Food Bioscience 57 (2024) 103533

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Influence of filtration treatments on grapevine DNA traceability in wine

Jianqiang Song [a,b,1], Camilla De Paolis [b,1], Paolo Boccacci [c,1], Lorenzo Ferrero [b], Amedeo Moine [c], Susana Río Segade [b], Simone Giacosa [b], Giorgio Gambino [c,*], Luca Rolle [b], Maria Alessandra Paissoni [b]

[a] School of Life Sciences, Ludong University, Yantai, 264025, China
[b] Department of Agricultural, Forest and Food Sciences, University of Torino, 10095, Grugliasco, Torino, Italy
[c] Institute for Sustainable Plant Protection, National Research Council of Italy (IPSP-CNR), Torino. Strada Delle Cacce 73, 10135, Torino, Italy

ARTICLE INFO

Keywords:
Wine
Clarification
Membrane filtration
Genetic traceability
Nebbiolo
SNPs

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 & Marmiroli, 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, Liò, & Scali, 2019). Whereas the

AbbreviationsAbbreviations: SSRs, Simple sequence repeats; SNPs, Single nucleotide polymorphisms; EDTA, Ethylene diamine tetraacetic acid; NaCl, Sodium chloride; CTAB, Cetyltrimethylammonium bromide; EIPC, TaqMan® exogenous internal positive control; KI, Kieselguhr; PE, Perlite; CE, Cellulose; PES, Polyethersulfone; PVDF, Polyvinylidene difluoride; PTFE, Polytetrafluoroethylene; CO, Control; HPLC, High performance liquid chromatography; CI, Color intensity; TP, The total polyphenol index; TF, Total flavonoids; TNA, Total non-anthocyanins flavonoids; TA, Total monomeric anthocyanins; qPCR, Quantitative PCR; NTU, Nephelometric turbidity units.

* Corresponding author.
E-mail address: giorgio.gambino@ipsp.cnr.it (G. Gambino).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.fbio.2023.103533
Received 1 November 2023; Received in revised form 21 December 2023; Accepted 22 December 2023
Available online 28 December 2023

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-Carrazana et al., 2005; Doulia et al., 2016; Prodanov 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-*O*-glucoside chloride 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), 1.4 M NaCl, 1M 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). NucleoSpin® Plant II were purchased from Macherey-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 IMERYS 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/hL). 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 PMA 4 pneumatic press (Velo SpA, Altivole, Italy). Malolactic fermentation was induced by the inoculation of *Oenococcus oeni* (Malotabs™, Lallemand Inc., Montreal, Quebec, Canada). After malolactic fermentation, 50 mg/L SO_2 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 SO_2. 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 case 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, Tuttlingen, 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, Weihheiim, 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 a UV-1800 spectrophotometer (Shimazdu, Kyoto, Japan). Wine color intensity (CI) and hue were determined by measuring the absorbance at 420, 520, 620 nm, and expressed as $A_{420}+A_{520}+A_{620}$ on 10 mm path length and the ratio between A_{420} and

A_{520}, respectively, according to OIV-MA-AS2-07B method (OIV, 2020). CIELab values including L^*, a^* and b^* color components, were determined, and Chroma (C^*) and hue angle (h) and the total color difference (ΔE^*) between CO and filtration treated wines was calculated according to OIV-MA-AS2-11 method (OIV, 2020).

The total polyphenol index (TP) was determined by measuring A_{280} and reported as mg/L of (−)-epicatechin (Scalzini et al., 2020). Total 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.

2.3.3. Individual anthocyanins

Wine samples were diluted one time with a HCl solution (pH 0.5), then filtered using PTFE 0.45 μm syringe filters (Lab Logistics Group GmbH, Meckenheim, Germany) before HPLC injection. Fifty μL of the samples were injected in a HPLC system (Agilent 1260, Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector. Compound separation was performed using a LiChroCART analytical column (25 cm × 0.4 cm) obtained from Merck (Darmstadt, Germany). Mobile phase A and B were 10% formic acid aqueous solution and 10% formic acid aqueous solution containing 50% methanol, respectively. The following gradient was adopted: 0–15 min, 28%–45% B; 15–35 min, 45%–70% B; 35–45 min, 70%–90% B; 45–48 min, 99% B; 48–58 min, 28% B, with a flow rate of 1.0 mL/min. The individual anthocyanins were identified and quantified as described by Río Segade et al. (2014). Total monomeric anthocyanin content (TA) was calculated as the sum of individual anthocyanins (mg/L of malvidin-3-*O*-glucoside chloride).

