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From grape to wine: A thorough compound specific isotopic, enantiomeric and quali-quantitative investigation by means of gas chromatographic analysis

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ABSTRACT

In this study, multiple analytical approaches, including simultaneous enantiomeric and isotopic analysis, were employed to thoroughly investigate the volatile fraction in *Moscato giallo* grape berries and wines. For the qualitative and quantitative profiling, a fast GC-QqQ/MS approach was successfully utilized. However, prior to isotopic analysis, the extracts underwent an additional concentration step, necessitating an assessment of isotopic fractionation during the concentration process. Once the absence of carbon isotopic fractionation was confirmed, this research aimed to develop a suitable gas chromatographic method for the simultaneous detection of both enantiomeric and isotopic ratios of target monoterpenoids in *Moscato giallo* samples. To address the limitations associated with a one-dimensional approach, multidimensional gas chromatography was employed to enhance separation before IRMS and qMS detections. Utilizing a Deans switch transfer device, the coupling of an apolar column in the first dimension and a chiral cyclodextrin-based stationary phase in the second dimension proved effective for this purpose. The data obtained from the analysis of *Moscato giallo* samples allowed for the assessment of natural isotopic and enantiomeric distributions in grapes and wines for the first time in the literature. Significant enantiomeric excesses were observed for the target terpenoids investigated. Regarding isotopic distribution, a consistent trend was observed for all detected target terpenols, including the linalool enantiomers. To date, this study represents the first investigation of simultaneous $\delta^{13}C$ and chiral investigation of the main terpenoids in oenological products in the literature.

1. Introduction

Volatile organic compounds (VOCs) in grapevines (*Vitis vinifera* L.) encompass a broad spectrum of analytes that contribute to the distinctive aroma of each grape variety. With regard to Moscato varieties, it can be observed that the majority of volatile compounds are terpenoids, with monoterpenes and sesquiterpenes representing the primary constituents. Terpenoids composition typically exists in two forms: free or glycosylated derivatives. Free terpenoids are known to directly contribute to grape aroma. Conversely, terpenoids in glucosidic form, do not exert an immediate impact on flavour.

Upon glycoside hydrolysis, the aroma perception is significantly enhanced due to the increased volatile content. Qualitatively and quantitatively similar profiles are reported for free and bound forms in oenological products obtained from the same variety [1]. As extensively documented in the literature [2,3], various factors, including fruit ripeness stage and grape variety, influence the differing terpenoid compositions in grapes and wines. In the case of Moscato cultivars, the

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aroma is predominantly composed by linalool, geraniol, nerol, citronellol, imparting a characteristic floral flavor. However, variations in their quantitative distribution often account for distinct sensory perceptions of specific varieties, such as linalool in *Moscato giallo* [2–4]. Gas chromatography coupled to mass spectrometry (GC–MS) is widely employed to evaluate VOC distribution in grape and wine samples [2]. However, due to the relatively low terpenoid concentrations in oenological products (≤ 1 mg kg^{-1}), a concentration step is typically required post-extraction. Modern analytical methods now offer solutions to overcome this limitation. Recently, Paolini *et al.* described a rapid gas chromatographic approach combined with tandem mass spectrometry for determining the main VOCs in oenological products [5]. The enhanced sensitivity obviates the need for additional concentration steps, saving time and chromatographic runtime. Given the significant interest in oenological products, alongside qualitative and quantitative characterization, more advanced GC-based approaches are necessary to investigate the biochemical pathways of grapevines as the plant of origin, and wine as the transformation product. Among these techniques, enantioselective gas chromatography (Es-GC) stands out as a recognized method for these purposes [6–9]. As plants biosynthetize their chiral metabolites with specific enantiomeric excesses, it is possible to assess typical trends by evaluating their enantiomeric distribution on a chiral GC column. While numerous studies have focused on the qualitative and quantitative characterization of VOCs in grapes and wines, their enantiomeric distributions have been relatively unexplored [10, 11]. Most studies have discussed the enantiomeric distribution of target volatiles in grapes or wines according to the variety investigated [10, 11]. However, few studies have evaluated potential variations in enantiomeric ratios throughout winemaking [12]. Besides Es-GC, the investigation of the isotopic ratio through gas chromatography coupled to isotopic ratio mass spectrometry (GC—C-IRMS) is similarly acknowledged as a reliable method for genuineness validation [13].

Specifically, δ^{13}C evaluation enables differentiation between C_3 and C_4 plants, as well as, in many cases, synthetic products, based on differential CO_2 uptake by the plant [14,15]. Concerning oenological products, very few studies have explored the isotopic distribution of target VOCs [16–18]. To date, no literature exists evaluating the chiral and isotopic distribution of terpenols throughout winemaking, thus examining the transition from grape to wine. In this research, to simultaneously determine the chiral and isotopic data of target VOCs in oenological products, a novel analytical approach was developed. As reported by our research group [19], to overcome limitations associated with co-elutions, enantioselective multidimensional gas chromatography (Es-MDGC) employing a heart-cut transfer device was utilized before IRMS and qMS detection. The coupling of an apolar column and a chiral phase in the first and second dimensions allowed a higher resolution power, and the δ^{13}C evaluation of the enantiomers separated. In this research work, seventeen grape samples of the *Moscato giallo* variety were provided by local farmers from Trentino and Veneto regions (Italy) between September and October 2021. For characterization of the wines' volatile composition, all samples underwent microvinification using a *Saccharomyces cerevisiae* yeast strain. Qualitative and quantitative investigations of all samples, both grapes and wines, were conducted using a fast GC-QqQ/MS approach. Simultaneous detection of the enantiomeric and isotopic ratios of the main aromatic terpenoids was performed through *enantio* selective multidimensional gas chromatography coupled to isotopic ratio mass spectrometer and single quadrupole mass spectrometer (Es-MDGC—C-IRMS/qMS).

