoots were cut into 3-4 cm pieces and ground with a hammer miller (Dietz-motoren KG, Elektromotorenfabrik, 7319 Dettingen-teck, Germany) to a particle size around 2-20 mm (similar to commonly used oak chips). The chips obtained from this process were treated in three different ways:

- 1) Toasted vine-shoot chips (T): chips were subjected to a toasting process at 180 °C using a thermostatic oven (TFC 120 Forced air Oven, ArgoLab), as previously reported (Cebrián-Tarancón et al., 2018) with a slight increase of the toasting time after preliminary trials (1 h instead of 45 min, data not shown) (Noviello, Paradiso, et al., 2024).
- 2) Boiled and toasted vine-shoot chips (BT): vine-shoot pieces were immersed in boiling water for 5 min, dried overnight at 45 °C in a thermostatic oven, and toasted as described before. The concentration of trace elements in the water used for boiling the vine-shoot chips is: S (15 mg L⁻¹), Cl (38 mg L⁻¹), K (6 mg L⁻¹), Ca (65 mg L⁻¹), Ti (15 µg L⁻¹), Br (70 µg L⁻¹), Rb (3.5 µg L⁻¹) and Sr (411 µg L⁻¹).
- 3) Steam-treated and toasted vine-shoot chips (ST): vine-shoot pieces were steam-treated for 30 min, dried at 55 °C in a thermostatic oven for 2 h, and toasted at 180 °C for 1 h as described before.

In addition, as a comparison, commercial toasted oak chips (O distributed by I-OAK srl, (Dello, BS, Italy) were used.

2.2. Vine shoots characterization

2.2.1. Analysis of the chips' microstructure

Three vine-shoot chip samples (V1, V2, V3) having a length of approximately 10 mm and showing all branch anatomical parts (from bark to pith) were selected for the analysis with high resolution micro-X-ray computed tomography (µCT).

To assess structural, morphological and morphometric variations occurring due to the underwent treatment, vine-shoot chips were analyzed before and after each of the studied treatments, e.g. toasting (T), boiling and toasting (BT), steam treatment and toasting (ST) (Fig. S1).

A SkyScan 1272 µCT instrument (Bruker Nano GmbH, Berlin, Germany) equipped with a W micro-focus source (40 kV, 250 µA, <5 µm spot size) and a 16 M-pixel CCD detector was used. Radiographies were acquired setting a pixel size of 2.0 µm, a rotation step of 0.2° (from 0 to 180°) and an exposure time of 2100 ms, for a total scan duration of 3h. To improve data quality, flat field correction, averaging of 3 frames, and random movement of 10 pixels were applied during acquisition. The reconstruction of radiographies was performed using the software NRecon version 1.6.10.4. The software DataViewer (version 1.5.2.4 64bit, Bruker µCT®) and CTvox (version 3.1.1 r1191, Bruker µCT®) were used for the 2D and the 3D rendering, respectively.

2.2.2. Analysis of the chips' surface morphology

After µCT analysis, the surface morphology of the untreated and T, BT, ST chips was further studied at the micrometric scale using a Zeiss Sigma 300 VP field emission gun scanning electron microscope (SEM) (Zeiss Oberkochen, Germany). For the analysis, an electron acceleration voltage of 10 kV, an aperture of 30 µm and a working distance of 3 to 6 mm was used. Chips were fixed up on an aluminum stub with an adhesive graphite disc. The analyses were performed under variable pressure (P = 133 Pa), and micrographs were acquired with a variable-pressure secondary-electron detector (VPSED).

2.2.3. Analysis of the micro and macro elements, total phenolic content and antioxidant activity of vine-shoot chips

The concentration of micro and macro elements in both vine-shoot chips and oak chips was determined by total-reflection X-ray fluorescence spectrometry (TXRF). Approximately, 10 g of chips were pulverized using a vibromilling system (MM 400, Retsch GmbH, Haan, Germany) equipped with zirconium oxide jars and balls to avoid contamination. Each sample was ground using two cycles (30 Hz for 1 min, each), each followed by a stirring phase. Then, 100.0 ± 5.0 mg of pulverized sample were dispersed into 5 mL of Triton X-100 1% aqueous solution. An aliquot of 10 μ L of Ga solution (Gallium Standard for AAS, TraceCERT®, 1000 mg L⁻¹) was added as an internal standard, and the suspension was vortexed for 30 s. Each sample was prepared and analyzed in triplicate. The suspensions were placed in an ultrasonic bath for 15 min, then vortexed for a few seconds. After this step, 10 μ L was deposited at the center of a siliconized (SERVA Electrophoresis GmbH, Heidelberg, Germany) quartz reflector and dried at 50 °C on a hot plate. To avoid possible contamination, sample suspension deposition and drying were performed under a laminar flow hood. Measurements were performed with an S2 PICOFOX TXRF spectrometer (Bruker Nano GmbH, Berlin, Germany), equipped with a Mo source (30 W, 50 kV, 600 μ A), a multilayer monochromator with an energy resolution better than 150 eV (@Mn-K α , 10 kcps) and an Xflash® SDD detector (30 mm²). The acquisition time for all spectra was set to 1000 s. Spectra were analyzed with SPECTRA 7® software (Bruker Nano GmbH, Berlin, Germany).

The phenolic content and antioxidant activity of vine-shoot and oak chips were determined after extraction as previously reported (Dorosh et al., 2020), with some modifications. All samples (2 g) were extracted with 100 mL of a 50:50 ethanol:water (v/v) solution in an ultrasonic bath using a Bandelin Sonoplus GM3200 sonicator (Bandelin electronic GmbH & Co KG Heinrichstraße 3-4D, Berlin, Germany), connected to a probe with a diameter of 13 mm (Bandelin Sonoplus 497 Titanteller TT13) at a frequency of 20 kHz, 150 W, 60 °C for 60 min. The extracts obtained were centrifuged at 16,000 \times g (Mod. SL 16R Centrifuge, Thermo Fisher Scientific, Massachusetts, USA) for 15 min at 4 °C. The total phenolic content (TPC, mg of gallic acid equivalents g⁻¹) and antioxidant activity (DPPH assay and ABTS assay, μ mol Trolox Equivalent g⁻¹) were determined as described in section 2.3.2.

2.3. Vine-shoot chips application in winemaking

2.3.1. Vineyard's characteristics and winemaking

The research was carried out on Aglianico (A) and Nero di Troia (NT) (*Vitis vinifera* L.) grapes which were obtained from two different commercial vineyards located in Castel del Monte area (Apulia region, Southern Italy, near Corato; 41°04'35" N; 16°21'45" E – Aglianico; 41°04'46" N; 16°21'34" E – Nero di Troia). Approximately 200 kg of grapes of each variety were handpicked and packed in 20 kg perforated plastic boxes and transferred to the experimental winery of the CREA (Council for Agricultural Research and Economics, Research Center for Viticulture and Enology - Bari, Italy) for the winemaking trials. The experimental design of trials is shown in Fig. 1, and the following winemaking technologies were tested.