2.4. DNA traceability analysis

2.4.1. DNA extraction from wines

CTAB based method 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 KL refrigerated centrifuge (Sigma Laborzentrifugen, Osterode am Harz, Germany), then the wine pellet was dissolved in 5 mL of TEX buffer, which was incubated for 1 h at 65 °C with mixing at the interval of 10–15 min. Five mL of chloroform: isoamyl alcohol (24:1) was added into the sample, then sequentially homogenized and centrifuged (Sigma 3-16 KL centrifuge) for 10 min (8000×*g*, 4 °C). The supernatant added with 0.1 volume of pre-warmed 10% CTAB (65 °C) was extracted again with 1 volume of chloroform: isoamyl alcohol. The aqueous phase added with 2 volumes of cold ethanol was store in freezer (−25 °C) overnight. The precipitated DNA was obtained after centrifugation (10,000×*g*, 30 min, 4 °C) using a Hermle Z216-MK Refrigerated Microcentrifuge (Wehingen, Germany), suspended in TE buffer (250 μL) and incubated (30 min, 48 °C) with the addition of proteinase K (20 μL, 20 mg/mL). Then, the sample was added with 1 volume of phenol:chloroform:isoamyl alcohol (25:24:1), homogenized and centrifuged (Z216-MK Microcentrifuge) (11,000×*g*, 15 min, 4 °C). The aqueous phase was added with 2 volumes of cold ethanol and 2.5 mol/L of ammonium acetate, and store in freezer (−25 °C) for 2 h. The pellets were obtained after centrifugation (Z216-MK Microcentrifuge) for 30 min (20,000×*g*, 4 °C) and washed with cold ethanol (500 μL, 70%, v/v). The extracted DNA was dissolved in 100 μL of ultrapure sterile water, then purified with the NucleoSpin® Plant Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. DNA quantity and quality were evaluated by measuring the absorbance at 230, 260, 280 nm, using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA was stored at −20 °C until analysis.

2.4.2. Grapevine DNA quantification and PCR inhibitors determination

Quantitative PCR (qPCR) amplification of *VvNCED2* (VIT_10s0003g03750) was used for grapevine DNA quantification following the method reported by Savazzini and Martinelli (2006). TaqMan® EIPC reagents were used for the evaluation of the presence of PCR inhibitors in the extracted DNA (Boccacci et al., 2020). The qPCR mixture was composed of the extracted DNA (2.5 μL), TaqMan® Environmental Master Mix 2.0 (5 μL), 0.4 μL of EIPC DNA, 2 μL of EIPC 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 *VvNCED2* TaqMan® 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), 40X TaqMan® SNP Genotyping Assay mix (0.25 μL), and sterile water (2.25 μL). The amplification cycles were the same reported in Section 2.4.2. Allelic discrimination was performed using the CFX Maestro Software version 2.0 (Hercules, CA, USA). All the extracted DNA samples were determined 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.

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%, 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 (ranged from 0.09 to 1.47 NTU) indicate a wine clarification (Ribéreau-Gayon et al., 2006).

CF-KI, CF-PE, CE-KI and CE-PE had no significant influence on phenolic compounds including TP, TF and TNA, indicating the minor adsorbent capacity of depth filter agents on wine phenolic compounds (Ribéreau-Gayon et al., 2006). Similarly, compared to the unfiltered control, membrane filtration treatments showed no significant influence on the phenolic compounds including TP, TF and TNA, in accordance with Buffon et al. (2014) who found that cross-flow microfiltration had no significant influence on wine phenolic profile. However, reductions of polyphenols in membrane filtered wine were observed in previous studies because of strong adsorption of membranes on these compounds (Arriagada-Carrazana et al., 2005; Prodanov et al., 2019). Rosária et al. (2022) revealed that the influence of filtration on wine phenolic composition was dependent on filtration type and initial wine composition.

In most cases, the hue of the filtered wines was not influenced except

Table 1
Turbidity, phenolic composition, color parameters of 'Nebbiolo' wines subjected to filtration treatments.

Treatment	Turbidity	TF	TNA	TP	L^*	a^*	b^*	C^*	h	CI	Hue
CO	12.28 ± 0.23a	2608 ± 34a	2447 ± 34a	4836 ± 75a	34.7 ± 0.1d	53.71 ± 0.22e	42.88 ± 0.20d	68.72 ± 0.30e	38.60 ± 0.02cde	4.51 ± 0.01 ab	1.03±0a
CF-KI	4.60 ± 0.32c	2637 ± 72a	2484 ± 64a	4919 ± 102a	35.6 ± 0.1c	54.83 ± 0.12d	43.74 ± 0.07c	70.14 ± 0.13d	38.58 ± 0.03de	4.45 ± 0.01bc	1.03±0 ab
CF-PE	8.77 ± 0.69b	2638 ± 27a	2479 ± 25a	4809 ± 59a	35.7 ± 0.3bc	54.84 ± 0.19d	43.77 ± 0.22c	70.16 ± 0.28cd	38.60 ± 0.07cde	4.44 ± 0.04c	1.03±0 ab
CE-KI	0.19 ± 0.21e	2618 ± 34a	2450 ± 31a	4732 ± 85a	36.3 ± 0.1 ab	55.76 ± 0.11abc	44.59 ± 0.08b	71.40 ± 0.13 ab	38.65 ± 0.05cd	4.42 ± 0.01cd	1.02±0bc
CE-PE	0.24 ± 0.13e	2604 ± 29a	2434 ± 27a	4866 ± 75a	36.1 ± 0.1abc	55.72 ± 0.10abc	44.64 ± 0.06 ab	71.4 ± 0.11 ab	38.70 ± 0.01bc	4.44 ± 0.01bc	1.02 ±0abc
CE45	0.23 ± 0.08e	2596±7a	2434 ± 10a	4825 ± 33a	36.1 ± 0.4abc	55.28 ± 0.30cd	43.97 ± 0.21c	70.63 ± 0.37cd	38.50 ± 0.02ef	4.40 ± 0.05cd	1.03±0 ab
PES45	0.81 ± 0.13de	2598 ± 20a	2446 ± 13a	4877 ± 53a	36.4 ± 0.1a	55.46 ± 0.14bc	44.04 ± 0.13c	70.81 ± 0.19bc	38.45 ± 0.01f	4.37 ± 0.02e	1.03±0 ab
PES22	0.23 ± 0.21e	2586 ± 28a	2417 ± 21a	4754 ± 21a	36.4 ± 0.0a	55.88 ± 0.23 ab	44.77 ± 0.30 ab	71.61 ± 0.37a	38.70 ± 0.08bc	4.41 ± 0.01cd	1.02 ±0acbc
PVDF45	0.30 ± 0.11e	2635 ± 29a	2456 ± 27a	4727 ± 70a	36.4 ± 0.1a	55.72 ± 0.05abc	44.58 ± 0.06b	71.36 ± 0.08 ab	38.66 ± 0.01cd	4.40 ± 0.01cd	1.03±0 ab
PVDF22	0.09 ± 0.03e	2635 ± 13a	2464 ± 11a	4888 ± 152a	36.1 ± 0.0abc	55.92 ± 0.02 ab	44.95 ± 0.03 ab	71.74 ± 0.04a	38.79 ± 0.01 ab	4.45±0bc	1.02±0bc
PTFE45	1.47 ± 0.15d	2637 ± 15a	2473 ± 19a	4765 ± 87a	36.7 ± 0.2bc	56.01 ± 0.15a	45.10 ± 0.16a	71.91 ± 0.22a	38.84 ± 0.03a	4.52 ± 0.03a	1.02±0c