2. Materials and methods

2.1. Samples and chemicals

Seventeen different *Moscato giallo* grape samples were kindly provided by local farmers in Trentino Alto Adige and Veneto (Italy), in September and October 2021. Starting from these samples, seventeen wine samples were produced through a microvinification process (Section 2.2). Dichloromethane, linalool, geraniol, eucalyptol, and anhydrous sodium sulfate were purchased from Merck (Merck Life Science, Darmstadt, Germany). Isolute ENV+ solid phase extraction (SPE) cartridges (1 g) were supplied by Biotage (Uppsala, Sweden). Glycosidic enzyme (AR 2000) was purchased from Gist-Brocades (France). For the calibration of the δ^{13}C values with respect to the Vienna PeeDee Belemnite (VPDB) scale, three certified alkanes from Indiana mix A7 were selected, viz. hexadecane (δ^{13}C −26.15 ‰), nonadecane (δ^{13}C-31.99 ‰) and eicosane (δ^{13}C −40.91 ‰) (Indiana University, Bloomington, IN). Isotopic ratio measurements of the samples of interest were made by the following formula:

$$\delta^{13}\mathrm{C} = \frac{\mathrm{Rsample} - \mathrm{Rstandard}}{\mathrm{Rstandard}}$$

where *R* is the heavier carbon isotope's abundance ratio compared to the lighter one (^{13}C/^{12}C).

2.2. Winemaking, extraction, and concentration

For winemaking process, the microvinification was carried out on 500 mL of grape juice, inoculated with the laboratory yeast strain *Saccharomyces cerevisiae* W303. The alcoholic fermentation occurred at 25 °C, and was monitored by measuring CO_2 production, indicated by weight loss until it stabilized. Completion of fermentation was confirmed by measuring glucose and fructose concentrations using a WineScan™ FT 120 spectrophotometer (Foss, Hillerød, Denmark). After fermentation, the wine was recovered, and yeast cells were removed by centrifugation.

For grapes extraction, 50 g of frozen berries were homogenized, and mixed with a solution of 1-heptanol and ethyl 3-hydroxybutyrate, 0.5 g of gluconolactone, and 0.2 mL of nonyl-β-D-glucopyranoside, according to Paolini et al. [18]. Dealing with wine samples, 50 mL were diluted to 100 mL with Milli-Q water after the addition of 100 µL of n-Heptanol and 100 µL of Nonyl-β-d-glucopyranoside, as internal standards. For the extraction step, the same procedure was exploited for both grape and wine. After being eluted with 30 mL of dichloromethane from SPE cartridges, the free VOCs fraction was dried with anhydrous sodium sulfate. Subsequently, an aliquot was employed for GC–MS/MS analysis. VOCs in glycosidic form were primarily eluted with 30 mL of methanol, drying this solution with a rotary evaporator. In a second step, it was dissolved in 4.5 mL of citrate buffer at pH 5 by adding 200 µL AR 2000 (70 mg mL^{-1} in water), aiming to release the VOCs from their glycosidic bound. A solution of 0.2 mL containing 1-heptanol and ethyl 3-hydroxybutyrate was then added, and the VOCs were extracted through SPE cartridges and eluted with dichloromethane. In the same way as the free forms, an aliquot was selected for GC–MS/MS analysis. For more details about the extraction procedure the reader is directed to Paolini et al. [18].

In the current research, the analysis were carried out by blending free and bound VOCs. Before the isotopic analysis, a further concentration step was needed. In particular, 60 mL of pentane were added to the glass boiling flask to form a low-boiling azeotropic mixture. Concentration was carried out in a 40 °C bath until a volume of 0.5 mL was reached, prior to Es-MDGC—C-IRMS/qMS analysis.

2.3. GC–MS/MS conditions

Quali-quantitative analysis was carried out through an Agilent Intuvo 9000 gas chromatograph coupled to Agilent 7000 Series triple quadrupole MS (Agilent). GC separation was performed by means of a DB-wax ultra inert (20 *m* × 0.18 mm I.D. × 0.18 µm d_f) capillary column, and ramping the oven as follows: 40 °C (2 min), to 55 °C at 10 °C min^{-1}, to 165 °C at 20 °C min^{-1}, and finally to 240 °C (5 min) at 40 °C min^{-1}. The split/splitless injector was set at 250 °C, split mode (1:5), delivering a constant He flow of 0.8 mL min^{-1}. From the MS side, the filament

current was 50 μA, and mass spectra were acquired in dynamic multiple reaction monitoring (dMRM) mode, by using N_2 as collision gas (flow of 1.5 mL min^{-1}), and in full scan mode (mass range 33 - 400 *m/z*). The source temperature and transfer line were maintained at 230 °C and 250 °C, respectively. For more details about method development, the reader is directed to Paolini et al. [5].

Agilent Technologies MassHunter workstation software – data acquisition (ver. B.07.06) and the Agilent MassHunter workstation software – quantitative analysis (ver. B.08.00) were used to acquire and process the analytical data.

2.4. GC—C-IRMS conditions

The GC—C-IRMS system was composed by a Trace GC Ultra (GC IsoLink + ConFlo IV, Thermo Scientific) coupled to an isotope ratio mass spectrometer (DELTA V, Thermo Scientific), and to a single-quadrupole MS (ISQ Thermo Scientific). GC analyses were carried out by using a Phenomenex Zebron-Wax column (30 *m* × 0.32 mm I.D. × 0.5 μm d_f), which was ramped as follows: 40 °C (3 min), to 55 °C at 3 °C min^{-1}, then to 165 °C at 5 °C min^{-1}, and finally to 240 °C (8.5 min) at 10 °C min^{-1}. The split/splitless injector port was set at 260 °C, injection volume 2 μL, splitless mode, delivering helium as the carrier gas at a flow rate of 2 mL min^{-1}. The combustion chamber was set at 1000 °C. The ion source and transfer line temperatures were maintained at 250 °C.