- Control (C): Grapes of the two varieties were crushed, de-stemmed and the must poured into 100 L stainless steel vats. The musts were sulphated (6 g K₂S₂O₅ hL⁻¹), inoculated with the commercial *Saccharomyces cerevisiae* (20 g hL⁻¹) and yeast activator (SafCENO™NDA 21, Fermentis, Marquette-lez-Lille, France). Maceration was performed for 7 days with 2 punch-downs per day. Then, draining was applied to recover the free-run wine, and the grape pomace was gently pressed to recover press-run wine, by using an 80 L stainless-steel hydro press (Enotecnica Pillan, Camisano Vicentino, Italy). The free-run and press-run wines were blended and raked after 2 weeks to eliminate gross lees. Then, to promote the malolactic fermentation a yeast parietal extract (Enolis Scorza P, L'Enotecnica s. r.l.) (30 g hL⁻¹), the commercial *Oenococcus oeni* (Enobacter red boost, L'Enotecnica s. r.l.) (1 g hL⁻¹), combined with a formulation composed of yeast peel, vitamins (B₂, B₅, B₆) and potassium caseinate (Enoactiv Bacter, L'Enotecnica s. r.l.) (30 g hL⁻¹) were added. The fermentation took place under controlled conditions at 20 °C. The malic acid decrease was monitored using a Foss WineScan FT 120, as described by the manufacturer (Foss, Hillerød, Denmark).
- Maceration with chips (M): as control, but with addition of toasted (T), boiled and toasted (BT), steam-treated and toasted (ST), and oak chips (O) in maceration (12 g L⁻¹, before alcoholic fermentation).
- Aging with chips (A): as control, but with addition of toasted (T), boiled and toasted (BT), steam and toasted (ST), and oak chips (O) in

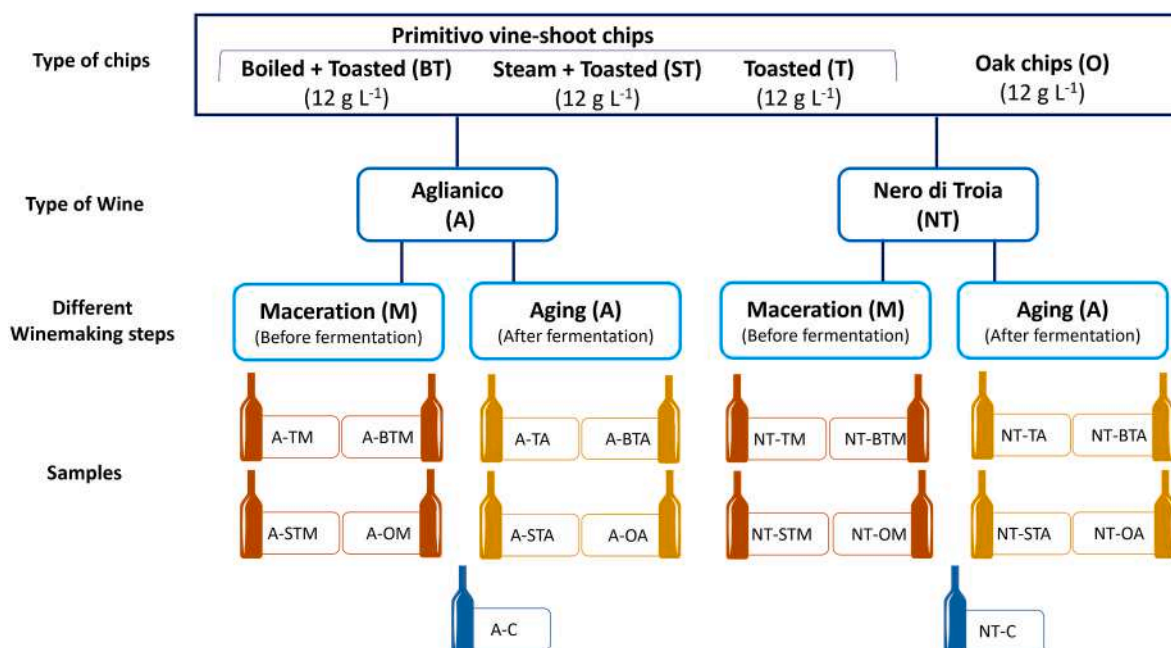


Fig. 1. Winemaking trials scheme.

aging (12 g L⁻¹, after alcoholic and malolactic fermentation). All the types of chips were kept for 35 days in bottles of 2 L.

At the end of the experimental winemaking process, wines were racked into dark green Bordeaux bottles. The oenological parameters of wines, such as ethanol (E, % v/v), pH, titratable acidity (TA, g L⁻¹), volatile acidity (VA, g L⁻¹ acetic acid), malic acid (MA, g L⁻¹) and lactic acid content (LA, g L⁻¹), dry reduced extract (DRE, g L⁻¹), and ashes (g L⁻¹) were analyzed by using a Foss WineScan FT 120 (Foss, Hillerød, Denmark) and are reported in Table 1.

A detailed list of the samples obtained with their acronyms used throughout the text is reported in Table S1. All tests were done in triplicate and analyzed simultaneously.

2.3.2. Assessment of phenolic composition, color indices and antioxidant activity

Total phenolic content (TPC as mg L⁻¹ of gallic acid equivalents), flavonoids (F, as mg L⁻¹ of (+)-catechin), anthocyanins (A, as mg L⁻¹ of malvidin-3-glucoside), vanillin-reactive flavans (VRF, as mg L⁻¹ of (+)-catechin) and proanthocyanidins (Pr, as mg L⁻¹ of cyanidin chloride) and color indices (CD, color intensity; H, hue) were analyzed by using an Evolution 60S UV-Vis spectrophotometer (Thermo Fisher Scientific, Rodano, Italy) (Gambacorta et al., 2011; Glories, 1984).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed on the wines according to the procedure of Tarantino et al. (2020). Briefly, each extract (50 µL) was combined with 950 µL DPPH* solution (0.08 mmol L⁻¹ in ethanol). The decrease in absorbance was read at 517 nm using the UV-Vis spectrophotometer. The results were expressed in µmol Trolox equivalents L⁻¹ for all wines (µmol TE L⁻¹). All the determinations were carried out in triplicate. Antioxidant activity was also determined by ABTS-TEAC assay (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)/Trolox Equivalent Antioxidant Capacity) (Tarantino et al., 2020). For spectrophotometry determinations, the reaction took place directly in cuvettes by adding 50 µL of each sample to 950 µL of final ABTS*⁺ solution. After 8 min, the decrease in absorbance was measured at 734 nm, using the UV-visible spectrophotometer. The results were expressed as µmol Trolox equivalents (TE) L⁻¹ for all wines (µmol TE L⁻¹). Each sample was analyzed in triplicate.

The quantitative analysis of phenolic compounds was performed according to the procedure previously reported in the literature (Noviello, Antonino, et al., 2024) and using an ultra-high performance liquid chromatography (UHPLC) Ultimate 3000RS (Dionex), composed by LPG-3400RS quaternary pump, WPS-3000 TRS autosampler, TCC-3000RS column compartment, photodiode array (PDA) 3000RS

detector (Thermo Fisher Scientific, Waltham, MA, USA). The analytical separation of phenols was achieved using a Hypersil Q C18 column 100 mm of length, 2.1 mm internal diameter and 1.9 µm of particle size, held at 30 °C and at a constant flow of 0.3 mL min⁻¹ with water-formic acid (90:10 v/v) (solvent A) and acetonitrile-formic acid (99.9:0.1 v/v) (solvent B). The gradient program of solvent A was: 0-20 min from 98% to 30%; 20-24 min isocratic at 30%. Then equilibration at the initial conditions for 9 min. The PDA detector was set to scan from 220 to 600 nm of wavelength managed by a 3D field.

