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Application of Stir Bar Sorptive Extraction (SBSE) to Evaluate the Volatile Compounds' Profile of Primitivo Wine

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Abstract: SBSE combined with GC/MS is a very promising tool for the identification of volatile compounds involved in wine's aroma profile, at levels below those previously obtained by conventional methods. At today a strictly absolute quantitative evaluation of all flavour components of wine's volatile fraction is not really feasible, however it's useful to produce aroma profiles which allow to identify or to compare the compounds responsible for the wine's tipycality. This paper shows first data concerning the use of SBSE extraction method to characterize Primitivo wine samples produced in an experimental farm of the countryside near Manduria(Puglia, Italy) through various production technologies. The data permit to obtain sufficiently typical volatile compound profiles, which show some differences due to the various production technologies or to the wine aging time. The evaluation of data has been done comparing the abundance indexes, estimated from the ratios of the area of each compound to that of the most relevant peak in the graph. The reported data show that the SBSE extraction method results sufficiently right for the purpose.

Key words: SBSE, wine, flavor, Primitivo, volatile profile

INTRODUCTION

When studying the compounds responsible for wine aroma, one of the main problems is the choice of a suitable extraction procedure to qualitatively and quantitatively represent the original flavor profile. Several methods have been developed and all of them present advantages and disadvantages: some of them are based on extraction procedures foreseeing the previous isolation of volatile compounds from the matrix wine, as for example liquid-liquid extraction or stripping with nitrogen[1]. Other methods permit to study static or dynamic headspace and more recently some properly sorption criteria have been adopted. Referring to these last ones, the sensitivity of solid phase microextraction (SPME) is limited by the small amount of sorptive material that can be coated on the fibres, instead the technique of stir bar sorptive extraction (SBSE) seems to be preferable since the compounds responsible for flavour or off-flavour of wine are often present at very low concentration (e. g. less than 1 $\mu g\ L^{-1}$).

At today a strictly quantitative evaluation, from the analytical point of view, of all or of the main flavour components of wine's volatile fraction is not really effectively feasible, considering the high number of molecules, their wide range of polarity, solubility and volatility and the unstability of many of them which can give rise to appearance of artifacts.

From the practical point of view, the analysis of wine's volatile fraction is useful to produce a finger print that allows to evidence: profile's changes correlated to sensorial characters, flavour composition and aromatic characters influenced by the origin or by the adopted technologies, or correlation between the presence of some molecules and sensorial descriptors. However the investigation concerning standardized criteria able to produce an analytical flavor profile becomes more and more actual to identify feasible differentiated characters correlated with wine's typicality.

The often verified repeatability of data achieved with stir bar sorptive extraction (SBSE) technique suggests to adopt this method to produce a first research about volatile compounds' characterization of Primitivo, a wine produced in an experimental farm of the countryside near Manduria (Puglia, Italy).

The paper shows the results concerning the analytical characterization of volatile compounds by SBSE, evaluating three different Primitivo wine obtained

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with three different production parameters from grapes harvested in the same area and on the same day. The paper has been realized with the purpose to verify if the SBSE applied on the three Primitivo wines evidence profiles' data which can be considered as distinctive for Primitivo, at the same time showing differences referable to the applied vinification technology or to the aging of the wine.

MATERIALS AND METHODS

Samples: Primitivo is the name of a vineyard which produces early grapes, tilled in the whole Puglia area in the south of Italy. Nobody knows the historical origin of this grape variety in Italy: at first it was confused with a Bourgogne Pinot, but in 1967 the wine was recognized to look like Zinfandel from California and in 1994 by DNA analysis it was cleared up that Primitivo and Zinfandel are two clones of the same grape variety[2].

The samples of wine objects of the experimentation derived from three different ways of vinification, conduced by V. Liuzzi in a farm placed in the Manduria D.O.C. area in 2002: the first wine (A) derived from an industrial type technology, i.e. using potassium metabisulphite after the pick from the bunch and press phase, starter yeast during the fermentation phase and adopting mechanical devices. The second (B) and third (C) wine derived from two pilot experimentation: the second using mechanical systems without yeast, the third adopting pick by hand and tread without yeast too.

Two series of the three samples were analysed: one was analysed in January 2003 and one in March 2003.

