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Optimization of a Solid-Phase Microextraction method for the Gas Chromatography–Mass Spectrometry analysis of blackberry (Rubus ulmifolius Schott) fruit volatiles

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Abstract

Solid-Phase Microextraction method for the Gas Chromatography–Mass Spectrometry analysis of blackberry (Rubus sp.) volatiles has been fully optimized by means of a Box–Behnken experimental design. The optimized operating conditions (Carboxen/Polydimethylsiloxane fiber coating, 66 °C, 20 min equilibrium time and 16 min extraction time) have been applied to the characterization for the first time of the volatile composition of Rubus ulmifolius Schott blackberries collected in Italy and Spain. A total of 74 volatiles of different functionality were identified; esters and aliphatic alcohols were the pre- dominant classes in both sample types. Methylbutanal (2.02–25.70%), ethanol (9.84–68.21%), 2,3-butanedione (2.31–14.71%), trans-2-hexenal (0.49–17.49%), 3-hydroxy-2-butanone (0.08–7.39%), 1-hexanol (0.56–16.39%), 1-octanol (0.49–10.86%) and methylbutanoic acid (0.53–21.48%) were the major com- pounds in most blackberries analyzed. Stepwise multiple regression analysis of semiquantitative data showed that only two variables (ethyl decanoate and ethyl acetate) were necessary for a successful differentiation of blackberries according to their harvest location.

Keywords:

Blackberry (Rubus ulmifolius Schott) Solid-Phase Microextraction (SPME)

Gas Chromatography–Mass Spectrometry (GC–MS)

Volatiles Experimental design

1. Introduction

Rubus ulmifolius Schott, a perennial shrub belonging to the Rosaceae family, grows in many areas worldwide and it is popularly well known by its edible fruits, the blackberries. Wild or cultivated fruits are highly appreciated by the combination of their appealing color and desirable flavor and taste, as well as for the reported benefits on human health (antioxidant, anticancer, anti-inflammatory and anti-neurodegenerative activities) associated to their con- sumption (Heinonen, Meyer, & Frankel, 1998; Seeram et al., 2006). Furthermore, fresh or processed (frozen, dehydrated, etc.) blackberries are also used in the industrial elaboration of a wide variety of foodstuffs such as breakfast cereals, dairy products, juices, jams, liquors, etc. (Morales, Albarracín, Rodríguez, & Duque, 1996). Although food aroma is widely recognized as an important sensorial attribute and its study can be considered as a valuable approach for its objective characterization, a limited number of references deal with the analysis of volatiles from berries of the Rubus genus (Blanch, Flores, & Ruiz del Castillo, 2011; Casabianca & Graff, 1994; Du, Finn, & Qian, 2010a; Du, Kurnianta, McDaniel, Finn, & Qian, 2010b; Georgilopoulos & Gallois, 1987; Ibañez, López- Sebastián, Ramos, Tabera, & Reglero, 1998; Klesk & Qian, 2003a; Klesk & Qian, 2003b; Malowicki, Martin, & Qian, 2008; Meret, Brat, Mertz, Lebrun, & Günata, 2011; Morales et al., 1996; Qian & Wang, 2005; Turemis, Kafkas, Kafkas, Kurkcuoglu, & Baser, 2003; Wang, Finn, & Qian, 2005).

The high separation power of capillary Gas Chromatography (GC) and the high sensitivity and useful qualitative information provided by Mass Spectrometry (MS) have made the coupling GC–MS the technique of choice for the analysis of the complex mixtures of volatiles present at low concentration in Rubus fruits. However, different procedures such as liquid–liquid extraction (Georgilopoulos & Gallois, 1987; Qian & Wang, 2005), Stir Bar Sorptive Extraction (SBSE) (Du et al., 2010b) and Purge-and-Trap (P&T) (Klesk & Qian, 2003a) have been assayed for the required fractionation/enrichment of volatiles prior to their chromatographic analysis.

Solid-Phase Microextraction (SPME) emerged in the 90s (Arthur & Pawliszyn, 1990) as an alternative technique for fractionation of volatiles from interfering non-volatile matrix compounds. Among other advantages, SPME can be considered as a fast, simple, afford- able, sensitive, solvent-free and easy-to-automate technique, in which the recovery of volatiles is mainly modulated by the selection of the SPME fibre coating. Although SPME has been widely used for the analysis of food flavor compounds (Jelen´ , Majcher, & Dziadas, 2012; Kataoka, Lord, & Pawliszyn, 2000), it has scarcely been applied to the study of the aroma of berries belonging to the Rubus genus (Blanch et al., 2011; Ibañez et al., 1998; Meret et al., 2011; Osorio et al., 2007; Turemis et al., 2003). In most of these papers, the optimization of SPME methods does not include the evaluation of different SPME fiber coatings or the developed methods are only applied to a single or a few Rubus samples. Furhermore, no previous study addresses the characterization of the volatile composition of R. ulmifolius Schott fruits.

The aim of this work was the development of a SPME GC–MS method for the analysis of blackberry (Rubus sp.) volatiles. After selection of the most appropriate fiber coating and optimization by means of an experimental design of the most relevant SPME operating factors, this method was applied to the characterization for the first time of the volatile composition of R. ulmifolius Schott samples collected in Italy and Spain.

2. Materials and methods

2.1. Samples

Commercial frozen blackberries (Rubus sp.) from La Cuerva (Cáceres, Spain) (sample BLACK) were used for optimization of SPME method. Full ripe R. ulmifolius Schott samples collected in June–August 2012 were analyzed as an example of application of the previously optimized SPME conditions. Table 1 lists the sample code and harvesting location (Italy or Spain) of the thirteen black-berry samples under study.

For sample homogeneity, whole blackberries were freeze-dried, powdered and sieved (<0.5 mm) prior to analysis.

2.2 Solid-Phase Microextraction (SPME)

Fractionation of volatiles from freeze-dried blackberries was done using a SPME fiber attached to a manual SPME holder (both from Supelco, Bellefonte, PA). Five SPME fiber coatings with different polarity and extraction mechanism were evaluated for optimization of headspace sampling: CAR/PDMS (85 lm CarboxenTM–Polydimethylsiloxane StableFlex, medium polarity, adsorption/partition), PDMS/DVB (65 lm Polydimethylsiloxane/ Divinylbenzene, low polarity, partition), PDMS (100 lm Poly- dimethylsiloxane, low polarity, partition), PA (85 lm Polyacrylate, high polarity, partition), and CAR/PDMS/DVB (50/30 lm Carboxen/ Polydimethylsiloxane/Divinylbenzene, adsorption/partition). All fibers were conditioned before use according to the manufacturer's recommendations until no interfering peaks were obtained in blank runs.

Blackberry powders (0.2 g) were exactly weighted into a 5 mL vial sealed with a screw cap provided with a predrilled Teflon-faced septum. After the equilibrium time (teq), the SPME fiber was exposed to the headspace of the blackberry sample for the extraction time (text) at the extraction temperature (T). Values of experimental parameters evaluated in the optimization process are detailed in Section 2.3.