Quantitative analysis was performed according to the external standard method based on calibration curves obtained by injecting different concentrations of standard solutions. Specifically, the standard used were: (+)-catechin, (+)-epicatechin, malvidin-3-O-glucoside, quercetin were phyproof® reference substances (PhytoLab, Dutendorfer, Germany); gallic acid, caftaric acid, syringic acid, p-coumaric acid, rutin hydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA); trans-resveratrol was purchased from United States Pharmacopeia (USP, Maryland, United States).

2.3.3. Micro and macro elements

The elemental composition of the produced wines was studied by TXRF. Samples were prepared as follow: 10 µL of a Ga solution (100 mg L⁻¹) were added to 990 µL of each wine sample. After homogenization, 10 µL of the sample were transferred onto a quartz reflector (previously made hydrophobic) and dried at 50 °C for 10 min on a heating plate. Samples were analyzed in triplicates. The instrumental and analytical specifications are reported in section 2.2.3.

2.4. Statistical analysis

One-way analysis of variance (ANOVA) was applied to evaluate the differences among the different samples of vine-shoot chips. Tukey's test was employed for the post-hoc comparisons of the means ($p < 0.05$). In order to assess the effect of the treatments on the final wines, in the first instance cluster analysis was performed by means of heatmaps. In a second step, sparse partial least square (sPLS) analysis was carried out to identify the most relevant variables which can describe each class of wine. Both heatmaps and sPLS analyses were performed using Metaboanalyst 6.0 (<https://www.metaboanalyst.ca/>).

Table 1

Average concentration and standard deviation (n = 3) of TPC (mg of gallic acid equivalents g⁻¹), DPPH (µmol Trolox Equivalent g⁻¹), ABTS (µmol Trolox Equivalent g⁻¹) and elements' concentration (mg kg⁻¹) in oak and vine-shoot chips.

Parameter	O	WT	T	BT	ST
TPC	86.8 ± 4.8 ^a	33.2 ± 0.7 ^b	31.5 ± 3.0 ^b	21.3 ± 5.2 ^b	25.1 ± 0.8 ^b
DPPH	555 ± 57 ^a	236 ± 54 ^b	136 ± 46 ^b	104 ± 41 ^b	96 ± 15 ^b
ABTS	830 ± 149 ^a	306 ± 62 ^b	279 ± 70 ^b	199.±6 ^b	235 ± 35 ^b
Element					
P	< LOD	1196 ± 189 ^a	924 ± 131 ^a	969 ± 192 ^a	1183 ± 42 ^a
S	45.4 ± 6.8 ^b	445 ± 34 ^a	473 ± 56 ^a	501 ± 18 ^a	479 ± 38 ^a
Cl	<LOD	98.7 ± 5.1 ^{bc}	88.7 ± 8.6 ^c	200 ± 10 ^a	115.2 ± 10 ^b
K	730 ± 53 ^c	7131 ± 240 ^{ab}	6778 ± 385 ^b	6873 ± 287 ^b	7830 ± 474 ^a
Ca	685 ± 56 ^d	6310 ± 108 ^c	7676 ± 297 ^b	8406 ± 311 ^a	7402 ± 81 ^b
Mn	29.6 ± 2.2 ^c	40.0 ± 0.4 ^b	47.8 ± 3.1 ^a	52.2 ± 4.2 ^a	45.4 ± 2.2 ^{ab}
Fe	8.8 ± 1.6 ^b	21.3 ± 0.6 ^a	21.5 ± 2.6 ^a	21.9 ± 1.8 ^a	17.0 ± 1.0 ^a
Cu	2.0 ± 0.2 ^b	6.8 ± 0.1 ^a	7.5 ± 0.3 ^a	7.4 ± 0.4 ^a	6.9 ± 0.3 ^a
Zn	1.0 ± 0.4 ^c	15.1 ± 0.3 ^b	16.3 ± 0.9 ^b	23.0 ± 0.8 ^a	16.9 ± 0.7 ^b
Br	<LOD	1.6 ± 0.1 ^c	2.2 ± 0.1 ^{ab}	2.5 ± 0.2 ^a	2.1 ± 0.1 ^b
Rb	0.6 ± 0.1 ^b	6.3 ± 0.3 ^a	5.8 ± 0.3 ^a	5.9 ± 0.2 ^a	6.7 ± 0.7 ^a
Sr	1.5 ± 0.3 ^c	20.2 ± 1.4 ^b	23.3 ± 1.3 ^b	27.7 ± 1.6 ^a	23.1 ± 1.3 ^b

The different letters indicate statistically significant differences according to the Tukey's test ($p < 0.05$). Results are expressed as mean ± standard deviation (n = 3). Abbreviations: O, oak chips; WT, vine-shoot chips without thermal treatments; T, toasted vine-shoot chips; BT, boiled and toasted vine-shoot chips; ST, steam-treated and toasted vine-shoot chips; <LOD, concentration lower than the limit of detection.

3. Results and discussion

3.1. Characterization of vine-shoot and oak chips

3.1.1. Microstructural characterization of vine-shoot chips

To assess possible morphological and structural modifications occurring after each different treatment, a combination of μ CT and SEM analyses was performed.

In Fig. 2, μ CT (Fig. 2A–E) and SEM (Fig. 2F and G) micrographs of an untreated vine-shoot chip sample are reported. For the visualization of μ CT micrographs the greyscale was chosen, with black voxels corresponding to the lowest-density sub-volumes of the sample (e.g. the air inside vessels and fractures of the chip). Grey regions (intermediate density fraction) depict the woody part of the chip while white voxels are attributable to more dense tissues or domains. The 3D renderings in Fig. 2A, D and 2E show a side view (with a detail zoomed in Fig. 2B), a frontal view of the bark and a whole view of the vine-shoot chip, respectively. In particular, Fig. 2B allows to clearly visualize the sequence pith-xylem-bark, with a residue of parenchyma cells from a broken medullary ray, remaining attached to the xylem vessels visible behind them. The 3D micrograph of a cross section of the chip (Fig. 2C) offers a comprehensive description of the chip anatomy. Pith cells, along with those of medullary rays appear spongy, in some cases broken (Fig. 2F), and often of a lighter grey color compared to both sap-conducting tissues and bark. Indeed, pith consists of undifferentiated parenchyma cells with a function of storage of nutrients (Kirkendall et al., 2015), including starch (Urry et al., 2020), which can lead to an increase of local density. White spots, likely starch grains, are also visible between the phloem and the cortical tissue. Moreover, the absence of annual rings proves that chips have been obtained from one-year vine shoots, while the presence of small fractures should be due to the mechanical stress of the hammer milling. Finally, it deserves attention that, as inferable from Fig. 2B and C, the chopping of the chips

preferentially occurs by the breaking of pith and medullary rays, which are known to deteriorate over time and to be a common cause of wood weakness. Differently, woody tissues' surfaces of the chip appear intact and free of fractures, as shown in the SEM micrograph in Fig. 2G.