Apparatus and principles of use: A small stir bar (Gerstel, Mülheim a/d Ruhr-Twister, film thickness 0.5 mm, 10 mm length) coated with polydimethylsiloxane was placed directly in 5 mL of the sample (in a corked vial) and stirred for 1 h on a magnetic stirrer. The stir bar, removed and rinsed with distilled water, was placed into a thermodesorption unit (TDS-2, Gerstel). Heating, the stir bar releases the sorbed compounds into a GC/MS system (HP 5890/5971A, Hewlett Packard, Little Falls, CA).

The thermal desorption was carried out with a temperature program from 20°C, ramped at 60°C min^{-1} to 240°C and held for 20 min, carrier gas was helium at 50 kPa at constant pressure; desorption was in splitless mode; the TDS transfer line was set at 280°C.

The PTV (Programmed Temperature Vaporiser) injector (CIS-3, Gerstel) was held at -50°C for the total desorption time and then ramped at 12°C s^{-1} in split mode (split ratio: 1/20) to 240°C and held for 2 min. The pneumatics configuration during desorption allows a combination of splitless desorption and high flow which gives optimum transfer of compounds from the stir bar to the GC/MS system.

The GC was fitted with a SupelcoWax-10 polar capillary column (30 m×0.25 mm i.d., 0.25 μm film thickness, Supelco, Bellefonte, PA). Helium was used as carrier gas at 50 kPa constant pressure (column flow rate of 1 mL min^{-1}). The GC oven temperature was programmed from 50°C held for 10 min, ramped at 2°C min^{-1} to 200°Cand held for 35 min. The MS transfer line was set at 280°C.

The MS was operated in EI mode and positive ions at 70 eV were recorded with a scan range from m/z 40 to m/z 300 at 2 scans s^{-1}.

Data expression: Some preliminary remarks are useful to justify the adoption of the calculus criteria adopted in this paper.

The theory of SBSE is rather straightforward and similar to that of SPME[3]. The only parameter governing the recovery of the analyte from the sample is the ratio of the partitioning constant and the phase ratio between the polydimethylsiloxane (PDMS) on the stir bar and the water sample. In the case of SBSE the phase ratio is about 100 times higher than in SPME. It is also known that the recovery for SBSE result very higher than the recovery for SPME: this is attributed to the phase ratio between the PDMS extraction phase and the water sample.

The analytes are sorbed into the bulk of the PDMS phase and not adsorbed: the degradation of unstable analytes is significantly less on PDMS compared to adsorbents and the losses of thermolabile solutes are minimized.

On the other hand some experiments show[4] that various compounds extracted to a similar extent in SBSE are not extracted to similar extent in SPME, where more apolar compounds are extracted in significant higher amounts than the least apolar ones. The differences can be attributed to the difference in the octanol-water partitioning coefficients among the various compounds: in fact the teory of the recovery for SBSE has been developed with the approximation that the partitioning coefficients between PDMS and water are proportional to octanol-water partitioning coefficients.

On the theme of application of stir bar sorptive extraction for wine analysis, Y. Hayasaka *et al.*[5] published data concerning the analysis of the composition of a Cabernet Sauvignon wine: the autors estimated the relative levels of the volatile compounds from the ratios of their areas to that of a relevant

Table 1: Repeatability of abundance indexes Rn for 12 molecules identified in the SBSE/TDS/GC/MS chromatograms

	Rn (abdundance indexes)							
	Replication							
Compound	1	2	3	4	5	Mean	SD	RSD (%)
Isoamyl acetate	21.2	22.8	22.4	22.3	21.8	22.1	0.55	2.49
amyl alcohol + isoamyl alcohol	331.4	338.5	344.8	346.2	328.9	338.0	6.93	2.05
ethyl hexanoate	52.4	54.8	54.3	55.4	53.2	54.0	1.09	2.01
Hexanol	56.4	58.6	59.8	59.8	59.7	58.9	1.31	2.23
ethyl octanoate	450.3	459.9	448.2	456.9	449.2	452.9	4.64	1.02
Benzaldehyde	11.2	10.8	11.1	10.8	11.4	11.1	0.23	2.11
ethyl decanoate	110.8	115.9	113.9	115.4	110.1	113.2	2.37	2.09
diethyl succinate	542.3	558.2	560.1	542.3	541.4	548.9	8.43	1.54
ethyl phenylacetate	255.8	264.8	258.7	263.4	266.1	261.8	3.89	1.49
Phenylethyl acetate	218.1	226.1	218.4	223.5	225.1	222.2	3.36	1.51
Geraniol	56.8	59.8	57.9	56.4	58.4	57.9	1.21	2.09
ethyl-3-methyl-butanoate	154.2	156.8	160.5	158.9	160.2	158.1	2.35	1.49

