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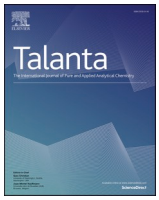

Flow-modulation comprehensive two-dimensional enantio-gas chromatography: A valid and flexible alternative to heart-cutting multidimensional enantio-gas chromatography

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ABSTRACT

The present research is focused on the proposal of use of flow-modulation comprehensive two-dimensional enantio-gas chromatography (FM eGC × GC) as a valid, flexible, and possibly superior alternative to heart-cutting multidimensional enantio-GC (eMDGC). The latter, a technique of demonstrated utility, is used specifically for the targeted separation of chiral compounds, whereas FM eGC × GC can produce both targeted and high-resolution untargeted information in a single run. It is clearly possible to use eMDGC for untargeted analysis, often with a flame ionization detector (stand-by analysis), to monitor a first-dimension (^{1}D) separation, of much lower peak capacity compared to FM eGC × GC. If eMDGC is used with mass spectrometry (MS), it is normally exploited to monitor the second-dimension (^{2}D) separation.

The analytical instrument consisted of automated solid-phase microextraction (SPME), and a low duty-cycle FM eGC × GC system (with time-of-flight MS), equipped with an enantioselective ^{1}D column (2,3-di-*O*-methyl-6-*t*-butyl silyl β-cyclodextrin derivative) and a ^{2}D polyethylene glycol one. Ten Marsala wines were subjected to analysis, for the determination of chiral lactones (many at the low ppb level, due to the high concentration capacity of SPME) and for general analyte profiling. In many instances, highly complex chromatograms were attained, with statistical analysis (ANOVA-simultaneous component analysis and partial least squares discriminant analysis) used for sample differentiation.

1. Introduction

Heart-cutting enantioselective multidimensional gas chromatography (eMDGC) is a technique of established utility for the targeted analysis of specific chiral compounds, often contained in food samples [1]. Commonly, a Deans switch is used as transfer device, with an achiral separation performed in the first dimension (^{1}D) and a chiral one in the second dimension (^{2}D). Additionally, an initial "stand-by" ^{1}D analysis is performed to monitor the whole sample chromatogram, and to enable the selection of the heart-cut time windows. After, a "cut" analysis is carried out, during which the target chiral compounds are separated on the ^{2}D column. A flame ionization detector (FID) is often used for the "stand-by" analysis, while mass spectrometry, or in alternative a FID, are used for the "cut" analysis. In this way, enantiomer ratios can be determined in a reliable manner, greatly reducing the occurrence of chromatographic interferences, often observed in eGC [2]. Though variations do exist, the aforedescribed instrumental configuration is the most popular among eMDGC analysts [3].

Enantioselective comprehensive two-dimensional gas chromatography (eGC × GC) has been previously reported, with both cryogenic and flow modulation (CM/FM) [4,5]. With regard to CM, experiments with either ^{1}D and ^{2}D enantioselective columns have been made [6–8];

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the choice of an enantioselective ^{1}D column is the best choice, due to a much higher separation power compared to a short ^{2}D one. Proceeding onto FM, then it appears to be a better choice (compared to CM) for three reasons: 1) the increase of signal-to-noise ratio (*s/n*) values provided by CM is not necessary in enantioselective analyses; 2) FM systems have a lower economical cost; 3) if required, brief (<500 ms) modulation periods (P_M) can be applied, which becomes an important factor for closely-eluting enantiomers.

The present investigation involves the targeted and untargeted headspace analysis of Marsala wine. Marsala wine (or "Marsala") is one of the four most important fortified aged wines, with classification related to aging, colour and sugar [9,10]. Considering aging, Marsala can be distinguished in "Fine" (min. 1 year), "Superiore" (min. 2 years), "Superiore Riserva" (min. 4 years.), "Vergine" (min. 5 years) and "Stravecchio" (min. 10 years). The beverage is available in three different colours namely gold, amber, and ruby, and is also classified as dry, semi-dry, and sweet according to the sugar content [11]. Finally, Marsala wines are classified as Marsala Vergine (Marsala Vergine and Stravecchio) and Marsala Conciati (Fine, Superiore and Superiore Riserva) on the basis of the production process. Aging is carried out using oak and/or cherry barrels [12].

The study of wine aroma is an important factor in quality control. In particular, γ- and δ-lactones are important constituents of beverage aromas, especially barrel-aged ones [13]. These compounds are often potent and pleasant odorants, that contribute to a variety of aromas, including "coconut", "fatty" and "sweet fruit" [14]. Almost all lactones are chiral, and the abundance of the single enantiomers varies significantly, although the (*R*)-enantiomer is dominant [15,16]. Furthermore, lactones can be exploited as markers for beverages aged in wood barrels, and for possible cases of adulteration [17].

The use of oak wood aging and specific fermentation methods cause different changes in the beverage chemical composition and overall sensory properties [17]. Among the compounds found in oak wood, *cis*- and *trans*-β-methyl-γ-octalactones (commonly known as whisky lactones) are key compounds that significantly contribute to the final aroma, with their concentration increasing proportionally to the aging time. Specifically, whisky lactones can exist in the form of four stereoisomers - two enantiomeric pairs of two diastereomers. Each isomer has its own characteristic coconut-based odour (*cis*-, with additional earthy, hay-like notes, while *trans*-with celery-like ones) and specific odour thresholds; furthermore, the *cis* isomer is a stronger odorant than the *trans* one [18,19]. It should be noted that in nature, oak wood contains only *trans*-(+)-(3S,3R) and *cis*-(−)-(3S,4S) whisky lactone isomers [20].

The present research is based on the use of (low duty-cycle) FM eGC × GC, as a valid (and potentially superior alternative) to heart-cutting eMDGC. Ten Marsala wine samples were subjected to headspace solid-phase microextraction (HS SPME), while detection was carried out by using time-of-flight mass spectrometry (ToFMS) - hydrogen was used as carrier gas [21,22]. Fifteen target chiral lactones were determined, with 14 as enantiomers. The lactones were quantified by constructing matrix-matched calibration curves; moreover, instrumental detection limits (IDL) were calculated. The developed method was also exploited to investigate the Marsala volatilomes, highlighting their highly complex nature, with over 300 compounds tentatively-identified. The samples were differentiated through statistical analysis, specifically by means of ANOVA-simultaneous component analysis and partial least squares discriminant analysis. It is noteworthy, that the Marsala volatilome has been previously investigated by using HS SPME cryogenic-modulation GC × GC-MS [9]; that research, however, was based only on untargeted profiling using achiral columns.

2. Materials and methods

2.1. Samples and chemicals

Ten Marsala wines were obtained directly from commercial activities located in Messina. The bottles were stored at ambient temperature prior to analysis. They belonged to three different types of Marsala; specifically, six Fine, three Superiore and one Stravecchio.

The following lactones, available as standard compounds, were investigated: γ-valerolactone (purity ≥99 %), γ- and δ-hexalactone (purity ≥98 %), γ-heptalactone (purity ≥98 %), γ- and δ-octalactone (purity ≥97 %), γ- and δ-nonalactone (purity ≥98 %), γ- and δ-decalactone (purity ≥98 %), γ- and δ-undecalactone (purity ≥98 %), γ- and δ-dodecalactone (purity ≥97 %), *cis*- and *trans*-whisky lactone (purity ≥98 %). The standards, sodium chloride, ethanol, and 3-octanol, used as internal standard (IS), were purchased from Merck Life Science (Merck KGaA, Darmstadt, Germany). The IS was solubilized in ethanol and was added to each sample at a concentration of 170 μg L^{-1}. Individual stock standard solutions were prepared in ethanol, for all the lactones.

2.2. Instrumentation

The HS SPME extractions were performed automatically by using an L-PAL3 GC Autosampler (LECO, Mönchengladbach, Germany). The extraction procedure was based on a previously-published paper [18]. Briefly, 5 mL of each sample were place in a 20 mL headspace vial, along with 0.5 g of NaCl and 5 μL of the IS solution. A 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber was conditioned according to the manufacturer's guidelines. The samples were incubated for 30 min at 60 °C, followed by 30 min of extraction at the same temperature. The agitation speed was 500 rpm. After extraction, the analytes were desorbed for 2 min at 220 °C, in the splitless mode. After each extraction and desorption procedure, the fiber was reconditioned for 5 min at 220 °C, to avoid analyte carryover between sequential extractions. Three replicates for each sample were carried out.

The FM eGC × GC-ToFMS applications were performed on a Pegasus® BT 4D GC × GC-ToFMS system equipped with a Flux™ modulator (LECO). The GC × GC column set was: Astec CHIRAL DEX B-DM [2,3-di-*O*-methyl-6-*t*-butyl silyl derivative of β-cyclodextrin phase] with dimensions 30 m × 0.25 mm ID × 0.12 μm d_f employed as ^{1}D column, while the ^{2}D column was a Supelcowax 10 [polyethylene glycol] with dimensions 1.30 m × 0.10 mm ID × 0.10 μm d_f and with 0.3 m located inside the MS transfer line (220 °C). All columns were provided by Merck Life Science.

The carrier gas used was H_2, delivered at a constant flow of 0.8 ml min^{-1}.

The main GC oven was held at 50 °C for 2 min, then ramped up to 220 °C at 2 °C min^{-1}, with a secondary oven temperature offset of +10 °C. The P_M was set at 4 s, with a re-injection period of 80 ms. The auxiliary pressure unit (EPC) provided a constant flow of 3.5 ml min^{-1}. The ToFMS parameters were as follows: acquisition delay was 240 s; acquisition rate was 150 spectra s^{-1}; electron ionization was performed at 70 eV, while mass spectra were acquired in the mass channel range *m/z* 40–400. Unmodulated analyses were also performed, using an acquisition rate of 5 spectra s^{-1}.

Data were acquired and processed by using the ChromaTOF software v. 5.50.55.0.63466 (LECO). The mass spectral databases used were the Flavour and Fragrance Natural and Synthetic Compounds FFNSC 4.0 (Chromaleont s.r.l. Messina, Italy) and the NIST 2017 Mass Spectral Library (NIST 17) purchased by Chromaleont s.r.l.

2.3. Data analysis

For "hit" recognition the LECO ChromaTOF Tile-computed v.1.01 (LECO Corporation, St. Joseph, MI) software was used. Normalization was performed using the 3-octanol IS peak signal ($^{1}t_R$ = 1594.03 s, $^{2}t_R$ = 1.6881 s), extracted at *m/z* 59. Tile sizes of 3 modulations for the ^{1}D separation and 23 spectra for the ^{2}D were selected, to encompass the average peak widths along both dimensions. A *s/n* threshold of 10 was applied to exclude low signal hits from the hitlist [23]. No F-ratio

threshold was set herein; the entire *m/z* range was included for tile-based F-ratio analysis.

After hit list generation, compound tentative identification was performed using the FFNSC 4.0 and the NIST 17 databases. The sample files were labeled according to Marsala type, resulting in ten classes for F-ratio analysis, with three replicates *per* class.

2.4. ANOVA simultaneous component analysis - ASCA

ASCA performs a multivariate analysis of variance (ANOVA) - even when the number of measured variables is higher than the available samples - by applying principal component analysis (PCA) to each of the factors under study [24,25]. Such an approach allows to determine if a given factor is significant relative to the residual error. ASCA models show if the factor under study has a significant role in explaining the variance structure of the experimental data. Cases in which more than one factor is involved can also be studied. For significance estimation, the *p*-value is calculated for each factor based on a test consisting in repeated random permutation of the factor levels. ASCA tests a null hypothesis H_0 of no experimental effect of the factor of interest against the alternative hypothesis (H_1) of an experimental effect, at a pre-selected significance level of *p*.

In the present study the factor considered is the type of Marsala wine, encoded at three levels: Fine, Superiore, Stravecchio. Descriptor variables are the FM eGC × GC-ToFMS data.