Note: Same letter in the same columns indicate no significant difference between different filtration treatments by Tukey test ($P < 0.05$). Turbidity is expressed as nephelometric turbidity units (NTU). TP, Total phenolic index, expressed as (−)-epicatechin/L. TF, total flavonoids, expressed as (+)-catechin/L. TNA, total non-anthocyanins 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. PVDF45, 0.45 μm pore size polyvinylidene difluoride. PVDF22, 0.22 μm pore size polyvinylidene difluoride. PTFE45, 0.45 μm pore size polytetrafluoroethylene.

for CE-KI, PVDF22 and PTFE45 treatments (only decreased by 1%), indicating the unchanged proportion between yellow and red in these wines. Arriagada-Carrazana et al. (2005) reported a slight decrease of hue in membrane filtered wines. A slight reduction in CI between1.2%–3.0% was observed in filtered wines, but not after PTFE45 treatments (Table 1). Similarly, Oberholster et al. (2013) demonstrated a CI reduction in membrane filtered wines, although Buffon et al. (2014) reported that there was no significant influence on wine color between the control and cross-flow microfiltration treated wines.

A minor difference in the CIELab coordinates (L^*, a^* and b^*) for different wines after filtration was obtained. All the filtration treatments showed significant increase in L^*, but minor decrease in a^* and b^* with respect to the control. Rosária et al. (2022) found a decrease in the a^* coordinate, but a slight increase in the L^* coordinate of filtration treated wines. The color differences were visually confirmed in Fig. 1. Wines treated with the two depth filtration treatments (CE-KI and CE-PE) and membrane filters (PVDF45, PVDF22, PES22 and PTFE45) showed higher ΔE^* values than the generally accepted visual recognition threshold (3.0) of wine color differences (Pérez-Magariño & González-Sanjosé, 2003).

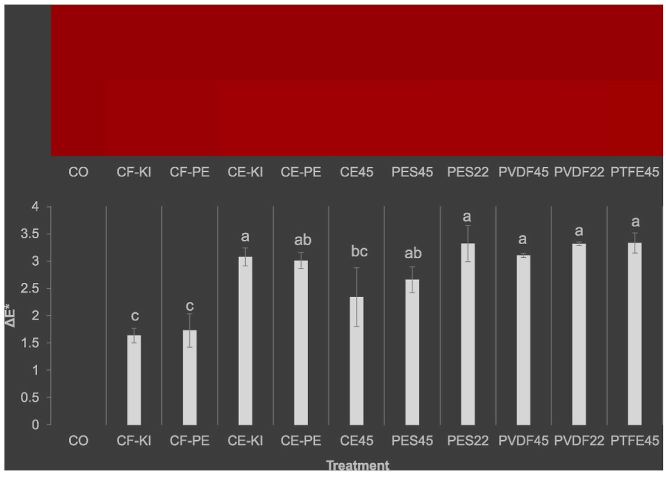

Fig. 1. RGB color of 'Nebbiolo' wines subjected to filtration treatments. Same letter in the same columns indicate no significant difference between different filtration treatments by Tukey test ($P < 0.05$). CO, control. CF-KI, kieselguhr accompanied by centrifugation. CF-PE, perlite accompanied by 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. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Filtration treatment on individual anthocyanins

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 by peonidin-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 PVDF45 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 SO_2 from anthocyanins (Berké 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.