Rn=Cn/f.e. × 1000, where Cn= single compound area count , f.e.= phenylethyl alcohol area count, The values of RSD (%) for the others compounds not cited in this table are included in the range 2.5-3.5

internal standard rather than using absolute quantitative values. These Authors conclude that with SBSE it is possible to analyze complex samples such as wine by scan mode, with better confirmation of identity and without sacrificing sensitivity. Moreover, althoug the solid phase was the same (PDMS), differences in area ratio are evidenced between the SPME and SBSE extraction, due to difference in volatility: this happens for example for compounds as ethyl succinate and ethyl octanoate since SPME was adopted with headspace technique, while with the SBSE the stirring bar was immersed in the wine.

J. Pawliszyn writes that the type sample matrix can change not only the distribution coefficient, but also the equilibration time in sorptive extraction; to reduce the matrix effect[6] suggests the dilution of the sample 100-fold with water. Other Authors suggest 5 or 10% alcoholic solutions (E. Pfannkoch *et al.*, 2001) as dilution phase for the matrix.

N. Ochiai to validate the method for determination with SBSE of preservatives in beverages, vinegar and aqueous sauces, shows that an increase in the extraction temperature caused an increase in the rate of extraction for all compounds, but simultaneously a decrease in the responses between 15 and 120 min extraction, because of a decrease in the distribution coefficients.

Moore, a note of E. Pfannkoch *et al.* concerning criteria to enhancing selectivity of the PDMS phase, shows the effect of pH to enhance PDMS partitioning and the effect of methanol to eliminate some interferences ad the effect of acetonitrile to enhance extraction of very non polar compounds.

Considering the cited notes and the content of others papers, it is possible to deduce that:

The reproducibility of a flavor profile obtained using the Gerstel Twister technique depends strictly from the riproducibility of the operative conditions of extraction (time of contact with PDMS, temperature, pH of the extraction medium, composition of the extraction medium, dilution of the sample, etc.).

To compare matrices using flavor profiles or to verify the possibility to identify some flavor peculiarities for a specific matrix or to verify the existing influence of technological parameters on a fixed matrix, it doesn't need to determine the absolute content of single flavor compounds in a flavor profile but the use of reproducible parameters is enough; adopting the ratio between the area counts of each volatile compound and the area of the most represented compound in the chromatogram, it is possible to characterize or compare flavor profiles, avoiding the problems deriving from the difficulty of repeating the operative conditions at very high extent.

Due to variability of absolute quantitative values, in the sense explaided above, it is preferable to use more reproducible data: the abundance indexes Rn defined in this paper represent more significative data for flavor profile.

The SBSE is not a technique devoted to total and undifferentiated extraction of all volatile compounds in a flavor profile and so the recovery values of single volatiles have not a practical utilization. The calculation of recovery and the conventional validation has practical utilization when it is important to know the content of an additive or of a product which could have toxic activity[7].

The sensitivity obtained using the SBSE methodology varied somewhat from compound to compound[5] and results much greater than using conventional methods by approximately 10-100 times. The lower detection limits[8] were calculated to be in the range from 0.08 to 2 ppt, depending on the matrix and on the extraction conditions.

Table 2: Evaluation by SBSE/TDS/GC/MS of volatile compounds in three Primitivo wine samples produced with 3 different technologies A, B, C and analysed in January and March 2003. The compounds' area count have been reported as Rn abundance indexes in accordance with the formula in the text