2.5. Es-MDGC—C-IRMS/qMS conditions

The design of the MDGC—C-IRMS/qMS system has been already reported in previous studies [14,19]. For this application, a SLB-5 ms 30 *m* × 0.25 mm I.D. × 0.25 μm d_f (Merck Life Science, Darmstadt, Germany) column was used in the ^{1}D, ramping the temperature oven as follows: 40 °C (1 min) to 184 °C at 3 °C min^{-1}, finally to 330 °C at 15 °C min^{-1}. The split/splitless injector was set at 280 °C, splitless 1 min, delivering a constant helium flow at 1.0 mL min^{-1}, by using a pressure program: 184 kPa (1 min) to 275 kPa at 1.89 kPa min^{-1} and finally to 330 kPa at 5.68 kPa min^{-1}. The ^{1}D flame ionization detector conditions were 330 °C, H_2 flow 40.0 mL min^{-1}, air flow rate 400 mL min^{-1}, operating at a sampling rate of 80 ms. The MEGA-DEX ASX 1 chiral phase 25 *m* × 0.25 mm I.D. × 0.25 μm d_f (MEGA, Milano, Italy) was exploited as ^{2}D capillary column, and the GC oven was programmed as follows: 40 °C (11 min) to 142 °C (5 min) at 3 °C min^{-1} and finally to 210 °C at 8 °C min^{-1}. As well as for the injector side, a pressure program was applied to the auxiliary pressure control (APC) in order to achieve a similar carrier flow in the ^{2}D dimension (≈1 mL min^{-1}), as follows: 140 kPa (11 min) to 193 kPa at 1.39 kPa $min^{-1}$and finally to 265 kPa at 9.8 kPa min^{-1}. After the ^{2}D separation, the flux was divided through a tee-union to the combustion chamber (850 °C), and thus to the IRMS system, via a 0.7 × 0.32 mm I.D. uncoated column and to the qMS via a 3.5 *m* × 0.1 mm I.D. uncoated column. From the qMS side, ion source and interface temperature were maintained at 200 °C, acquiring a 40–400 *m/z* mass range at an acquisition speed of 10 Hz. From the GC side, all the data were collected through the MDGC solution control software package (Shimadzu Europa). From the IRMS side, the following settings were used for an Elementar VisION system (Elementar Analysensysteme GmbH, Langenselbold, Germany): acceleration voltage: 3795.805 V; trap current, 600 mA; magnet current, 3700.000 mA. IRMS results were handled by the lyticOS stable isotope data processing software (Elementar Analysensysteme GmbH, Langenselbold, Germany). All the multidimensional analyses were carried out in triplicates, and the standard deviations for IRMS results were found to be < 0.5 ‰.

3. Results and discussion

3.1. Quali-quantitative analysis through fast GC-QqQ/MS

In the current study, after the extraction process (Section 2.2), the

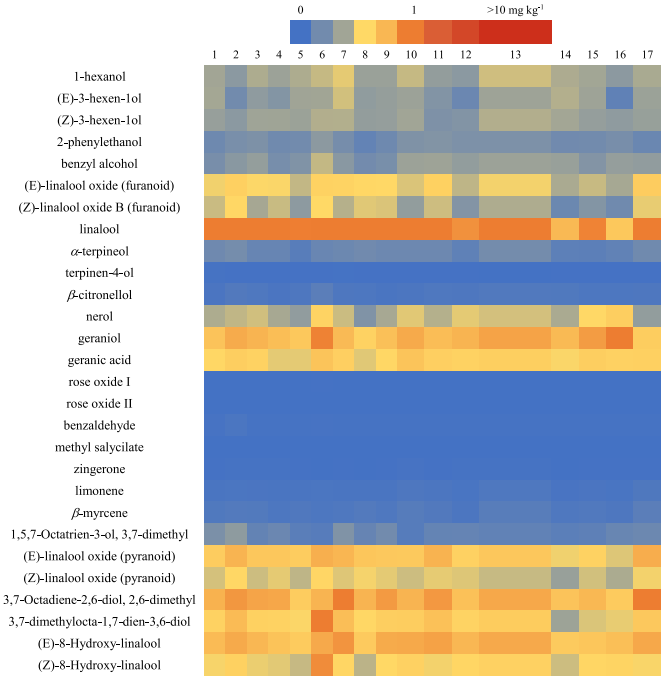

Fig. 1. Fast GC-QqQ/MS quali-quantitative composition of the volatile fraction of the seventeen grape samples investigated.

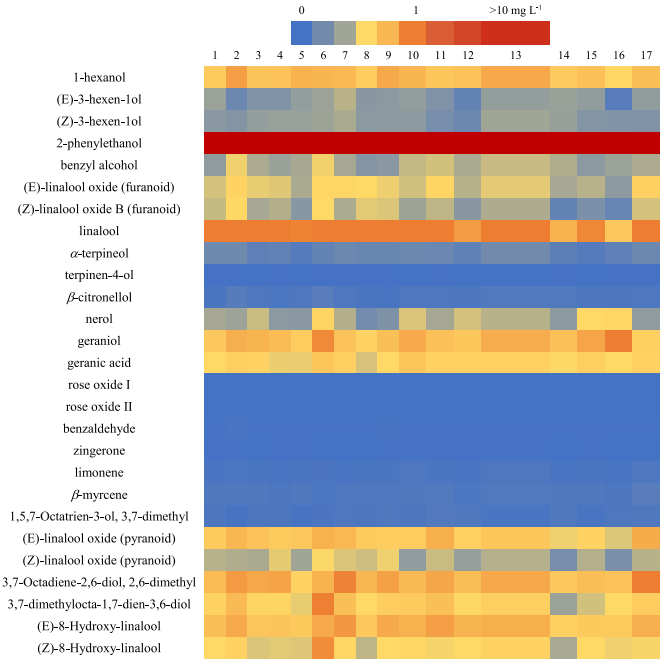

Fig. 2. Fast GC-QqQ/MS quali-quantitative composition of the volatile fraction of the seventeen wine samples investigated.