In all treatments, the toasting process was responsible for the expansion of previous fractures within the chips, as well as for the formation of new ones. This can be undoubtedly visualized by comparing the corresponding reconstructed cross sections (2D renderings) obtained through μ CT analyses of the same chips before and after the treatments. In particular: Fig. 3A vs Fig. 3B and Fig. 3E vs Fig. 3F and Fig. 3I vs Fig. 3J compare the pre vs post treatment of the T, ST and BT chips, respectively. However, while in the case of T the treatment did not modify the size and the shape of the chip, in ST the steam treatment (data not shown) provoked a remarkable shrinkage of the whole structure, as well as the wrinkling of the outer surfaces. Such effects are visible also in BT, although with a pretty lower shrinkage, along with the exfoliation of the bark. In particular, the crushing and disruption of the medullary cells is recognizable after ST and BT treatments (Fig. 3F and J), which in turn results in the distortion of the xylem. Surface micrographs obtained by SEM provided further insights on the damage suffered by the chips. The T sample showed the initiation of the cracking of the bark, with new fractures perpendicular to the main axis direction (Fig. 3C). Conversely, as yet observed in μ CT, medulla and xylem cells (Fig. 3D) almost maintained the same morphology of the untreated sample (Fig. 2F). For ST and BT chips instead, the situation completely worsens, with the decay of medullary cells that become unrecognizable (Fig. 3H and L). SEM micrographs of ST and BT samples (Fig. 3G and K) show rippled surfaces, with many fissures and, especially for BT, outer woody fibers look breaking and exfoliating. Furthermore, after SEM analysis it has been observed that the grainy looking surface of the BT sample could be attributed to Ca-rich precipitates (likely CaCO_3 , data not shown) formed after the boiling treatment during chips drying. Indeed, such Ca deposits were not detected in a second test in which

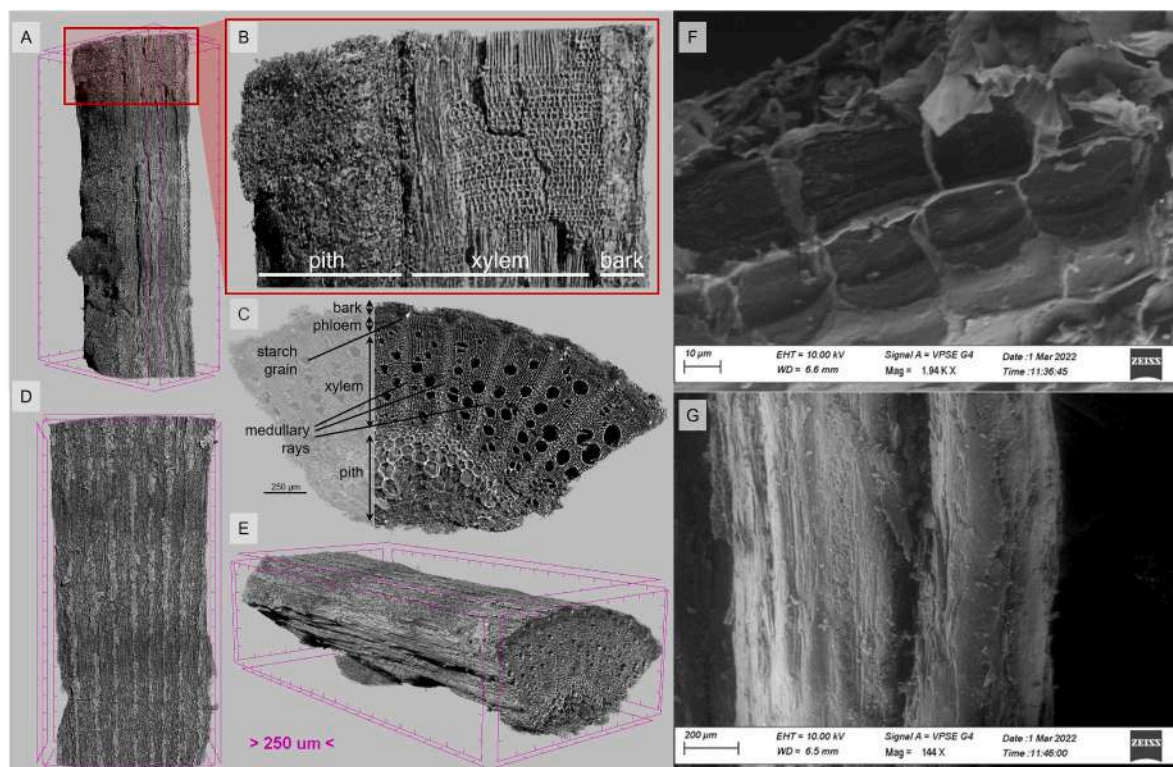


Fig. 2. Micro-CT 3D renderings and SEM micrographs of a representative untreated vine-shoot chip. In detail: A) side view (μ CT); B) enlarged view of A (μ CT); C) cross section view (μ CT); D) frontal view of the bark (μ CT); E) whole view (μ CT); F) detail of parenchyma cells of the pith (SEM); G) surface detail of the xylem region (SEM).

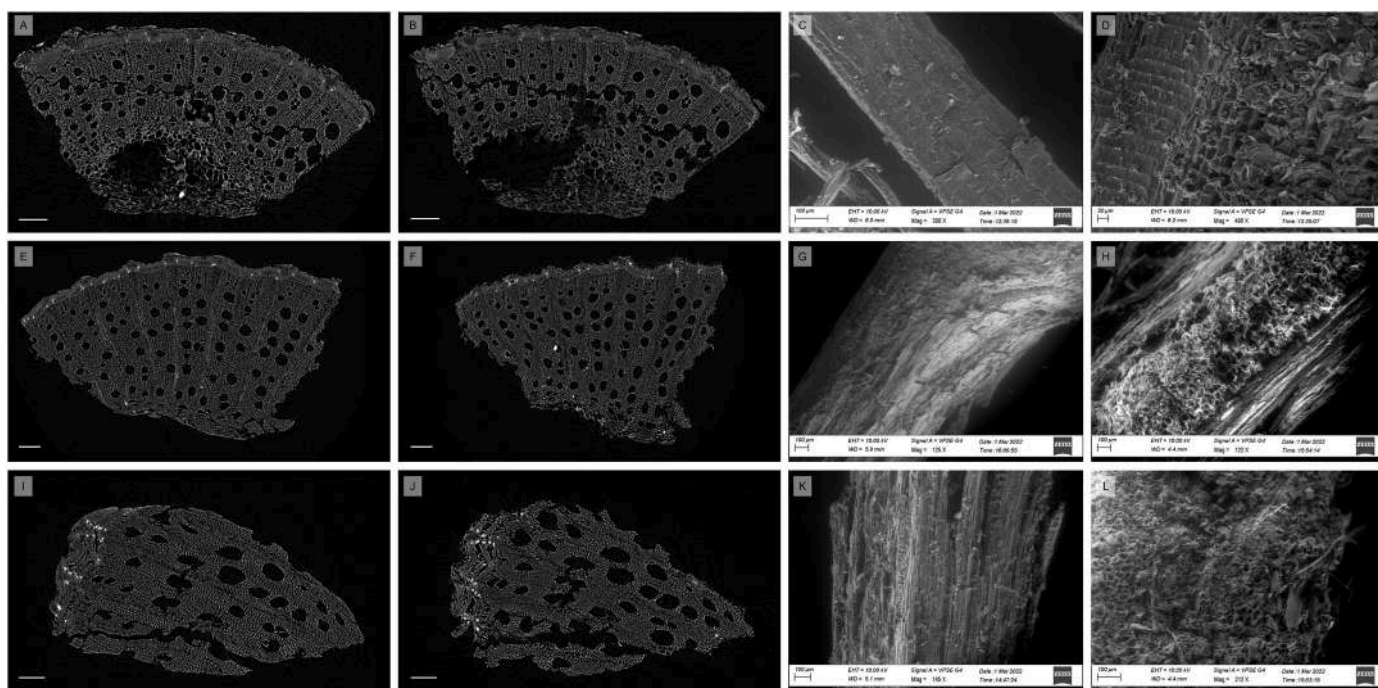


Fig. 3. Micro-CT 2D reconstructed cross sections (A, B, E, F, I, J) and SEM (C, D, G, H, K, L) micrographs. A) T, pre-treatment; B) T, post-treatment; C) T, post-treatment, detail of the bark; D) T, post-treatment, detail of the pith; E) ST, pre-treatment; F) ST, post-treatment; G) ST, post-treatment, surface detail of the xylem region; H) ST, post-treatment, detail of the pith; I) BT, pre-treatment; J) BT, post-treatment; K) BT, post-treatment, surface detail of the xylem region; L) BT, post-treatment, detail of the pith.

chips were boiled in distilled water.