Treatment	TA	Delphinidin-3-*O*-glucoside	Cyanidin-3-*O*-glucoside	Petunidin-3-*O*-glucoside	Peonidin-3-*O*-glucoside	Malvidin-3-*O*-glucoside	∑ Delphinidin derivatives	∑ Cyanidin derivatives	∑ Petunidin derivatives	∑ Peonidin derivatives	∑ Malvidin derivatives	∑ simple glycosides	∑ acetyl glucosides	∑ cinnamoyl glucosides
CO	44.01 ± 0.10a	1.93 ± 0.03	1.34 ± 0.11a	3.05 ± 0.02	7.79 ± 0.03c	22.80 ± 0.12cde	1.93 ± 0.03	2.29 ± 0.11a	3.54 ± 0.03a	9.64 ± 0.02a	26.35 ± 0.12a	36.9 ± 0.1cd	3.74 ± 0.06a	3.37 ± 0.05
CF-KI	43.54 ± 0.21 ab	1.9 ± 0.02	1.22 ± 0.13 ab	3.04 ± 0.01	7.77 ± 0.06c	22.72 ± 0.11e	1.9 ± 0.02	2.13 ± 0.13 ab	3.51 ± 0.01a	9.58 ± 0.07 ab	26.16 ± 0.12 ab	36.65 ± 0.16d	3.51 ± 0.08b	3.38 ± 0.06
CF-PE	43.24 ± 0.17bc	1.92 ± 0.04	1.17 ± 0.01b	3.06 ± 0.02	7.75 ± 0.09c	22.72 ± 0.09de	1.92 ± 0.04	2.07 ± 0.01b	3.51 ± 0.02a	9.48 ± 0.09abc	26.00 ± 0.08abc	36.62 ± 0.16d	3.25 ± 0.09c	3.38 ± 0.04
CE-KI	42.75 ± 0.2cd	1.88 ± 0.03	1.26 ± 0.03 ab	3.05 ± 0.14	8.12 ± 0.06a	23.17 ± 0.17a	1.88 ± 0.03	2.02 ± 0.04b	3.39 ± 0.13 ab	9.49 ± 0.06abc	25.72 ± 0.17c	37.47 ± 0.21a	1.96 ± 0.04hi	3.32 ± 0.03
CE-PE	42.57 ± 0.02d	1.89 ± 0.01	1.28 ± 0.03 ab	2.97 ± 0.06	8.03 ± 0.12 ab	23.24 ± 0.08a	1.89 ± 0.01	2.03 ± 0.03b	3.25 ± 0.11b	9.40 ± 0.11c	25.74 ± 0.07c	37.41 ± 0.08 ab	1.86 ± 0.10i	3.31 ± 0.03
CE45	43.05 ± 0.19bcd	1.91 ± 0.05	1.27 ± 0.03 ab	3.05 ± 0.07	8.09 ± 0.06a	23.27 ± 0.1a	1.91 ± 0.05	2.04 ± 0.01b	3.41 ± 0.07 ab	9.51 ± 0.07abc	25.92 ± 0.12bc	37.6 ± 0.16a	2.12 ± 0.04gh	3.34 ± 0.05
PES45	43.53 ± 0.21 ab	1.93 ± 0.01	1.23 ± 0.02 ab	3.09 ± 0.05	7.89 ± 0.06bc	23.15 ± 0.07 ab	1.93 ± 0.01	2.09 ± 0.03b	3.51 ± 0.05a	9.52 ± 0.06abc	26.21 ± 0.10 ab	37.29 ± 0.14abc	2.83 ± 0.08d	3.4 ± 0.03
PES22	42.91 ± 0.15cd	1.85 ± 0.02	1.17 ± 0.01b	3 ± 0.01	7.77 ± 0.05c	22.81 ± 0.11bcde	1.85 ± 0.02	2.03 ± 0.02b	3.42±0 ab	9.42 ± 0.04bc	25.93 ± 0.11bc	36.60 ± 0.15d	2.95 ± 0.05d	3.35 ± 0.01
PVDF45	42.99 ± 0.05cd	1.89 ± 0.03	1.23 ± 0.01 ab	3.06 ± 0.01	8.06 ± 0.01 ab	23.12 ± 0.03abc	1.89 ± 0.03	2.04±0b	3.43 ± 0.01 ab	9.52±0abc	25.86 ± 0.02bc	37.36 ± 0.04 ab	2.26 ± 0.02 fg	3.36 ± 0.02
PVDF22	42.79 ± 0.01cd	1.87 ± 0.02	1.20 ± 0.02 ab	2.97 ± 0.07	7.91 ± 0.03bc	23.07 ± 0.07abcd	1.87 ± 0.02	2.01 ± 0.02b	3.35 ± 0.06 ab	9.42 ± 0.03bc	25.89 ± 0.09bc	37.02 ± 0.05bcd	2.43 ± 0.04f	3.35 ± 0.05
PTFE45	43.19 ± 0.31bc	1.86 ± 0.02	1.22 ± 0.01 ab	3.05 ± 0.1	8.01 ± 0.02 ab	23.05 ± 0.22bcde	1.86 ± 0.02	2.05 ± 0.01b	3.45 ± 0.1 ab	9.57 ± 0.02 ab	25.99 ± 0.25abc	37.19 ± 0.27abc	2.63 ± 0.07e	3.37 ± 0.03

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 coumaroylated 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-Carrazona 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_{280} and A_{260}/A_{230}), 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 & Martinelli, 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
Quantity and quality extracted DNA from 'Nebbiolo' wines subjected to filtration treatments.

