Influence of filtration treatments on grapevine DNA traceability in wine

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ABSTRACT

Wine authentication through grapevine DNA traceability could be affected by wine technological processing treatments. In this study, filtration treatments including depth filter treatment using kieselguhr, perlite and membrane filtration using different types and pore sizes (0.22 and 0.45 μm) were applied on ‘Nebbiolo’ wine at an experimental scale. We used ‘Nebbiolo’ because it is an important Italian winegrape variety used to produce high-quality wines. Phenolic composition and color properties of the treated wines were examined using spectrophotometric and HPLC methods, while grapevine DNA traceability was evaluated using PCR based assays. The filtration treatments, as expected, significantly decreased turbidity compared to the unfiltered control, although total phenolics, total flavonoids and total non-flavonoids were not significantly affected. A significant reduction of acetylated anthocyanins by 6.1%–43.3% was observed in filtered wines, which could account for the reduced in total anthocyanins and color intensity of these wines. Grapevine DNA was significantly reduced in filtered wine by 37.2%–99.7%, with the reduction rate depending mainly on the properties of filter material. Polyvinylidene difluoride (PVDF) membranes with a pore size of 0.22 μm showed highest reduction of grapevine DNA in wine, resulting in the failure of TaqMan® single nucleotide polymorphisms (SNPs)-based assays used to detect grape DNA in wines.

1. Introduction

Wine industry is one of the most profitable sectors, consequentially wine fraud including adulteration and counterfeiting can occur, damaging the image and market of premium wines (Ranaweera et al., 2021). In the past decades, wine fraud and mislabeling were preferentially detected using chemical-based techniques including mass spectrometry, spectroscopic methods and chromatography techniques combined with chemometrics (Sun et al., 2022; Villano et al., 2017). However, these methods are affected by various factors that impact on wine composition such as climate seasonal conditions, soil characteristics, vineyard management and enological practices (Boccacci et al., 2020; Sun et al., 2022).

DNA based methods are reported to be accurate and proficient for variety identification of wine because grapevine DNA is more resistant to the winemaking process than other wine components. DNA markers, including nuclear and chloroplast simple sequence repeats (SSRs) (Agrimonti & Marmirolı, 2018; Baleiras-Couto & Eiras-Dias, 2006; Boccacci et al., 2012; García-Beneytez et al., 2002) and single nucleotide polymorphisms (SNPs) (Catalano et al., 2016), are tested for DNA authentication of wine (Boccacci et al., 2020; Galstyan et al., 2021). Failure of amplification and inconsistent results were observed for wine varietal authentication using SSR genotyping due to DNA degradation (Savazzini & Martinelli, 2006; Vignani, Lio, & Scali, 2019). Whereas the...
high sensitivity of SNP-based assay can be observed in low-quality fragmented DNA, resulting in potential application in quantitative varietal authentication of blended wine and against fraud (Boccacci et al., 2020; Zambianchi et al., 2021).

Among wine production chain, residual DNA in wine is influenced by many factors such as wine making practice, clarification agents, aging, and yeast/bacterial activity (Catalano et al., 2016; García-Beneytez et al., 2002; Villano et al., 2017; Zambianchi et al., 2021, 2022). Regarding wine clarification, filtration treatments are usually applied to eliminate suspended and colloidal particles in wine. These treatments, including depth filtration and membrane filtrations, can reduce in wine the content of aroma compounds, phenolics, pesticide residues, sulfide-bound copper, and other wine constituents due to the adsorption properties of filter aids and membranes (Arriagada-Garrazana et al., 2005; Douila et al., 2016; Prodano et al., 2019; Zhang et al., 2022). The adsorption capacity is reported to be dependent on the formation of hydrogen bonds and electrostatic interaction (Cai, Xie, Zhong, Tian, & Yang, 2021; Cassano et al., 2017). Despite research on the effects of filtration on wines, to our knowledge the detailed response of filtration treatments on DNA traceability in wine has not been studied. Vitis vinifera L. cv. Nebbiolo is widely planted in northwestern Italy, where high-quality ‘Nebbiolo’ wines are produced under Protected Denomination of Origin (DOC and DOCG) (Raimondi et al., 2020). The misrepresentation of the origin and variety on ‘Nebbiolo’ wine labels can occur due to its high quality and economic value (Miglietta & Morrone, 2018). Cultivar-specific SNP technique was optimized for varietal authentication of ‘Nebbiolo’ wines (Boccacci et al., 2020; Gambino et al., 2022). However, there is limited research literature on the influence of wine processing treatments, especially stabilization treatments, on varietal identification efficiency of this cultivar wine. Gambino et al. (2022) revealed that DNA concentration was reduced by using different fining agents, especially bentonite and gelatine, which drastically reduced grapevine DNA below identification threshold. Given that filtration treatments can modify wine chemical-physical parameters depending on the filtration method and the characteristic of the filter material, it is hypothesized that DNA traceability in wine could be influenced by filtration treatments. Therefore, the impact of commonly used filtration treatments at an experimental scale on DNA traceability of ‘Nebbiolo’ wine were determined in this study using TaqMan® genotyping assay. The result will provide useful information on varietal identification of wines.