Data processing was performed by means of PLS_Toolbox® (Version 9.2, Eigenvector Research Inc.) that allows the calculation of ASCA + models [26], suitable also for cases in which unbalanced classes (different number of samples in each level of the factor) are considered.

2.5. Partial least squares discriminant analysis - PLS-DA

PLS is a widespread linear classification technique particularly useful in cases in which the number of samples is lower than the number of measured variables. The method applies PLS regression using a dummy index (*e.g.*, encoded by 0 and 1) as the response variable [27]. When more than two classes are involved, it is necessary to apply a PLS-2 algorithm, which allows to predict one matrix of response variables, with as many columns as classes.

PLS-DA can be used for building predictive models, which can be applied to determine class membership of new samples, and/or to assess the importance of descriptor variables in characterizing the different classes under study. This latter aim can be achieved by computing the VIP (variable importance in projection) scores, a value computed for each descriptor variable in each class [28]. A VIP score higher than one indicates that the corresponding variable is important in the model obtained for the characterization of the specific class.

3. Results and discussion

The aim of the present investigation was the development of an HS SPME FM eGC × GC-ToFMS method for the simultaneous determination of fifteen target chiral lactones and of the volatile fraction of Marsala wines. Hence, the instrumental approach is of a both untargeted and targeted nature, and is herein proposed as a valid, if not better alternative to heart-cutting eMDGC.

The HS SPME method used has been previously published [18], while the FM eGC × GC-ToFMS one was optimized, in particular, to avoid the loss of ^{1}D resolution, potentially leading to an incorrect measurement of enantiomeric ratios. Moreover, lactones are generally present at the μg L^{-1} concentration levels, thus attention was also devoted to the IDLs, considering that a low duty-cycle modulator was used. A P_M of 4 s was applied with a re-injection pulse of 80 ms, leading to a theoretical duty cycle of 0.02 (*circa* 2 % of the ^{1}D eluate reaches the detector). The resolution between all the enantiomers was satisfactory, as can be seen in Fig. 1 (standard compounds); it is noteworthy that δ-hexalactone (peak 3) is present as a single enantiomer. Table 1 reports IDLs, coefficient of variation (CV%) values, full widths at half maximum, tailing factors and ^{1}D resolution values (n = 3), the latter calculated without performing the modulation process.

The IDLs were calculated by multiplying the standard deviation of the analyte area at the lowest concentration level (1 μL liquid injection, in the splitless mode) by the statistical confidence factor *t*. The confidence factor *t* is determined using Student's t-distribution with a 99 % confidence level and *n*-1 degrees of freedom. For each pair of enantiomers, the average IDLs are reported. The average IDL values ranged from 0.02 ng for δ-undecalactone, δ-dodecalactone, and *cis*-whisky lactone to 0.17 ng for δ-hexalactone. The tailing factor values ranged from 1.2 to 1.6. With regard to enantiomeric resolution, values ranged from 1.4 for δ-decalactone and δ-dodecalactone to 12.0 for *trans*-whisky lactone.

Particular attention was devoted to determining the enantiomeric ratios of the target chiral lactones. Indeed, the enantiomeric ratio values, calculated by performing an unmodulated analysis of the standard components, were compared with those obtained through eGC × GC. The values obtained in both modes were in agreement with each other, demonstrating that the modulation process had no influence on the calculation of the enantiomeric ratios.

The quantification of all the target compounds was carried out by using the multiple standard addition method, with each sample spiked at five different concentration levels, according to the initial amount of

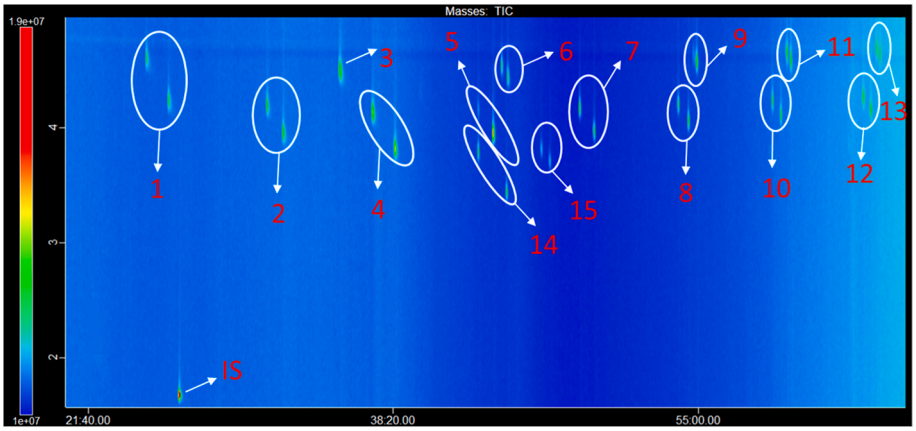

Fig. 1. FM GC × GC-ToFMS chromatogram of a mixture of 15 chiral lactones (50 ppm), and the IS. Peak identification: 1. γ-valerolactone; 2. γ-hexalactone; 3. δ-hexalactone; 4. γ-heptalactone; 5. γ-octalactone; 6. δ-octalactone; 7. γ-nonalactone; 8. γ-decalactone; 9. δ-decalactone; 10. γ-undecalactone; 11. δ-undecalactone; 12. γ-dodecalactone; 13. δ-dodecalactone; 14. *trans*-whisky lactone; 15. *cis*-whisky lactone.

Table 1
List of the analyzed lactones with concentration range, average IDLs, CV% values, full widths at half maximum (FWHM), tailing factors (TF) and ^{1}D resolution (R_S) calculated without performing the modulation process.

Compound	On-column injected amount range (ng)	IDL (ng)	CV%	FWHM (s)	TF	R_S	Enantiomeric ratio
γ-valerolactone: 1	0.24–5.02	0.03	12.1	0.142	1.4	6.4	50/50
γ-valerolactone: 2	0.26–4.98		14.8	0.132	1.5		
γ-hexalactone: 1	0.25–4.92	0.05	11.9	0.129	1.5	5.7	50/50
γ-hexalactone: 2	0.25–5.08		8.3	0.123	1.5		
δ-hexalactone	0.50–10.0	0.17	14.9	0.136	1.5	–	–
γ-heptalactone: 1	0.25–5.09	0.14	15.0	0.125	1.5	8.5	50/50
γ-heptalactone: 2	0.25–4.91		14.9	0.118	1.5		
γ-octalactone: 1	0.04–1.09	0.13	14.3	0.124	1.5	6.1	10/90
γ-octalactone: 2	0.46–8.91		12.3	0.122	1.5		
δ-octalactone: 1	0.23–5.36	0.03	14.9	0.130	1.4	2.6	44/56
δ-octalactone: 2	0.27–4.64		15.0	0.129	1.4		
γ-nonalactone: 1	0.24–5.15	0.08	13.8	0.125	1.5	5.8	50/50
γ-nonalactone: 2	0.26–4.85		13.0	0.118	1.5		
γ-decalactone: 1	0.26–5.01	0.05	13.1	0.123	1.4	4.4	49/51
γ-decalactone: 2	0.24–4.99		12.4	0.118	1.4		
δ-decalactone: 1	0.08–1.54	0.03	11.1	0.135	1.3	1.4	15/85
δ-decalactone: 2	0.42–8.46		11.1	0.132	1.3		
γ-undecalactone: 1	0.24–4.96	0.03	9.2	0.121	1.4	3.7	50/50
γ-undecalactone: 2	0.26–5.04		11.6	0.119	1.4		
δ-undecalactone: 1	0.25–5.06	0.02	10.2	0.131	1.2	1.6	48/52
δ-undecalactone: 2	0.25–4.94		9.0	0.129	1.2		
γ-dodecalactone: 1	0.24–5.02	0.04	14.6	0.120	1.3	3.1	48/52
γ-dodecalactone: 2	0.26–4.98		13.9	0.117	1.3		
δ-dodecalactone: 1	0.24–4.98	0.02	14.4	0.128	1.2	1.4	48/52
δ-dodecalactone: 2	0.26–5.02		13.9	0.129	1.2		
trans-whisky lactone: 1	0.25–5.04	0.03	12.9	0.118	1.5	12.0	51/49
trans-whisky lactone: 2	0.25–4.96		12.2	0.109	1.5		
cis-whisky lactone: 1	0.24–5.08	0.02	14.5	0.120	1.6	3.6	50/50
cis-whisky lactone: 2	0.26–4.92		12.1	0.114	1.5		

lactones present in the Marsala sample.

3.1. Target analyses

Ten Marsala wines were then analyzed by using the HS SPME FM eGC × GC-ToFMS method, for the determination of the chiral lactones. All the investigated Marsala samples were amber in color, while the Marsala Superiore, Stravecchio, and Fine 6 were dry, and the other Marsala Fine samples were semi-dry. Table 2 reports the chiral lactone amounts ($n = 3$), involving a total of six different compounds. The absolute quantities of each compound are reported, along with the % values of each enantiomer, with these showing a great variability. The CV% values are satisfactory, always lower than 15 %.

Whisky lactones have the highest concentration among the investigated lactones. In particular, the *trans* isomer ranged from 0.33 to 145.75 μg L^{-1} (Marsala Superiore 2 and Marsala Stravecchio, respectively), while the *cis* isomer ranged from 8.50 to 496.00 μg L^{-1} (Marsala Fine 5 and Marsala Stravecchio, respectively). Whisky lactones are the most important volatile compounds present in oak wood, because they contribute to the final aroma of the product, and they are considered as oak markers [8]. No whisky lactones were found in Marsala Fine 1, 2 and 6, while only the *cis* isomer was determined in Marsala Fine 5. Different factors, such as the wood structure, toasting technique, and aging time, affect the whisky lactone amount in the product.

The first (eluting) γ-nonalactone enantiomer (ranging from 3.00 to 6.00 μg L^{-1}) was always present in excess with respect to the second one (ranging from 0.03 to 3.18 μg L^{-1}). In Marsala Fine 3, Fine 4, and Superiore 1, the γ-nonalactone enantiomers were not determined. Both γ-decalactone enantiomers were observed in five samples, mainly those with more aging (Marsala Superiore 2 and 3, Stravecchio), apart from Marsala Fine 1 and 2. Low concentrations of both γ-undecalactone isomers were observed in Marsala Superiore 2 and 3, and Stravecchio (in the ranges 0.15–1.36 and 0.04–1.09 μg L^{-1}, for enantiomers 1 and 2, respectively). Only two aged samples (Marsala Superiore 2 and 3) contained both γ-dodecalactone isomers.

The results indicate a steady, albeit slightly irregular increase in lactones with aging. Moreover, enantiomer 1 was always present in higher amounts, apart for γ-decalactone and γ-dodecalactone in Marsala Superiore 2. According to previously published papers [15,16], the first eluting enantiomer can be tentatively-identified as the (R)-enantiomer form. Enantiomer % values varied greatly - *e.g.*, the γ-nonalactone enantiomers were determined in seven samples, with the first present in the range 55–99 %.

3.2. Untargeted analysis

The method developed enabled also an in-depth study of the volatile composition of the ten Marsala wine samples. To illustrate the high complexity of Marsala wines, the chromatogram of the Stravecchio sample is shown in Fig. 2, in which the eight lactones determined are indicated; slight variations in analyte retention times between the standard solution (Fig. 1) and the beverage are presumably due to the different injection procedures - liquid injection and HS SPME. Specifically, for the identified lactones, the (total) retention time shift ranged from 0.02 to 0.06 min. Overall, 331 compounds were tentatively identified (apart from the targeted lactones, no other pure standard compounds were used), and are listed in Table S1.

Absolute quantification was not performed, it being outside the scope of the present investigation; however, normalized MS (to the IS) areas can be exploited to evaluate concentration trends of the same compound in the different samples. In general, enantiomer ratios can be determined through MS peak areas.