	Samples of January			Samples of March		
	Vinification			Vinification		
compound	A	B	C	A	B	C
isoamyl acetate	18	20	12	22	19	26
limonene	nd	nd	nd	nd	nd	46
amyl alcohol + isoamyl alcohol	412	173	83	338	155	221
ethyl hexanoate	nd	42	36	54	42	125
ethyl heptanoate	21	50	18	48	61	13
ethyl lactate	140	nd	205	118	102	315
hexanol	52	54	54	59	51	79
ethyl octanoate	56	206	95	453	181	226
heptanol	21	nd	13	25	nd	nd
benzaldehyde	26	9	74	11	23	25
ethyl nonanoate	nd	32	nd	60	50	18
ethyl 2-hydroxy-4-methyl-pentanoate	38	18	41	20	39	35
linalool	18	23	22	22	29	25
octanol	48	16	34	nd	31	nd
ethyl decanoate	33	181	57	113	87	81
gamma-butyrolactone	33	70	57	37	74	89
ethyl benzoate	nd	34	nd	29	34	17
nonanol	36	37	38	74	47	26
furfuryl alcohol	nd	31	nd	nd	nd	nd
diethyl succinate	380	101	432	549	397	411
citronellol	13	19	69	29	23	nd
ethyl phenylacetate	203	379	115	262	438	43
(E)-beta damascenone	19	62	230	82	66	142
phenylethyl acetate	56	100	245	222	131	105
ethyl laurate	nd	142	93	nd	73	55
geraniol	31	33	105	58	45	52
benzyl alcohol	nd	nd	nd	nd	14	28
ethyl-3-methyl-butanoate	64	23	67	158	87	108
gamma nonalactone	nd	nd	nd	nd	nd	49
nerolidol	nd	nd	nd	nd	169	96

From the preliminary experiences carried out in the present paper, considering the molecules identified at lower levels in the wine samples analyzed, the lower detection limit was 1.0 $\mu g\ L^{-1}$.

Table 1 reports the repeatibility data of Rn abundance indexes for the molecules identified in the SBSE/TDS/GC/MS replicate chromatograms concerning, for example, the vinification A (march) (Table 2).

RESULTS AND DISCUSSION

A typical chromatogram produced by SBSE/TDS/GC/MS from a sample of Primitivo wine A is shown in Fig. 1 (January) and the data of Table 2 permit to compare the flavor profiles of three samples of Primitivo wine deriving from three different vinification experiences as described in metarials and methods. The same table reports the data corresponding to the same wines analysed in January and March 2003. Each Rn value is the mean of 5 replications.

Figure 2 shows the comparison of chromatograms SBSE/TDS/GC/MS corresponding to wines analysed in March 2003. It is possible to show that, even if it's possible to notice some quantitative differences attributable to the production technologies, the graphs show a fundamental similarity to ascribe to variety features.

Comparable data are well done using the abundance indexes (Rn) defined as follows:

$$Rn=Cn/f.e. \times 1000$$

Where, Cn = single compound area count, f.e.= phenylethyl alcohol area count.

The phenylethyl alcohol has been considered as reference peak to evaluate the Rn indexes since this molecule shows in all chromatograms the most representative area count value.

From a close examination of data reported in Table 2, considering not only the abundance of the peaks most quantitatively represented but also the minor peaks, it is possible to define the profile of Primitivo wine obtained by SBSE as sufficiently characteristic. The flavor profiles evidently shows some quantitative data differentiated because of the influence of vinification conditions and at the same time, as foreseen, of the maturation.

Considering the Fig. 3, elaborated from the abundance indexes of Table 2, it is possible to evidence