2. Material and methods

2.1. Chemicals, reagents and wine sample

Chemical reagents including ethylene diamine tetraacetic acid (EDTA), sodium chloride (NaCl), Tris-HCl, cetyltrimethylammonium bromide (CTAB), β-mercaptoethanol and ethanol were purchased from Merck KGaA (Darmstadt, Germany). Malvidin-3-β-galactoside was purchased from Extrasynthese (Genay Cedex, France). Ultrapure water was produced by a Milli-Q system (Merck Millipore, Darmstadt, Germany). TEX buffer containing 20 mM EDTA (pH 8.0), 1 M Tris-HCl (pH 8.0), 3% CTAB and 1% β-mercaptoethanol and TE buffer containing 1 mM EDTA (pH 8.0) and 10 mM Tris-HCl (pH 8.0) were prepared according to Gambino et al. (2022). Proteinase K was sourced from Thermo Fisher Scientific (Waltham, MA, USA). Nucleo-Spin® Plant II were purchased from Machery-Nagel GmbH & Co. KG (Düren, Germany). TaqMan® Environmental Master Mix 2.0, TaqMan® Exogenous Internal Positive Control (EIPC) reagents (containing primers, VIC probes specific for EIPC), TaqMan® 9-cis-epoxycarotenoid-dioxygenase gene (VvNCED2) assay and TaqMan® SNP Assay (containing primers, FAM and VIC probes) were from Thermo Fisher Scientific (Waltham, MA, USA). All other chemicals and reagents were supplied by Merck KGaA (Darmstadt, Germany). Kieselguhr (KI) and Perlite (PE) were provided by IMEWS Filtration EMEA (Milan, Italy).

Membrane sheets (47 mm of diameter) of different material and pore size were purchased as follows: cellulose nitrate (CE, 0.45 μm pore size) membranes were from Sartorius Stedim Biotech (Goettingen, Germany), polyethersulfone (PES, 0.22 and 0.45 μm) membranes were from Pall Corporation (Port Washington, NY, USA), polyvinylidene difluoride (PVDF, 0.22 and 0.45 μm) membranes were from Merck Millipore (Darmstadt, Germany), and polytetrafluoroethylene (PTFE, 0.45 μm) membranes were from Hermann Bohlender (Gruensfeld, Germany).

The wine used for the experiment was produced in 2022 from about 200 kg of ‘Nebbiolo’ grapes from Roero wine area, Cuneo, Italy. The must was inoculated with Saccharomyces cerevisiae active dry yeast (FERMOL Premier Cru, AEB, Brescia, Italy; 20 g/L). Maceration lasted for 10 days, the cap was punched down once the first day, and two punches down were carried out daily until the 4th day. At the end of maceration, free-run wine was obtained, and then the pomace cap was gently pressed using a FMA 4 pneumatic press (Velo SpA, Altiare, Italy). Malolactic fermentation was induced by the inoculation of Oenococcus oeni (Malolab™, Lallemand Inc., Montreal, Quebec, Canada). After malolactic fermentation, 50 mg/L SO2 were added, and the wine was subsequently racked to remove the lees. The wine was stored in a steel tank in the winery for nine months until the beginning of the filtration treatments. The wine sample contained 10 mg/L of free SO2.

Wine basic physical-chemical parameters are available in Table S1.

2.2. Filtration treatments

Eleven filtration treatments using different depth filter aids and filter membranes were conducted on the same wine sample at a laboratory scale. Four different types of membranes filters including CE, PES, PVDF and PTFE with 0.45 μm pore size were applied (CE45, PES45, PVDF45, and PTFE45, respectively). PES and PVDF membranes with 0.22 μm pore size were also used for filtration treatments (PES22 and PVDF22, respectively). In addition, cellulose membrane was used in combination with two filtration aids: Kieselguhr (KI) and Perlite (PE). In both cases KI and PE were used to create an alluviation panel above the cellulose membrane, to depth filtrate the wine samples. Moreover, KI and PE were used without other membranes (CF-KI and CF-PE): 1.4 g of KI or PE per liter of wine were added to the wine samples, stirred for 1.5 min and successively centrifuged at 3000 × g for 5 min at 20 °C (Hettich 32R, Tuttingen, Germany). The control (CO) was unfiltered wine. All the treatments were carried out in triplicate using a vacuum filtration system (VWR International, Milan, Italy), and collecting 500 mL of wine for each replication: 50 mL of treated wine were used for physical-chemical determination, while the remaining wine (450 mL) was bottled and stored at −20 °C until DNA extraction.

2.3. Wine chemical-physical analysis

2.3.1. Basic chemical-physical parameters

Total acidity was analyzed by titration using OIV-MA-AS313-01 method (OIV, 2020). pH and turbidity were determined using an inolab® pH 730 pH meter (WTW, Weilheim, Germany) and a TB1 portable turbidimeter (Velp Scientifica, Usmate, Italy), respectively. Individual acids (malic acid, lactic acid, tartaric acid, citric acid, and acetic acid), alcohol, glycerol, fructose and glucose were evaluated by high performance liquid chromatography (HPLC, Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector and a refractive index detector (Giordano et al., 2009).