Among the tentatively-identified compounds, fatty acid esters comprise a significant group of compounds in Marsala wines. The most abundant esters were ethyl octanoate and diethyl succinate, that confer fruity and tropical notes; specifically, their amounts increased from the youngest to the oldest sample. Also, ethyl acetate (ethereal, fruity, sweet, weedy, green odour) exhibited the highest amount in Marsala Stravecchio and lower amount in the youngest wines. Other esters found in the investigated samples were isoamyl acetate, ethyl butyrate, ethyl hexanoate, ethyl decanoate and ethyl lactate. The first compound showed the highest amount in Marsala Fine 1 and Marsala Stravecchio,

Table 2
Analyzed samples along with lactone concentrations, enantiomer % values reported in parenthesis, and CV% values.

Sample	*trans*-whisky lactone		*cis*-whisky lactone		γ-nonalactone: 1		γ-nonalactone: 2		γ-decalactone: 1		γ-decalactone: 2		γ-undecalactone: 1		γ-undecalactone: 2		γ-dodecalactone: 1		γ-dodecalactone: 2	
	ppb	CV%	ppb	CV%	ppb	CV%	ppb	CV%	ppb	CV%	ppb	CV%	ppb	CV%	ppb	CV%	ppb	CV%	ppb	CV%
Fine 1	–	–	–	–	3.91 (55)	7.1	3.18 (45)	3.1	3.89 (54)	8.0	3.28 (46)	10.9	–	–	–	–	–	–	–	–
Fine 2	–	–	–	–	6.00 (96)	5.5	0.25 (4)	8.6	0.35 (68)	14.3	0.17 (32)	14.1	–	–	–	–	–	–	–	–
Fine 3	10.00	14.9	66.50	14.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fine 4	39.50	9.2	227.00	14.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fine 5	–	–	8.50	14.7	3.23 (78)	0.5	0.93 (22)	14.2	–	–	–	–	–	–	–	–	–	–	–	–
Fine 6	–	–	–	–	5.91 (73)	13.3	2.15 (27)	5.6	–	–	–	–	–	–	–	–	–	–	–	–
Superiore 1	52.71	9.9	154.00	10.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Superiore 2	0.33	14.5	12.00	14.0	3.00 (99)	7.5	0.03 (1)	5.3	0.68 (42)	13.1	0.94 (58)	14.4	1.29 (54)	10.4	1.09 (46)	14.4	0.90 (45)	14.5	1.12 (55)	11.7
Superiore 3	16.20	5.8	136.28	1.7	4.55 (70)	13.2	1.91 (30)	14.4	0.79 (92)	11.1	0.07 (8)	12.5	1.36 (82)	11.3	0.31 (18)	13.5	0.73 (95)	4.8	0.04 (5)	14.7
Stravecchio	145.75	2.4	496.00	7.1	4.77 (81)	2.3	1.14 (19)	8.4	0.67 (79)	14.3	0.18 (21)	8.4	0.15 (79)	12.4	0.04 (21)	11.7	–	–	–	–

ethyl butyrate in Marsala Fine 5, ethyl hexanoate in Marsala Superiore 1, and ethyl decanoate in Marsala Superiore 1. Finally, ethyl lactate - an important compound which contributes to the broader, fuller taste of wine - showed the highest amount in Marsala Fine 6 [29].

The most abundant alcohol (excluding ethanol) was isopentyl alcohol, providing chemical notes, detected in comparable amounts in all samples except for Marsala Fine 4, where its level was the lowest [2]. Isobutyl alcohol was found in similar quantities in all the investigated samples.

A wide range of aldehydes with diverse odour notes were also found. Increasing values of furfural that confers sweet, woody and roasty notes were found from Marsala Fine 1 (2.95) to Marsala Superiore 1 (66.62) [29].

Untargeted enantiomers were also detected and separated, such as for example 2-ethyl hexanol, with similar amounts for the two enantiomers.

3.3. Multivariate data processing

The multivariate data processing performed in the present study is aimed at evaluating, from an exploratory point of view, the possibility of differentiating Marsala wine samples according to three different types (Fine, Superiore and Stravecchio) and to identify which compounds, among the ones tentatively-identified, are the most informative for such a profiling. The chemometric methods applied (ASCA and PLS-DA) were limited to the exploratory domain and were not aimed at building predictive models, also considering the reduced sample size.

ASCA was applied considering as the data matrix the FM eGC × GC-ToFMS data and, as the factor under study, the class membership at three possible levels: Fine, Superiore and Stravecchio.

The effect of the factor "Marsala type" contributes to the overall sum of squares of the data matrix with a 22.73 % of explained variance. Such a factor indicates that this information is not predominant in the dataset, but significant and useful for sample differentiation at a 5 % significance level ($p\text{-}value = 0.02$).

Fig. 3 shows that the different Marsala types (three replicates *per* sample) are characterized by a different average score value on PC1 of ASCA decomposition. In more detail, Fine samples are described by the lowest average score value, at around −1. Superiore samples present again a negative average score value, but higher (around −0.5). Stravecchio wine, in turn, is characterized by positive score value (on average about 1.25).

Fig. S1 reports the analytical descriptors that presented the most important contribution on defining the lowest-order principal component in the ASCA decomposition. In particular, among the 1186 variables analyzed, only the ones with an associated loading absolute value higher than 0.1 are represented (for the sake of clarity). The same variables are also listed in Table S2, reporting the variable number, the compound identity and the associated numerical loading value. It must be highlighted that the higher the absolute value of the loading, the more important is the contribution of the corresponding variable for the differentiation of the Marsala type. Moreover, from a joint interpretation of ASCA scores and loadings, it can be concluded that compounds with a negative loading value present a higher concentration in Marsala types with negative scores: Fine and Superiore. Conversely, Stravecchio Marsala wine (with positive scores) is characterized by a higher concentration of compounds with positive loadings.

To verify the consistency of the chemical characterization performed by means of ASCA decomposition, PLS-DA was carried out considering the three Marsala types as three classes to be modelled. Afterwards, a variable evaluation was performed by analysing the VIP profiles and selecting the most informative analytical descriptors for discrimination of the three classes. The selection criterion was defined as VIP value higher than 4 for all the three classes. In this way, eleven descriptors were selected; they are listed in Table S3, indicating the detail of the VIP score value for each of the three classes. Variables selected by both of the

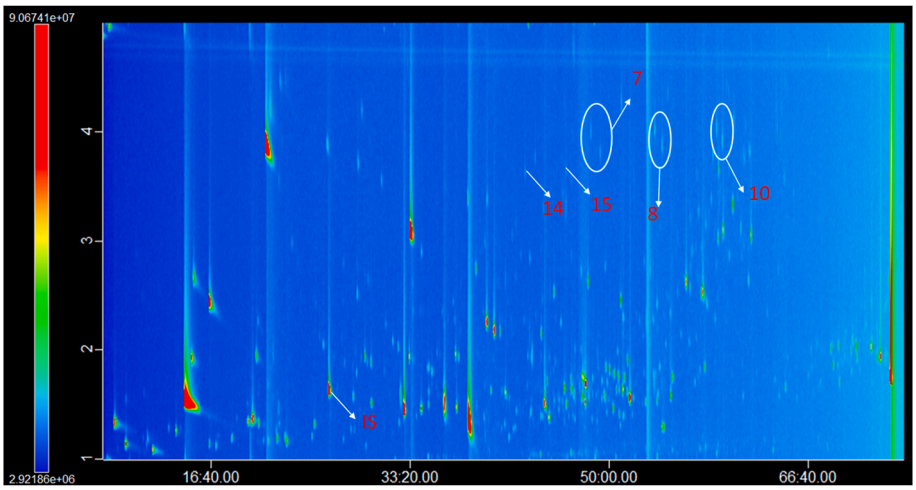

Fig. 2. FM GC × GC-ToFMS chromatogram of the Marsala Stravecchio headspace. Refer to the legend of Fig. 1 for peak identification.

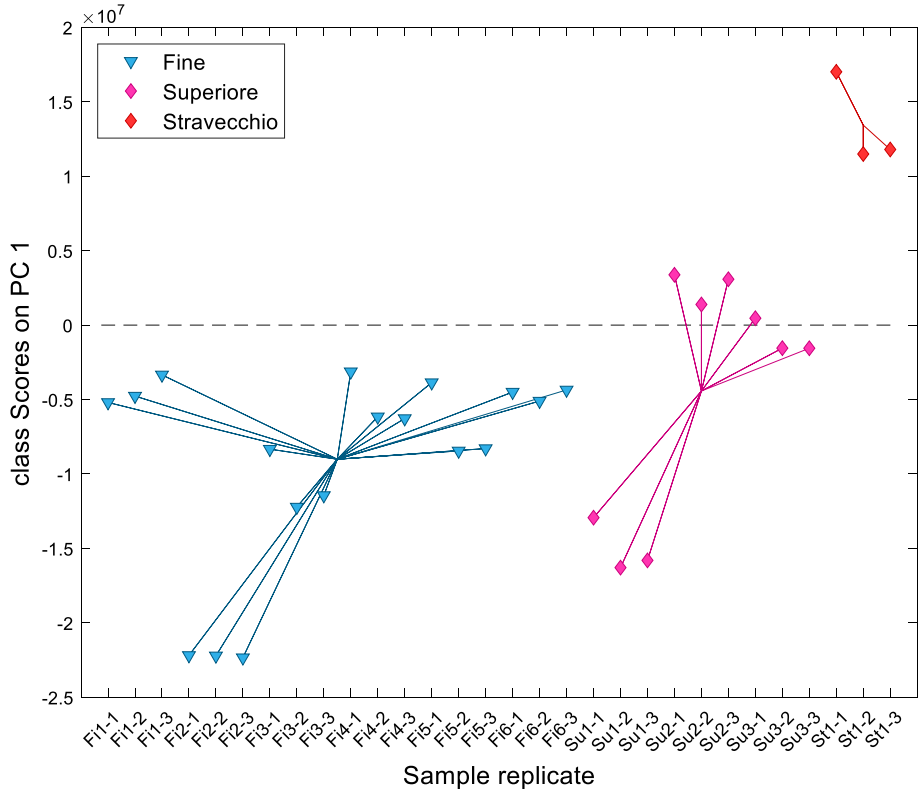

Fig. 3. Scores on PC1 of ASCA decomposition for the factor "Marsala type" (Fine - Fi; Superiore - Su; Stravecchio - St).

approaches (ASCA and PLS-DA) are highlighted in bold, confirming a good consistency between the two outcomes. It is also noticeable that the commonly selected variables are the ones with the highest absolute value of the ASCA loadings and of the PLS-DA VIP scores. These can be, therefore, considered as the most important compounds for the characterization and differentiation of the three Marsala wines.

The normalized areas of the six most informative compounds in the Marsala wines, defined by both statistical approaches, are shown in Fig. 4 (in both a bar chart and box plot).

The Stravecchio sample is the one with the higher amounts of ethyl benzoate, ethyl 2-furoate, and ethyl phenylacetate. The first gives a pleasant fruity odour, the second has a balsamic smell, while the third has a honey-like aroma. Diethyl malate is present in similar quantities in the Stravecchio and Superiore 2 samples, with caramel and fruity notes. Phenethyl alcohol, which gives a floral (rose) odour, is present in higher quantities in all Fine samples (except Fine 1) and in the Superiore 1 and 2 samples [29]. As for diethyl malate, also 2-oxopentanedioic acid is present in similar amounts in the Stravecchio and Superiore 2 samples, and it is an important sulfur-binding compound [30]. Considering the median values of the six compounds: phenethyl alcohol is the most abundant compound, followed by ethyl phenylacetate; ethyl benzoate, ethyl 2-furoate and diethyl malate are present in similar amounts, while 2-oxopentanedioic acid is the least abundant.