2.2. Experimental design

The effect of three independent factors (T, teq and text) on the SPME fractionation of blackberry volatiles was studied using a Box–Behnken design. A total of 15 experiments (3-level design including a subset of the runs in the full three-level factorial and 3 centerpoints per block to estimate the experimental error) were carried out in randomised order. Experimental ranges for factors evaluated were: T = 40–80 °C, teq = 10–20 min, text = 10–30 min.
The quadratic model proposed was:

$$R= \beta_0 + \beta_1 T + \beta_2 t_{eq} + \beta_3 t_{ext} + \beta_{1,1} T^2 + \beta_{2,2} t^2_{eq} + \beta_{3,3} t^2_{ext} + \beta_{1,2} T t_{eq} + \beta_{1,3} T t_{ext} + \beta_{2,3} t_{eq} t_{ext} + \varepsilon$$

where β_0 is the intercept, bi are the first-order coefficients, $\beta_{i,i}$ the quadratic coefficients for ith factors, $\beta_{i,j}$ the coefficients for the interaction of factors i and j and ε is the error.
Two response (R) variables were individually considered in the optimization of the SPME method: R1, total volatile amount/g of sample, and R2, furan derivative amount/g of sample. The parameters of the model were estimated by multiple linear regression (MLR) using StatGraphics Centurion XV software (Statistical Graphics Corporation, Rockville, MD, USA). The experimental con- ditions that independently maximized R_1 and minimized R_2 were obtained from the fitted models. A desirability function (RD) (Derringer & Suich, 1980) was also used as a response that simultaneously maximizes R1 and minimizes R2; this function takes values between 0 (completely undesirable value) and 1 (completely desirable or ideal response). Optimization of this multiple response provides SPME experimental conditions that give rise to the ‘‘most desirable’’ response values.

2.3. Gas Chromatography–Mass Spectrometry (GC–MS) analysis

GC–MS analyses were performed on an Agilent 6890 (Palo Alto, CA, USA) gas chromatograph coupled to a Hewlett-Packard 5973 quadrupole mass detector. The SPME fiber was desorbed into the injection port at 250 °C in splitless mode (2–3 min). Compounds were resolved on a Supelcowax column (27.2 m × 0.25 mm i.d. 0.25 lm film thickness; Supelco (Bellefonte, PA, USA)) using helium as carrier gas (1 mL min—1). The oven was temperature programmed from 40 °C (splitless time) to 220 °C (60 min) at 3 °C min—1. Mass spectra were recorded in electron impact (EI) mode at 70 eV within the m/z range 35–350. The transfer line and ionization source were thermostated at 280 and 230 °C, respectively. Acquisition was done using MSD ChemStation soft- ware (Agilent Technologies, Palo Alto, CA, USA). All analyses were performed in duplicate.

Qualitative analysis was based on the comparison of experi- mental spectra with those of the Wiley mass spectral library (McLafferty & Stauffe, 1989), and was further confirmed by using linear retention indices (I^T) (d'Acampora Zellner et al., 2008) and published data (Meret et al., 2011; Morales et al., 1996; Osorio et al., 2007; Qian & Wang, 2005; etc).
Semiquantitative data (percentage of total volatile composition) were directly calculated from total ion current (TIC) peak areas, assuming no differences in response factor for all volatiles quantified.

2.4. Statistical data analysis

Statistical data analysis (correlation matrix, Principal Compo- nent Analysis (PCA), Cluster Analysis and Stepwise Multiple Regression) was carried out by using the Statistica software v. 7.1 (StatSoft, 2005).

3. Results and discussion

3.1. Selection of the SPME fibre coating

As selectivity of SPME fractionation markedly depends on the fiber coating selected, five SPME fibers with different characteristics (polarity and retention mechanism) were evaluated under identical experimental conditions. Average values (T = 60 °C, t_{eq} = 15 min and t_{ext} = 20 min) for ranges of SPME variables further optimized in Section 3.2 were chosen for the fractionation/enrichment of 0.2 g of BLACK sample. As shown in Fig. 1, the highest total volatile amount was extracted by the CAR/PDMS and PA fibers, whereas PDMS and CAR/PDMS/DVB extracted the lowest (7–8% of CAR/PDMS fiber). PA fiber was better for extraction of polar or medium volatility compounds such as 1-decanol, myrtenol, p-cymen-8-ol, ethyl dodecanoate, etc. CAR/PDMS showed a selective recovery towards compounds of low molecular weight such as methylbutanal (sum of 2-methyl- and 3-methyl-isomers), ethanol, 2,3-butanedione, limonene, trans-2-hexenal, 3-hydroxy- 2-butanone, 2-heptanol, 1-hexanol, nonanal, etc. and provided the highest sensitivity for fractionation of both erythro- and threo-2,3-butanediol, ethyl 3-hydroxy-butanoate and other com- pounds eluting at middle retention times. A similar GC–MS profile to that of CAR/PDMS was obtained by using PDMS/DVB fiber but with a lower sensitivity. Therefore, and considering the intended application of the SPME method here developed for the non- targeted characterization of blackberry samples, CAR/PDMS fiber was selected for further optimization.

3.2. *Experimental design for optimization of T, t_{eq} and t_{ext}*

SPME recovery of volatiles is highly influenced by the operating conditions (T, t_{eq} and t_{ext}). Thus, the influence of these three inde- pendent variables on sampling of blackberry volatiles was studied using a Box–Behnken design. The ranges for experimental conditions assayed were selected based on previous references on the SPME fractionation of volatiles from blackberries (Blanch et al., 2011; Meret et al., 2011) and from other food matrices (Soria, Martínez-Castro, & Sanz, 2003; Soria, Sanz, & Villamiel, 2008).

Two dependent variables were individually considered: first, as optimization of SPME method was aimed to its further application to the overall characterization of R. ulmifolius Schott samples from different locations, total volatile amount for fifteen selected vola- tiles/g of blackberry (R_1) was selected as variable to be maximized. Compounds included in R_1 were chosen to consider blackberry volatiles of different functionality (alcohols: 1-hexanol, 2-heptanol, 1-octanol, trimethylbenzenemethanol; aldehydes: hexanal, trans- 2-hexenal, nonanal, decanal, benzeneacetaldehyde; ketones: 2-pentanone, 3-hydroxy-2-butanone; esters: ethyl 3-hydroxybut- anoate, lactones: butyrolactone, etc.) present in a wide range of rel- ative concentrations, and compounds of organoleptic importance in this berry such as myrtenol. Second, as several compounds in the GC–MS profiles might derive from blackberry carbohydrate matrix through temperature-depending degradation, their concentration could be related to the experimental SPME conditions being used. Therefore, the amount of furan derivatives (2-furancarboxal- dehyde and 5-methyl-2-furancarboxaldehyde) per gram of sample (R_2) was also selected as a response variable to be minimized, as it was related to the unwanted thermal degradation of blackberry matrix.

Response surface methodology was applied to calculate the coefficients of the quadratic models proposed and to estimate the statistical significance of the estimated regression coefficients. Regarding R_1 model, the most significant ($P < 0.05$) coefficients were T, t_{ext}, T^2 and T t_{ext}, whereas T, T^2 and T text were those for R_2 model. Table 2 lists the model equations and fit quality for both R_1 and R_2 after excluding non-significant ($P > 0.05$) terms in the model. As shown by the adjusted R-squared values (R^2_{adj}) and standard deviation of the residuals obtained, the quadratic models pro- posed accurately described the variability of both R_1 and R_2. As expected, the optimal set of operating conditions (Table 2) was different when considering the optimization of R_1 and R_2, dependent variables to be maximized and minimized, respectively. Temperature was the factor showing the main differences regarding optimal conditions for R_1 and R_2. Increasing extraction temperature is known to be a good way to improve recovery, but high temperatures are also associated with the unwanted generation of artifacts.

When using RD (weights of 0.3 and 1 for R_1 and R_2, respectively) as the response to be optimized, a maximum value of 0.82 was obtained by using the following SPME conditions: T = 66 °C, t_{eq} = 20 min and text = 16 min.