2.3.2. Phenolic composition and color characteristics

For each treatment replicate, 50 mL of wine samples were collected for color and phenolic analysis. All spectrophotometric measurements were performed using an UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Wine color intensity (CI) and hue were determined by measuring the absorbance at 420, 520, 620 nm, and expressed as A420/A520+A620 on 10 mm path length and the ratio between A420 and


2.3.3. Individual anthocyanins expressed as mg/L of (analyzed based on the method reported by Petrozziello et al. (2018) and flavonoids (TF) and total non-anthocyanins flavonoids (TNA) were analyzed based on the method reported by Petrozziello et al. (2018) and expressed as mg/L of (–)-catechin.

2.4. DNA traceability analysis

2.4.1. DNA extraction from wines

CTAB method based was conducted as described by Siret et al. (2002) with some modifications (Agrimonti & Marmiroli, 2018) as reported by Gambino et al. (2022). Wines were frozen at –20 °C for 15 days before analysis. One hundred mL of wine was centrifuged for 1 h (4000 × g, 4 °C) using a Sigma 3-16 K centrifuge (Sigma Laborzentrifugen, Osterode am Harz, Germany), then the wine pellet was dissolved in 5 mL of TE buffer (pH 8.0) and centrifuged (20 min, 13,000 × g) to obtain the supernatant.

Amplification cycles were as follows: the initial denaturation step set at 95 °C for 15 min, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C, and a final extension step 10 min at 60 °C.

3. Result and discussion

3.1. Filtration treatment on turbidity, phenolic composition and color characteristics

In this study, a ‘Nebbiolo’ wine aged for 9 months was used for filtration treatments, and the influence of these treatments on wine turbidity, phenolic composition and color parameters were assessed. The chemical results are shown in Table 1. CF-KI and CF-PE significantly decreased wine turbidity by 62.5% and 28.6%, respectively, whereas CE filter membrane pre-coated with KI and PE treatments and other membrane filtration treatments showed a significant decrease of between 88.5% and 99.3%. Low turbidity values of wines submitted to different filtration treatments were separated with different letters using Tukey post-hoc test at p-value ≤ 0.05.

For the wine variables, one-way analysis of variance was conducted using SPSS Version 22 (IBM, Armonk, New York, USA). The differences among the filtration treatments were separated with different letters using Tukey post-hoc test at p-value ≤ 0.05.

A 20% decrease in the initial denaturation step set at 95 °C for 10 min, then 55 cycles of 95 °C for 15 s and 60 °C for 1 min. DNA standard was extracted from 'Nebbiolo', 'Barbera' and 'Freisa' young leaf using NucleoSpin® Plant Kit. Grapevine DNA and the percentage of qPCR inhibition were quantified from the calibration curves of the VnNCE2 DNA assay and EIPC, respectively, using the CFX96 Detection System from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). All extracted DNA samples were analyzed in triplicate.

2.4.3. SNP genotyping

Two markers, SNP_15082 and SNP_14783, were analyzed in extracted DNA as reported by Boccacci et al. (2020). FAM and VIC probes were designed using Primer Express version 3.0 (Thermo Fisher Scientific) to genotype non-'Nebbiolo' alleles ('Barbera' and 'Freisa') and 'Nebbiolo' alleles (Table S2). The qPCR mixture consisted of the extracted DNA (2.5 μL), TaqMan® Environmental Master Mix 2.0 (5 μL), 0.4 μL of EIPC DNA, 2 μL of qPCR mix (containing premixed forward, reverse primers, and VIC probe specific for EIPC) and sterile water (0.1 μL). Amplification cycles were as follows: the initial denaturation step set at 95 °C for 10 min, then 55 cycles of 95 °C for 15 s and 60 °C for 1 min. DNA standard was extracted from 'Nebbiolo', 'Barbera' and 'Freisa' young leaf using NucleoSpin® Plant Kit. Grapevine DNA and the percentage of qPCR inhibition were quantified from the calibration curves of the VnNCE2 DNA assay and EIPC, respectively, using the CFX96 Detection System from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). All extracted DNA samples were analyzed in triplicate.