Sample	DNA yield ng/mL wine	A_{260}/A_{280}	A_{260}/A_{230}	VvNCED2 quantification DNA yield, pg/mL of wine	% Grapevine DNA	Loss of DNA after treatment, %	SNP_14783			SNP_15082		
							R1	R2	R3	R1	R2	R3
CO	21.56 ± 1.98a	1.91 ± 0.14a	1.53 ± 0.71bc	9.73 ± 0.80a	0.05 + 0.0006	–	+	+	+	+	+	+
CF-KI	18.53 ± 2.92a	2.02 ± 0.02a	1.88 ± 0.06 ab	3.79 ± 0.21cde	0.02 + 0.004	−60.6 ± 5.3	+	+	+	+	+	+
CF-PE	2.54 ± 0.16c	2.02 ± 0.36a	2.55 ± 0.70a	5.68 ± 0.85bc	0.23 + 0.04	−40.8 ± 13.0	+	+	+	+	+	+
CE-KI	1.76 ± 0.09c	1.70 ± 0.29 ab	0.67 ± 0.03cd	4.80 ± 1.42bcd	0.27 + 0.07	−50.5 ± 13.8	+	+	+	+	+	+
CE-PE	2.03 ± 0.25c	1.19 ± 0.47b	0.61 ± 0.05cd	4.03 ± 0.76bcd	0.20 + 0.03	−58.5 ± 7.4	+	+	+	+	+	+
CE45	1.98 ± 0.04c	1.78 ± 0.10 ab	0.61 ± 0.0cd	6.03 ± 0.99b	0.30 + 0.05	−37.2 ± 14.8	+	+	+	+	+	+
PES45	2.06 ± 0.86c	1.78 ± 0.28 ab	1.13 ± 0.324bcd	2.65 ± 0.47def	0.13 + 0.04	−72.2 ± 6.9	+	+	+	+	+	+
PES22	2.09 ± 0.07c	1.39 ± 0.16 ab	0.52 ± 0.18d	1.58 ± 0.44 fg	0.08 + 0.02	−83.7 ± 3.4	+	+	+	+	+	+
PVDF45	1.58 ± 0.36c	1.50 ± 0.27 ab	0.55 ± 0.14d	1.86 ± 0.72efg	0.12 + 0.04	−80.4 ± 8.3	+	+	+	+	+	+
PVDF22	2.04 ± 0.25c	1.33 ± 0.16 ab	0.54 ± 0.07d	0.01 ± 0.02g	0 + 0	−99.7 ± 0.3	nd	nd	+	+	nd	nd
PTFE45	8.48 ± 0.70b	1.91 ± 0.11 ab	1.55 ± 0.17bc	1.23 ± 0.36 fg	0.02 + 0.004	−87.0 ± 4.2	+	+	+	+	+	+

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 'nd' 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 in wines subjected to PVDF22 treatment was not linked to the presence of PCR inhibitors, but was due to the low DNA yield in the extracted samples. Gambino et al. (2022) previously reported that loss of identification efficiency 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.