3.3. *Characterization of the volatile composition of Italian and Spanish blackberries*

The optimized SPME GC–MS method was applied to the characterization of *R. ulmifolius* Schott blackberries collected in Italy and Spain. As an example of the results obtained, Fig. 2 shows the total ion current (TIC) chromatograms obtained for samples SEG and FIL (for sample identification, see Table 1). Percent quantitative data together with linear retention indices for a total of 74 volatiles identified/characterized based on GC and MS data in all samples under study are listed in Table 3. Average relative standard deviation of data for all Italian and Spanish blackberries analyzed was 14.3%.

As shown in Fig. 2, SPME GC–MS profiles of *R. ulmifolius* Schott fruits were higly complex irrespective of the harvest location. Although a wide variability was found for the relative concentrations of volatiles in the thirteen blackberries analyzed, methylbutanal (2.02–25.70%), ethanol (9.84–68.21%), 2,3-butane-dione (2.31–14.71%), trans-2-hexenal (0.49–17.49%), 3-hydroxy-2-butanone (0.08–7.39%), 1-hexanol (0.56–16.39%), 1-octanol (0.49–10.86%) and methylbutanoic acid (0.53–21.48%) were the major compounds in all samples analyzed, representing on average 76.4% and 65.1% of the total TIC profiles of Italian and Spanish blackberries studied. VER blackberry showed the richest volatile composition followed by NIC and FIL samples; LEG and SEG blackberries showed the poorest volatile TIC profiles.

Similarly to other Rubus species, esters and aliphatic alcohols were the predominant chemical classes, followed by terpenic and aromatic compounds, aldehydes and ketones. In contrast to Andean (*Rubus glaucus* Benth.) blackberries fractionated by SPME (Meret et al., 2011; Osorio et al., 2007), in which aromatic esters such as ethyl and methyl benzoate were predominant, aliphatic esters such as ethyl acetate, hexyl butanoate, hexyl hexanoate, 2-methylbutyl-3-methylbutyrate and ethyl and methyl esters of hexanoic, octanoic, decanoic and dodecanoic acids were the main contributions to this class in R. ulmifolius Schott fruits. Several of these esters have previously been found in the volatile fractions of different red fruits including strawberries (*Fragraria* × *ananassa*), raspberries (*Rubus idaeus*), etc. analyzed by HS-SPME (Blanch et al., 2011; Ibañez et al., 1998). Except for ethyl acetate which was detected at high relative concentrations (1.18–3.93%) in most of Italian blackberries analyzed, the remaining esters were present at low percent concentrations irrespective of the sample considered (Table 3). Butyl benzoate was the only aromatic ester detected in *R. ulmifolius* Schott in concentrations up to 0.13%. Similarly to Andean blackberries (Meret et al., 2011; Morales et al., 1996), 3- hydroxyesters were detected in a wide range of relative concentrations (0–0.87%) in *R. ulmifolius* Schott collected both in Spain and Italy, whereas 5-hydroxyesters (characteristic of *Rubus laciniata* L. (Thornless Evergreen), Georgilopoulos & Gallois, 1987) were not detected in any of the samples analyzed.

A wide number of very volatile alcohols (IT < 1520), which are preferentially recovered by the CAR/PDMS fiber, were determined in *R. ulmifolius* Schott samples here analyzed. Although most of these compounds have previously been reported in homogenates of other species of the *Rubus genus* such as *R. laciniata* L., *R. glaucus* Benth., *R. arcticus* and *R. idaeus*, etc. analyzed by SPME, liquid–liquid extraction, SBSE and Dynamic Headspace (Du et al., 2010a,b; Georgilopoulos & Gallois, 1987; Meret et al., 2011; Morales et al., 1996; Pyysalo, Suihko, & Honkanen, 1977; Qian & Wang, 2005, three other (4-methyl-1-pentanol, 3-ethyl-4-methyl- pentanol and 3-ethylphenol) were identified for the first time in this paper; the different species and extraction procedure would justify the differences in composition observed. C6 alcohols such as 1-hexanol (0.56–16.39%), 3-hexen-1-ol (0.04–0.34%) and 2-hex-en-1-ol (0.13–0.75%), which have been described to arise from enzymatic oxidative degradations of fatty acids, were detected in both Italian and Spanish *R. ulmifolius* Schott samples, their contents being probably related with those of the corresponding aldehydes. Whereas 2-heptanol has been described as one of the main aroma contributors in *R. laciniata* L. and *R. glaucus* Benth., this alcohol showing sweet, fruity and green notes was only present at percent concentrations ranging 0–43–3.10% in blackberries under study.

With some exceptions (e.g. 4.72% limonene in SAN, 2.62% linalool in SEG, 2.48% trans-caryophyllene in ROS, etc.), terpenic compounds were present at relative concentrations below 1% in all blackberries analyzed. p-Cymen-8-ol, also known as cherry propanol, was detected at concentrations below 0.05% in only six of the blackberries here analyzed (Table 3). This compound, with a sweet, fruity and cherry odor, has previously been reported in blackberries fractionated by SPME (e.g. concentrations of 2% in *R. glaucus* Benth.) (Ibañez et al., 1998; Meret et al., 2011), and it has also been described as one of the most important components in the fraction obtained by continuos liquid–liquid and further fractionation on silica gel of *R. laciniata* L. juice (Georgilopoulos & Gallois, 1987). Myrtenol, a monoterpenoid alcohol with woody/pine/balsam odor, was quantified by SPME GC–MS in the range 0.04–0.84% and 0.02– 0.22% in Spanish and Italian *R. ulmifolius* Schott fruits, respectively. Using a similar approach but with a different SPME fiber coating (PDMS/DVB), Meret et al. (2011) reported relative abundances of 0.9% in Andean blackberries from Ecuador. Terpinen-4-ol, described as one of the main terpenic alcohols in Andean and Thornless Evergreen blackberries, was not detected in *R. ulmifolius* Schott.

2-Heptanone (present in relative concentrations higher than 3% in SEG and LEG) and nonanal (>6.7% in SEG and ESP) have previ- ously been reported as major components within their classes in *Rubus* sp. samples analyzed by SPME (Meret et al., 2011) or liquid–liquid extraction (Georgilopoulos & Gallois, 1987). 5,5- Dimethyl-2-cyclopenten-1-one (0.01–0.26%) was the only car- bonyl compound tentatively identified in *R. ulmifolius* Schott and not previously reported in other Rubus species.

Several furan derivatives such as 2-furancarboxaldehyde (furfural), 5-methylfurfural, dihydro-2(3H)-furanone, 2-furanmethanol and 5-hydroxymethylfurfural (HMF) were determined at different percentages in *R. ulmifolius* Schott fruits analyzed. BOV blackberry showed the highest furfural relative concentration (7.75%) and HMF was only detected at very low concentrations in this sample. Turemis et al. (2003), in a study on the use of SPME in immersion mode (Im-SPME) for analysis of the aroma composition of 5 Turkish blackberry cultivars, found that furans were the most abundant aromatic compounds and that HMF, present at relative concentrations higher than 80% in all cultivars analyzed, was the main specific blackberry-like aromatic compound. Furans, and particularly furfural which represents a third of the total odorous profile, have also been described as characteristic volatiles isolated by Simulta- neous Distillation–Extraction (SDE) from concentrated *R. laciniata* L. juice (Georgilopoulos & Gallois, 1988). Klesk and Qian (2003a,b) also reported several hydroxyfuranones as the most significant odour active volatiles in Marion (*Rubus* spp. hyb) and Thornless Evergreen cultivars fractionated by dynamic headspace and by solvent-assisted flavor extraction (SAFE), whereas only dihydro-2(3H)-furanone was detected in *R. ulmifolius* Schott. In addition to other factors such as the *Rubus* specie analyzed, the differences observed between our study and previous results regarding the content of furan derivatives could be mainly due to the more drastic experimental conditions used in those techniques, and probably giving rise to a higher degradation of carbohydrate matrix of blackberries.