2.5. Statistical analysis

For the wine variables, one-way analysis of variance was conducted using SPSS Version 22 (IBM, Armonk, New York, USA). The differences among the filtration treatments were separated with different letters using Tukey post-hoc test at p-value ≤ 0.05.
increased malvidin-3-glucoside (17.7%–34.8%) in ‘Nebbiolo’ wines, followed bypeonidin-3-glucoside (13.2%–20.0%) and malvidin-3-glucoside (9.8%–17.7%). The sum of total anthocyanin contents in all wines exceeded 100%.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Turbidity</th>
<th>TF</th>
<th>TNA</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>C*</th>
<th>h</th>
<th>CI</th>
<th>Hue</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>12.28±0.23a</td>
<td>2608±34a</td>
<td>2447±37a</td>
<td>4836±37a</td>
<td>34.7±37a</td>
<td>53.7±37a</td>
<td>42.88±37a</td>
<td>68.72±37a</td>
<td>38.60±37a</td>
<td>4.51±0.03a</td>
</tr>
<tr>
<td>CF-KI</td>
<td>4.60±0.32c</td>
<td>2637±34a</td>
<td>2484±37a</td>
<td>4919±37a</td>
<td>35.6±37a</td>
<td>54.8±37a</td>
<td>43.74±37a</td>
<td>70.14±37a</td>
<td>38.58±37a</td>
<td>4.45±0.03a</td>
</tr>
<tr>
<td>CF-PE</td>
<td>8.77±0.52d</td>
<td>2638±34a</td>
<td>2479±37a</td>
<td>4899±37a</td>
<td>35.7±37a</td>
<td>54.8±37a</td>
<td>43.77±37a</td>
<td>70.16±37a</td>
<td>38.60±37a</td>
<td>4.44±0.10b</td>
</tr>
</tbody>
</table>

Note: Sample in the same columns indicate no significant difference between filtration treatments by Tukey test (P < 0.05). Turbidity is expressed as nephotometric turbidity units (NTU). TF, Total phenolic index, expressed as (+)-epicatechin/L. TF, Total flavonoids, expressed as (+)-catechin/L. CI, color intensity, expressed as absorbance units on 10 mm path length. CO, control. CF-KI, kieselguhr accompanied by centrifugation. CF-PE, perlite accompanied by centrifugation. CE-KI, Cellulose membrane pre-coated with kieselguhr. CE-KI, cellulose membrane pre-coated with perlite. CE45, 0.45 μm pore size cellulose. PES45, 0.45 μm pore size polyethersulfone. PES22, 0.22 μm pore size polyethersulfone. PTFD45, 0.45 μm pore size polyvinylidene difluoride. PTFD22, 0.22 μm pore size polyvinylidene difluoride. PTFE45, 0.45 μm pore size polytetrafluoroethylene.

For the individual anthocyanins quantified in filtration treated wines (Table 2), malvidin-3-O-glucoside represents the majority of anthocyanins (51.8%–54.6%) in ‘Nebbiolo’ wines, followed bypeonidin-3-O-glucoside (17.7%–19.0%). The sum of the concentrations of simple glycoside anthocyanins were found higher than those of acetyl- and cinnamoyl-glucoside anthocyanins, in agreement with previous studies (Paissoni et al., 2020; Río Segade et al., 2014).

Although a high decrease of malvidin-3-O-glucoside in wines submitted to cross-flow microfiltration was previously observed (Cameira-dos-Santos et al., 1994), CF-KI and CF-PE had no significant influence on total simple glycoside anthocyanins, and some other membrane filtration treatments including CE-KI, CE-PE, CE45, and PTFD45 slightly increased malvidin-3-O-glucoside and peonidin-3-O-glucoside up to 4.2%. The increased glucoside anthocyanins in filtered wines could be regenerated from decolorized anthocyanins by bisulfite addition since that vacuum filtration treatments caused the release of free SO2 from anthocyanins (Berke et al., 1998; Ribereau-Gayon et al., 2006).

Compared to the control, CF-KI and CF-PE treatments showed a reduction of acetylated glucosides by 6.1% and 13.1%, respectively, and CE-KI and CE-PE treatments led to significant decreases of these compounds by 47.6% and 50.3%, indicating the co-adsorption of CE and
### Table 2