VOCs profile of both grapes and wines was evaluated in its entirety, composed of both free and bound components. The combination of fast GC and triple quadrupole MS allowed a fast and reliable characterization of 28 target VOCs in grapes and wines. The GC-QqQ/MS method was optimized using commercially available standards by acquiring three MS/MS transitions for each compound. For more details the reader is directed to Paolini et al. [5].

In the current study, starting from grapes (Fig. 1 and Table S1), linalool was the principal compound in the majority of *Moscato giallo*

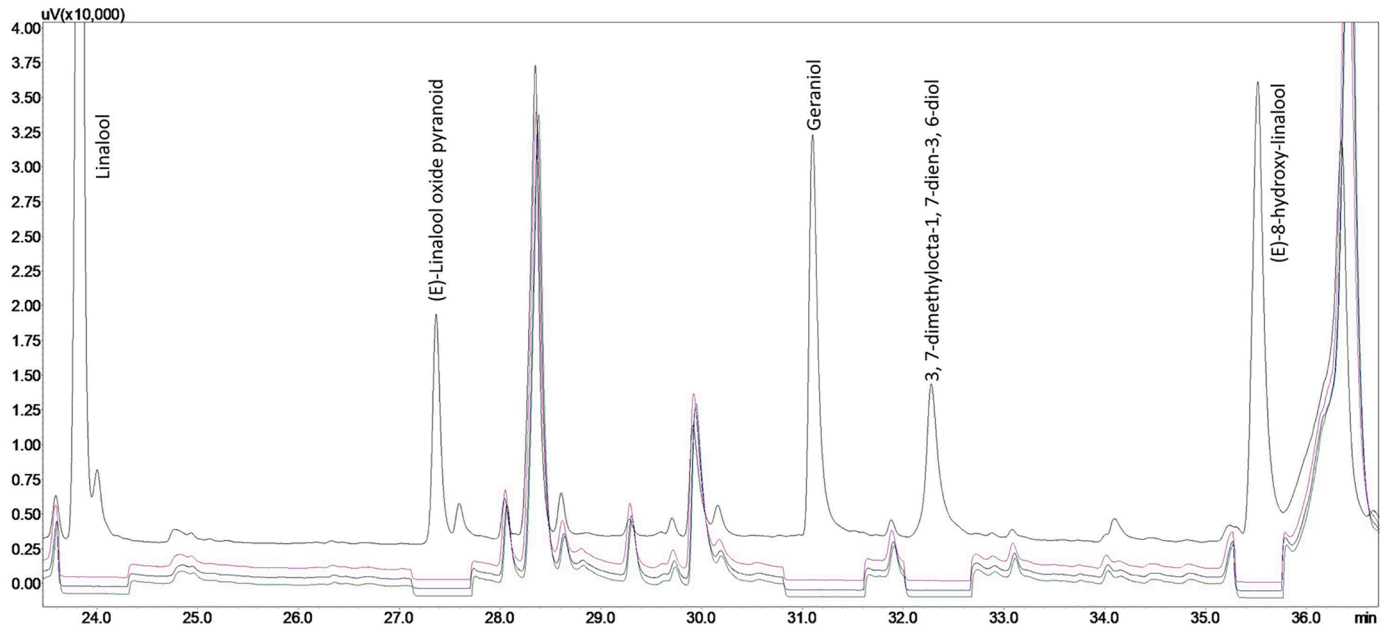

Fig. 3. Data comparison between a ^{1}D stand-by analysis (black trace) and the relative ^{1}D cut ones (colored traces) for a *Moscato giallo* grape sample.

samples, with the exception of sample 16, where geraniol was found to be present in higher concentrations. Furthermore, the linalool oxide pyranoid forms exhibited higher concentrations than the furanoid forms. In detail, the amount of the (E)-linalool oxide forms, both furanoid and pyranoid, were higher than the relative (Z) ones. Moreover, (E)−8-hydroxy-linalool was the predominant form in the majority of the samples, in comparison to the (Z) isomer.

As described in Section 2.2, all the grapes were subjected to winemaking by exploiting a *Saccharomyces cerevisiae* yeast strain. This procedure allowed the evaluation of the flavour components in grapes and wines, outlining the varietal profile of the *Moscato giallo* samples. While most of the components analyzed were found in a similar amount between grape and wine (Fig. 2 and Table S2), it is worth of interest the quantitative of phenetyl alcohol found in the wine samples, ranging from 14 to 33 mg L^{-1}.

These data were consistent with previous studies carried out by Ugliano et al. and Fernandez et al., which identified phenetyl alcohol as one of the principal components after *Saccharomyces cerevisiae* fermentation [20,21].

In a second step, the main terpenoid components in grapes and wines were further investigated via isotopic and chiral analyses, in accordance with the quali/quantitative findings of the volatile fraction (Section 3.3).

3.2. Evaluation of carbon isotopic fractionation during the concentration step for standards

While samples were evaluated for qualitative and quantitative aims through GC–MS/MS analysis after the extraction process, an additional concentration step was necessary for isotopic analysis. In detail, an isotopic ratio mass spectrometer has higher detection limits than a conventional MS. This issue is often limiting for the analysis of target components present at very low concentrations, such as terpenoids in oenological products. In order to obtain a sufficient amount of VOCs prior to isotopic analysis, a concentration step was essential after the extraction process.

This procedure (Section 2.2) allowed increasing the concentration of terpenoids, before the IRMS detection. Nevertheless, this process may involve solute losses, in addition to solvent evaporation, potentially resulting in carbon isotopic fractionation [22].

To address this issue, according to Paolini et al. [18], preliminary tests were conducted, before analyzing real samples by means of multidimensional gas chromatography (Section 3.3). A solution of three terpenoid standards having different boiling points, i.e. eucalyptol, geraniol, and linalool, was prepared in dichloromethane in order to emulate the sample concentration process. As shown in Fig. S1, during the concentration step, aliquots were collected at different times (from t_1 to t_5) until reaching the final step (t_6).