Although acids have been reported as aroma components of different *Rubus* species analyzed by solvent extraction or by SBSE (Du et al., 2010b; Morales et al., 1996; Qian & Wang, 2005), they were poorly recovered or even not detected by SPME (Meret et al., 2011). In agreement with this, several acids (acetic, butanoic, hexanoic, etc) and both diastereomers of 2,3-butanediol were detected in most of *R. ulmifolius* Schott blackberries under study. However, as they eluted as very broad non-gaussian peaks, they were not considered for quantitation.

Quantitative data listed in Table 3 were subjected to statistical analysis in order to get insight into the compounds more useful for the characterization of the Italian/Spanish blackberries under study. First, and with the aim of studying the unsupervised grouping of samples according to their volatile composition, data were subjected to exploratory techniques such as PCA in the correlation mode.

Spanish samples were differentiated from Italian ones based on their negative scores for PC2 (Fig. 3).

As expected for relative data, in which an increase in the per- cent concentration of a compound is directly related to the decrease of others, most volatiles showed negative loadings for first principal components. Compounds with high loadings (absolute value > 0.6) were generally associated with volatiles of similar functionality. PC1, explaining 20.7% of data variance, was negatively associated with alcohols of the same homologous series (from 1-butanol to 1-decanol) and positively with furan derivatives (5-methylfurfural, furfural, 2-furanmethanol and HMF) and hydroxyesters (methyl-3-hydroxybutanoate and ethyl-3-hydroxy- butanoate). Regarding PC2 (17.0% data variance), ethyl esters of acetic, decanoic and dodecanoic acids together with ethanol showed the highest positive loadings for this principal component, while aldehydes such as hexanal, decanal and phenylacetaldehyde, and compounds with a terpenic structure (p-cymene, limonene, camphor, anethole (1-methoxy-4-(1-propenyl)-benzene), a-terpineol and myrtenol) afforded the most significant negative contributions. Sesquiterpenes with similar retention such as α-cubebene, α -ylangene, α -copaene were the compounds more correlated (loadings with absolute value > 0.84) with PC3 (15.5% data variance). Similar grouping of volatiles was also found in the cluster analysis of variables (Ward's method, Euclidean linkage distance) of these data (data not shown).

When supervised correlation among individual volatiles and the collection place of blackberries was studied, compounds present at higher percent concentration in Spanish samples were 3-penten-2-one, nonanal, 2,4-heptadienal, decanal, α-terpineol and 2-phenyl-ethanol, whereas for Italian samples were ethyl acetate, ethanol, 2-methylbutyl pentanoate, ethyl decanoate and a non-identified compound (m/z: 112, 55, 142, 84) with $I^T = 2038$. The stepwise multiple regression analysis of these data showed that only two variables (ethyl decanoate and ethyl acetate) were enough to differentiate Italian and Spanish blackberries with statistical significance (R^2_{adj} 0:822, $P < 0.00007$).

4. Conclusions

The SPME GC–MS method here optimized is shown as an afford- able, fast and solvent-free approach which can be performed with low sample amounts and be easily implemented at the food industry for quality control purposes. In addition to the capability of prediction of the harvest location (Italy/Spain) of *R. ulmifolius* Schott blackberries, relative data gathered by the optimized SPME GC–MS method might also be used for the characterization of other *Rubus* species and/or for the evaluation of the changes in their aroma associated with different factors (e.g. harvest year, growing conditions, etc).

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Table 1

Blackberry samples under study.

Sample code	Location
NIC	Nicotera (Calabria, Italy)
CIC	Cicerna (Calabria, Italy)
FIL	Filadelfia (Calabria, Italy)
GRAN	Granatara (Calabria, Italy)
ROS	Rosarno (Calabria, Italy)
COS	Cosoleto (Calabria, Italy)
BOV	Bovalino (Calabria, Italy)
LEG	Leganés (Madrid, Spain)
CAS	Castañar de Ibor (Cáceres, Spain)
SAN	Santander (Spain)
VER	La Vera (Cáceres, Spain)
ESP	El Espinar (Segovia, Spain)
SEG	Segovia (Spain)

Table 2

Summary of the results obtained in the optimization of SPME operating conditions by experimental design.

Model equation	R^2_{adj} (%)	Residuals	Optimal conditions		
			T (°C)	t_{eq} (min)	t_{ext} (min)
$R_1 = 7.49 \times 10^9 – 2.86 \times 10^8 \cdot T - 1.12 \times 10^8 \cdot t_{ext} + 2.69 \times 10^6 \cdot T^2 + 2.58 \times 10^6 \cdot T \cdot t_{ext}$	96.88	271	80	15	30
$R_2 = 1.09 \times 10^{10} – 3.49 \times 10^8 \cdot T + 2.79 \times 10^6 \cdot T^2 + 3.62 \times 10^6 \cdot T \cdot t_{ext}$	83.95	480	43	11	30

Table 3

Percent quantitative data (average for n = 2 replicates and relative standard deviation (%) in brackets) obtained in the SPME GC–MS analysis of the volatile composition of Italian/Spanish blackberry samples under study.