Anthocyanin profile of ‘Nebbiolo’ wines subjected to filtration treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TA</th>
<th>Delphinidin derivative 3-O-glucoside</th>
<th>Cyanidin-3-O-glucoside</th>
<th>Petunidin-3-O-glucoside</th>
<th>Peonidin-3-O-glucoside</th>
<th>Malvidin-3-O-glucoside</th>
<th>∑ Delphinidin derivatives</th>
<th>∑ Cyanidin derivatives</th>
<th>∑ Petunidin derivatives</th>
<th>∑ Peonidin derivatives</th>
<th>∑ Malvidin derivatives</th>
<th>∑ simple glycosides</th>
<th>∑ acetyl glucosides</th>
<th>∑ cinnamoyl glucosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>44.01 ± 0.03</td>
<td>1.34 ± 0.03</td>
<td>3.05 ± 0.02</td>
<td>7.79 ± 0.03</td>
<td>22.80 ± 0.01</td>
<td>1.93 ± 0.03</td>
<td>2.19 ± 0.01</td>
<td>3.54 ± 0.01</td>
<td>9.64 ± 0.07</td>
<td>26.35 ± 0.01</td>
<td>36.89 ± 0.01</td>
<td>3.74 ± 0.01</td>
<td>3.37 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>0.10a</td>
<td>0.11a</td>
<td>0.03c</td>
<td>0.12cde</td>
<td>0.03c</td>
<td>0.12cde</td>
<td>0.03c</td>
<td>0.12cde</td>
<td>0.03c</td>
<td>0.12cde</td>
<td>0.03c</td>
<td>0.12cde</td>
<td>0.03c</td>
<td>0.12cde</td>
<td>0.03c</td>
</tr>
<tr>
<td>CF-KI</td>
<td>43.54 ± 0.19</td>
<td>1.22 ± 0.02</td>
<td>3.04 ± 0.01</td>
<td>7.77 ± 0.02</td>
<td>22.72 ± 0.01</td>
<td>1.91 ± 0.02</td>
<td>2.13 ± 0.13</td>
<td>3.51 ± 0.13</td>
<td>9.58 ± 0.07</td>
<td>26.16 ± 0.01</td>
<td>36.65 ± 0.01</td>
<td>3.51 ± 0.01</td>
<td>3.38 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>0.17bc</td>
<td>0.06c</td>
<td>0.11e</td>
<td>0.01a</td>
<td>0.01a</td>
<td>0.01a</td>
<td>0.01a</td>
<td>0.01a</td>
<td>0.01a</td>
<td>0.01a</td>
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<td></td>
</tr>
<tr>
<td>CE-KI</td>
<td>42.25 ± 0.02c</td>
<td>1.26 ± 0.03</td>
<td>3.05 ± 0.14</td>
<td>8.12 ± 0.21</td>
<td>23.17 ± 0.02</td>
<td>1.88 ± 0.03</td>
<td>2.02 ± 0.04</td>
<td>3.39 ± 0.13</td>
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<td>25.72 ± 0.01</td>
<td>37.47 ± 0.01</td>
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<td>3.32 ± 0.01</td>
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<tr>
<td>CE-PE</td>
<td>42.57 ± 0.02c</td>
<td>1.28 ± 0.01</td>
<td>2.97 ± 0.06</td>
<td>8.03 ± 0.01</td>
<td>23.24 ± 0.01</td>
<td>1.91 ± 0.01</td>
<td>2.03 ± 0.04</td>
<td>3.25 ± 0.04</td>
<td>9.40 ± 0.06</td>
<td>25.74 ± 0.01</td>
<td>37.41 ± 0.01</td>
<td>1.86 ± 0.01</td>
<td>3.31 ± 0.01</td>
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<tr>
<td>CE45</td>
<td>43.05 ± 0.02c</td>
<td>1.27 ± 0.01</td>
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<td>23.27 ± 0.01</td>
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<td>3.41 ± 0.04</td>
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<td>25.92 ± 0.01</td>
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<tr>
<td>PES45</td>
<td>43.53 ± 0.03c</td>
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<td>3.09 ± 0.05</td>
<td>7.89 ± 0.05</td>
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<td>1.93 ± 0.01</td>
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<tr>
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<td>0.02ab</td>
<td></td>
</tr>
<tr>
<td>PES22</td>
<td>42.91 ± 0.03c</td>
<td>1.17 ± 0.01</td>
<td>3 ± 0.01</td>
<td>7.77 ± 0.01</td>
<td>22.81 ± 0.01</td>
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<td>25.93 ± 0.01</td>
<td>36.60 ± 0.01</td>
<td>2.95 ± 0.01</td>
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<tr>
<td>0.15cd</td>
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<td>0.05c</td>
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<td>0.02b</td>
<td>0.13bcde</td>
<td>0.02b</td>
<td>0.13bcde</td>
<td>0.02b</td>
<td>0.13bcde</td>
<td>0.02b</td>
<td>0.13bcde</td>
<td>0.02b</td>
<td>0.13bcde</td>
<td>0.02b</td>
</tr>
<tr>
<td>PVDF45</td>
<td>42.99 ± 0.03c</td>
<td>1.23 ± 0.01</td>
<td>3.06 ± 0.01</td>
<td>8.01 ± 0.01</td>
<td>23.05 ± 0.01</td>
<td>1.86 ± 0.02</td>
<td>2.05 ± 0.03</td>
<td>3.45 ± 0.03</td>
<td>9.57 ± 0.02</td>
<td>25.99 ± 0.01</td>
<td>37.19 ± 0.01</td>
<td>2.63 ± 0.01</td>
<td>3.37 ± 0.01</td>
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<tr>
<td>0.05cd</td>
<td>0.01c</td>
<td>0.01ab</td>
<td>0.03abc</td>
<td>0.01ab</td>
<td>0.03abc</td>
<td>0.01ab</td>
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<tr>
<td>PVDVF2</td>
<td>42.79 ± 0.02c</td>
<td>1.20 ± 0.02</td>
<td>2.97 ± 0.07</td>
<td>7.91 ± 0.01</td>
<td>23.07 ± 0.01</td>
<td>1.87 ± 0.02</td>
<td>2.01 ± 0.03</td>
<td>3.35 ± 0.03</td>
<td>9.42 ± 0.06</td>
<td>25.89 ± 0.01</td>
<td>37.02 ± 0.01</td>
<td>2.43 ± 0.01</td>
<td>3.35 ± 0.01</td>
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</tr>
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<td>0.02ab</td>
<td>0.03bc</td>
<td>0.07abcd</td>
<td>0.02ab</td>
<td>0.03bc</td>
<td>0.02ab</td>
<td>0.03bc</td>
<td>0.02ab</td>
<td>0.03bc</td>
<td>0.02ab</td>
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<td>0.03bc</td>
<td></td>
</tr>
<tr>
<td>PTFE45</td>
<td>43.19 ± 0.06c</td>
<td>1.23 ± 0.01</td>
<td>3.05 ± 0.1</td>
<td>8.01 ± 0.1</td>
<td>23.05 ± 0.1</td>
<td>1.86 ± 0.02</td>
<td>2.05 ± 0.04</td>
<td>3.45 ± 0.04</td>
<td>9.57 ± 0.02</td>
<td>25.99 ± 0.1</td>
<td>37.19 ± 0.1</td>
<td>2.63 ± 0.01</td>
<td>3.37 ± 0.01</td>
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<tr>
<td>0.31bc</td>
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<td>0.22bcde</td>
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<td>0.22bcde</td>
<td>0.01b</td>
<td>0.22bcde</td>
<td>0.01b</td>
<td>0.22bcde</td>
<td>0.01b</td>
<td>0.22bcde</td>
<td>0.01b</td>
<td>0.22bcde</td>
<td>0.01b</td>
</tr>
</tbody>
</table>