Overall, from a microstructural point of view:

- the toasting process (independently of any pre-treatment) can cause the expansion and propagation of the fractures previously present in the vine-shoot chip and the formation of new ones;
- steam and boiling were responsible for a partial collapse of the chip's structures, which caused the shrinkage of the chips, with a consequent reduction of total porosity.

3.1.2. Chemical composition

Table 1 reports the chemical characterization of the oak chips (O) and of the vine-shoot chips before (WT) and after each treatment. Oak chips showed the highest total polyphenol content (TPC) and the highest antioxidant activity (DPPH and ABTS). Lower values of TPC, DPPH and ABTS were recorded for all the Primitivo vine-shoot chips. Moreover, no differences in these three parameters were observed among the different types of vine-shoot chips.

The mineral elements detected in the oak chips are both qualitatively and quantitatively different from the ones found in the vine-shoot samples. In fact, while in the chips produced from Primitivo shoots P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Br, Rb and Sr were found, in the oak chips, P and halogens were not detected. In oak chips, all the elements are generally present in lower concentrations with respect to vine shoots. In particular, S, K, Ca, Zn, Rb, and Sr are at least an order of magnitude less concentrated in oak chips. The most concentrated macronutrients of Primitivo vine shoots were K and Ca. These data are in accordance with the ones reported for vine shoots of Tempranillo, Cencibel and Cabernet Sauvignon but with different concentrations (Cebrián-Tarancón et al., 2021).

The applied treatments did not cause significant changes in the concentration of the various elements (**Table 1**). However, the boiling process (BT) caused a statistically significant increase in Cl, Ca, Br, and Sr compared to the WT sample, probably caused by the presence of these elements in the water used for the boiling process. In addition, a

significant increase in Zn concentration in BT shoots was observed. Zinc could have been released by the aluminum container (containing 0.3% of Zn) used during the boiling process (Vitali Cepo et al., 2022).

3.2. Characterization of wines

The oenological parameters of Aglianico and Nero di Troia wines together with their phenolic composition, antioxidant activity, color indices, elemental and organic compounds composition were studied. All the data are reported in **Table S2, S3 and S4** (Supplementary materials) and summarized in the heatmaps reported in **Fig. 4**. The heatmaps pointed out that the final characteristics of the wines strongly depend on the stage of chips addition in the production process. For both Aglianico and Nero di Troia, wines produced after the addition of chips in maceration are more similar to control wines than those produced with the addition of chips during aging. In general, with the addition of chips in maceration, ethanol, titratable acidity and tartaric acid decreased, as also observed by Fanzone et al. (2021) on Malbec wines. In the case of the addition of chips during aging, a reduction of the concentration of some mineral elements (S, P, K, Cl, and Fe), quercetin, color intensity, vanillin reactive flavans (VRF), antioxidant activity (DPPH) and anthocyanins was recorded respect to the control wine. Together with these general aspects, some interesting specific results were observed in each wine production. In Aglianico wines, A-C and A-OM were the wines with the highest concentration of polyphenols, usually quite scarce in the other wines. A-OM wines had also the highest concentration of (+)-catechin, lactic and syringic acids. Only in A-STA high concentrations of petunidin 3-(6'-p-coumaroyl)-glucoside, vitisin A and B were found. A-TM showed the highest concentrations of Ca, Mn, Fe, Sr and Br, while A-STM showed the highest concentrations of Rb and caftaric acid. Moreover, when vine-shoot chips were added during aging, the Aglianico wine showed higher values of Cu, Zn, dry reduced extracts, ashes, hue, pH, volatile acidity, titratable acidity, malic acid and resveratrol. Finally, only in A-OA a high concentration of gallic acid and rutin hydrate was found.

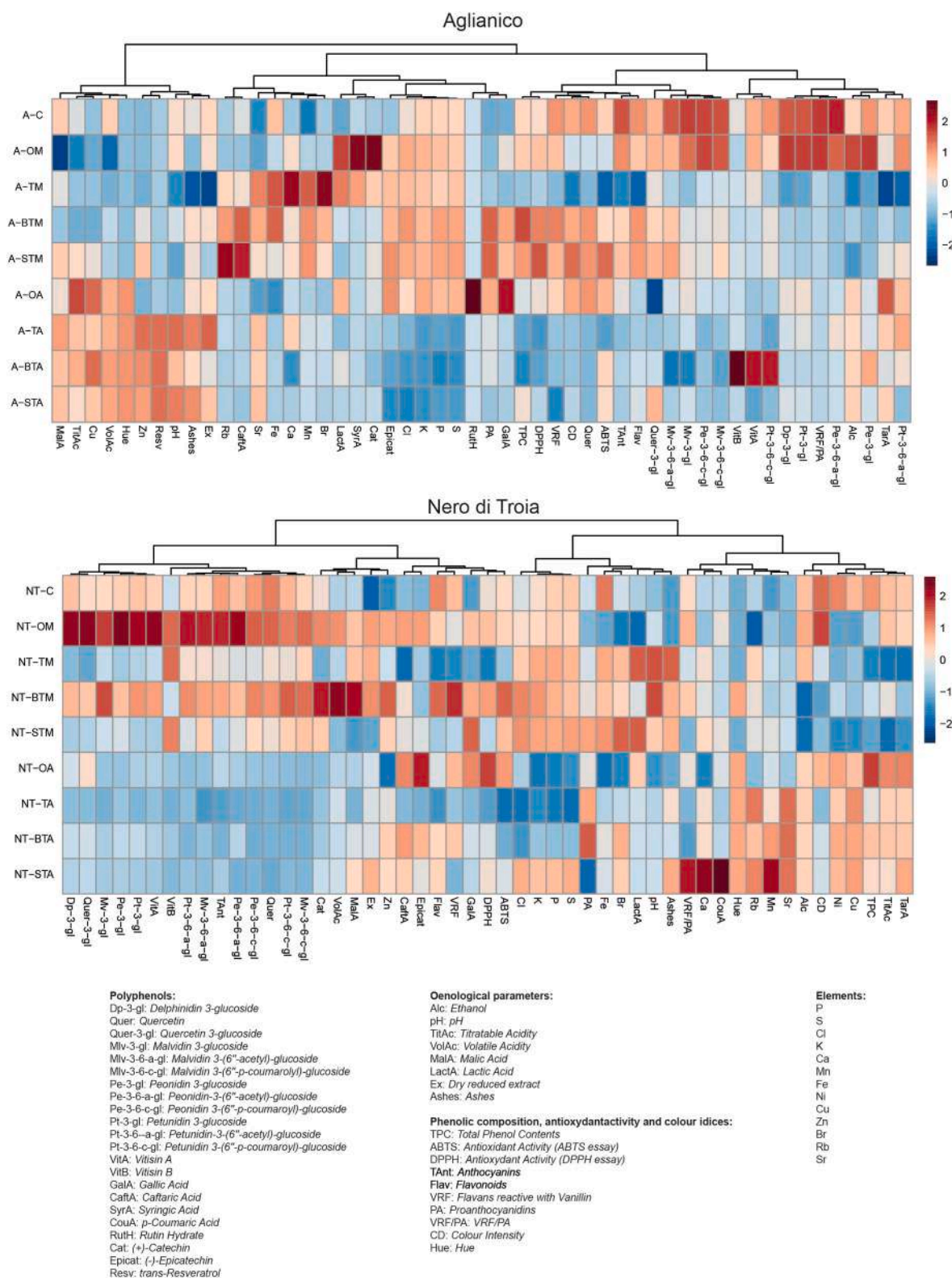


Fig. 4. Heatmaps and dendrograms derived from the hierarchical clustering of the Aglianico and Nero di Troia wines produced using the different treatments.