No relevant isotopic differences (always $< \pm 0.5$ ‰) were found concerning the starting solution (t_0) for all the terpenoids. These results confirmed the lack of isotopic fractionation during the concentration step and allowed the reliable isotopic measurements of the oenological products (Section 3.5).

3.3. Enantio-selective multidimensional gas chromatographic analysis coupled to MS and IRMS detection

As previously outlined in Section 3.1, for quali-quantitative analysis, tandem mass spectrometry coupled with monodimensional GC provided increased resolution power through the selection of specific multiple reaction monitoring (MRM) transitions for the key VOCs. However, for IRMS detection, it was not possible, as δ^{13}C is determined after the combustion of all the VOCs to carbon dioxide. Furthermore, undesired overlappings compromise a reliable measurement, according to the well-known chromatographic isotopic effect along a CO_2 peak [23]. As already stated by our research group, monodimensional conditions (GC—C-IRMS) would lead to several limitations in cases of co-elutions, needing MDGC—C-IRMS approaches for the analysis of real samples [19,24]. This issue is even trickier in the case of the direct coupling of enantio-selective gas chromatography to IRMS. In this field, although the chosen chiral column can efficiently separate the enantiomers of interest, unexpected overlappings can involve other undesired sample components [19]. In this regard, even slight co-elution may result in unreliable isotopic results and reduced repeatability. To address these limitations, an MDGC method was developed to achieve an efficient separation. In detail, MDGC configuration was carried out with complementary stationary phase, to ensure the baseline resolution of the components of interest, aiming also to the separation of each chiral terpenoid of interest into its two enantiomers. Accordingly, MDGC conditions were optimized using an apolar column in the ^{1}D and the

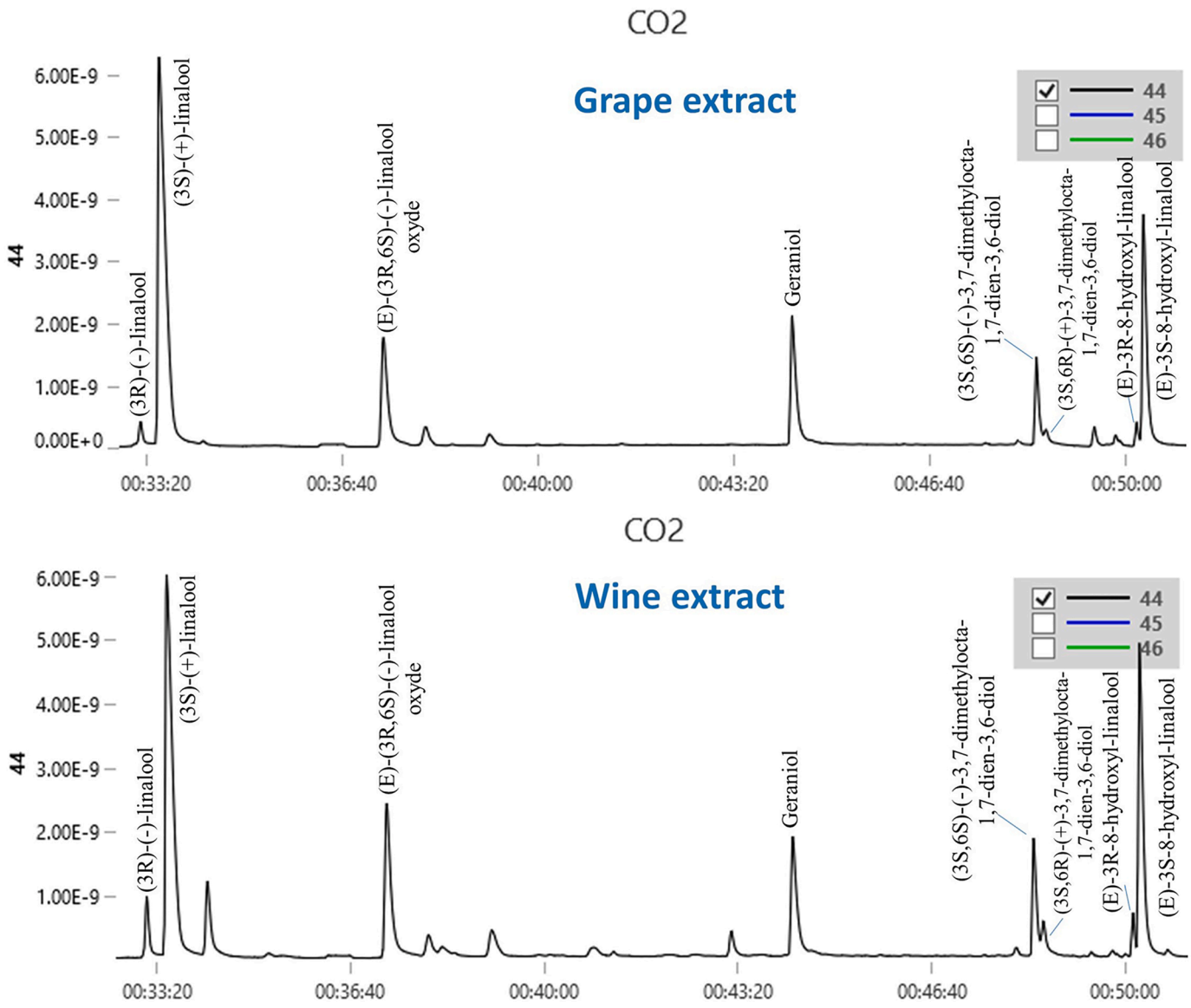

Fig. 4. ^{2}D chiral IRMS profile of a *Moscato giallo* grape extract (upper chromatogram) and the relative wine (lower chromatogram) after the multidimensional separation. Note: the elution order of (E)−8-hydroxy-linalool was tentatively assigned according to the discussion provided in Section 3.4.