**Note:** Same letter in the same columns indicate no significant difference between different filtration treatments by Tukey test (P < 0.05). All data is expressed as mg malvidin-3-O-glucoside chloride/L. TA, total monomeric anthocyanins. CO, control. CF-KI, kieselguhr accompanied by centrifugation. CF-PE, perlite accompanied by centrifugation. CE-KI, Cellulose membrane pre-coated with kieselguhr. CE-KI, cellulose membrane pre-coated with perlite. CE45, 0.45 μm pore size cellulose. PES45, 0.45 μm pore size polyethersulfone. PES22, 0.22 μm pore size polyethersulfone. PVDF45, 0.45 μm pore size polyvinylidene difluoride. PVDF22, 0.22 μm pore size polyvinylidene difluoride. PTFE45, 0.45 μm pore size polytetrafluoroethylene.
depth filtration aids on these compounds. Moreover, acetylated glucosides in wines subjected to membrane filtration treatments were also significantly reduced by 21.1%–43.3%, but these treatments had no significant influence on cinnamoyl-glucosides. However, Gonçalves et al. (2012) reported significant adsorption of membrane filter on cinnamoyl anthocyanins compared to other forms of anthocyanins. Vieira et al. (2018) demonstrated that anthocyanins content variations in membrane filtered wines were impacted by membrane texture surface properties with different adsorption capacity.

Compared to the control, CF-KI and PES45 treatments had a minor effect on TA with a reduction by 1.1%, whereas CF-PE treatment caused significant reduction of TA by 1.7%. Moreover, in most cases, membrane filters resulted in significant reduction of TA reaching 3.3%. The lower TA in filtration treated wines could be partially responsible for the lower CI of these wines compared to the control, given that a correlation on these two parameters was found on a large set of red wines (Giacosa et al., 2021). The decreases of TA and CI in treated wines could be partially due to the adsorption of the depth filter aids and membrane filters on acetyl anthocyanins (Arriagada-Carrarozana et al., 2005; Oberholster et al., 2013; Vieira et al., 2018). In general, compared to the untreated sample, the filtration treatments caused also some modifications in the anthocyanin profile of ‘Nebbiolo’ wines, with the highest impact provided by CE-KI and CE-PE treatments (Table 2).

### 3.3. Filtration treatment on grapevine DNA traceability

DNA in wine was extracted using a CTAB based method (Gambino et al., 2022) and the results were reported in Table 3. The DNA yield and two absorbance ratios ($A_{260}/A_{230}$ and $A_{260}/A_{280}$), generally used to estimate the quality of extracted DNA, were determined through a spectrophotometric analysis. High quality DNA was obtained in CO and after some filtration approaches (CF-KI and PTFE45). However, after many filtration treatments the absorbance ratios, in particular $A_{260}/A_{230}$, were low indicating a high content of polyphenols and carbohydrates in the extracted DNA (Table 3), in accordance with previous results (Agrimonti & Marmiroli, 2018; Gambino et al., 2022; Zambianchi et al., 2021, 2022).

Further, the presence of yeast DNA and phenolic substances in the extracted DNA from wine resulted in overestimation of spectrophotometric quantified grapevine DNA yield (Boccacci et al., 2020; Gambino et al., 2022). The more reliable grapevine DNA content could be quantified by qPCR using specific DNA makers as the VvNCED2 amplified by TaqMan® probe (Fig. S2), as previously suggested (Savazzini & Martelli, 2006). The grapevine DNA, expressed as VvNCED2, accounts for up to 0.30% of the extracted DNA samples in CO and filtration treated wines, in agreement with Boccacci et al. (2020) and Gambino et al. (2022) who also confirmed the overestimation by spectrophotometric analysis of DNA extracted from ‘Nebbiolo’ wines.