Peak no.	Compound	I^T	Relative data (%)*												
			GRA	CIC	BOV	NIC	FIL	ROS	COS	SEG	ESP	LEG	CAS	SAN	VER
1	Ethyl acetate	–	0	0	3.93 (13.6)	1.18 (6.8)	1.93 (2.6)	2.65 (0.2)	1.85 (2.8)	0	0	0	0	0	0
2	Methylbutanal (sum of isomers)	–	15.08 (6.5)	6.55 (2.7)	2.02 (16.5)	8.53 (0.4)	5.70 (9.1)	13.56 (1.9)	10.03 (0.2)	9.42 (2.4)	7.80 (3.5)	8.32 (0.8)	7.45 (21.2)	25.70 (9.5)	10.14 (3.5)
3	Ethanol	–	12.46 (10.7)	35.78 (6.0)	68.21 (0.8)	50.44 (1.6)	50.76 (1.6)	24.88 (26.9)	38.63 (8.3)	17.74 (1.0)	31.38 (17.8)	14.88 (11.0)	17.75 (33.1)	13.70 (7.9)	9.84 (15.1)
4	2,3-Butanedione	–	4.62 (2.5)	8.70 (3.2)	2.31 (4.1)	5.59 (0.5)	7.06 (5.8)	5.67 (2.1)	9.14 (5.2)	7.87 (3.2)	5.23 (6.3)	5.93 (4.0)	14.71 (25.6)	13.46 (20.1)	7.29 (6.0)
5	Hexanal	1111	3.04 (9.5)	2.70 (1.0)	1.14 (7.5)	1.44 (5.2)	0.77 (5.3)	1.20 (6.8)	1.43 (8.0)	2.23 (3.3)	3.32 (11.2)	5.09 (4.1)	2.44 (5.0)	3.87 (14.6)	1.32 (3.3)
6	2-Methyl-2-butenal	1118	0.12 (11.8)	0.10 (2.4)	0	0.13 (2.8)	0.09 (5.3)	0.09 (4.9)	0.18 (0.3)	0.13 (8.0)	0.13 (26.7)	0.06 (12.6)	0.17 (23.7)	0.61 (68.9)	6.36 (1.1)
7	3-Penten-2-one	1141	1.23 (9.7)	0.77 (0.4)	0.97 (5.5)	0.44 (1.6)	0.33 (0.8)	1.16 (45.2)	1.16 (12.8)	1.84 (8.9)	1.13 (20.4)	1.93 (1.6)	0.80 (1.7)	2.07 (40.6)	3.60 (5.3)
8	1-Butanol	1158	1.20 (4.3)	2.70 (7.3)	0.97 (2.2)	1.56 (6.3)	2.68 (0.2)	2.88 (6.5)	2.36 (6.5)	2.50 (6.3)	2.99 (6.2)	2.38 (8.3)	5.23 (1.5)	0.37 (18.2)	4.51 (6.7)
9	2-Heptanone	1188	1.26 (20.3)	0.35 (3.4)	0.03 (30.0)	0.79 (4.9)	0.68 (5.9)	1.34 (12.2)	1.84 (32.1)	3.11 (0.6)	0.52 (6.1)	3.05 (1.5)	0.34 (25.6)	0.06 (12.8)	0.97 (19.1)
10	Methyl hexanoate	1194	0.11 (14.0)	0.03 (19.4)	0	0.11 (6.8)	0.08 (5.2)	0.05 (19.0)	0.02 (9.1)	0.05 (0.7)	0.02 (10.9)	0.07 (14.8)	0.08 (26.3)	0.06 (51.9)	0.15 (5.5)
11	Limonene	1197	0.01 (61.7)	0	0.12 (4.9)	0.03 (41.6)	0.01 (57.2)	0.02 (61.1)	0	0.87 (23.5)	0.07 (61.2)	0	0.45 (55.1)	4.72 (29.4)	0.12 (5.7)
12	1,8-Cineole	1203	0	0	0	0	0	0.23 (47.7)	0	0	0	0	0	0	tr**
13	3-Methyl-1-butanol	1213	0.31 (2.4)	1.22 (7.4)	0.05 (2.0)	1.41 (2.9)	0.91 (87.3)	1.32 (0.8)	1.01 (1.0)	0.20 (0.1)	0.48 (13.0)	0.38 (12.9)	1.99 (36.6)	0.23 (39.1)	1.43 (1.7)
14	*trans*-2-Hexenal	1219	11.11 (3.4)	8.48 (2.4)	0.49 (17.2)	2.89 (6.4)	2.13 (53.3)	5.32 (7.1)	4.94 (1.4)	6.51 (0.7)	12.95 (31.3)	17.49 (0.9)	7.97 (5.2)	8.15 (30.4)	8.55 (9.3)
15	Ethyl hexanoate	1238	0.06 (13.1)	0.05 (17.5)	0.02 (25.9)	0.25 (7.8)	0.12 (5.5)	0.07 (1.6)	0.06 (27.8)	0.12 (1.1)	0.07 (45.6)	0.04 (8.7)	0.03 (19.6)	0.06 (53.0)	0.08 (10.8)
16	1-Pentanol	1255	0.42 (8.6)	0.58 (11.6)	0.39 (15.5)	0.32 (14.1)	0.58 (4.6)	0.65 (21.1)	0.48 (18.3)	0.59 (16.4)	0.54 (2.4)	0.59 (31.6)	0.49 (3.5)	0.27 (5.4)	0.58 (14.7)
17	*p*-Cymene	1268	0.04 (0.7)	0.02 (10.3)	0.16 (11.8)	0.02 (1.6)	0.02 (0.7)	0.14 (1.5)	0.02 (30.2)	0.43 (39.2)	0.06 (15.1)	0.06 (0.7)	0.09 (46.2)	3.56 (20.1)	0.04 (11.8)
18	3-Hydroxy-2-butanone	1289	0.08 (5.3)	6.03 (5.1)	1.33 (11.5)	2.25 (47.2)	4.16 (3.0)	2.40 (0.6)	3.78 (8.7)	1.50 (1.5)	3.37 (12.0)	3.30 (3.4)	7.39 (31.8)	2.52 (30.3)	1.22 (9.2)
19	2-Methylbutyl-3-methylbutyrate	1298	0.06 (10.6)	0.10 (14.2)	0.13 (14.3)	0.12 (4.4)	0.08 (12.8)	0.21 (13.3)	0.14 (26.1)	0.04 (21.5)	0.07 (0.2)	0.07 (10.1)	0.10 (29.9)	0.02 (48.9)	0.02 (9.0)
20	4-Methyl-1-pentanol	1318	0.05 (0.7)	0.10 (17.0)	0	0.09 (6.3)	0.07 (8.6)	2.16 (8.3)	0.26 (25.0)	0.09 (3.2)	0.05 (26.4)	0.08 (1.1)	0.45 (6.2)	0	0.36 (7.8)
21	2-Heptanol	1325	0.78 (0.5)	0.57 (6.9)	0.43 (16.1)	0.92 (3.8)	1.64 (82.2)	2.33 (11.8)	2.30 (31.6)	1.51 (0.4)	2.10 (1.0)	3.10 (0.0)	1.10 (26.4)	1.75 (30.0)	0.60 (7.0)
22	6-Methyl-5-hepten-2-one	1339	0.17 (5.9)	0.17 (0.1)	0.39 (0.7)	0.10 (0.5)	0.09 (3.4)	0.12 (3.0)	0.11 (24.1)	0.23 (29.1)	0.35 (4.2)	0.23 (40.3)	0.28 (0.9)	0.15 (5.3)	0.23 (13.6)
23	1-Hexanol	1357	7.23 (5.8)	5.39 (5.7)	0.56 (4.0)	4.39 (2.9)	5.02 (3.2)	8.78 (11.6)	4.15 (28.3)	7.66 (0.5)	4.71 (11.1)	7.59 (2.3)	6.38 (19.0)	1.95 (51.9)	16.39 (4.9)
24	*trans*-3-Hexen-1-ol	1368	0.20 (0.7)	0.06 (7.8)	0.06 (10.2)	0.29 (4.8)	0.34 (2.6)	0.24 (10.7)	0.12 (29.9)	0.04 (1.2)	0.16 (9.6)	0.22 (20.0)	0.14 (31.5)	0.08 (11.7)	0.31 (3.0)
25	Methyl octanoate	1391	0.02 (4.7)	0.01 (11.6)	0.01 (25.1)	0.02 (7.7)	0.01 (17.9)	0.01 (9.0)	0.01 (23.3)	0.01 (5.4)	0.01 (23.1)	0.01 (37.7)	0.02 (33.3)	0.01 (39.6)	0.03 (7.1)