MEGA DEX ASX-1 chiral phase in the ^{2}D one. Fig. 3 shows the data comparison between the stand-by analysis and the cut replicates, realized on the ^{1}D apolar column, having a very high reproducibility in terms of retention times [25]. By these means, the most representative *Moscato giallo* terpenoids were chosen to be transferred through the Deans switch system to the complementary ^{2}D chiral column, namely linalool, (E)-linalool oxide pyranoid, geraniol, 3,7-dimethylocta-1, 7-dien-3,6-diol, and (E)−8-hydroxy-linalool. After the ^{2}D separation, the components were split into the parallel qMS and IRMS detectors. The simultaneous ^{2}D qMS detection allowed confirmation of the compounds transferred from the ^{1}D exploiting the FFNSC 4.0 MS database.

Fig. 4 shows ^{2}D profile achieved in the chiral column, by comparing grape and wine extracts' profile. As visible, a higher resolution was achieved (Fig. 4), by reducing the overlappings involved in the ^{1}D stand-by on the apolar column (Fig. 3). Thanks to the MDGC separation, linalool enantiomeric forms were completely separated from the co-eluted compound in the ^{1}D stand-by analysis, allowing the right evaluation of isotopic and enantiomeric ratio, in both grapes and wines. Similarly, (E)-linalool oxide pyranoid was baseline separated from the interference in the stand-by analysis. Unfortunately, as shown in Fig. 4, the enantiomers of 3,7-dimethylocta-1,7-dien-3,6-dien were not resolved at baseline, making it possible only to evaluate the enantiomeric ratios.

3.4. Enantiomeric data in grape and wine

The seventeen *Moscato giallo* grape and wine samples were analyzed following the method described in Section 3.3. To provide a proper signal for isotopic analysis, the extraction process involved the same additional concentration step described in Section 3.2.

From a chiral standpoint, characteristic trends were found for the target terpenoids in all the *Moscato giallo* samples investigated (Table 1).

In this respect, previous works on chiral terpenoids [10,26–29] allowed assigning the specific identity of each enantiomer investigated. Only for (E)−8-hydroxy-linalool, due to the lack of literature data, the elution order and the optical rotation were unknown. As reported in Table 1, a marked enantiomeric excess was always observed, outlining a typical behavior. Starting with grape extracts, linalool was predominant in the (3S)-(+) form (94.3–97.7 %), as well as the second eluting enantiomer of (E)−8-hydroxy-linalool (89.8–96.3 %). As already stated,

Table 1
Enantiomeric ratios (ER) for the four chiral terpenoids investigated, from grapes to wines.

	Linalool				(E)-linalool oxide pyranoid				3,7-dimethylocta-1,7-dien-3,6-diol				(E)−8-hydroxy-linalool			
	Grape		Wine		Grape		Wine		Grape		Wine		Grape		Wine	
ID	3R (-)	3S (+)	3R (-)	3S (+)	3R,6S (-)	3S,6R (+)	3R,6S (-)	3S,6R (+)	3S,6S (-)	3S,6R (+)	3S,6S (-)	3S,6R (+)	3R*	3S*	3R*	3S*
1	2.3	97.7	11.0	89.0	100	0	100	0	82.3	17.7	85.9	14.1	7.5	92.5	17.8	82.2
2	2.4	97.6	10.7	89.3	100	0	100	0	84.8	15.2	88.0	12.0	6.9	93.1	16.1	83.9
3	3.3	96.7	13.2	86.9	100	0	100	0	86.3	13.7	90.2	9.9	8.9	91.1	14.8	85.2
4	3.4	96.6	12.7	87.4	100	0	100	0	86.5	13.5	92.8	7.2	8.2	91.8	14.0	86.0
5	3.6	96.4	16.3	83.7	100	0	100	0	83.1	16.9	92.3	7.8	7.6	92.4	11.3	88.7
6	3.8	96.2	14.9	85.1	100	0	100	0	78.2	21.8	87.3	12.7	3.7	96.3	15.2	84.8
7	2.6	97.4	7.7	92.4	100	0	100	0	82.2	17.8	88.5	11.5	7.0	93.0	9.8	90.2
8	2.4	97.6	10.5	89.5	100	0	100	0	83.7	16.3	90.3	9.7	7.8	92.2	16.0	84.0
9	2.7	97.3	14.6	85.4	100	0	100	0	86.2	13.8	89.9	10.1	9.7	90.3	18.5	81.6
10	4.5	95.5	11.8	88.2	100	0	100	0	80.6	19.4	92.8	7.2	6.6	93.4	10.5	89.5
11	2.6	97.4	9.4	90.7	100	0	100	0	85.4	14.6	90.0	10.0	7.1	92.9	12.0	88.0
12	3.9	96.1	13.4	86.6	100	0	100	0	86.0	14.0	89.3	10.7	7.8	92.2	12.1	87.9
13	3.1	96.9	14.4	85.6	100	0	100	0	82.4	17.6	91.1	8.9	8.0	92.0	16.9	83.1
14	5.2	94.8	11.2	88.8	100	0	100	0	84.5	15.5	89.2	10.9	10.1	89.9	18.3	81.7
15	3.8	96.2	17.9	82.2	100	0	100	0	79.1	20.9	90.6	9.4	8.4	91.6	22.2	77.9
16	5.7	94.3	13.6	86.4	100	0	100	0	85.4	14.6	90.8	9.2	10.2	89.8	15.3	84.7
17	2.4	97.6	10.7	89.3	100	0	100	0	81.9	18.1	90.5	9.5	8.5	91.5	12.5	87.5

Notes:.
* tentatively assigned according to the similar enantiomeric ratios between linalool and (E)−8-hydroxy-linalool from grape to wine.

Table 2
$\delta^{13}C$ values for the target terpenes investigated in the seventeen *Moscato giallo* grape and wines.