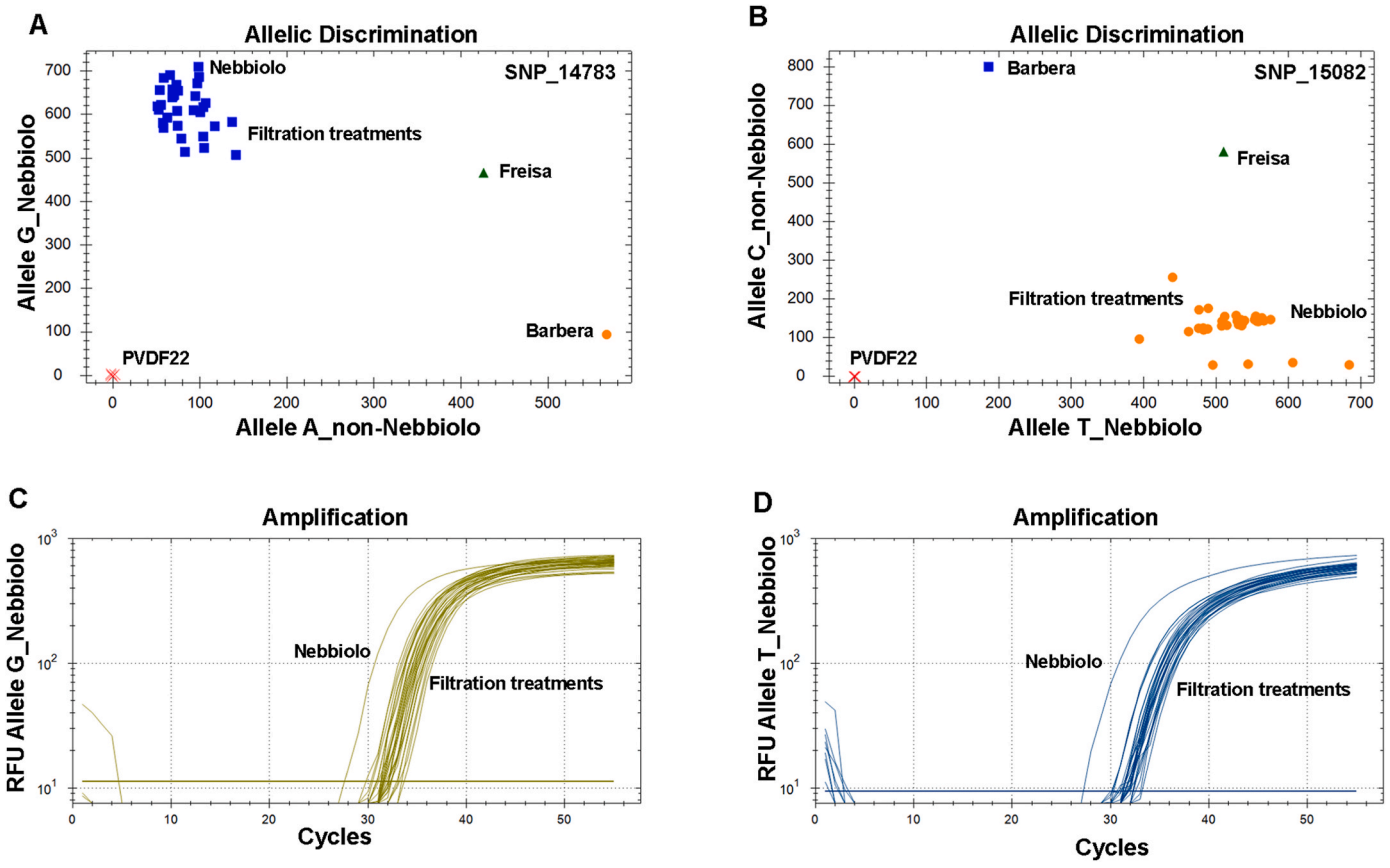

Fig. 2. SNP genotyping in 'Nebbiolo' wines subjected to filtration treatments. (A, B) Scatterplots of TaqMan® SNP_14783 and TaqMan® SNP_15082 genotyping assays with 'Nebbiolo' wines. (C) Relative fluorescence unit (RFU) of the TaqMan® SNP_14783 probe tagged with VIC dye (allele G 'Nebbiolo'). (D) Relative 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.

Funding

This publication is part of the project NODES which has received funding from the MUR – M4C2 1.5 of PNRR with grant agreement no. ECS00000036.

CRediT authorship contribution statement

Jianqiang Song: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Camilla De Paolis:** Validation, Investigation, Formal analysis, Data curation. **Paolo Boccacci:** Validation, Investigation, Formal analysis, Data curation. **Lorenzo Ferrero:** Validation, Resources, Investigation, Formal analysis, Data curation. **Amedeo Moine:** Investigation, Formal analysis, Data curation. **Susana Río Segade:** Writing – review & editing, Validation, Methodology, Investigation. **Simone Giacosa:** Writing – review & editing, Validation, Resources, Methodology. **Giorgio Gambino:** Writing – review & editing, Visualization, Supervision, Methodology, Funding acquisition, Conceptualization. **Luca Rolle:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Maria Alessandra Paissoni:** 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.

Acknowledgements

Jianqiang Song would like to thank all the authors for their support and contributions for this research and acknowledge the financial support of Shandong Provincial Education System Government Sponsored Study Abroad Program.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2023.103533.

References

Agrimonti, C., & Marmiroli, N. (2018). PCR analysis of experimental and commercial wines by means of nuclear and chloroplast SSRs. *European Food Research and Technology, 244*(12), 2127–2140. https://doi.org/10.1007/s00217-018-3121-5

Arriagada-Carrazana, J. P., Sáez-Navarrete, C., & Bordeu, E. (2005). Membrane filtration effects on aromatic and phenolic quality of Cabernet Sauvignon wines. *Journal of Food Engineering, 68*(3), 363–368. https://doi.org/10.1016/j.jfoodeng.2004.06.011

Baleiras-Couto, M. M., & Eiras-Dias, J. E. (2006). Detection and identification of grape varieties in must and wine using nuclear and chloroplast microsatellite markers. *Analytica Chimica Acta, 563*(1), 283–291. https://doi.org/10.1016/j.aca.2005.09.076

Berké, B., Chèze, C., Vercauteren, J., & Deffieux, G. (1998). Bisulfite addition to anthocyanins: Revisited structures of colourless adducts. *Tetrahedron Letters, 39*(32), 5771–5774. https://doi.org/10.1016/S0040-4039(98)01205-2

Boccacci, P., Akkak, A., Torello Marinoni, D., Gerbi, V., & Schneider, A. (2012). Genetic traceability of Asti Spumante and Moscato d'Asti musts and wines using nuclear and chloroplast microsatellite markers. *European Food Research and Technology, 235*(3), 439–446. https://doi.org/10.1007/s00217-012-1770-3

Boccacci, P., Chitarra, W., Schneider, A., Rolle, L., & Gambino, G. (2020). Single-nucleotide polymorphism (SNP) genotyping assays for the varietal authentication of 'Nebbiolo' musts and wines. *Food Chemistry, 312*. https://doi.org/10.1016/j.foodchem.2019.126100. Article 126100.

Buffon, P., Heymann, H., & Block, D. E. (2014). Sensory and chemical effects of cross-flow filtration on white and red wines. *American Journal of Enology and Viticulture, 65*(3), 305–314. https://doi.org/10.5344/ajev.2014.13090

Cai, M., Xie, C., Zhong, H., Tian, B., & Yang, K. (2021). Identification of anthocyanins and their fouling mechanisms during non-thermal nanofiltration of blueberry aqueous extracts. *Membranes, 11*(3), Article 200. https://doi.org/10.3390/membranes11030200

Cameira-Dos-Santos, P. J., Cheynier, V., Brillouet, J. M., Cadot, Y., Escudier, J. L., Roesink, H. D. V., & Moutounet, M. (1994). Cross-flow filtration of wines with polymeric membranes: The influence of wine making technology and backpulse on the fouling process. *Prehrambeno Tehnologije Biotehnologije, 32*(2–3), 77–83.