26	Nonanal	1393	1.76 (2.4)	2.14 (1.2)	2.14 (5.8)	1.52 (5.1)	1.35 (13.2)	1.71 (10.5)	2.65 (24.3)	7.20 (7.2)	6.71 (2.7)	4.35 (1.4)	3.83 (2.8)	1.79 (21.9)	2.47 (12.1)
27	2-Hexen-1-ol	1411	0.40 (3.9)	0.27 (2.1)	0.60 (6.9)	0.61 (6.0)	0.41 (1.2)	0.42 (6.5)	0.40 (22.9)	0.16 (8.7)	0.54 (11.2)	0.75 (17.9)	0.18 (4.5)	0.64 (31.3)	0.13 (3.7)
28	Hexyl butanoate	1417	0.46 (12.8)	0.23 (6.3)	0	0.09 (2.8)	0.12 (7.7)	0.19 (16.1)	0	0	0.06 (6.4)	0.20 (0.5)	0.28 (46.7)	0.45 (35.5)	0.12 (10.5)
29	Ethyl octanoate	1437	0.13 (11.4)	0.07 (4.9)	0.05 (9.1)	0.14 (7.7)	0.07 (11.4)	0.06 (15.6)	0.14 (27.3)	0.08 (4.9)	0.04 (0.6)	0.05 (2.6)	0.05 (38.7)	0.05 (26.3)	0.05 (9.3)
30	α-Cubebene	1449	0.27 (14.2)	0.09 (2.3)	0	0.26 (6.0)	0.15 (3.3)	0.15 (10.5)	0.02 (2.2)	0	0	0.02 (12.7)	0.05 (9.0)	0.14 (15.0)	0.08 (1.2)
31	Unknown (43 (100), 45 (28), 58 (22), 84 (18), 69 (13))***	1456	2.13 (2.0)	1.67 (1.3)	0.22 (15.6)	0.66 (1.3)	0.63 (3.3)	1.67 (0.9)	1.21 (4.7)	2.64 (1.1)	0.75 (27.9)	1.22 (1.3)	0.40 (3.3)	0.85 (14.0)	0.23 (8.9)
32	1-Heptanol	1460	0.34 (5.6)	0.26 (7.3)	0.09 (14.0)	0.26 (14.2)	0.19 (8.8)	0.51 (16.2)	0.59 (7.3)	0.80 (1.6)	0.25 (9.5)	0.45 (5.4)	0.35 (17.4)	0.15 (21.2)	0.54 (5.0)
33	2-Furancarboxaldehyde	1467	1.75 (2.6)	0.65 (22.2)	7.75 (7.7)	0.62 (6.9)	0.09 (5.3)	0.28 (4.4)	0.23 (14.3)	0.34 (4.8)	1.01 (27.9)	1.47 (2.0)	0.26 (34.4)	3.02 (86.8)	0.14 (15.1)
34	α-Ylangene	1470	0.15 (10.9)	0.04 (2.2)	0	0.11 (6.3)	0.06 (2.6)	0.06 (0.7)	0.01 (5.0)	0	0	0	0.01 (84.1)	0.14 (59.1)	0.03 (9.0)
35	5,5-Dimethyl-2-cyclopenten-1-one	1474	0.26 (26.5)	0.07 (8.2)	0.02 (22.9)	0.05 (4.8)	0.01 (17.8)	0.06 (7.7)	0.04 (4.3)	0.18 (9.9)	0.08 (29.4)	0.09 (6.7)	0.12 (9.5)	0.12 (64.7)	0.02 (25.5)
36	α-Copaene	1478	1.25 (13.0)	0.34 (5.4)	0.09 (13.5)	0.76 (7.6)	0.46 (1.9)	0.46 (2.6)	0.12 (0.3)	0.16 (12.9)	0.06 (15.6)	0.18 (1.6)	0.26 (22.5)	0.48 (28.1)	0.23 (7.1)
37	Methyl-3-hydroxybutanoate	1484	0	0	0.01 (21.5)	tr	0.01 (20.2)	tr	0	0	0	0	0	0.01 (10.5)	0
38	2,4-Heptadienal	1491	0.06 (9.9)	0.04 (5.4)	0.08 (19.4)	0.03 (30.6)	0.03 (16.7)	0.06 (6.2)	0.02 (7.5)	0.10 (2.1)	0.06 (13.8)	0.09 (3.7)	0.08 (29.6)	0.09 (31.5)	0.22 (16.1)
39	Decanal	1497	0.16 (9.2)	0.17 (0.6)	0.41 (15.3)	0.22 (20.0)	0.12 (16.6)	0.11 (14.2)	0.05 (17.4)	0.47 (6.2)	1.10 (25.0)	0.51 (1.2)	1.03 (24.4)	0.84 (58.6)	0.26 (10.4)
40	Camphor	1498	0	0	0	0	0.01 (10.6)	0	tr	tr	0	0	0	0.04 (14.7)	0
41	3-Ethyl-4-methylpentanol	1512	0.31 (6.5)	0.10 (4.0)	0	0.14 (3.3)	0.05 (15.3)	0.20 (10.2)	0.18 (21.3)	0.18 (7.3)	0.12 (3.9)	0.26 (4.4)	0.27 (7.3)	0.03 (2.9)	0.27 (9.6)
42	Benzaldehyde	1517	0.25 (6.4)	0.27 (0.7)	0.14 (5.5)	0.20 (4.8)	0.26 (4.9)	0.30 (12.8)	0.22 (1.8)	0.25 (1.4)	0.21 (0.4)	0.47 (7.0)	0.54 (24.1)	0.26 (21.4)	0.43 (0.2)
43	Ethyl-3-hydroxybutanoate	1520	0.02 (4.8)	tr	0.87 (5.1)	0.09 (1.8)	0.08 (0.8)	0.04 (14.2)	0.01 (59.5)	0	0.03 (39.6)	0	0	0.15 (13.5)	0
44	Epizonarene	1536	0.47 (14.1)	0.12 (4.6)	tr	0.40 (9.6)	0.24 (0.5)	0.23 (6.2)	0.05 (0.7)	0	0.01 (78.7)	0.01 (71.3)	0.07 (77.9)	0.15 (20.9)	0.12 (9.2)
45	Linalool	1554	0.38 (6.1)	0.04 (41.2)	0.02 (10.4)	0.21 (20.0)	0.13 (10.9)	0.17 (23.1)	0.16 (5.5)	2.62 (3.2)	1.21 (10.8)	0.33 (13.1)	0.48 (28.0)	0.31 (25.5)	0.74 (3.8)
46	1-Octanol	1562	3.45 (8.0)	3.38 (4.7)	0.69 (2.4)	2.98 (10.5)	3.84 (7.1)	5.39 (17.2)	4.16 (7.0)	10.86 (3.7)	2.60 (3.8)	5.95 (4.2)	2.78 (10.3)	0.49 (13.5)	8.69 (8.9)
47	5-Methylfurfural	1572	0.10 (7.1)	0.03 (4.3)	0.26 (1.8)	0.04 (5.9)	0.01 (7.5)	0.02 (6.4)	0.01 (1.0)	0.08 (13.4)	0.04 (34.5)	0.08 (0.6)	0.02 (1.9)	0.07 (77.4)	0.01 (10.1)
48	*trans*-Caryophyllene	1579	0.04 (19.4)	0	0	0	0	2.48 (91.2)	0	0	0	0.45 (8.2)	0.08 (97.4)	0.05 (78.0)	0.17 (3.1)
49	Methyl decanoate	1596	0.01 (6.4)	0.01 (18.7)	tr	0.01 (31.5)	0.02 (1.8)	0.01 (1.0)	0.02 (11.1)	0.01 (0.4)	tr	0.01 (10.0)	0.01 (27.5)	0.04 (8.9)	0.02 (2.4)
50	Hexyl hexanoate	1611	0.34 (11.6)	0.37(0.3)	0.01 (13.5)	0.10 (12.6)	0.05 (3.6)	0.14 (10.6)	0.02 (14.5)	0.11 (11.0)	0.04 (2.6)	0.07 (0.2)	0.08 (38.4)	0	0.17 (1.3)
51	Dihydro-2(3H)-furanone	1617	0.59 (2.1)	1.75 (7.2)	0.69 (0.5)	0.86 (13.9)	0.82 (1.5)	0.69 (4.8)	0.59 (0.0)	0.37 (14.2)	0.61 (8.1)	0.67 (7.3)	0.52 (16.7)	0.83 (21.9)	0.54 (4.7)
52	Phenylacetaldehyde	1638	0.97 (5.3)	0.16 (11.4)	0.11 (10.1)	0.30 (12.1)	0.17 (5.3)	0.26 (6.3)	0.20 (6.9)	0.66 (2.8)	0.54 (7.0)	0.45 (3.5)	0.38 (11.8)	1.24 (16.2)	0.96 (1.8)