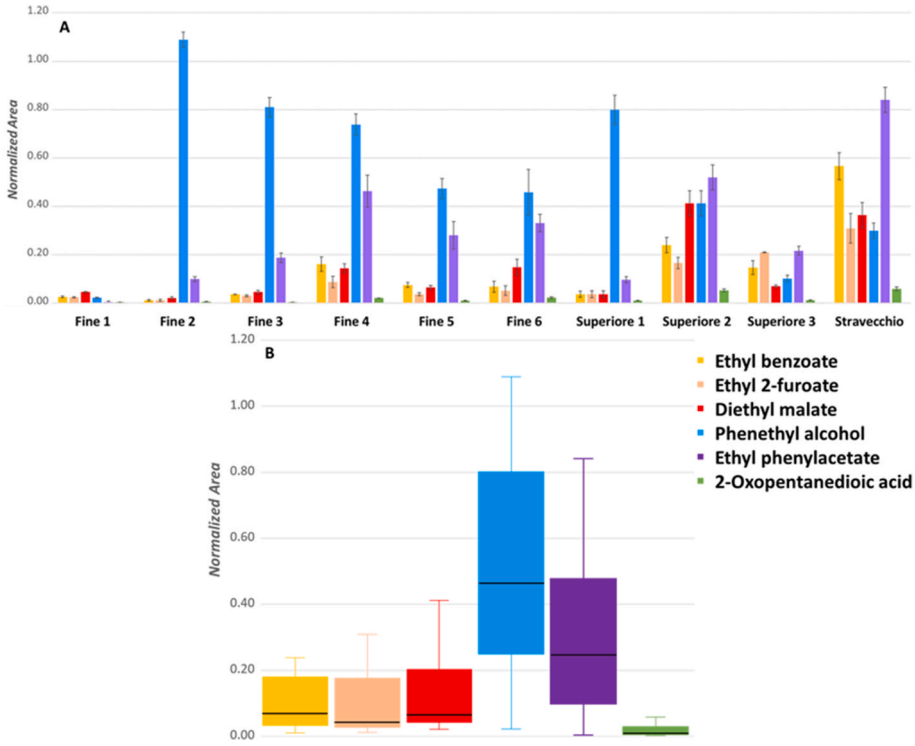

Fig. 4. Bar chart (A), and box plot (B) reporting the normalized areas of the most informative compounds in the investigated Marsala wines.

4. Conclusions

The scope of the present research is to propose the use of FM eGC × GC as a prime choice for the determination of both chiral and untargeted volatile compounds. For such a demonstrative objective, a complex sample - Marsala wine - was subjected to analysis. In such a respect, the results attained confirm the high usefulness of the analytical platform employed, inasmuch that two types of information can be produced in a single run. Even though a low duty cycle modulator was used, inevitably causing a great reduction of the analyte amounts reaching the MS system, the chiral lactones were detected at the low ppb level. Moreover, the headspace chromatogram profiles were of high complexity and detail. Even though a reduced number of samples was subjected to investigation, statistical analysis enabled a clear differentiation between Marsala types, and the highlighting of six compounds which differed most. The superiority of FM eGC × GC over classical eMDGC, in untargeted analysis, is clear. Future research will be devoted to a side-by-side comparison between FM eGC × GC and eMDGC in the chiral-only analysis of well-known food samples, such as Citrus essential oils.

CRediT authorship contribution statement

Micaela Galletta: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mariosimone Zoccali:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Data curation, Conceptualization. **Cristina Malegori:** Writing – original draft, Software, Formal analysis, Data curation. **Paolo Oliveri:** Writing – original draft, Software, Formal analysis, Data curation. **Peter Q. Tranchida:** Writing – review & editing, Writing – original draft, Conceptualization. **Luigi Mondello:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Monica Mondello:** Writing – review & editing, Writing – original draft, 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2024.126137.

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Flow-modulation comprehensive two-dimensional enantio-gas chromatography: a valid and flexible alternative to heart-cutting multidimensional enantio-gas chromatography

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Table S1. Tentative identification with MS similarities and normalized areas relative to the ten Marsala samples.

Compound	Fine 1		Fine 2		Fine 3		Fine 4		Fine 5	
	MS similarity	Normalized area	MS similarity	Normalized area	MS similarity	Normalized area	MS similarity	Normalized area	MS similarity	Normalized area
2-(1-Ethylpentyl)-1,3-dioxolane		-		-		-	770	0.06		-
2,4-Dimethyl-1,3-dioxolane		-		-		-	751	0.42		-
14-Methyl-8*E*-hexadecenal	749	2.05	765	3.42	754	2.15	760	3.96	765	5.96
2,5-Dimethyl-5-hexen-3-ol	851	0.02	863	0.05	870	0.06	855	0.04	865	0.08
2H-2,4a-Methanonaphthalen-1(5H)-one, hexahydro-5,5-dimethyl isomer I		-	762	2.99		-		-		-
2H-2,4a-Methanonaphthalen-1(5H)-one, hexahydro-5,5-dimethyl isomer II	766	0.71	761	0.96	764	0.84		-		-
2-Oxopentanedioic acid	749	0.01	751	0.02	754	0.01	760	0.02	758	0.02
2-Phenylethyl formate	823	0.01	815	0.01	810	0.09	818	0.07	830	0.22
3-Butyn-1-ol	700	2.58	700	0.01	706	1.51	700	1.77	702	2.43
Methyl octyl acetaldehyde		-		-		-		-	786	0.32
2-Ethylhexyl acetate		-		-		-		-		-
2-Methylbutyl acetate	867	6.96	849	0.46	870	2.67	868	0.62	865	2.81
1,4,4-Trimethyl-3,5-dimethylidenecyclopentene	832	0.05	830	0.09	840	0.02	815	0.03	810	0.06
3,5,5-Trimethylhexyl acetate	880	0.25		-	880	0.25		-		-
Butyl acetate		-		-		-	870	0.25		-
Ethyl acetate	944	27.22	913	9.98	912	347.70	887	300.37	903	1053.22
Hexyl acetate	896	0.59		-	897	0.44		-	898	0.29
Isoamyl acetate	888	23.39	932	1.86	897	10.32	885	2.56	895	12.39
Isobutyl acetate	889	1.93		-	904	1.29		-	916	1.21
Acetic aldehyde	894	14.13	915	7.47	917	35.65	904	40.51	958	41.44

Acetic anhydride	929	472.43		-		924	238.22	918	4.01		-	
Acetoin:1		-		-		710	0.16	680	0.07	703	0.24	
Acetoin:2		-		-		733	0.10	733	0.10		-	
Acetone	920	0.52		-		876	0.78	831	1.04		-	
Acetophenone		-		-		795	0.21	795	0.21		-	
2',4'-Dihydroxy acetophenone		-		-			-		-		-	
4'-Hydroxyacetophenone		-		-			-	868	0.12		-	
Acetylpropionyl		-		-			-	896	0.20		-	
Diethyl adipate		-		-			-		-		-	
Benzaldehyde	936	2.38	942	3.66		921	9.14	900	12.05	940	9.57	
Butyl benzoate	862	0.08	923	0.25		877	0.35		-	867	0.46	
Ethyl benzoate	873	0.03	907	0.01		896	0.04	880	0.16	945	0.07	
Isobutyl benzoate		-		-			-		-		-	
Propyl benzoate		-		-			-		-		-	
Benzosulfonazole		-	779	1.22		779	1.22		-		-	
Benzyl alcohol	804	2.35	855	1.10		816	3.92	797	1.39	813	5.19	
Benzyl ether	856	0.11		-		856	0.11		-		-	
Cis-α-bergamotene		-		-			-		-		-	
Bicyclogermacrene		-		-			-		-		-	
Biphenyl oxide	866	0.14	757	0.08		830	0.23		-		-	
(*Z*)-α-Bisabolene		-		-			-		-		-	
β-Bisabolene:1		-		-			-		-		-	
β-Bisabolene:2		-		-			-		-		-	
Bois de rose oxide:1	886	0.19	831	0.32		851	0.20	855	0.11	826	0.17	
Bois de rose oxide:2	876	0.31	877	0.38		844	0.24	844	0.06	819	0.07	
(*E*)-2-Butenal	771	0.06		-		788	0.39	805	0.72		-	
1-Ethoxy-3-methyl-2-butene	795	0.24	820	0.47		789	0.31	777	0.14	753	0.22	
2,3-Butanediol:1	818	8.17	787	22.96		811	35.09	831	18.75	821	59.98	
2,3-Butanediol:2	812	7.39	765	5.78		810	60.19	831	44.65	827	108.57	
Butan-2-ol		-		-		813	2.69	813	2.69		-	

2-Methylbutyl butanoate		-			-		770	0.09	752	0.03	787	0.15
Butyl alcohol	873	1.44		897	0.46		871	1.52	855	1.54	883	2.25
Benzyl butyl ether		-			-		781	0.06	781	0.06		-
Sec-butylamine		-		789	22.49		789	22.49		-		-
2-Methylbutyraldehyde		-			-		784	1.41		-		-
Ethyl 2-methylbutyrate	903	1.45		940	1.77		910	1.75	903	1.06	888	1.76
2-Phenylethyl 2-methylbutanoate		-			-			-		-		-
Benzyl butyrate		-			-		833	0.44		-	833	0.44
Ethyl butyrate	911	5.68		918	4.32		924	15.07	935	13.21	943	28.70
Isopentyl butyrate	865	0.08		784	0.07		844	0.17	825	0.08	877	0.44
γ-Butyrolactone	909	0.80			-		886	1.32	898	0.75	932	1.43
Cadalene	763	0.10		828	0.63		789	0.33		-		-
α-Calacorene	758	0.02		851	0.35		800	0.15		-		-
β-Calacorene	755	0.43			-		755	0.43		-		-
Cis-calamenene		-			-			-		-		-
Trans-calamenene		-			-			-		-		-
Camphor:1		-			-			-		-		-
Camphor:2		-			-			-		-		-
Sec-butyl carbinol		-			-			-		-		-
δ-Cadinene	929	0.01		920	0.01		930	0.01	918	0.01	910	0.01
δ-2-carene:1		-		756	0.22			-		-		-
δ-2-carene:2		-		837	0.30			-		-		-
Caryolan-8-ol		-			-			-		-		-
4,8-α-Epoxycaryophyllene		-			-			-		-		-
(*E*)-Caryophyllene:1		-			-			-		-		-
(*E*)-Caryophyllene:2		-			-			-		-		-
(*Z*)-Caryophyllene:1		-			-			-		-		-
(*Z*)-Caryophyllene:2		-			-			-		-		-
4,8-β-Epoxycaryophyllene		-			-			-		-		-

9-Epi-(*E*)- caryophyllene		-			-			-			-	
Caryophyllene acetate isomer II		-			-			-			-	
α-Chamigrene	768		0.10		-			-			-	
1,4-Cineole		-			-			-			-	
α-Copaene		-			-			-			-	
2-Phenylcrotonaldehyde		-			-			-	840	0.52		-
Ethyl crotonate	825		0.09		-		850	0.10	875	0.11		-
4-Ethyl cumene		-			-			-			-	
Cyclobutane-1,1-dimethyl-2-octyl	734		0.03	736		0.07	740	0.09	730	0.05	745	0.17
Cis-2-tert-butylcyclohexanol	803		0.15		-		803	0.15		-		-
Trans-4-tert-butylcyclohexanol	852		0.59		-		852	0.59		-		-
Trans-4-tert-butylcyclohexyl acetate	834		2.22		-		834	2.22		-		-
Cyclomyral		-			-		763	1.14		-	763	1.14
1,4,4-trimethyl-3,5-dimethylenecyclopentene	846		0.36		-		818	0.55	765	0.24	844	1.05
2-Prop-1-enyl-p-cymene		-		764		0.12	788	0.14		-	813	0.17
p-Cymene	806		0.08	797		0.35	821	0.61	766	0.08	841	0.55
(*Z*)-β-Damascenone	761		0.58	815		1.48	797	1.16		-	771	1.06
(*Z*)-4-decenal		-			-		753	6.75		-	753	6.75
1-Decen-3-ol		-			-		776	0.30		-	776	0.30
1-Decene		-			-		899	1.23		-	899	1.23
3-Decanone		-			-			-		-		-
Decanal	855		0.17	876		0.09	869	0.27	872	0.13	871	0.41
Ethyl decanoate	907		8.13	932		139.44	913	58.57	905	4.67	912	59.81
Isoamyl decanoate		-			-			-		-		-
Methyl decanoate		-		782		0.21	782	0.21		-		-
(*E*)-Ethyl 4-decenoate		-			-		812	4.95		-	811	7.61
Decyl alcohol		-		883		1.08	894	0.91	904	0.21		-
Decyl methyl ketone		-			-		819	1.18	820	0.45		-