All the filtration treatments reduced the DNA recovery from ‘Nebbiolo’ wine from 37.2% to 99.7% depending on the filter characteristics (Table 3). For the two depth filtration aids, CF-KI and CF-PE, treatments showed significant reductions by 60.6% and 40.8% of the concentrations of grapevine DNA with respect to the control, confirmed the different absorption capacities of KI and PE on grapevine DNA. For membranes with pore size of 0.45 μm, PTFE provided the highest grapevine DNA removal (87.0%), followed by PVDF (80.4%) and PES (72.2%). Moreover, CE45 treatment showed the lowest reduction (37.2%) of grapevine DNA. The losses of grapevine DNA in wines subjected to 0.22 μm pore size membrane filters (PVDF and PES) were higher compared to the loss due to 0.45-μm corresponding membrane filters, but significant differences were not found. It should be mentioned that the 0.22-μm PVDF membrane filter significantly removed most of grapevine DNA (99.7%). Our results confirmed preliminary observation of Catalano et al. (2016), who reported that filtration with perlites caused significant reduction of DNA yield in wine. Although DNA molecules can pass through filter used in this work, the membrane filter treatments showed a reduction of DNA yield compared to unfiltered control (CO), suggesting that DNA molecules could be

### Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA yield ng/μL</th>
<th>A$<em>{260}$/A$</em>{320}$</th>
<th>A$<em>{260}$/A$</em>{280}$</th>
<th>VvNCED2 quantification DNA yield, pg/mL of wine</th>
<th>% Grapevine DNA</th>
<th>Loss of DNA after treatment, %</th>
<th>SNP_14783 R1</th>
<th>SNP_14783 R2</th>
<th>SNP_15082 R1</th>
<th>SNP_15082 R2</th>
<th>SNP_15082 R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>21.56 ± 1.98a</td>
<td>1.91 ± 0.14a</td>
<td>1.53 ± 0.71bc</td>
<td>9.73 ± 0.80a</td>
<td>0.05 ± 0.006</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>CF-KI</td>
<td>18.53 ± 2.92a</td>
<td>2.02 ± 0.02a</td>
<td>1.88 ± 0.37bc</td>
<td>3.79 ± 0.21cd</td>
<td>0.02 ± 0.004</td>
<td>–60.6 ± 5.3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CF-PE</td>
<td>2.54 ± 0.18c</td>
<td>2.02 ± 0.36a</td>
<td>2.55 ± 0.70a</td>
<td>5.68 ± 0.85bc</td>
<td>0.23 ± 0.04</td>
<td>–40.8 ± 13.0</td>
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<td>+</td>
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<tr>
<td>CE-KI</td>
<td>1.76 ± 0.09c</td>
<td>1.70 ± 0.29a</td>
<td>0.67 ± 0.03cd</td>
<td>4.80 ± 1.42bcd</td>
<td>0.27 ± 0.07</td>
<td>–50.5 ± 13.8</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>CE-PE</td>
<td>2.03 ± 0.25c</td>
<td>1.19 ± 0.47b</td>
<td>0.61 ± 0.05cd</td>
<td>4.03 ± 0.76bcd</td>
<td>0.20 ± 0.03</td>
<td>–58.5 ± 7.4</td>
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<tr>
<td>CE45</td>
<td>1.98 ± 0.04c</td>
<td>1.78 ± 0.10a</td>
<td>0.61 ± 0.00cd</td>
<td>6.03 ± 0.99b</td>
<td>0.30 ± 0.05</td>
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<td>PES45</td>
<td>2.06 ± 0.86c</td>
<td>1.78 ± 0.28a</td>
<td>1.13 ± 0.394cd</td>
<td>2.65 ± 0.47def</td>
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<tr>
<td>PES22</td>
<td>2.09 ± 0.07c</td>
<td>1.39 ± 0.16ab</td>
<td>0.52 ± 0.18d</td>
<td>1.58 ± 0.44f</td>
<td>0.08 ± 0.02</td>
<td>–83.7 ± 3.4</td>
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<tr>
<td>PVDF45</td>
<td>1.58 ± 0.36c</td>
<td>1.50 ± 0.27ab</td>
<td>0.55 ± 0.14d</td>
<td>1.86 ± 0.72ef</td>
<td>0.12 ± 0.04</td>
<td>–80.4 ± 8.3</td>
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<tr>
<td>PVDF22</td>
<td>2.04 ± 0.25c</td>
<td>1.33 ± 0.16ab</td>
<td>0.54 ± 0.07d</td>
<td>0.01 ± 0.02g</td>
<td>0.0 ± 0</td>
<td>–99.7 ± 0.3</td>
<td>nd</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>PTFE45</td>
<td>8.48 ± 0.70b</td>
<td>1.91 ± 0.11a</td>
<td>1.55 ± 0.17bc</td>
<td>1.23 ± 0.36 fg</td>
<td>0.02 ± 0.004</td>
<td>–87.0 ± 4.2</td>
<td>+</td>
<td>+</td>
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</table>