	(3S)-(+)-linalool		(E)-(3R,6S)-(-)-linalool oxide pyranoid		geraniol		(E)-(3S)−8-hydroxy-linalool*	
	$\delta^{13}C$		$\delta^{13}C$		$\delta^{13}C$		$\delta^{13}C$	
ID	Grape	Wine	Grape	Wine	Grape	Wine	Grape	Wine
1	−35.2	−34.5	−34.4	−33.4	−33.3	−32.3	−33.0	−32.5
2	−33.4	−33.6	−32.6	−31.9	−31.2	−31.5	−30.8	−31.0
3	−37.6	−36.5	−36.6	−35.6	−35.4	−33.5	−34.9	−34.4
4	−37.1	−36.9	−36.2	−35.8	−34.9	−34.4	−34.3	−34.5
5	−35.6	−35.5	−34.6	−34.3	−33.8	−33.1	−33.5	−34.2
6	−38.3	−37.9	−36.8	−36.5	−37.2	−37.0	−35.2	−36.7
7	−39.7	−39.3	−38.5	−37.9	−36.4	−35.9	−36.6	−36.7
8	−35.5	−34.4	−34.6	−33.7	−32.9	−32.1	−32.7	−32.4
9	−37.8	−36.7	−36.9	−36.1	−35.2	−34.3	−34.8	−34.5
10	−36.4	−35.8	−35.1	−34.7	−34.1	−33.0	−33.6	−33.9
11	−37.3	−36.4	−36.9	−36.0	−35.2	−34.2	−35.0	−35.0
12	−37.6	−36.1	−36.6	−35.2	−35.5	−33.8	−35.3	−34.6
13	−36.4	−34.4	−35.1	−33.6	−33.9	−31.0	−32.8	−31.5
14	−39.8	−37.0	−38.7	−36.2	−37.5	−34.8	−36.3	−35.0
15	−36.8	−35.7	−35.7	−34.5	−34.4	−33.0	−34.7	−34.0
16	−39.3	−38.5	−38.1	−37.1	−35.9	−35.3	−35.5	−36.5
17	−36.7	−36.2	−35.8	−35.8	−34.0	−33.4	−34.1	−33.5

Notes:
* enantiomer tentatively assigned according to the similar enantiomeric ratios between linalool and (E)−8-hydroxy-linalool from grape to wine.

(E)-linalool oxide pyranoid was always found as enantiomeric pure component in the 3R,6S-(-) form [10]. For 3,7-dimethylocta-1,7-dien-3,6-diol, the (3S,6S)-(-) form was more predominant (78.2–86.5 %) than the 3S, 6R-(+) one. These enantiomeric distributions for *Moscato giallo* grapes were in agreement with respect to previous literature studies about other Moscato grape varieties [10,26].

Dealing with wine samples, as previously discussed in Section 3.1, winemaking can lead to variations in the volatile fraction due to yeast activity. Regarding chiral GC application, several studies have noted a racemic form of linalool, regardless of the type of wine investigated [11,30]. For instance, Khvalbota *et al.* and Song *et al.* highlighted a racemic distribution of linalool in traditional Slovak wines [30] and various white wine varieties [11], respectively. In the case of Moscato samples, Askari et al. studied the variation in the enantiomeric distribution of linalool throughout the fermentation process, from fresh grape juices to three-year bottle-matured wine, observing a progressive racemization process [12]. This shift was attributed to fermentation processes during winemaking and the activity of yeast. Following the microvinification process described in Section 2.2, which involved starting from berry juices, a similar phenomenon was observed in this study. Specifically, (3S)-(+)-linalool was consistently predominant in all the wines, albeit with reduced abundance compared to grapes (Table 1). This behavior is clearly illustrated in Fig. 4. Interestingly, a very similar trend was registered also for the second eluting enantiomer of (E)−8-hydroxy-linalool, since both the analytes registered the highest and the lowest shifts in Sample 7 and Sample 15, respectively. This very similar behavior may suggest the 3S-form as the predominant enantiomer of (E)−8-hydroxy-linalool, according to the shifts registered in linalool enantiomers from grape to wine. For the other analytes investigated, a comparable enantiomeric distribution was noted between grapes and wines for 3,7-dimethylocta-1,7-dien-3,6-diol forms and (E)-linalool oxide pyranoid (Table 1).

Table 3
Enantiomeric ratios (ER)% and $\delta^{13}C$ values for (3R)-(-) and (3S)-(+) linalool in the seventeen *Moscato giallo* grape and wine samples.

	Linalool							
	Grape				Wine			
	3R (-)		3S (+)		3R (-)		3S (+)	
ID	ER	$\delta^{13}C$	ER	$\delta^{13}C$	ER	$\delta^{13}C$	ER	$\delta^{13}C$
1	2.3	−36.8	97.7	−35.2	11.0	−36.3	89.0	−34.5
2	2.4	−34.6	97.6	−33.4	10.7	−35.1	89.3	−33.6
3	3.3	−37.3	96.7	−37.6	13.2	−37.3	86.9	−36.5
4	3.4	−36.9	96.6	−37.1	12.7	−37.9	87.4	−36.9
5	3.6	−36.2	96.4	−35.6	16.3	−36.5	83.7	−35.5
6	3.8	−38.1	96.2	−38.3	14.9	−38.8	85.1	−37.9
7	2.6	−39.0	97.4	−39.7	7.7	−39.8	92.4	−39.3
8	2.4	−35.9	97.6	−35.5	10.5	−35.9	89.5	−34.4
9	2.7	−37.8	97.3	−37.8	14.6	−37.4	85.4	−36.7
10	4.5	−36.7	95.5	−36.4	11.8	−37.2	88.2	−35.8
11	2.6	−37.2	97.4	−37.3	9.4	−37.6	90.7	−36.4
12	3.9	−37.9	96.1	−37.6	13.4	−37.1	86.6	−36.1
13	3.1	−37.7	96.9	−36.4	14.4	−36.5	85.6	−34.4
14	5.2	−39.5	94.8	−39.8	11.2	−38.1	88.8	−37.0
15	3.8	−37.5	96.2	−36.8	17.9	−37.0	82.2	−35.7
16	5.7	−38.9	94.3	−39.3	13.6	−38.3	86.4	−38.5
17	2.4	−37.1	97.6	−36.7	10.7	−37.8	89.3	−36.2

3.5. Isotopic data in grape and wine

According to the need for a baseline separation of each target component prior to IRMS detection, the carbon isotopic ratios of (3R)-(-)-linalool, (3S)-(+)-linalool, the second eluting enantiomer of (E)−8-hydroxy-linalool, (E)-(3R,6S)-(-)linalool oxide pyranoid and geraniol were assessed.