Dihydromyrcenyl formate	830	0.32		-		830	0.32		-			-	
Dimethyl disulfide		-		-			-		-			-	
Docosane		-		-			-		-			-	
1-Dodecene	897	1.08		-		892	1.12	880	0.22		899	0.91
Dodecanal	852	0.07	878	0.11		874	0.12	881	0.07		903	0.17
Dodecane	865	0.09	813	0.07		868	0.28	874	0.18		874	0.44
Ethyl dodecanoate	899	1.16	924	2.35		895	1.16	858	0.13		901	0.60
Dodecanol	868	1.16	906	0.86		887	1.01		-			-
2-Dodecanol		-		-		793	0.65		-		793	0.65
Eicosane		-		-			-		-			-
β-Elemene		-		-			-		-			-
Epicubenol		-		-			-		-			-
Eremophilene		-		-			-		-			-
Estragole		-		-			-		-			-
(*Z*)-2-(3,3-dimethylcyclohexylidene)ethane		-		-			-		-			-
Eucalyptol	863	0.12		-		863	0.12		-			-
10-Epi-γ-eudesmol		-	755	0.08			-		-			-
γ-Eudesmol	823	0.05		-		823	0.05		-			-
Eugenol		-		-			-		-			-
(*E*)-β-farnesene		-		-			-		-			-
Endo-fenchol		-		-			-		-			-
Ethyl formate		-		-			-		-			-
Heptyl formate	873	0.42	880	0.14		888	0.24	884	0.15		913	0.27
Isopentyl formate		-		-		854	0.18	877	0.17		817	0.19
Octyl formate	888	0.99		-		882	0.68		-			-
2-Acetyl-5-methylfuran	800	0.16		-		846	0.21	891	0.25			-
2-Acetyl furan	778	0.09	803	0.26		804	0.26	782	0.44		854	0.24
2-Pentylfuran		-		-		838	0.45	887	0.31		848	0.38

2,2-Dimethyl-5-(1-methyl-1-propenyl)-tetrahydrofuran		-		-		-		-		-		
2(5H)-Furanone	754	0.04		-	754	0.04		-		-		
3-Furaldehyde	938	0.81	878	2.87	868	1.80	788	1.70		-		
Furfural	761	2.95	812	8.95	800	13.42	807	11.44	821	12.23		
5-Methylfurfural	800	0.58	922	1.40	829	2.81	818	1.64	821	4.57		
Ethyl 2-furoate	933	0.02	886	0.01	887	0.03	927	0.09	831	0.04		
Germacrene B		-		-		-		-		-		
Germacrene D		-		-		-		-		-		
Methylphenyl glyoxylate		-		-	922	0.14	922	0.14		-		
4-ethyl guaiacol	894	15.29	870	5.23	877	6.93	874	1.57	878	4.45		
4-propyl guaiacol	839	0.15		-	839	0.15		-		-		
Guaiazulene		-		-		-		-		-		
Heneicosane		-	820			-		-		-		
(*E*)-Heptadec-4-enol		-		-		-		-		-		
Heptadecane	909	2.06	948	4.73	911	3.58	901	1.80	906	4.65		
Ethyl heptanoate	897	0.22	879	0.41	893	0.63	900	0.29	875	1.60		
Heptyl methyl ketone	809	0.48	831	1.12	808	0.89		-	784	1.06		
Ethyl hex-(2*E*)-enoate	883	0.20	797	0.25	840	0.22		-		-		
Hex-(3*E*)-enol		-		-		-		-		-		
Hex-(3*Z*)-enol	815	0.09		-	799	0.22		-	782	0.35		
Hexadecane	877	0.26	938	0.58	882	0.49	820	0.04	874	0.74		
Hexadecene	904	0.14	923	0.19	910	0.22	898	0.13	919	0.30		
Hexanal		-		-		-		-		-		
Ethyl hexanoate	902	21.48	945	38.38	908	28.89	900	9.12	902	41.60		
Isopentyl hexanoate	862	0.21	876	0.52	844	0.24	776	0.03	851	0.23		
2-Ethyl hexanol:1	840	0.53	860	1.20	847	2.19	853	0.70	848	5.87		
2-Ethyl hexanol:2	857	0.55	883	1.35	855	1.82	847	0.72	848	3.96		
Hexanol	858	10.97	909	10.01	864	9.99	836	3.68	853	13.60		
α-Himachalene:1		-		-		-		-		-		

α-Himachalene:2		-		-	771	0.16		-	771	0.16
α-Dehydro-ar-himachalene		-		-	813	0.87		-		-
α-Humulene		-		-		-		-		-
Humulol		-		-		-		-		-
Ethyl hydrocinnamate	785	0.12	771	0.11	831	0.31	851	0.23	909	0.55
Isobutenyl methyl ketone		-	783	0.10	802	0.16	840	0.08		-
Isobutyl alcohol	853	24.95	927	28.78	871	25.39	855	9.30	855	24.37
Butyl isobutyrate		-		-		-		-		-
Ethyl isobutyrate	916	3.12	903	5.87	910	5.01	903	1.55	931	7.07
Isopentyl alcohol	917	418.27	939	290.04	899	359.10	883	150.76	872	401.61
Ethyl isovalerate		-	894	2.63	882	2.64	887	1.23	881	1.93
Jasmacyclene	849	0.59		-	849	0.59		-		-
Junenol		-		-		-		-		-
Ethyl lactate	785	6.95		-	776	22.29		-		-
Isopropyl laurate		-		-		-		-		-
Tetrahydro lavandulyl acetate	784	0.10		-	784	0.10		-		-
Lavandulyl isobutyrate	778	0.10		-	778	0.10		-		-
Limonene	866	0.16	920	0.41	886	0.60	871	0.11	869	0.51
Linalool		-		-		-		-		-
Tetrahydrolinalyl acetate:1	756	0.55		-	756	0.55		-		-
Tetrahydrolinalyl acetate:2	763	0.52		-	763	0.52		-		-
Cis-linalool oxide	811	0.09		-	787	0.08	763	0.06		-
Trans-linalool oxide		-		-		-		-		-
Linalyl anthranilate		-		-		-		-		-
Ethyl linoleate		-		-		-		-		-
Diethyl malate	815	0.05	812	0.02	806	0.05	810	0.14	803	0.07
Massoia lactone		-		-		-		-		-
p-Menth-3-ene		-		-		-		-		-
Menthol	889	0.15		-	889	0.15		-		-
Menthone	803	0.14	839	0.19	808	0.20	782	0.08	817	0.28

Mesitylene		-		795		0.17		785		0.18		-		775		0.20
Diphenyl methane		-			-				-			-			-	
Methyl nonyl carbinol		-			-			832		0.63	832		0.63		-	
Methyl nonyl ether	821		0.27		-			812		0.27	803		0.27		-	
Methyl propenyl ketone		-			-			869		0.30	869		0.30		-	
Methyl undecyl ether	851		0.27		-			851		0.27		-			-	
α-Muurolene		-		830		0.17		830		0.17		-			-	
Myrcene		-			-			787		0.20		-		787		0.20
Myrcenol	764		0.10		-			764		0.10		-			-	
Myrcenone					-			781		0.23	787		0.11		-	
Naphthene	843		0.38	819		0.38		834		0.47		-		832		0.65
2-Methyl napthalene		-			-			834		0.21		-			-	
Neryl butyrate		-			-			774		0.45		-		774		0.45
Nonadecane		-			-				-			-			-	
Nonan-2-ol		-		826		0.20		815		0.42		-			-	
Nonan-3-one	821		0.14	788		0.37		796		0.37		-		799		0.25
Nonanal	844		0.36	886		0.29		856		0.56	855		0.27	849		0.76
Ethyl nonanoate	850		0.59	893		1.24		879		0.84	875		0.20	897		0.99
Nonanol		-		896		0.57		895		0.69	900		0.26	873		0.72
Nonene	859		2.63		-			859		2.63		-			-	
Nonyl methyl ketone	788		0.11	794		0.18		784		0.19	796		0.09	756		0.36
(*E*)-β-ocimene		-			-			773		0.08		-		773		0.08
(*Z*)-oct-2-enal	784		0.32		-			784		0.32		-			-	
(*E*)-Oct-4-ene		-			-				-			-			-	
Ethyl (*E*)-oct-4-enoate		-			-				-			-			-	
Oct-2-en-4-one		-			-			786		3.35	803		0.84	779		1.21
Octadecane	868		0.09		-			843		0.20	889		0.27		-	
Octan-3-one		-		881		0.50		822		0.49	764		0.25	869		0.56
2-Methyl octanal		-			-			761		0.12	767		0.05		-	
Ethyl octanoate	922		73.04	944		287.90		936		156.99	932		28.40	935		241.49

Isobutyl octanoate		-			-			-			-	
Isopentyl octanoate	862		0.51	901		0.95	862		0.59		-		831		0.27
Methyl octanoate	843		0.13	837		0.43	840		0.28		-			-	
Phenylethyl octanoate	770		0.05		-		770		0.05		-			-	
Octanol		-		910		0.64	888		0.53	870		0.25	883		0.70
(*E*)-oct-2-ene		-			-			-			-			-	
Ethyl oleate	818		3.17		-		818		3.17		-			-	
Trans-2,3-dimethyloxirane	751		0.94		-		779		0.65		-		806		0.36
Ethyl palmitate	875		5.93	854		0.68	856		2.27	839		0.19		-	
β-Patchoulene		-			-			-			-			-	
Pelargol	879		0.23		-		879		0.23		-			-	
Pent-4-enophenone	820		0.06		-		817		0.09	814		0.11		-	
Pentadecane	882		0.31	938		0.72	888		0.48	900		0.35	872		0.84
Pentadecanolide		-			-			-			-			-	
2-Methyl pentanal		-			-			-			-			-	
Pentane		-			-		777		0.31	760		0.10	793		0.52
3-Methyl pentanol	877		0.47	918		0.29	889		0.30	918		0.16	863		0.39
4-Methyl-1-pentanol	846		0.20		-		851		0.14	855		0.08		-	
Pentyl alcohol	780		1.81		-		810		0.99		-			-	
2-Phenethyl acetate	880		7.91	909		0.85	879		4.07	861		1.06	880		5.75
Phenethyl alcohol	866		0.02	892		1.09	866		0.81	862		0.74	856		0.47
α-Phellandrene:1		-			-			-			-			-	
α-Phellandrene:2		-			-			-			-			-	
3-Ethyl phenol		-			-			-			-			-	
4-Ethyl phenol	871		59.69	810		1.15	864		16.85	877		3.97	895		7.49
4-Tert-butyl phenol		-			-		791		3.12	778		0.38	802		4.01
Phenylacetaldehyde	777		1.55	804		2.34	785		3.31	759		1.11	793		4.37
Benzyl phenylacetate		-			-		806		0.22	806		0.22		-	
Ethyl phenylacetate	842		0.01	891		0.10	859		0.19	854		0.46	847		0.28
Phenethyl phenylacetate		-			-			-			-			-	