Cassano, A., De Luca, G., Conidi, C., & Drioli, E. (2017). Effect of polyphenols-membrane interactions on the performance of membrane-based processes. A review. *Coordination Chemistry Reviews, 351*, 45–75. https://doi.org/10.1016/j.ccr.2017.06.013

Catalano, V., Moreno-Sanz, P., Lorenzi, S., & Grando, M. S. (2016). Experimental review of DNA-based methods for wine traceability and development of a single-nucleotide polymorphism (SNP) genotyping assay for quantitative varietal authentication. *Journal of Agricultural and Food Chemistry, 64*(37), 6969–6984. https://doi.org/10.1021/acs.jafc.6b02560

Doulia, D. S., Anagnos, E. K., Liapis, K. S., & Klimentzos, D. A. (2016). Removal of pesticides from white and red wines by microfiltration. *Journal of Hazardous Materials, 317*, 135–146. https://doi.org/10.1016/j.jhazmat.2016.05.054

Galstyan, A. G., Semipyatniy, V. K., Mikhailova, I. Y., Gilmanov, K. K., Bigaeva, A. V., & Vafin, R. R. (2021). Methodological approaches to DNA authentication of foods, wines and raw materials for their production. *Foods, 10*(3). https://doi.org/10.3390/foods10030595. Article 595.

Gambino, G., Ferrero, L., Scalzini, G., De Paolis, C., Paissoni, M. A., Segade, S. R., Giacosa, S., Boccacci, P., & Rolle, L. (2022). Impact of oenological processing aids and additives on the genetic traceability of 'Nebbiolo' wine produced with withered grapes. *Food Research International, 151*. https://doi.org/10.1016/j.foodres.2021.110874. Article 110874.

García-Beneytez, E., Moreno-Arribas, M. V., Borrego, J., Polo, M. C., & Ibáñez, J. (2002). Application of a DNA analysis method for the cultivar identification of grape musts and experimental and commercial wines of *Vitis vinifera* L. using microsatellite markers. *Journal of Agricultural and Food Chemistry, 50*(21), 6090–6096. https://doi.org/10.1021/jf0202077

Giacosa, S., Parpinello, G. P., Río Segade, S., Ricci, A., Paissoni, M. A., Curioni, A., Marangon, M., Mattivi, F., Arapitsas, P., Moio, L., Piombino, P., Ugliano, M., Slaghenaufi, D., Gerbi, V., Rolle, L., & Versari, A. (2021). Diversity of Italian red wines: A study by enological parameters, color, and phenolic indices. *Food Research International, 143*. https://doi.org/10.1016/j.foodres.2021.110277. Article 110277.

Giordano, M., Rolle, L., Zeppa, G., & Gerbi, V. (2009). Chemical and volatile composition of three Italian sweet white Passito wines. *Oeno One, 43*(3), 159–170. https://doi.org/10.20870/oeno-one.2009.43.3.796

Gonçalves, F. J., Rocha, S. M., & Coimbra, M. A. (2012). Study of the retention capacity of anthocyanins by wine polymeric material. *Food Chemistry, 134*(2), 957–963. https://doi.org/10.1016/j.foodchem.2012.02.214

Liang, Z., & Keeley, A. (2013). Filtration recovery of extracellular DNA from environmental water samples. *Environmental Science & Technology, 47*(16), 9324–9331. https://doi.org/10.1021/es401342b

Miglietta, P. P., & Morrone, D. (2018). Quality, prices and production efficiency: An exploratory study of Italian wines with appellation of origin. *New Medicine, 17*(1), 73–89. https://doi.org/10.30682/nm1801g

Oberholster, A., Carstens, L. M., & du Toit, W. J. (2013). Investigation of the effect of gelatine, egg albumin and cross-flow microfiltration on the phenolic composition of Pinotage wine. *Food Chemistry, 138*(2), 1275–1281. https://doi.org/10.1016/j.foodchem.2012.09.128

OIV. (2020). *Compendium of international methods of analysis of wines and musts.* Paris: International Organisation of Vine and Wine.

Paissoni, M. A., Río Segade, S., Carrero-Carralero, C., Montanini, C., Giacosa, S., & Rolle, L. (2020). Role of anthocyanin traits on the impact of oenological tannins addition in the first stage of red winegrape skin simulated maceration. *Food Chemistry, 320*. https://doi.org/10.1016/j.foodchem.2020.126633. Article 126633.

Pérez-Magariño, S., & González-Sanjosé, M. L. (2003). Application of absorbance values used in wineries for estimating CIELAB parameters in red wines. *Food Chemistry, 81*(2), 301–306. https://doi.org/10.1016/S0308-8146(02)00509-5

Petrozziello, M., Torchio, F., Piano, F., Giacosa, S., Ugliano, M., Bosso, A., & Rolle, L. (2018). Impact of increasing levels of oxygen consumption on the evolution of color, phenolic, and volatile compounds of Nebbiolo Wines. *Frontiers in Chemistry, 6*. https://doi.org/10.3389/fchem.2018.00137. Article 137.