Table 2 describes the δ ^{13}C values obtained for the components investigated, while a deeper discussion about the linalool enantiomers is provided in Table 3.

Looking at the isotopic data for grapes and wines, although different absolute $\delta^{13}C$ values were found among the samples investigated, a consistent relative trend was observed among the target terpenols. These results were satisfying given that monovarietal samples were examined. Moreover, isotopic data were found to be very similar throughout winemaking for most of the samples investigated. Specifically, (3S)-(+)-linalool consistently exhibited the most negative $\delta^{13}C$ values, ranging from −33.4 ‰ (Sample 2) to −39.8 ‰ (Sample 14) in grapes, and from −33.6 ‰ (Sample 2) to −39.3 ‰ (Sample 7) in wines. Similarly, the (E)-(3R,6S)-(-)-linalool oxide pyranoid $\delta^{13}C$ values ranged from −32.6 ‰ (Sample 2) to −38.7 ‰ (Sample 14) in grapes, and from −31.9 ‰ (Sample 2) to −37.9 ‰ (Sample 7) in wines. On the other hand, geraniol and the second eluting enantiomer of (E)−8-hydroxy-linalool had the most positive values in *Moscato giallo* samples, both in grapes and wines (Table 2).

Following the photosynthetic cycle of C_3 plants, as reported in a review by van Leeuwen et al. [31], the components produced generally exhibit $\delta^{13}C$ values within the range of −24 to −34 ‰. However, as shown in Table 2, most of the $\delta^{13}C$, both for grapes and wines, especially for the enantiomers (3S)-(+)-linalool and (E)-(3R,6S)-(-)-linalool oxide pyranoid, fell outside this range. Nevertheless, from a biochemical standpoint, the biosynthesis of monoterpenoids in grapes accounts for the unusually more negative isotopic data observed. In a previous study, Luan et al. [32] demonstrated a characteristic incorporation of 1-deoxy-D-xylulose into (3S)-linalool and geraniol in grape berries. From an isotopic standpoint, the differences in metabolic pathways result in distinct isotopic fractionations for the secondary metabolites [33]. Specifically, this pathway leads to a greater depletion of ^{13}C in isoprenoids compared to the mevalonate acid pathway [33]. These considerations explain the negative $\delta^{13}C$ values recorded for terpenoids in grapes and wines in the current study. Consistently, samples with target volatiles exhibiting more negative $\delta^{13}C$ values compared to typical C_3 trend may be valuable for genuineness assessment, as reported in the literature [34].

As described in Section 3.3, the coupling of a chiral column to an isotopic ratio mass spectrometer allows for the evaluation of the $\delta^{13}C$ value of each separated enantiomer. As recently reported by Cucinotta et al. for lemon essential oils, this feature can help defining characteristic trends within a natural sample [35]. Table 3 summarizes the chiral and isotopic data obtained for linalool enantiomers.

Starting with grape samples, similar $\delta^{13}C$ values were found between linalool enantiomers, specifically the (3R)-(-) and (3S)-(+) forms. In detail, the difference between levo and dextrorotatory enantiomers was always within 1 ‰, except for samples 1, 2, and 13, where the (*R*)-(-) form showed more negative values. In the context of wine samples, $\delta^{13}C$ values of (3R)-(-) linalool were consistently more negative than those of the (3S)-(+) enantiomer, except for sample 16, which exhibited linalool values almost similar, at −38.3 ‰ and −38.5 ‰, respectively.

It is also noteworthy to observe the trend in the isotopic values of the same enantiomers, either (*R*)-(-) or (*S*)-(+) forms, during the winemaking process. Specifically, the $\delta^{13}C$ values of (3R)-(-) linalool were found to be very similar throughout winemaking (from grape to wine), always within 1 ‰, except for samples 13 and 14. On the other hand, more noticeable differences were highlighted for the (3S)-(+) enantiomer, from grape to wine.

4. Conclusions

This study represents the first instance in literature where the qualitative, quantitative, enantiomeric, and isotopic composition of target terpenoids throughout winemaking, from grapes to wines, has been comprehensively explored. In detail, an MDGC method was developed, integrating an apolar column in the ^{1}D and a chiral stationary phase in the ^{2}D, thereby enhancing the separation of target terpenoids before IRMS and qMS detection. This innovative approach enabled the simultaneous evaluation of four enantiomeric ratios and five compound-specific isotopic ratios in a single analytical run, encompassing both grapes and wines. While certain variations were observed in terms of enantiomeric distribution throughout winemaking, such as in the case of linalool, consistent results were obtained regarding isotopic ratios, even within the same enantiomeric pair. Ongoing efforts are directed toward expanding the database of grapes and wines under investigation to identify specific statistical trends that reaffirm the established authenticity range for natural samples.

CRediT authorship contribution statement

Lorenzo Cucinotta: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Francesca Cannizzaro:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Mauro Paolini:** Formal analysis. **Alberto Roncone:** Visualization, Formal analysis. **Federica Camin:** Supervision. **Luana Bontempo:** Supervision, Conceptualization. **Roberto Larcher:** Supervision, Conceptualization. **Danilo Sciarrone:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Luigi Mondello:** Supervision, Resources, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Supplementary materials

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