53	Ethyl decanoate	1640	0.14 (9.9)	0.15 (1.4)	0.07 (13.5)	0.17 (10.4)	0.14 (15.6)	0.11 (9.6)	0.16 (6.5)	0.07 (5.9)	0.08 (31.0)	0.04 (5.5)	0.06 (3.9)	0.05 (0.0)	0.11 (5.5)
54	1-Nonanol	1664	0.71 (13.8)	0.51 (2.3)	0.07 (20.3)	0.26 (3.3)	0.02 (1.9)	0.73 (18.5)	1.19 (2.0)	1.63 (8.2)	0.37 (2.7)	0.50 (7.2)	0.68 (33.7)	0.12 (22.4)	1.01 (3.2)
55	1-Methoxy-4-(2-propenyl)-benzene	1665	0.01 (13.9)	0	0	0.01 (6.8)	0.07 (13.8)	0.04 (5.0)	0	0.07 (18.2)	0	0	0.07 (67.7)	0	0
56	2-Furanmethanol	1667	0.17 (2.2)	0.24 (14.6)	0.70 (12.6)	0.18 (6.8)	0.04 (1.8)	0.09 (1.6)	0.07 (12.8)	0.09 (0.7)	0.14 (17.7)	0.28 (6.5)	0.21 (17.9)	0.19 (72.1)	0.05 (7.6)
57	α-Terpineol	1693	0.10 (18.4)	0.05 (3.5)	0.03 (10.5)	0.04 (0.8)	0.02 (7.6)	0.09 (30.3)	0.03 (5.4)	0.10 (10.8)	0.14 (14.3)	0.11 (0.7)	0.21 (11.4)	0.32 (39.2)	0.09 (5.7)
58	Methylbutanoic acid	1710	21.48 (1.8)	3.55 (5.1)	0.57 (5.8)	2.64 (5.3)	2.58 (2.8)	2.19 (1.0)	1.01 (6.7)	3.19 (1.9)	3.11 (7.6)	1.78 (1.4)	4.24 (18.1)	0.53 (13.1)	3.01 (7.2)
59	Cadinene	1746	0.04 (15.3)	0.01 (12.4)	0.01 (4.9)	0.09 (9.4)	0.03 (6.6)	0.04 (27.0)	tr	0	0	0.05 (6.9)	0.08 (80.2)	0.09 (15.6)	0.03 (9.1)
60	1-Decanol	1767	0.30 (7.0)	0.38 (2.5)	0.16 (8.2)	0.55 (14.1)	0.57 (4.2)	0.39 (2.6)	0.28 (8.8)	0.82 (10.4)	0.19 (0.4)	0.26 (1.1)	0.26 (12.9)	0.27 (21.0)	0.85 (6.1)
61	Myrtenol	1788	0.06 (1.4)	0.22 (114.2)	0.02 (26.3)	0.03 (4.8)	0.09 (113.5)	0.02 (12.6)	0.07 (131.5)	0.07 (24.7)	0.11 (31.6)	0.04 (14.4)	0.43 (36.9)	0.84 (46.5)	0.10 (5.1)
62	Methyl dodecanoate	1804	0	0.01 (26.4)	0.01 (16.9)	0.02 (12.9)	0.03 (9.6)	0.02 (10.5)	0.02 (11.1)	0.03 (2.7)	0.02 (6.0)	0.03 (23.9)	0.03 (22.0)	0.02 (8.2)	0.10 (1.7)
63	1-Methoxy-4-(1-propenyl)-benzene	1822	0	0	tr	tr	0.07 (17.8)	0.01 (11.9)	tr	0	0	0	0	0.52 (15.0)	tr
64	Ethyl dodecanoate	1845	0.01 (5.3)	0.07 (4.3)	0.02 (26.5)	0.09 (21.7)	0.10 (10.1)	0.06 (4.6)	0.05 (1.8)	0.06 (16.4)	0	0.01 (24.6)	0.02 (58.0)	tr	0.08 (0.3)
65	*p*-Cymen-8-ol	1849	0	0	0.05 (32.8)	0	0	0	0	0.04 (7.9)	0.03 (25.4)	0.02 (9.2)	0.02 (19.9)	0.03 (24.8)	0
66	Butyl benzoate	1854	0.01 (0.6)	0.01 (13.0)	0.01 (31.1)	0.01 (18.9)	tr	0.06 (0.9)	0.13 (5.1)	0.01 (13.2)	tr	tr	0.01 (51.4)	0.01 (32.4)	0.01 (13.0)
67	Benzyl alcohol	1874	0.72 (6.8)	1.41 (0.1)	0.03 (5.4)	0.83 (12.0)	0.97 (5.9)	1.43 (0.8)	1.08 (3.8)	1.25 (8.1)	0.91 (12.5)	2.55 (1.0)	4.01 (14.5)	0.60 (20.5)	1.65 (1.0)
68	2-Phenylethanol	1906	tr	0.33 (5.2)	0.03 (10.7)	0.81 (18.3)	0.33 (3.5)	0.94 (2.1)	0.57 (3.4)	0.52 (10.4)	1.30 (7.6)	1.25 (1.9)	1.59 (9.8)	0.59 (6.3)	1.74 (1.1)
69	2-Methyl-3-phenyl-1-propanol	1988	0.03 (15.0)	0.02 (22.3)	0	0.04 (24.0)	0.03 (2.5)	0.03 (4.7)	0.05 (1.3)	0.02 (12.2)	0.01 (10.6)	0.01 (15.7)	0.02 (41.2)	0.03 (31.3)	0
70	4-Ethyl-2-methoxyphenol	2026	0.17 (9.8)	0	0	tr	0.01 (10.7)	0.26 (4.1)	0.01 (46.5)	0	0	0	0	0	0.15 (0.6)
71	Unknown (112 (100), 55 (98), 142 (85), 84 (85), 56 (46), 117 (35))***	2038	0.15 (18.0)	0.25 (2.5)	0.18 (4.0)	0.20 (15.5)	0.19 (5.7)	0.01 (14.4)	0.01 (14.4)	0	0	0	0	0	0.01 (7.0)
72	Benzenepropanol	2040	0.13 (9.0)	0.05 (3.3)	tr	0.06 (19.3)	0.06 (6.3)	0.15 (0.3)	0.14 (3.6)	0.05 (10.4)	0.03 (45.6)	0.04 (4.3)	0.04 (38.4)	0.01 (18.6)	0
73	3-Ethylphenol	2175	0.04 (5.9)	tr	0	tr	0	0.20 (5.0)	tr	0	0	0	0	0	0.21 (0.6)
74	5-Hydroxymethylfurfural	2498	0	0	0.04 (17.4)	0	0	0	0	0	0	0	0	0	0