2-Phenyltoluene		-		-		-		-		-		
Di-isobutyl phthalate	850	7.07	840	1.72	844	4.82	835	2.48		-		
Phytone		-		-		-		-		-		
Ethyl propanoate	923	5.62		-	875	10.98	860	7.03	915	19.28		
Isopentyl propanoate	909	0.14		-	850	0.11	777	0.05	865	0.14		
3-Methylmercapto propanol	891	0.30	920	0.32	906	0.21		-	-	0.00		
Ethyl 3-acetyl propionate	921	0.81	904	1.21	884	1.16	885	1.48	847	1.05		
α-Pinene		-		-		-		-		-		
[2-[1-(3,3-dimethylcyclohexyl)ethoxy]-2-methylpropyl] propanoate		-		-		-		-		-		
2-methyl-1,3-dimethyl-3-butenyl propanoate		-		-		-		-		-		
Allyl propanoate		-		-	814	0.04	814	0.04		-		
Phenethyl propionate	816	0.05		-	816	0.03	-	0.00		-		
Propyl alcohol	796	1.93	822	3.07	817	3.29	819	2.93	829	5.05		
Propylene glycol		-		-	762	206.48	762	206.48		-		
Purolan		-		-		-		-		-		
Pyroracemic acid		-		-	761	0.15		-	761	0.15		
Ethyl pyruvate		-	895	2.15	919	1.75		-		-		
Pyruvophenone		-		-				-		-		
Sabinene		-		-				-		-		
Safranal		-		-				-		-		
Ethyl salicylate	903	0.33	915	1.51	909	0.90	923	0.48	919	0.88		
Methyl salicylate	838	1.36		-	890	1.19	913	0.52	916	0.92		
Sclareolate	788	0.42		-	788	0.42		-		-		
Selina-4,11-diene:1	891	0.09	806	0.19	831	0.12		-		-		
Selina-4,11-diene:2	759	0.03		-	759	0.03		-		-		
α-Selinene		-		-		-		-		-		
β-Selinene		-		-		-		-		-		
Sibirene		-		-		-		-		-		

Solusterol		-			-		893	0.44		-	893	0.44
Ethyl sorbate		-			-		843	0.46		-	826	0.43
Ethyl stearate		-			-			-		-		-
Styrene		-			-		776	1.50	784	1.05		-
Diethyl suberate		-			-			-		-		-
Diethyl succinate	913		137.65	891		213.92	902	196.09	910	151.31	912	132.84
Terpin-1-en-4-yl acetate		-			-			-		-		-
α-Terpinene	753		0.03	782		0.14	783	0.15		-		-
γ-Terpinene		-			-			-		-		-
α-Terpineol		-			-		797	0.64		-	824	0.67
Terpinolene	850		0.13		-		855	0.15		-	860	0.16
γ-Terpinyl acetate	814		0.26	817		0.14	818	0.24		-		-
α-Terpinyl formate	790		0.22	815		0.18	803	0.34		-	803	0.64
(*E*)-Tetradec-2-enol		-			-			-		-		-
Tetradec-1-ene	812		0.04		-		812	0.04		-		-
Tetradecane	891		0.18	917		0.50	880	0.44	849	0.24	871	0.63
Ethyl tetradecanoate	863		0.30		-		863	0.30		-		-
Isopropyl tetradecanoate		-			-			-		-		-
Trans-tetrahydro jasmone		-		765		5.91	772	3.38	778	0.84		-
2-Heptyl tetrahydrofuran		-			-			-		-		-
Trans-5-isopropenyl-2-methyl-2-vinyltetrahydrofuran:1		-			-			-		-		-
Trans-5-isopropenyl-2-methyl-2-vinyltetrahydrofuran:2		-			-			-		-		-
Thujopsan-2-α-ol	798		0.19		-		779	0.20	-		760	0.21
Cis-thujopsene	824		0.04	779		0.07	802	0.06		-		-
Thujyl acetate		-			-			-		-		-
Thymol		-			-		787	0.19	787	0.19		-
3(*E*)-hexenyl tiglate		-			-			-		-		-
Dodecyl tiglate		-			-		756	0.12	756	0.12		-

Phenylethyl tiglate		-			-		840	0.50	778	0.10	899	0.46
Tiglic aldehyde		-			-		786	0.25	799	0.21		-
Toluene		-			-		867	0.15	900	0.06	878	0.20
(*E*)-Tridecen-1-ol		-			-			-		-		-
Tridec-1-ene		-			-			-		-		-
Tridecane	774		0.04	903		0.18	845	0.17	797	0.12	889	0.29
Triethoxymethane	781		0.05		-		838	0.21	820	0.47	913	0.12
Undec-(2*E*)-enol		-			-			-		-		-
Ethyl undec-10-enoate	800		0.25	806		0.77	806	0.45	811	0.33		-
1-Hydroxy undec-2-ene		-			-		852	0.09		-	852	0.09
2-Methyl undecanal		-			-			-		-		-
Undecanal		-			-		875	0.18	889	0.17	866	0.11
Undecane		-		906		0.49	906	0.49		-		-
Ethyl undecanoate	835		0.66		-		835	0.66		-		-
Undecanol	875		1.02		-		873	1.07	871	1.12		-
Valencene		-			-			-		-		-
Ethyl valerate	758		0.11		-		821	0.35	796	0.11	866	0.82
Valeric anhydride	802		3.08	812		6.17	805	3.60	855	0.05	755	2.83
Viridiflorene		-			-			-		-	-	
cis-whisky lactone	905		0.01	910		0.02	915	0.11	903	0.43	900	0.02
trans-whisky lactone	885		0.02	891		0.06	882	0.43	884	0.34	890	0.03

Table S1 (continued).

Compund	Fine 6		Superiore 1		Superiore 2		Superiore 3		Stravecchio	
	MS similarity	Normalized area	MS similarity	Normalized area	MS similarity	Normalized area	MS similarity	Normalized area	MS similarity	Normalized area
2-(1-Ethylpentyl)-1,3-dioxolane	-		-		766	0.16	-		874	0.19
2,4-Dimethyl-1,3-dioxolane	758	0.60	-		-		-		-	
14-Methyl-8*E*-hexadecenal	760	9.69	750	7.90	755	4.34	764	4.61	752	3.90
2,5-Dimethyl-5-hexen-3-ol	850	0.16	848	0.06	855	0.09	846	0.03	853	3.69
2H-2,4a-methanonaphthalen-1(5H)-one, hexahydro-5,5-dimethyl isomer I	-		758	1.23	-		-		-	
2H-2,4a-Methanonaphthalen-1(5H)-one, hexahydro-5,5-dimethyl isomer II	-		-		-		-		-	
2-Oxopentanedioic acid	749	0.02	752	0.01	750	0.05	745	0.01	765	0.06
2-Phenylethyl formate	850	0.22	820	0.06	845	0.23	847	0.16	843	0.61
3-Butyn-1-ol	750	2.43	730	0.06	760	2.84	773	4.72	780	9.68
Methyl octyl acetaldehyde	-		-		777	0.17	-		-	
2-Ethylhexyl acetate	-		-		-		-		798	0.34
2-Methylbutyl acetate	902	2.49	903	2.35	854	1.83	856	2.41	858	5.25
1,4,4-Trimethyl-3,5-dimethylidenecyclopentene	834	0.03	840	0.10	857	0.35	825	0.01	852	0.12
3,5,5-Trimethylhexyl acetate	-		-		-		-		-	
Butyl acetate	-		-		-		-		836	0.22
Ethyl acetate	-		934	5.42	891	400.05	903	606.21	904	1487.12
Hexyl acetate	-		810	0.26	-		877	0.10	810	0.18
Isoamyl acetate	886	11.41	900	14.08	886	7.96	886	10.33	887	18.96
Isobutyl acetate	908	0.73	805	0.68	891	0.79	816	0.92	889	1.52
Acetic aldehyde	916	74.69	865	0.93	951	32.89	880	64.38	906	85.83

Acetic anhydride	-		801	1.17	923	0.68	931	90.13	929	23.18
Acetoin:1	-		-		806	0.50	-		782	0.51
Acetoin:2	-		-		772	0.15	-		-	
Acetone	-		-		-		911	1.67	777	4.83
Acetophenone	-		-		-		827	0.31	740	0.78
2',4'-Dihydroxy acetophenone	-		762	0.68	-		-		-	
4'-Hydroxyacetophenone	-		-		-		-		-	
Acetylpropionyl	-		-		-		909	0.25	906	0.28
Diethyl adipate	-		-		-		909	0.22	777	0.15
Benzaldehyde	885	18.04	901	13.61	901	135.66	909	11.95	905	20.80
Butyl benzoate	854	0.59	-		-		-		-	
Ethyl benzoate	877	0.07	909	0.04	952	0.24	898	0.15	892	0.57
Isobutyl benzoate	-		-		-		-		-	
Propyl benzoate	-		-		-		820	0.10	-	
Benzosulfonazole	-		-		-		-		823	1.64
Benzyl alcohol	809	9.56	-		837	19.74	815	1.75	832	5.96
Benzyl ether	-		-		-		-		817	0.31
Cis-α-bergamotene	-		-		927	0.49	-		-	
Bicyclogermacrene	-		-		849	0.11	-		-	
Biphenyl oxide	867	0.47	-		-		-		-	
(*Z*)-α-Bisabolene	-		-		899	0.54	-		-	
β-Bisabolene:1	-		-		917	0.73	-		-	
β-Bisabolene:2	-		-		815	0.11	-		-	
Bois de rose oxide:1	857	0.22	841	0.27	903	0.86	889	0.59	827	0.29
Bois de rose oxide:2	806	0.37	-		894	0.85	871	0.55	798	0.28
(*E*)-2-Butenal	-		-		754	0.27	835	0.65	-	
1-Ethoxy-3-methyl-2-butene	802	0.49	790	0.50	773	0.23	778	0.54	-	
2,3-Butadienol:1	800	65.58	806	24.41	825	40.38	829	24.92	823	75.38
2,3-Butadienol:2	815	134.55	809	31.20	831	100.25	827	40.98	826	144.72
Butan-2-ol	-		-		-		-		-	

2-Methylbutyl butanoate		-			-			-			-	
Butyl alcohol	848		1.89	840		1.45	866		1.11	879		1.89	879		4.33
Benzyl butyl ether		-		784		0.15		-			-			-	
Sec-butylamine		-			-			-			-		796		9.06
2-Methylbutyraldehyde	784		1.41	835		0.68	758		0.83		-			-	
Ethyl 2-methylbutyrate	917		2.71	904		1.91	926		1.02	960		3.73	928		4.04
2-Phenylethyl 2-methylbutanoate		-		793		0.12		-			-			-	
Benzyl butyrate		-			-			-			-			-	
Ethyl butyrate	913		23.41	932		11.65	943		22.19	942		13.44	929		17.36
Isopentyl butyrate	870		0.18	848		0.14	865		0.04		-		863		0.24
γ-Butyrolactone	806		2.31	922		2.07	895		1.55	936		1.59	929		3.86
Cadalene	777		0.25	817		0.44		-		820		0.47	807		0.69
α-Calacorene	790		0.06		-		888		4.63		-			-	
β-Calacorene		-		788		0.16		-			-		753		1.20
Cis-calamenene		-			-		841		4.52		-			-	
Trans-calamenene		-			-		836		1.30	759		0.23	756		0.42
Camphor:1		-			-		823		1.62		-			-	
Camphor:2		-			-		793		0.22		-			-	
Sec-butyl carbinol		-			-		780		0.15		-			-	
δ-Cadinene	925		0.01	935		0.01	915		0.01	929		0.02	923		0.01
δ -2-carene:1		-			-			-			-			-	
δ -2-carene:2		-			-			-			-			-	
Caryolan-8-ol		-			-		803		2.62		-			-	
4,8-α-Epoxycaryophyllene		-			-		870		0.41		-			-	
(*E*)-Caryophyllene:1		-			-		873		0.32		-			-	
(*E*)-Caryophyllene:2		-			-		933		1.09		-			-	
(*Z*)-Caryophyllene:1		-			-		866		0.24		-			-	
(*Z*)-Caryophyllene:2		-			-		809		0.15		-			-	
4,8-β-Epoxycaryophyllene		-			-		882		1.94		-			-	