Note: DNA purity and yield measured by NanoDrop; yield evaluated by a standard curve with FAM-labelled endogenous gene VvNCED2. Percentage ratio between DNA quantification by VvNCED2 and the yield measured by NanoDrop. Loss of DNA after treatment expressed as percentage ratio between the DNA (quantified by VvNCED2) of the control and the treated wine. Allelic profile of genotyping assay SNP_14783, SNP_15082 for each treatment replicate (R1, R2, and R3). ‘+’ indicates samples that correctly amplified, and ‘−’ stands for ‘not detected’. Same letter in the same columns indicate no significant difference between different filtration treatments by Tukey test (P < 0.05). CO, control. CF-KI, kieselguhr accompanied by centrifugation. CF-PE, perlite accompanied by centrifugation. CE-KI, Cellulose membrane pre-coated with kieselguhr. CE-KI, cellulose membrane pre-coated with perlite. CE45, 0.45 μm pore size cellulose. PES45, 0.45 μm pore size polyethersulfone. PES22, 0.22 μm pore size polyethersulfone. PVDF45, 0.45 μm pore size polyvinylidene difluoride. PVDF22, 0.22 μm pore size polyvinylidene difluoride. PTFE45, 0.45 μm pore size polytetrafluoroethylene.
adsorbed to the colloids in wine, and thus eliminated by filtration treatment. In general, all the filtration treatments lead to significant reduction of residual grapevine DNA in 'Nebbiolo' wine compared to the unfiltered control, and the reduction percentage depended on the treatment method and on the characteristic of material used, which have different adsorption characteristics on wine colloids and on DNA (Liang & Keeley, 2013).

'Nebbiolo' grape and wines can be distinguished from other grape cultivars using two previously validated 'Nebbiolo'-specific SNPs, SNP_15082 and SNP_14783 (Boccacci et al., 2020). The correct amplification of both SNPs by TaqMan® assays were observed in all wines except for the samples subjected to PVDF22 treatment (Fig. 2, Table 3). PCR inhibitors in extracted DNA can affect the efficiency of the genotyping assays for wine authenticity. Their presence in the samples was verified by adding an EIPC in all DNA extracts, the amplification efficiency of all wine samples ranged between 93% and 110%, without statistical differences when compared with the control containing DNA of optimal quality extracted from leaves and assuming an amplification efficiency of 100% (Fig. S1B). Therefore, the failure in varietal identification of grape DNA in 'Nebbiolo' wine treated with oenological processing aids and additives was observed in DNA samples with the concentration lower than 0.5 pg/mL of starting wine. This data was confirmed also in this work: after filtration of the 'Nebbiolo' wine, the PVDF22 was the only treatment with problems in SNP genotyping and with a DNA yield quantified by qPCR of VvNCED lower than 0.5 pg/mL of starting wine. Thus, the failure in recognizing DNA traces by TaqMan® assay in commercial 'Nebbiolo' wines after filtration depends on the combination of the pore size of the membranes (0.22 μm) and the characteristic of material, as polyvinylidene difluoride (PVDF22) was more effective at removing DNA than polyethersulfone (PES22).

4. Conclusions

In this study, the effects of filtration treatments using depth filtration aids and membrane filters on wine phenolic compounds and DNA traceability were evaluated. Although filtration treatments had no significant influence on TP, TF and TNA, those treatments decreased wine turbidity and CI. Filtration treatments showed significant reductions in the content of acetylated anthocyanins, leading to significantly decreased TA (with few exceptions), which could be responsible for the reduced CI in filtered wines. Filtration treatments can hinder genetic traceability of wine depending on the filtration method and the characteristic of material. 'Nebbiolo' was correctly identified by SNP based assay in wines subjected to depth filtration and membrane (CE, PES, PVDF, PTFE) filters with high pore size (0.45 μm) and low pore size (PES 0.22 μm). However, the membrane material PVDF with low pore size (0.22 μm) hindered TaqMan® assay, indicating that the uncertainty of authenticity of membrane-filtered wines by SNP-based assay was affected by the porosity and membrane material. This work, together with the previous study (Gambino et al., 2022), revealed that the combination of additives and filtration can make DNA recovery from wines very difficult, and future improvements of DNA extraction techniques from wine are needed.

Fig. 2. SNP genotyping in 'Nebbiolo' wines subjected to filtration treatments. (A, B) Scatterplots of TaqMan® SNP_14783 and TaqMan® SNP_15082 genotyping assays with 'Nebbiolo' wines. (C) Relative fluorescence unit (RFU) of the TaqMan® SNP_14783 probe tagged with VIC dye (allele G 'Nebbiolo'). (D) Relative fluorescence unit (RFU) of the TaqMan® SNP_15082 probe tagged with FAM dye (allele T 'Nebbiolo'). The control DNA from 'Nebbiolo', 'Barbera' and 'Freisa' were extracted from leaves. PVDF22: 0.22 μm pore size polyvinylidene difluoride.
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Paolo Bocciacci: Validation, Investigation, Formal analysis, Data curation.
Simone Giacosa: Writing – review & editing, Validation, Methodology, Investigation.
Simone Pace: Writing – review & editing, Visualization, Supervision, Methodology, Funding acquisition, Conceptualization.
Luca Rolle: Writing – review & editing, Supervision, Funding acquisition, Conceptualization.
Maria Alessandra Paisoni: Writing – review & editing, Validation, Investigation, Formal analysis, Data curation.

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.

Data availability
Data will be made available on request.

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Appendix A. Supplementary data
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