In the case of Nero di Troia, wines treated with both oak and vine-shoot chips during aging showed a decrease in polyphenols with respect to the control wine. Only in NT-OM and NT-BTM, an increase in some polyphenol compounds was observed. Vitisin B increased in NT-OM, NT-TM and NT-STM. A remarkable increase in volatile acidity, malic acid, (+)-catechin, flavonoids and flavans reactive with vanillin

was also observed in NT-BTM wines. When vine-shoot chips were added to Nero di Troia during aging, some elements (Mn, Sr and Rb) concentration and hue increased. The same was not observed when the wine was treated with oak chips during aging. The sample NT-STA showed the highest levels of Ca, Mn, p-coumaric acid and VRF/PA ratio.

Sparse partial least square analysis (sPLS) was used to identify the

most relevant variables which can describe each class of wine. Respecting classic PLS, sPLS performs variable selection by reducing the noise due to the overfitting caused by the high number of considered parameters (Hårdle et al., 2024). The sPLS results for Aglianico and Nero di Troia are reported in Fig. 5. In the case of Aglianico wines (Fig. 5A), a clear distinction between samples produced with different strategies can be observed by plotting component 1 vs component 2, which describe the 29.7% and 23.2% of the variance, respectively. Control samples and oak-treated wines (both added in maceration and during aging) are localized in the III quarter of the score plot. A-OA and A-OM chips are clearly separated, while the last one overlaps with the group of the A-C.

In the case of wine treated with vine-shot chips, samples treated during maceration are allocated in the II quarter, while samples treated during aging are localized in the I and IV quarter of the score plot. While the groups A-TA, A-BTA and A-STA overlap each other, A-TM can be clearly distinguished from the other two groups of wines (A-BTM and A-STM).

The control wines and the A-OM are the wines with the higher concentration of polyphenols (Pt-3-gl, Dp-3-gl, Pe-3-6-a-gl, Pe-3-gl and Mv-3-gl), a higher alcohol content and VRF/PA ratio (Fig. 5B). The use of oak chips during aging caused a reduction in these compounds and indexes. A further reduction in polyphenolic compounds, together with an increase in microelements concentration (Mn, Sr and Br), was also

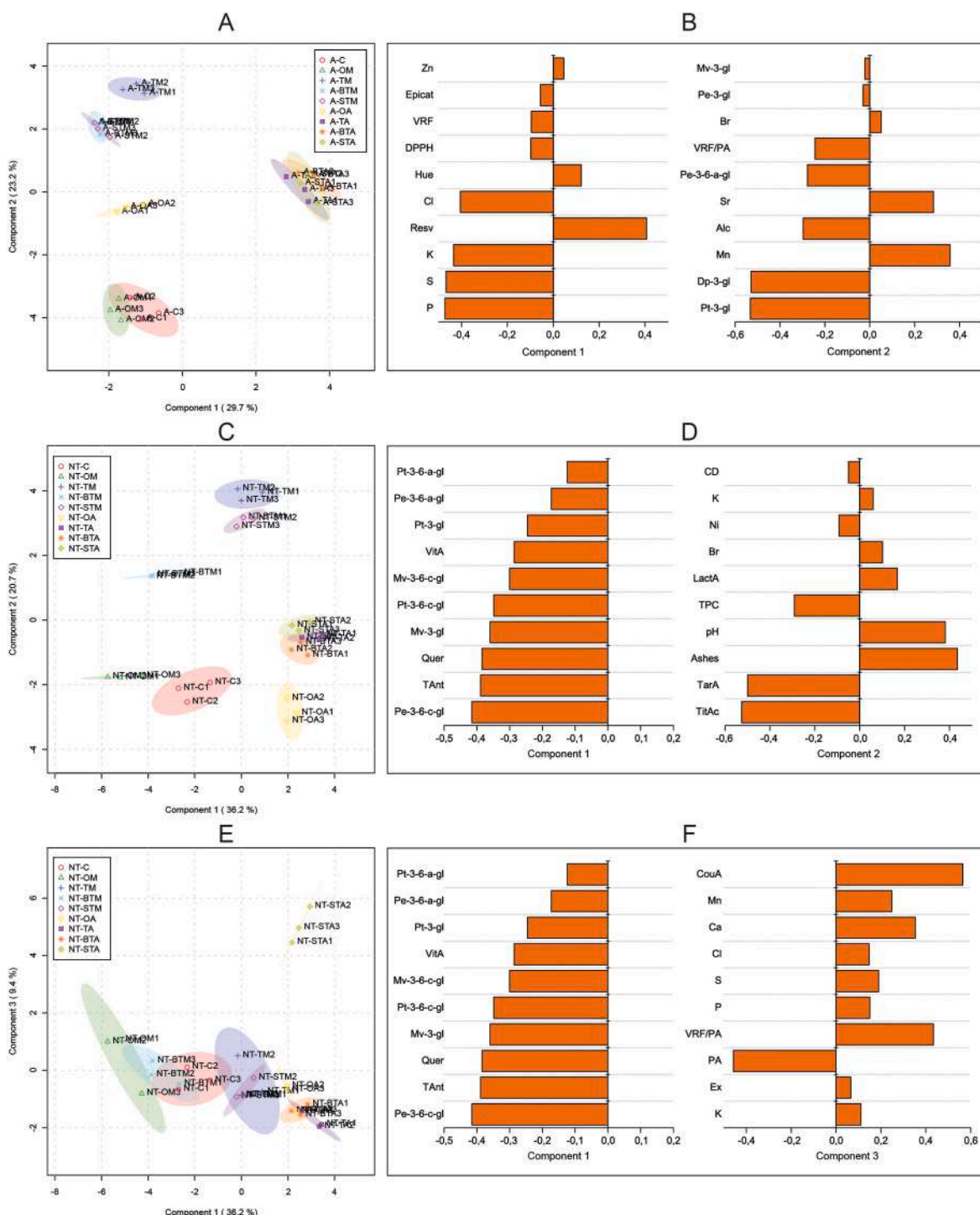


Fig. 5. Score and loading plots obtained via the sPLS analysis of Aglianico (A and B) and Nero di Troia (C, D, E and F) wines.

observed when vine-shoot chips were used in both aging and maceration. However, in wines produced by adding vine-shoot chips during aging, a decrease in macroelements concentration (K, S and P), together with Cl, associated with an increase in resveratrol (and consequently hue) was observed.