Prodanov, M., Aznar, M., Cabellos, J. M., Vacas, V., Lopez, F., Hernandez, M. T., & Estrella, M. I. (2019). Tangential-flow membrane clarification of malvar (Vitis vinifera L.) wine: Incidence on chemical composition and sensorial expression. *Oeno One, 53*(4), 725–739. https://doi.org/10.20870/oeno-one.2019.53.4.2480

Raimondi, S., Tumino, G., Ruffa, P., Boccacci, P., Gambino, G., & Schneider, A. (2020). DNA-based genealogy reconstruction of Nebbiolo, Barbera and other ancient grapevine cultivars from northwestern Italy. *Scientific Reports, 10*(1). https://doi.org/10.1038/s41598-020-72799-6. Article 15782.

Ranaweera, R. K. R., Capone, D. L., Bastian, S. E. P., Cozzolino, D., & Jeffery, D. W. (2021). A review of wine authentication using spectroscopic approaches in combination with chemometrics. *Molecules, 26*(14). https://doi.org/10.3390/molecules26144334. Article 4334.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). *Handbook of enology: The chemistry of wine stabilisation and treatments.* Chichester: John Wiley & Sons Ltd.. ISBN: 9780470010396.

Río Segade, S., Torchio, F., Giacosa, S., Ricauda Aimonino, D., Gay, P., Lambri, M., Dordoni, R., Gerbi, V., & Rolle, L. (2014). Impact of several pre-treatments on the extraction of phenolic compounds in winegrape varieties with different anthocyanin

profiles and skin mechanical properties. *Journal of Agricultural and Food Chemistry, 62*(33), 8437–8451. https://doi.org/10.1021/jf502656d

Rosária, M., Oliveira, M., Correia, A. C., & Jordão, A. M. (2022). Impact of cross-flow and membrane plate filtrations under winery-scale conditions on phenolic composition, chromatic characteristics and sensory profile of different red wines. *Processes, 10*(2). https://doi.org/10.3390/pr10020284. Article 284.

Savazzini, F., & Martinelli, L. (2006). DNA analysis in wines: Development of methods for enhanced extraction and real-time polymerase chain reaction quantification. *Analytica Chimica Acta, 563*(1–2), 274–282. https://doi.org/10.1016/j.aca.2005.10.078

Scalzini, G., López-Prieto, A., Paissoni, M. A., Englezos, V., Giacosa, S., Rolle, L., Gerbi, V., Río Segade, S., Pérez Cid, B., Moldes, A. B., & Cruz, J. M. (2020). Can a corn-derived biosurfactant improve colour traits of wine? First insight on its application during winegrape skin maceration versus oenological tannins. *Foods, 9*(12). https://doi.org/10.3390/foods9121747. Article 1747.

Siret, R., Gigaud, O., Rosec, J. P., & This, P. (2002). Analysis of Grape *Vitis vinifera* L. DNA in must mixtures and experimental mixed wines using microsatellite markers. *Journal of Agricultural and Food Chemistry, 50*(13), 3822–3827. https://doi.org/10.1021/jf011462e

Sun, X., Zhang, F., Gutiérrez-Gamboa, G., Ge, Q., Xu, P., Zhang, Q., Fang, Y., & Ma, T. (2022). Real wine or not? Protecting wine with traceability and authenticity for consumers: Chemical and technical basis, technique applications, challenge, and perspectives. *Critical Reviews in Food Science and Nutrition, 62*(24), 6783–6808. https://doi.org/10.1080/10408398.2021.1906624

Vieira, G. S., Moreira, F. K. V., Matsumoto, R. L. S., Michelon, M., Filho, F. M., & Hubinger, M. D. (2018). Influence of nanofiltration membrane features on enrichment of jussara ethanolic extract (*Euterpe edulis*) in anthocyanins. *Journal of Food Engineering, 226*, 31–41. https://doi.org/10.1016/j.jfoodeng.2018.01.013

Vignani, R., Liò, P., & Scali, M. (2019). How to integrate wet lab and bioinformatics procedures for wine DNA admixture analysis and compositional profiling: Case studies and perspectives. *PLoS One, 14*(2), Article e0211962. https://doi.org/10.1371/journal.pone.0211962

Villano, C., Lisanti, M. T., Gambuti, A., Vecchio, R., Moio, L., Frusciante, L., Aversano, R., & Carputo, D. (2017). Wine varietal authentication based on phenolics, volatiles and DNA markers: State of the art, perspectives and drawbacks. *Food Control, 80*, 1–10. https://doi.org/10.1016/j.foodcont.2017.04.020

Zambianchi, S., Soffritti, G., Stagnati, L., Patrone, V., Morelli, L., & Busconi, M. (2022). Effect of storage time on wine DNA assessed by SSR analysis. *Food Control, 142*. https://doi.org/10.1016/j.foodcont.2022.109249. Article 109249.

Zambianchi, S., Soffritti, G., Stagnati, L., Patrone, V., Morelli, L., Vercesi, A., & Busconi, M. (2021). Applicability of DNA traceability along the entire wine production chain in the real case of a large Italian cooperative winery. *Food Control, 124*. https://doi.org/10.1016/j.foodcont.2021.107929. Article 107929.

Zhang, X., Bowyer, P., Scollary, G. R., Clark, A. C., & Kontoudakis, N. (2022). Sulfide-bound copper removal from red and white wine using membrane and depth filters: Impacts of oxygen, H2S-to-Cu ratios, diatomaceous earth and wine volume. *Food Chemistry, 377*. https://doi.org/10.1016/j.foodchem.2021.131758. Article 131758.