* Semiquantitative data calculated excluding compounds eluting as broad non-Gaussian peaks (e.g. most organic acids and *erythro*- and *threo*-2,3-butanediol) (see Section 3.3).

** tr = trace (<0.01%).

*** Mass spectra (m/z, abundance (%)).

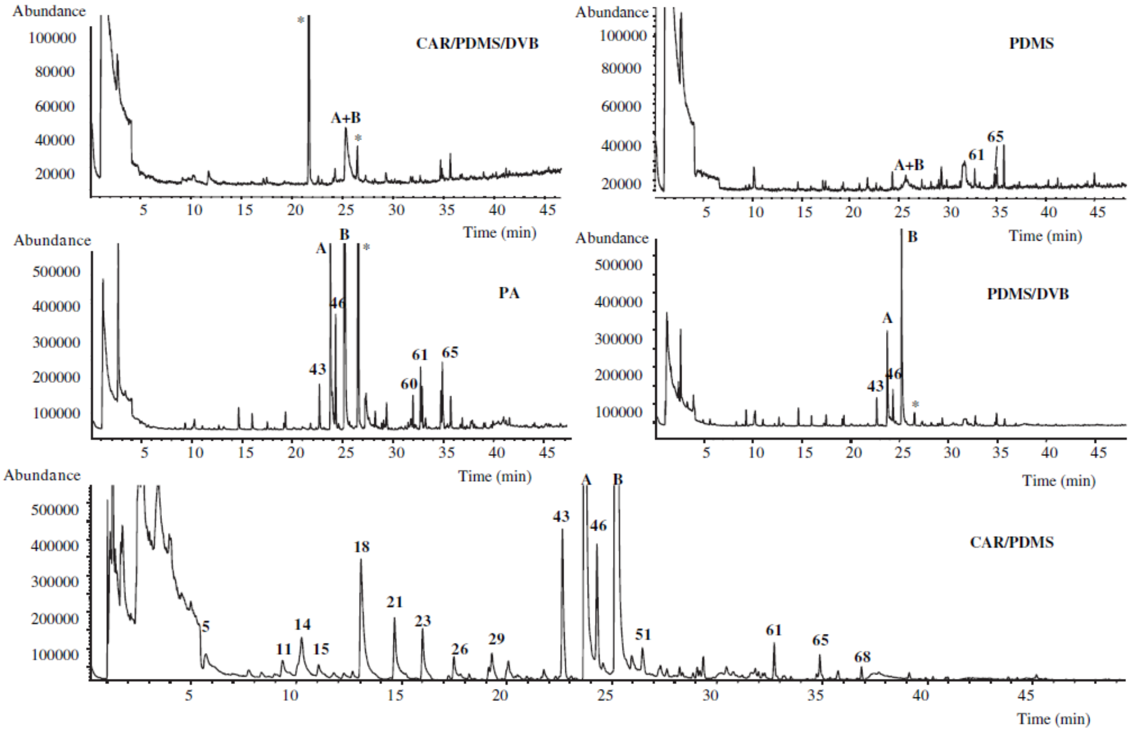

Fig. 1. GC–MS profiles of sample BLACK fractionated by using different SPME fibers. For peak identification, see Table 3. (A) and (B) are *threo*- and *erythro*-2,3-Butanediol, respectively. *Artifacts from the SPME fiber coating.

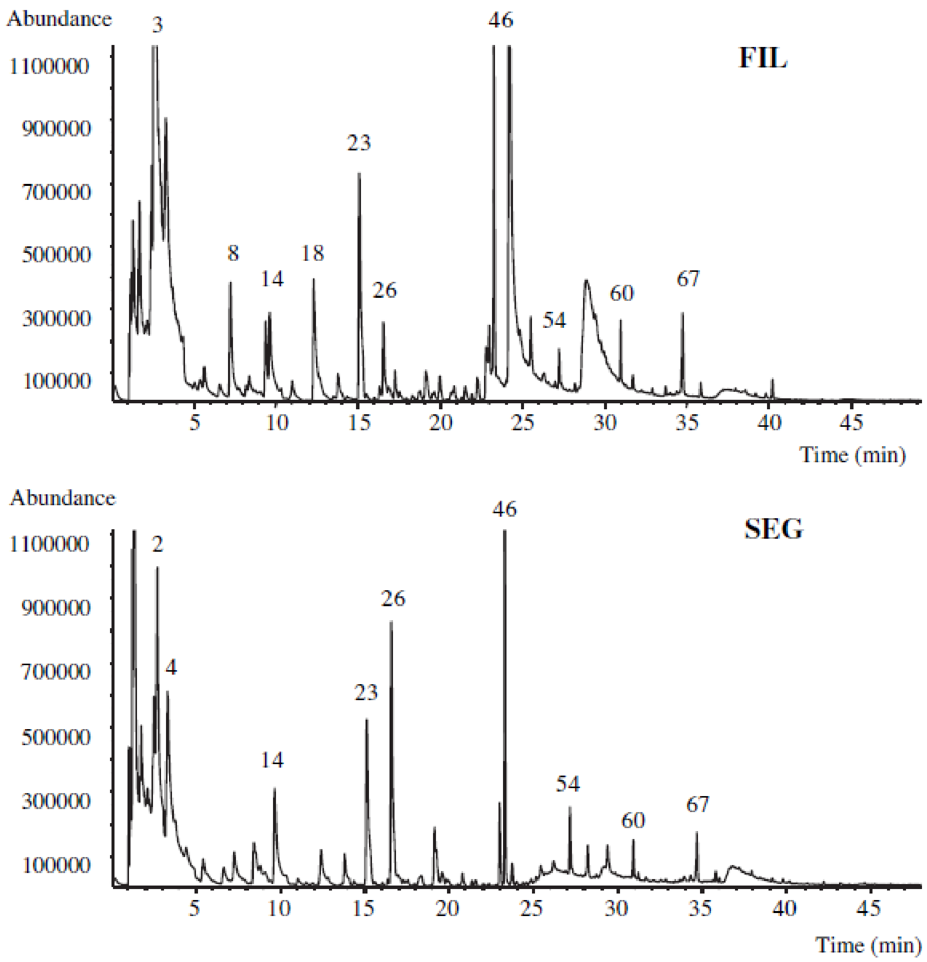

Fig. 2. SPME followed by GC–MS analysis of the volatile composition of blackberry samples SEG and FIL. For peak identification, see Table 3.

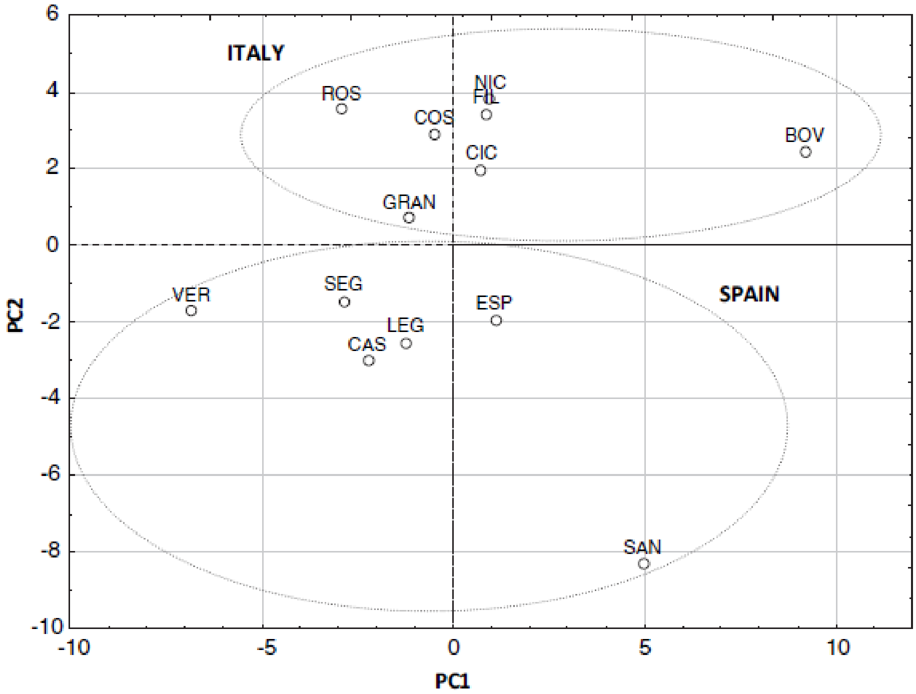

Fig. 3. PCA plot of volatile composition of Italian/Spanish blackberries under study. For sample identification, see Table 1.