A clear distinction among the different wines was also observed in the score plot of Nero di Troia samples (Fig. 5C), obtained by plotting component 1 vs component 2, which explain 36.2% and 20.7% of the variance, respectively. Also in this case, wines treated with oak chips in maceration are similar to control samples, and their scores are located in the III quarter of the graph. These samples are rich in polyphenolic compounds and vitisin A, and poor in macro and microelements, low in pH, color intensity, lactic acid and ash contents (Fig. 5D). Nero di Troia treated with oak chips during aging is clearly separated from these groups (IV quarter of the score plot) due to its lower concentration in polyphenolic compounds. The three groups of Nero di Troia wines obtained with the addition of vine-shoot chips during maceration are distinctly separated and are located in the II quarter of the score plot. N-BTM contains more polyphenols than the other NT wines, while NT-TM has higher pH, ash content and content of K, Br and lactic acid. Also in the case of Nero di Troia wines, all the wines produced with the addition of vine-shoot chips during aging are grouped together (IV quarter of the score plot) and are poor in polyphenolic compounds, as in the case of NT-OA wines. However, in this case, the component 1 vs component 3 score plot (Fig. 5E) allows separating sample NT-STA, due to its high content in p-coumaric acid, macro and microelements, and VLF/PA ratio (Fig. 5F).

All these results pointed out that the main factor affecting the final characteristics of the wine is the chips addition strategy. In fact, in both Aglianico and Nero di Troia this is described by the component 1 in sPLS. The environment of the two winemaking steps and the time of wine-chips contact were different in the two cases: during maceration, chips were in contact with a solution with increasing concentrations of alcohol and other different molecules for 7 days, while during aging chips were in contact with an alcoholic solution, rich in polyphenols and anthocyanins for 35 days. These different environments caused two different behaviors. The addition of vine-shoot chips in maceration produced an enrichment of the wine in microelements and, in the case of Nero di Troia, also an increase in pH, ashes and lactic acid content. With respect to oak chips, vine-shoot chips are richer in microelements and this could have been the cause of the observed enrichment. When chips were added during aging, a decrease in polyphenols (in the case of Nero di Troia) and macroelements (K, S, P and Cl in Aglianico) was observed. Even if oak chips are richer in polyphenolic compounds (TPC) than vine-shoot chips, a reduction of polyphenolic compounds was observed too when used in aging for both Aglianico and Nero di Troia wines. When added during maceration, oak chips caused an increase in the concentration of polyphenolic compounds, vitisin A and anthocyanins in Nero di Troia, while for Aglianico the addition of oak chips in maceration did not produce significant effects with respect to the control wine. A similar behavior was observed when toasted vine-shoot chips were added in Malbec and Bonarda wines: the wines produced after the addition of toasted chips in the aging step showed a lower concentration of polyphenols respect to the control wine and the one produced after the addition of toasted chips during the maceration step (Fanzone et al., 2021).

The results of sPLS show that when toasted vine-shoot chips were added during maceration, they affected more the final chemical composition of wines with respect to steam-treated and boiling-treated vine-shoot chips. This aspect depends on the chips microstructure, since the toasting process allowed the formation of cracks on the chip surface. Even if these cracks were observed in all the chips, the boiling and the steam treatment caused a collapse of the internal part of the chips, resulting in more compact structures and likely in the occlusion of potential infiltration routes; this features were not observed in simply toasted vine-shoot chips. Computational models demonstrated that the

extraction procedure from foodstuff with cracks and straight pores parallel to the major axis of the material (like in the case of toasted chips) is more efficient than flake structures with non-oriented pores (i.e. steam-treated and boiling-treated vine-shoot chips) (Crossley & Aguilera, 2001). The modification of the chip inner microstructure was stronger in boiled vine-shoot chips than in steam-treated ones. This could be the reason why A-STM and NT-STM wines showed intermediate chemical characteristics with respect to A-TM and A-BTM or NT-TM and NT-BTM. It can be hypothesized that when toasted chips are used, wine can more easily enter into the chips through the fractures on the chip surface and move through the chip using the internal structures of the vases. In the case of boiled and steam-treated chips, the contact of the wine with the inner part of the chip is more difficult since it is hindered by the more compact and less porous structure. This difference was not observed when chips were added during aging. In fact, both in the case of Aglianico and Nero di Troia wines, the groups of wines A-TA, A-BTA, A-STA and NT-TA, NT-BTA and NT-STA overlap in the score plots (Fig. 5A and C, respectively). This is probably due to the longer time of contact, which also facilitated the wine-chips contact and reduced the effect produced by the different microstructure of the chips. The microstructure of a material can influence the kinetic of the extraction (Aguilera et al., 1987; Crossley & Aguilera, 2001). For long periods of extraction (i.e. 35 days of aging) this effect can be neglected and the hydroalcoholic solution of the wine could penetrate in all the part of the vine-chip favoring the extraction. The three types of vine-shoot chips do not show any differences in terms of chemical composition (Table 1) and considering that the maximum extraction from wood in alcoholic beverage is achieved after one month (Smailagić et al., 2021), after 35 days of aging, the wine achieved the maximum extraction from all the type of vine-shoot chips. Even if the microstructure can influence the kinetics of the extraction process (Aguilera et al., 1987; Crossley & Aguilera, 2001), the long wine-chip contact time used in the aging cancel any effect of the vine-shoot chip pretreatment on the final chemical composition of the wine.

3.3. Oenological considerations

On the basis of all these data, some considerations can be made on the quality of the produced wines in dependence of the technological process used. Specifically, the lowest ethanol contents (Table S2) were recorded for all the Aglianico trials treated with vine-shoot chips during maceration, with a minimum observed for A-TM. This effect was less evident in the case of Nero di Troia variety, where, however, a slight reduction of the ethanol content was observed, also when the treatment was performed during aging. These results show a potential for using toasted vine-shoot chips to reduce the alcohol content of wines, that is facing a progressive increase as a result of global warming, due to earlier ripening and higher sugar concentrations at the time of grape harvest (van Leeuwen et al., 2024). In addition, lowering the alcohol content could be of interest for commercial purposes to meet the consumer's demand for wines with lower alcohol content (Ho, 2021; Saliba et al., 2013).

However, literature reports not-univocal effects of fermenting in contact with chips on the ethanol contents. Rodríguez-Bencomo et al. (2010) reported decreased ethanol content in a Tempranillo wine fermented with oak chips compared to control wine, though only data uncertainty was reported. On the other hand, Fanzone et al. (2021) observed a cultivar dependent effect, with an increase of ethanol content in Malbec wine when fermenting with chips, while no changes was observed on Bonarda. Authors hypothesized an effect on microbial metabolism (Fanzone et al., 2021; Rodríguez-Bencomo et al., 2010). Finally, Asproudi et al. (2025) found slightly higher fermentation yields in presence of chips, attributing this result to suspended substances, as in fermentation by immobilized yeasts. It seems that too many variables could play a potential role in regulating the effect of chips on ethanol contents and that further exploration of this effect could provide

winemakers a new opportunity to manage ethanol yields. In the Nero di Troia trials, titratable acidity decreased, especially in the NT-TM and NT-STM tests. A possible explanation for the decrease could be related to mineral element ions released from the chips that may have led to the precipitation of tartrate salts, and thus a decrease in acidity (Coulter et al., 2015). In fact, vine-shoot chips presented contents of K and Ca ten-fold higher than oak chips and this could have impacted on the supersaturation status of tartaric salts (Coulter et al., 2015; Fioschi et al., 2024). However, the changes of tartaric salts stability as affected by other than oak-derived chips deserve further investigation. It would be interesting to evaluate in further studies how the taste balance of the wines produced with these vine-wood chips might be modified.