9-Epi-(*E*)-caryophyllene		-			-		755	0.04		-		-	
Caryophyllene acetate isomer II		-			-		863	0.93		-		-	
α-Chamigrene		-			-			-		-		-	
1,4-Cineole		-			-		792	0.33		-		-	
α-Copaene		-			-		861	0.38		-		-	
2-Phenylcrotonaldehyde	791		0.94	792		0.47		-	830	0.80	772		0.30
Ethyl crotonate		-			-		920	1.28	826	0.30	864		0.80
4-Ethyl-cumene		-			-			-	779	0.50	780		0.70
Cyclobutane-1,1-dimethyl-2-octyl	734		0.37	736		0.17	715	0.27	720	0.13	765		3.67
Cis-2-tert-butylcyclohexanol		-			-			-		-		-	
Trans-4-tert-butylcyclohexanol		-			-			-		-		-	
Trans-4-tert-butylcyclohexyl acetate		-			-			-		-	790		0.30
Cyclomyral		-			-		760	0.52	764	0.70		-	
1,4,4-Trimethyl-3,5-dimethylenecyclopentene		-			-			-	869	3.30	846		2.2
2-Prop-1-enyl-p-cymene		-		833		0.24		-	762	0.10	765		0.31
p-cymene	897		1.98	796		0.52	875	1.09	837	0.30	834		0.41
(*Z*)-β-Damascenone	842		1.52	787		2.04		-		-		-	
(*Z*)-4-Decenal		-			-			-		-		-	
1-Decen-3-ol		-			-			-		-		-	
1-Decene		-			-			-		-	882		0.90
3-Decanone		-			-			-	765	0.31		-	
Decanal	873		0.56	893		0.32		-	861	0.12	853		3.19
Ethyl decanoate	909		80.81	935		342.16	934	9.83	915	97.25	913		19.69
Isoamyl decanoate		-		803		0.59		-	786	0.26		-	
Methyl decanoate		-		848		0.68		-		-		-	
(*E*)-Ethyl 4-decenoate	812		2.28	808		8.91		-	816	2.82		-	
Decyl alcohol	894		1.44	898		1.05	910	0.21	894	0.37		-	
Decyl methyl ketone	818		1.91		-			-	823	1.98		-	

Dihydromyrcenyl formate		-			-			-			-	
Dimethyl disulfide		-			-			-	855	0.39		-	
Docosane		-			-			-		-	874	0.30
1-Dodecene	893	2.25		908	0.70			-		-	903	16.72
Dodecanal	855	0.18		819	0.16			-		-	888	3.90
Dodecane	912	0.64		892	0.71		905	0.46	897	0.34	910	0.47
Ethyl dodecanoate	895	1.57		906	9.29		903	3.42	908	1.70	904	1.29
Dodecanol		-			-		909	0.35	895	0.41		-
2-Dodecanol		-		857	0.44			-		-	872	0.27
Eicosane		-		814	0.18			-		-	873	0.64
β-elemene		-			-		912	1.17		-		-
Epicubenol		-			-		830	0.40		-		-
Eremophilene		-			-		783	0.10		-		-
Estragole		-			-		908	0.79		-		-
(*Z*)-2-(3,3-Dimethylcyclohexylidene)ethanol		-			-		772	2.75		-		-
Eucalyptol		-			-		860	0.94		-		-
10-Epi-γ-eudesmol		-			-		869	0.41		-		-
γ-Eudesmol		-			-			-		-		-
Eugenol		-			-		780	0.35		-		-
(*E*)-β-farnesene		-			-		872	2.00		-		-
Endo-fenchol		-			-		826	0.26		-		-
Ethyl formate		-			-		774	0.90		-		-
Heptyl formate		-			-			-	881	0.21	871	0.43
Isopentyl formate	869	0.19		892	0.44		890	0.56	908	0.68	923	1.26
Octyl formate	875	0.37			-			-		-	781	0.30
2-Acetyl-5-methylfuran		-			-			-		-		-
2-Acetyl furan		-		856	0.50			-	779	0.34	838	1.10
2-Pentylfuran	779	0.67			-		929	5.83	757	0.23		-

2,2-Dimethyl- 5-(1-methylpropenyl)-tetrahydrofuran	-		-		-		810	0.09	814	0.22
2(5H)-Furanone	-		-		-		-		-	
3-Furaldehyde	-		-		754	0.98	889	0.08	-	
Furfural	801	31.53	812	66.62	814	8.18	818	9.55	835	43.75
5-Methylfurfural	783	5.86	778	3.70	854	2.41	809	1.69	805	2.24
Ethyl 2-furoate	856	0.05	882	0.04	830	0.17	932	0.21	934	0.31
Germacrene B	-		-		846	0.17	-		-	
Germacrene D	-		-		835	0.21	-		-	
Methylphenyl glyoxylate	-		-		-		-		-	
4-Ethyl guaiacol	870	8.11	-		782	1.03	875	3.56	880	4.22
4-Propyl guaiacol	-		-		-		-		-	
Guaiazulene	-		-		863	4.90	-		-	
Heneicosane	818		-		-		-		877	
Heptadec-(4E)-enol	-		-		-		-		813	
Heptadecane	893	4.65	920	4.83	899	0.42	-		757	0.10
Ethyl heptanoate	913	0.63	873	0.57	807	0.18	900	0.78	900	2.87
Heptyl methyl ketone	-		-		-		-		-	
Ethyl hex-(2*E*)-enoate	-		-		-		-		782	0.29
Hex-(3*E*)-enol	-		809	0.53	798	0.17	-		-	
Hex-(3*Z*)-enol	-		-		-		-		-	
Hexadecane	899	0.81	-		901	0.75	899	0.50	892	0.82
Hexadecene	904	0.33	897	0.38	-		-		859	0.12
Hexanal	-		859	0.31	883	1.44	-		773	0.19
Ethyl hexanoate	890	33.86	919	124.20	906	20.83	905	33.55	905	26.67
Isopentyl hexanoate	853	0.20	906	1.46	847	0.07	871	0.51	839	0.36
2-Ethyl hexanol:1	835	2.66	869	0.48	843	2.72	853	7.62	851	19.16
2-Ethyl hexanol:2	840	2.51	876	0.56	849	2.01	850	8.65	849	19.62
Hexanol	864	11.71	857	15.37	853	7.84	859	5.85	-	
α-Himachalene:1	-		-		847	0.16	-		-	

α-Himachalene:2		-		-		-		-		-
α-Dehydro-ar-himachalene	813	0.87	803	0.22	775	0.94		-		-
α-Humulene		-		-	900	3.66		-		-
Humulol		-		-	800	0.18		-		-
Ethyl hydrocinnamate	837	0.55		-		-	802	0.19	841	0.62
Isobutenyl methyl ketone	782	0.30		-		-	-	0.00	812	0.14
Isobutyl alcohol	866	39.54	886	26.56	859	30.16	859	25.12	856	37.81
Butyl isobutyrate		-		-	-	0.00	775	0.08	785	0.21
Ethyl isobutyrate	895	7.42	879	3.70	939	5.06	922	6.36	915	5.56
Isopentyl alcohol	886	534.81	901	613.85	882	554.26	884	467.23	887	597.38
Ethyl isovalerate	864	4.79	872	3.99	924	1.54	888	4.61	921	6.70
Jasmacyclene		-		-		-		-		-
Junenol		-		-	819	2.46		-		-
Ethyl lactate	767	37.64		-		-	767	36.56		-
Isopropyl laurate		-		-		-		-	827	1.16
Tetrahydro lavandulyl acetate		-		-		-		-		-
Lavandulyl isobutyrate		-		-	787	0.66		-		-
Limonene	903	1.84	850	0.47	959	4.16	884	0.20	857	0.14
Linalool		-		-	810	0.49		-		-
Tetrahydro linalool acetate:1		-		-		-		-		-
Tetrahydro linalool acetate:2		-		-		-		-		-
Cis-linalool oxide		-		-	794	0.22	775	0.11	818	0.19
Trans-linalool oxide		-		-	833	0.59		-		-
Linalyl anthranilate		-		-	821	0.45		-		-
Ethyl linoleate		-		-	864	6.40		-		-
Diethyl malate	792	0.15	797	0.04	820	0.41	811	0.07	816	0.36
Massoia lactone		-		-		-		-	775	0.46
p-Menth-3-ene		-		-		-		-	763	0.09
Menthol		-		-		-		-		-
Menthone	799	0.32	817	0.23	810	0.11		-		-

Mesitylene	-		790	0.27		-		789	0.29	801	0.39
Diphenyl methane	-			-		-			-	850	0.50
Methyl nonyl carbinol	-			-		-			-		-
Methyl nonyl ether	-			-		-		805	0.31	839	0.27
Methyl propenyl ketone	-			-		-		878	0.19	813	0.14
Methyl undecyl ether	-			-		-			-		-
α-Muurolene	-			-		910	8.09		-		-
Myrcene	-			-		884	1.37		-		-
Myrcenol	-			-		835	0.19		-		-
Myrcenone	775	0.34	777	0.50		-		772	0.35		-
Naphthene	841	0.44	764	0.39		-		877	0.55	824	0.47
2-Methyl napthalene	834	0.21		-		-		845	0.10		-
Neryl butyrate	-			-		-			-		-
Nonadecane	-			-		841	0.23		-		-
Nonan-2-ol	803	0.64		-			-	792	0.13		-
Nonan-3-one	777	0.72		-		781	0.06	785	0.69	773	0.44
Nonanal	845	1.11	843	0.28		832	0.78	846	0.22	826	2.66
Ethyl nonanoate	879	1.19	786	0.82		845	0.25	900	1.28	908	5.43
Nonanol	910	1.23	868	0.34			-	856	0.41		-
Nonene	-			-		890	0.32		-	892	1.17
Nonyl methyl ketone	-		811	0.68			-		-	783	0.27
(*E*)-β-Ocimene	-			-		903	0.59		-		-
(*Z*)-Oct-2-enol	-			-			-		-		-
(*E*)-Oct-4-ene	-			-			-		-	806	0.31
Ethyl-(*E*)-oct-4-enoate	-			-			-		-	804	1.11
Oct-2-en-4-one	777	8.00	802	0.28			-	792	0.35		-
Octadecane	772	0.25	812	0.11		893	0.38	888	0.20	903	0.40
Octan-3-one	774	0.66	910	0.44		815	0.41	776	0.45	793	0.37
2-Methyl octanal	755	0.20		-			-	811	0.06	804	0.12
Ethyl octanoate	945	154.13	915	780.26		915	37.53	913	224.58	927	72.03