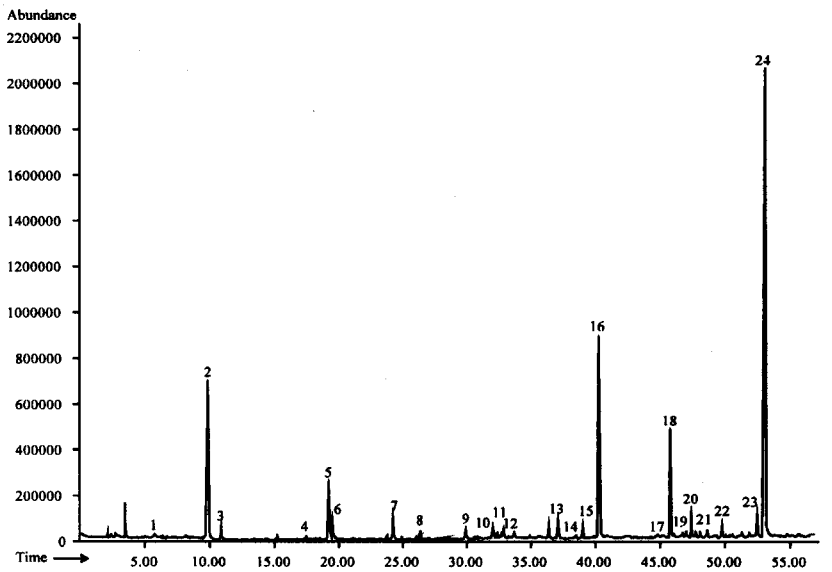

Fig. 1: Example of chromatogram SBSE/TDS/GC/MS related to the volatile compounds characterizing the Primitivo wine sample produced with A technology and analysed in January 2003. The peaks are identified as follows: isoamyl acetate (1) amyl alcohol + isoamyl alcohol (2) ethyl hexanoate (3) ethyl heptanoate (4) ethyl lactate (5) hexanol (6) ethyl octanoate (7) heptanol (8) benzaldehyde (9) ethyl 2-hydroxy-4-methyl-pentanoate (10) linalool (11) octanol (12) ethyl decanoate+γ-butyrolactone (13) ethyl benzoate (14) nonanol (15) diethyl succinate (16) citronellol (17) ethyl phenylacetate (18) (E)-β-damascenone (19) phenylethyl acetate (20) ethyl laurate (21) geraniol (22) ethyl-3-methyl-butanoate (23) phenylethyl alcohol (24)

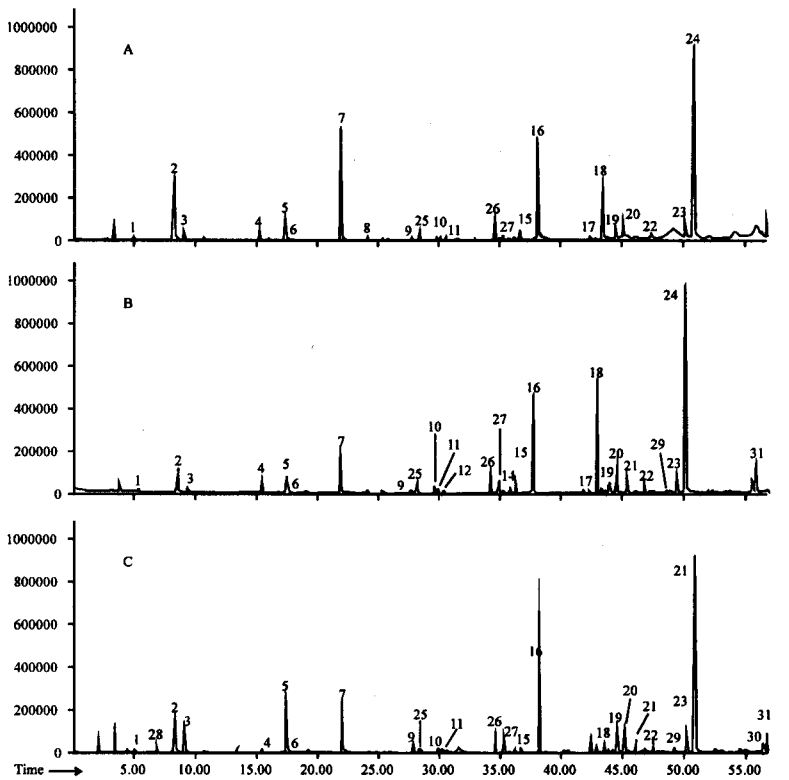

Fig. 2: Chromatograms SBSE/TDS/GC/MS corresponding to wines analysed in March 2003. The chromatogram on the top shows the volatile compounds of the sample produced with A technology, the one on the middle shows those obtained with B technology, the graph on the bottom shows the volatile fraction of the sample obtained adopting C technology. The peaks are identified as follows: isoamyl acetate (1) amyl alcohol+isoamyl alcohol (2) ethyl hexanoate (3) ethyl heptanoate (4) ethyl lactate (5) hexanol (6) ethyl octanoate (7) heptanol (8) benzaldehyde (9) ethyl 2-hydroxy-4-methyl-pentanoate (10) linalool (11) octanol (12) ethyl decanoate+ γ-butyrolactone (13) ethyl benzoate (14) nonanol (15) diethyl succinate (16) citronellol (17) ethyl phenylacetate (18) (E)-β-damascenone (19) phenylethyl acetate (20) ethyl laurate (21) geraniol (22) ethyl-3-methyl-butanoate (23) phenylethyl alcohol (24) ethyl nonanoate (25) ethyl decanoate (26) γ-butyrolactone (27) limonene (28) benzyl alcohol (29) γ nonalactone (30) nerolidol (31)