In terms of phenolic composition, antioxidant activity and color indices (Table S3), Aglianico wines showed a range of total phenolic content (TPC) values, with A-TM having the lowest value and A-BTM the highest. Nero di Troia wines generally showed higher TPC values compared to Aglianico, with NT-C showing the highest value and NT-TM the lowest. The ABTS and DPPH assays measure the antioxidant capacity, indicating a wine's ability to neutralize free radicals. For Aglianico, A-STM showed the highest ABTS value, while A-OM the lowest. Nero di Troia generally exhibited higher antioxidant activity compared to Aglianico, with NT-OA showing particularly high values in both ABTS and DPPH assays. Vine-shoot chips did not contribute to any increase in antioxidant activity. As regards anthocyanins, Aglianico showed a variation from about 290 mg L⁻¹ to over 460 mg L⁻¹, with A-C having the highest value. Anthocyanins decreased during maceration with toasted oak chips and this decrease is more evident in wines treated with vine-shoot chips (especially with toasted ones) during maceration. Wine treated with boiled and toasted vine-shoot chips showed a minimal decrease in anthocyanins content, potentially matching the results obtained with toasted oak chips, making them a practical alternative. Nero di Troia varied from about 330 mg L⁻¹ to nearly 500 mg L⁻¹, with NT-OM having the highest anthocyanin content. A similar situation was observed with boiled vine-shoot chips, maintaining anthocyanin levels close to those of the control and oak chips. It can be concluded that the anthocyanin retention effect during maceration with boiled and toasted vine-shoot chips is not cultivar dependent. This makes boiled vine-shoot chips a realistic choice for maintaining a good anthocyanin content during maceration. Regarding flavonoids (Flav), Aglianico wines ranged from about 1150 mg L⁻¹ to 1500 mg L⁻¹, with A-OM showing the lowest content, followed by A-TM. Nero di Troia ranged from about 1950 mg L⁻¹ to 2350 mg L⁻¹, with NT-TM having the lowest content. The decrease in flavonols is a positive result, as it reduces astringency. Therefore, the toasted vine-shoot chips can be useful for maintaining good final smoothness and reducing potential astringency (Picariello et al., 2020). Both wines showed similar concentrations of vanillin reactive flavans (VRF), related with bitter taste perception (Picariello et al., 2018). However, Nero di Troia generally showed higher values. Aglianico had the highest VRF content for A-BTM, similar to Nero di Troia, which showed the highest value for NT-BTM. Boiled chips caused an increase in bitter compounds, whereas toasted chips resulted in a decrease in bitter compounds in both vinified varieties. The proanthocyanidin content was significantly higher in Nero di Troia compared to Aglianico. NT-BTM had the highest proanthocyanidin content among the Nero di Troia treatments. It is not optimal to use boiled or steam-treated vine-shoot chips in wines intended to be consumed in a short period, as they would increase astringency; this effect would be desired to produce a wine capable of longer aging compared to wines not treated with these chips. Finally, Aglianico showed a range of CD values from 0.68 to 1.05, with A-C having the highest CD values. Regarding the tests with chips added during maceration, the values remained stable with boiled or steam-treated chips, while toasted chips caused a decrease in CD. Hue values do not seem to be compromised or highly variable compared to the control. From the experiments, the samples treated during maceration with OT still show the best and most desirable parameters currently in the field of winemaking. However, from a

sustainability and circular economy perspective, production with different treatments of chips (toasting, boiling + toasting, steam + toasting) from vine wood resulting from pruning can still be a viable alternative depending on the desired objective (Fanzone et al., 2021).

In terms of elemental composition (Table S4), previous research did not report significant changes in the elemental composition of wines (Cebrián-Tarancón et al., 2022). The oenological implications of these results are relevant in several aspects. The first focus is about the exchange process of K and Ca ions, as cations that could affect the stability of tartaric salts (Coulter et al., 2015; Fioschi et al., 2024). The treatment with chips determined different effects. As regards Aglianico wines, a significant decrease of K by about 35% was obtained when adding vine-shoot chips during aging, irrespective of their pre-treatment, while no change was observed in the other cases. Calcium either remained almost unchanged or slightly increased. In Nero di Troia wines, K increased when vine-shoot chips were added during maceration and significantly decreased when aged with oak chips and toasted vine-shoot chips, and in lower extent with boiled and toasted vine-shoot chips. Calcium remained almost unchanged, while increasing in NT-STA wines. As a consequence, an improvement of potassium bitartrate stability could be a possible side effect of vine-shoot chips used in wine aging. This could also affect the color intensity and longevity of the wine, considering the loss of color and acidity due to the tartaric instability in wine.

Relevant decreases were also observed for Fe when chips, whatever the typology, were used during aging. Considering the role of Fe as catalyzer of oxidation reactions in wines, this effect should be taken into account for the possible implications in oxidation phenomena (Danilewicz, 2013). On the other hand, the release of Cu was observed in the same conditions in Aglianico wines. Even though the observed levels were largely below the legal limits in Europe (1 mg L⁻¹) and the values reported in Italian wines (Wang et al., 2023), the possible effects related to the chemistry of this metal should not be disregarded (Wang et al., 2023).

Finally, some relevant variations in other microelements concentrations were also observed. In fact, the addition of chips in maceration enriched the wines with elements such as Mn, Sr and Rb. This effect was particularly evident in wines treated with vine-shoot chips compared to those treated with oak chips.

4. Conclusions

For the first time, this study provides detailed information on the microstructure and elemental composition of vine-shoot chips and on the effect of boiling and steam-treatment combined with toasting. Moreover, the impact of the addition of these chips before and after fermentation on the chemical composition of Aglianico and Nero di Troia red wines was evaluated. Generally, wines treated with oak chips were richer in polyphenolic compounds and anthocyanins with respect to the ones treated with vine-shoot chips. On the contrary, vine-shoot chips released some micro elements such as Mn, Sr and Br while decreasing in some cases cations involved in tartaric salts instability like Ca and K. The final chemical composition of the wines strongly depended on the chips addition strategy. In fact, both oak and vine-shoot chips can adsorb and/or absorb some polyphenolic compounds, anthocyanins and vitisin A when added during aging. When vine-shoot chips were added during maceration, the pre-treatment influenced also the final chemical characteristics of the wines. In fact, the different chips microstructures influenced the contact surface and the exchanges between wine and the vine-shoot chips. In particular, the collapse of the chip inner microstructure induced by boiling and steam-treatment, reduced the permeability of the chips to the wine, reducing color adsorption, maintaining anthocyanin levels comparable to oak-treated wines and improving antioxidant capacity.

Some aspects, related to ethanol yield, tartaric stability and sensory implications, require, however, further investigation.

This study confirmed therefore that vine-shoot chips can be a sustainable, in-house resource, that could provide an alternative to commercial oak chips, though requiring appropriate processing in order to better exert their potential and reduce intrinsic drawbacks.

CRedit authorship contribution statement

Mirella Noviello: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Carlo Porfido:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Chiara Di Natale:** Investigation, Formal analysis, Data curation. **Roberto Terzano:** Writing – review & editing, Resources. **Luigi Tarricone:** Writing – review & editing, Investigation. **Francesco Mazzone:** Writing – review & editing, Investigation. **Giuseppe Gambacorta:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Chiara Digiorio:** Writing – review & editing, Writing – original draft. **Francesco Caponio:** Writing – review & editing, Supervision, Resources. **Vito Michele Paradiso:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Ignazio Allegretta:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2026.119098>.

Data availability

Data will be made available on request.

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