Isobutyl octanoate		-		771		0.24		-		766		0.12		-	
Isopentyl octanoate	853		0.62	894		2.87	812		0.14	872		1.44	886		0.44
Methyl octanoate		-		883		1.14		-		763		0.17		-	
Phenylethyl octanoate		-			-			-			-			-	
Octanol		-		907		0.71	906		0.37	905		0.45	895		0.82
(*E*)-2-Octene		-		821		10.66		-		801		12.94		-	
Ethyl oleate		-			-		826		19.31		-			-	
Trans-2,3-dimethyloxirane		-			-			-			-			-	
Ethyl palmitate		-			-		895		119.98		-			-	
β-Patchoulene		-			-			-			-		878		0.92
Pelargol		-			-			-			-		845		0.42
Pent-4-enophenone		-			-			-			-			-	
Pentadecane	848		0.16	914		0.97	898		0.71	915		0.29	872		0.50
Pentadecanolide		-			-		777		0.19		-			-	
2-Methyl pentanal		-			-		793		0.26		-			-	
Pentane		-			-			-			-			-	
3-Methyl pentanol	871		0.21	890		1.13	844		0.38	882		0.41	848		0.39
4-Methyl-1-pentanol		-		828		0.52	893		0.14	860		0.27	874		0.25
Pentyl alcohol	840		0.16		-			-		804		0.35	804		0.48
2-Phenethyl acetate	864		4.77	891		2.82	873		1.88	873		2.29	864		7.00
Phenethyl alcohol	855		0.46	864		0.80	862		0.41	863		0.10	860		0.30
α-Phellandrene :1		-			-		898		0.14		-			-	
α-Phellandrene :2		-			-		867		0.24		-			-	
3-Ethyl phenol		-			-			-		857		9.20		-	
4-Ethyl phenol	865		11.97		-		835		1.68		-		899		13.41
4-Tert-butyl phenol	794		4.98		-			-			-			-	
Phenylacetaldehyde	791		7.17	784		4.89	782		3.12	806		1.94	782		1.51
Benzyl phenylacetate		-			-			-			-			-	
Ethyl phenylacetate	862		0.33	874		0.10	880		0.52	876		0.22	880		0.84
Phenethyl phenylacetate		-			-			-		873		0.20	872		0.73

2-Phenyltoluene		-			-			-			-		890		1.10
Di-isobutyl phthalate	851		8.00		-			-		845		3.19	836		2.85
Phytone		-			-			-			-		844		0.24
Ethyl propanoate	801		11.99		-		864		6.31	919		10.01	882		23.82
Isopentyl propanoate		-			-			-		775		0.09	796		0.33
3-Methylmercapto propanol		-			-			-			-		912		0.73
Ethyl 3-acetyl propionate	862		1.27	903		1.27	880		4.05	869		0.98	908		3.01
α-Pinene		-			-		860		0.47		-			-	
[2-[1-(3,3-dimethylcyclohexyl)ethoxy]-2-methylpropyl] propanoate		-			-			-			-		774		0.49
2-methyl-1,3-dimethyl-3-butenyl- propanoate		-			-		773		0.06		-			-	
Allyl propanoate		-			-			-			-			-	
Phenethyl propionate		-			-			-			-			-	
Propyl alcohol	817		3.46	856		4.13	793		4.32	800		4.40	805		8.25
Propylene glycol		-			-			-			-			-	
Purolan		-			-		759		0.09		-			-	
Pyroracemic acid		-			-			-			-		756		1.02
Ethyl pyruvate	943		1.35		-		771		3.89		-			-	
Pyruvophenone		-			-		823		7.85		-			-	
Sabinene		-			-		864		0.13		-			-	
Safranal		-		757		0.11		-			-			-	
Ethyl salicylate	887		1.28	799		0.36		-		934		0.77	918		1.98
Methyl salicylate	892		1.96		-		861		0.38	902		1.16	939		1.72
Sclareolate		-			-			-			-			-	
Selina-4,11-diene:1	796		0.09	784		0.09	894		1.99		-			-	
Selina-4,11-diene:2		-			-		902		0.19		-			-	
α- Selinene		-			-		908		0.63		-			-	
β-Selinene		-			-		918		1.59		-			-	
Sibirene		-			-		896		0.26		-			-	

Solusterol		-		-		-	803	0.06	835	0.10
Ethyl sorbate	859	0.48		-	763	0.07	818	0.23	755	0.16
Ethyl stearate		-		-	852	6.87		-		-
Styrene	768	1.94	881	0.48	917	0.21		-		-
Diethyl suberate		-		-		-		-	802	0.22
Diethyl succinate	882	344.73	912	255.09	913	124.60	879	266.07	914	539.06
Terpin-1-en-4-yl acetate		-		-	759	0.70		-		-
α-Terpinene	814	0.26	807	0.18	822	0.10	841	0.13		-
γ-Terpinene		-		-	865	0.95		-		-
α-Terpineol	770	0.62		-		-		-		-
Terpinolene		-		-	894	1.80		-		-
γ-Terpinyl acetate	823	0.32		-		-		-		-
α-Terpinyl formate		-		-	832	8.40		-		-
(*E*)-Tetradec-2-enol		-		-	827	0.11	828	0.09		-
Tetradec-1-ene		-		-	878	0.08	884	0.07	835	0.51
Tetradecane	874	0.63	903	0.67	895	0.96	881	0.23	897	0.39
Ethyl tetradecanoate		-		-	873	0.85		-	902	0.77
Isopropyl tetradecanoate		-		-	782	0.10		-	825	0.79
Trans tetrahydro jasmone		-		-		-	778	9.23	763	2.16
2-Heptyl tetrahydrofuran		-		-	796	0.54		-		-
Trans-5-isopropenyl-, 2-methyl-, 2-vinyltetrahydrofuran:1		-		-	843	0.22	848	0.16		-
Trans-5-isopropenyl-, 2-methyl-, 2-vinyltetrahydrofuran:2		-		-	760	0.10	757	0.08		-
Thujopsan-2-α-ol		-		-		-		-		-
Cis-thujopsene		-		-		-		-		-
Thujyl acetate		-		-	763			-		-
Thymol		-		-		-		-	869	2.77
3-(*E*)-Hexenyl tiglate		-		-		-		-	786	0.09

Dodecyl tiglate		-			-			-			-		754	1.20
Phenylethyl tiglate	843		0.94		-			-			-			-	
Tiglic aldehyde	773		0.28		-			-		767		0.37		-	
Toluene	823		0.19	903		0.85		-		815		0.16		-	
(*E*)-Tridecen-1-ol		-			-			-			-		910		3.44
Tridec-1-ene		-			-			-			-		910		1.24
Tridecane	861		0.23	876		0.20	873		0.17	834		0.07	854		0.13
Triethoxymethane		-		850		0.17	913		0.18	910		0.07		-	
Undec-(2*E*)-enol		-			-			-		858		0.08		-	
Ethyl undec-10-enoate		-			-		819		0.44		-		809		0.72
1-Hydroxy undec-2-ene		-			-		842		0.44		-		760		0.16
2-Methyl undecanal		-		784		1.51		-			-		805		0.92
Undecanal	871		0.26		-		869		0.08		-			-	
Undecane		-			-		777		0.14		-			-	
Ethyl undecanoate		-			-		857		11.62		-		828		0.41
Undecanol		-			-		816		0.12		-		891		1.26
Valencene		-			-		843		0.07		-			-	
Ethyl valerate	862		0.34		-		854		0.38	898		0.44		-	
Valeric anhydride	802		5.86	802		4.33		-		806		3.51	797		8.71
Viridiflorene	-				-		886		0.88		-		816		0.08
cis-whisky lactone	906		0.10	903		0.56	915		0.98	913		0.04	920		4.01
trans-whisky lactone	890		1.22	891		0.60	883		0.45	887		2.47	895		3.28

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Table S2. Variables with loading value on PC1 higher than 0.1 (absolute value).

Variable number	Compound	PC1 loading value
53	2,5-Dimethyl-5-hexen-3-ol	0.1130
129	**2-Oxopentanedioic acid**	**0.1897**
171	*Cis*-whisky lactone	0.1172
202	**Ethyl benzoate**	**0.4277**
206	Cyclobutane-1,1-dimethyl-2-octyl	0.1127
249	**Ethyl 2-furoate**	**0.2261**
267	*Trans*-whisky lactone	0.1038
347	**Diethyl malate**	**0.2427**
401	**Ethyl phenylacetate**	**0.4790**
513	2-Phenylethyl formate	0.1823
562	**Phenethyl alcohol**	**-0.3889**
812	14-Methyl-8*E*-hexadecenal	-0.1095
1178	3-Butyn-1-ol	0.1406

Table S3. Variables with VIP score values higher than 4 for all the three classes.

Variable number	Compound	VIP scores for class one (Fine)	VIP scores for class two (Superiore)	VIP scores for class three (Stravecchio)
15	Pent-4-enophenone	4.82	5.69	5.59
19	α-Terpineol	4.80	6.46	6.65
129	**2-Oxopentanedioic acid**	**5.83**	**4.15**	**4.93**
202	**Ethyl benzoate**	**10.37**	**8.08**	**10.39**
246	δ-Cadinene	5.84	6.99	7.11
249	**Ethyl 2-furoate**	**5.78**	**4.39**	**5.67**
296	1,4,4-Trimethyl-3,5-dimethylidenecyclopentene	9.31	11.68	8.49
347	**Diethyl malate**	**7.09**	**6.38**	**6.53**
401	**Ethyl phenylacetate**	**10.08**	**10.43**	**12.62**
477	2-Phenethyl acetate	6.83	7.02	4.61
562	**Phenethyl alcohol**	**10.88**	**6.46**	**9.46**

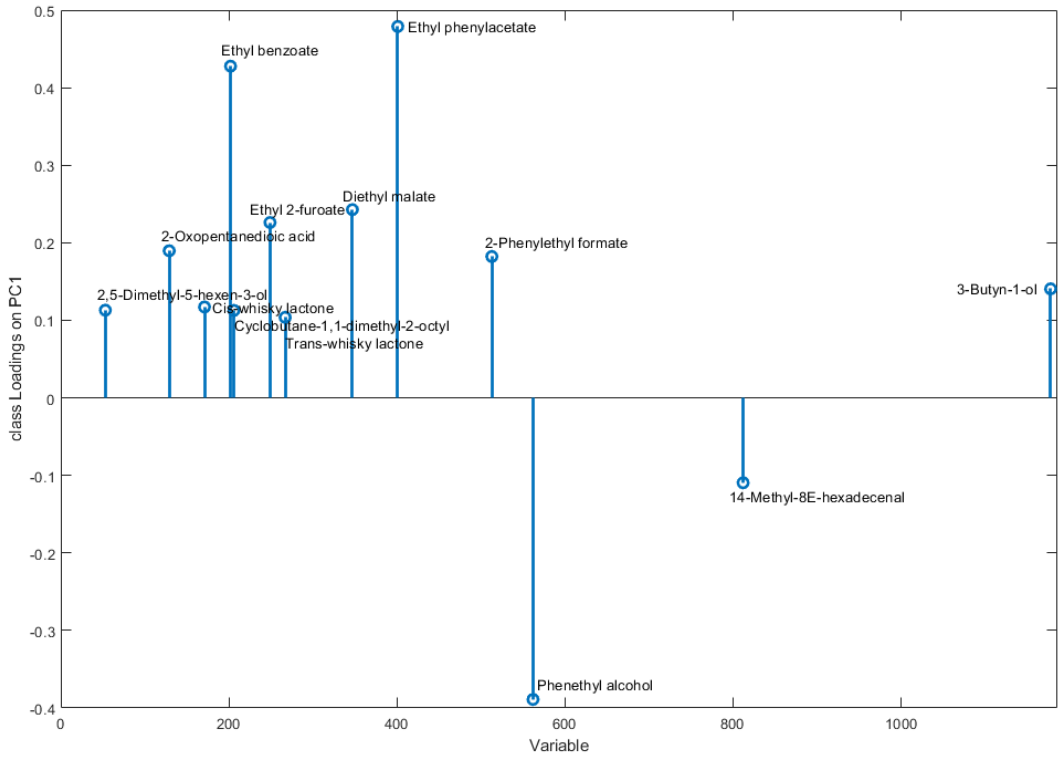

Figure S1. Loading on PC1 from ASCA decomposition of factor "Marsala type". Only variables with a loading value higher than 0.1 (absolute value) are represented.