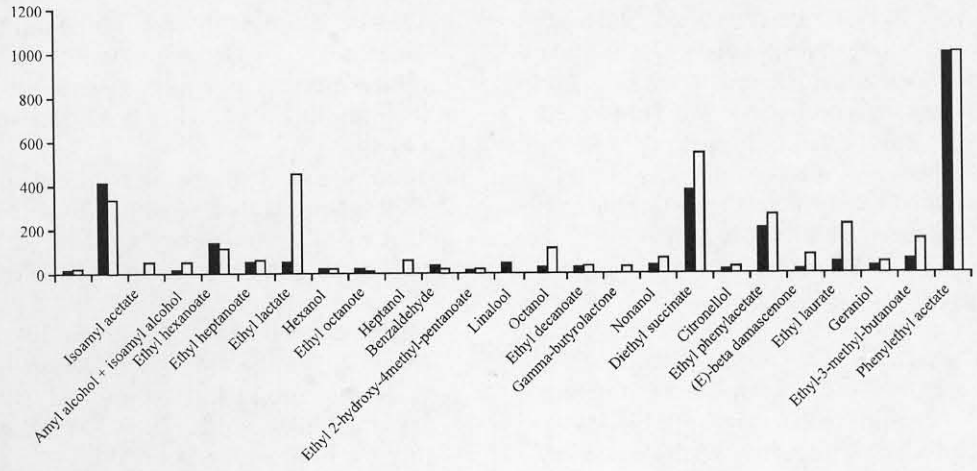

Fig. 3: Comparison between the Rn data for two aroma profiles of Primitivo wine produced with A technology, analysed in January (black) and March (white)

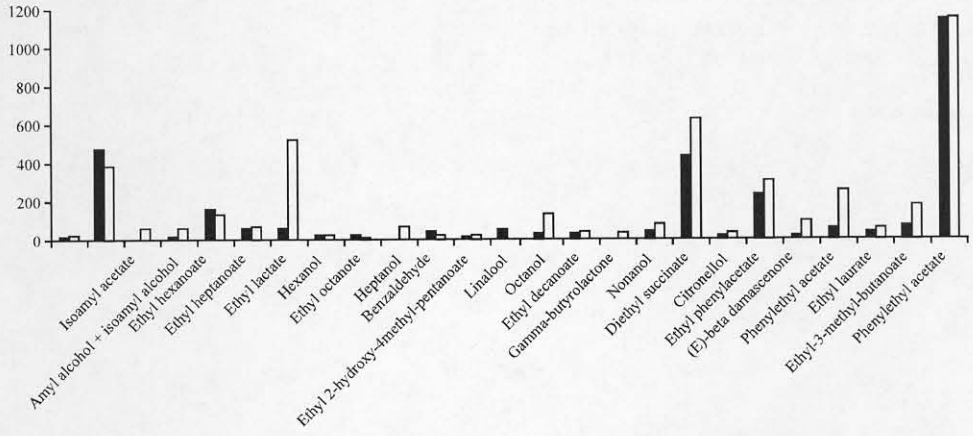

Fig. 4: Comparison between the Rn data for two aroma profiles of Primitivo wine produced with A (black) and C (white) technology, analysed in January

a volatile compounds profile sufficiently equivalent for the same wine produced with the A technology and analysed with a gap of two months: the quantitative difference for some molecules can be considered as consequence of the aging. Anyway the method is useful to study the volatile compounds' evolution and offers the basis to study the definitions of variety features.

At the same time, examining as further example the Fig. 4, also elaborated from data of Table 2 it is possible to evidence the substantial similarity of the aroma profile for the wine produced with the more industrial technology (A) with the aroma profile of the wine produced with the more traditional one (C). Some quantitative differences are only evident in the higher part of Fig. 4 and can be considered as deriving from the influence of the adopted technology.

These first experiences are to be considered as useful to motivate to a more large application of the SBSE for the analysis of various wines in order to create a map of abundance indexes to characterize or to compare flavor profiles.

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