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Volatile profile characterisation of Chilean sparkling wines produced by traditional and Charmat methods via sequential stir bar sorptive extraction

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ABSTRACT

The volatile compositions of Charmat and traditional Chilean sparkling wines were studied for the first time. For this purpose, EG-Silicone and PDMS polymeric phases were compared and, afterwards, the most adequate was selected. The best extraction method turned out to be a sequential extraction in the headspace and by immersion using two PDMS twisters. A total of 130 compounds were determined. In traditional Chilean sparkling wines, ethyl esters were significantly higher, while acetic esters and ketones were predominant in the Charmat wines. PCA and LDA confirmed the differences in the volatile profiles between the production methods (traditional vs. Charmat).

1. Introduction

Chile is currently among the top ten wine producing countries worldwide. Among the different types of wine produced in Chile, sparkling wine is becoming increasingly popular. It is estimated that its consumption will continue to grow rapidly, leading to a growth of Chile's wine production. For this reason, it is of great interest to characterise Chilean sparkling wines.

The sparkling wine production process is based on the second fermentation of base wine in which yeast produces a significant quantity of CO2 (Liger-Belair, 2005; Martínez-Rodríguez & Pueyo, 2009). There are two main production processes: Traditional and Charmat methods. In the traditional procedure, the second fermentation of the base wine is carried out within the bottle and results in high quality wines (Torresi, Frangipane, & Anelli, 2011). Some of the most popular sparkling wines, such as Champagne and Cava, are produced by the traditional method. Regarding the Charmat method, the second fermentation is carried out in hermetically sealed tanks. This process involves faster and cheaper production techniques than the traditional method. In Chile, most sparkling wines are produced employing the Charmat method. Depending on the method employed, the sparkling wine has different characteristics (Caliari, Panceri, Rosier, & Bordignon, 2015; Stefenon et al., 2014).

Aroma is one of the most important indicators of sparkling wine quality (Kemp, Alexandre, Robillard, & Marchal, 2015). Therefore, due to the relevance of the aroma in the acceptability of a product by consumers, it is very interesting to know what volatile compounds are involved in its aroma. In general, the volatile profile of sparkling wines produced by the traditional or Charmat method is mainly composed of esters, alcohols, and acids, and also some terpenes, such as limonene, linalool, or lilial have an important role in the overall aroma (Bosch-Fusté et al., 2007; Coelho, Coimbra, Nogueira, & Rocha, 2009; Riu-Aumatell, Bosch-Fusté, López-Tamames, & Buxaderas, 2006). In this context, comparative studies on the effects of the two types of production methods on the volatile compositions of sparkling wines are scarce. A recent publication showed that the sparkling wine produced by the traditional method has higher concentrations of terpenes, alcohols, acids, and especially, ethyl esters (Caliari et al., 2015).

The determination of volatile compounds may require an extraction stage prior to analysis. To date, different extraction techniques have been employed to study the volatile profiles of

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sparkling wines: Liquid–liquid extraction (Perez-Magarino, Ortega-Heras, Martinez-Lapuente, Guadalupe, & Ayestaran, 2013), solid phase extraction (Caliari, Burin, Rosier, & Bordignon-Luiz, 2014), stir bar sorptive extraction (SBSE) with liquid desorption (Coelho et al., 2009), and headspace solid phase microextraction (SPME) (Gallardo-Chacón, Vichi, López-Tamames, & Buzaderas, 2009; Ganss, Kirsch, Winterhalter, Fischer, & Schmarr, 2011). The headspace SPME method is the most employed extraction technique for this purpose. However, SBSE has a greater extraction capacity than SPME (David & Sandra, 2007). In the SBSE technique, the analyte can be extracted by a direct immersion of the sorptive stir bar into the sample (Zalacain, Marin, Alonso, & Salinas, 2007) or placing the stir bar into the headspace (HSSE) (Callejón et al., 2010). This technique is primarily performed by employing a stir bar known as the Twister®, which is traditionally coated with polydimethylsiloxane (PDMS) as a non-polar phase. Different types of extraction phases have been synthesized in-house to improve the extraction of more polar compounds. Among these phases, monolithic materials (Huang, Lin, & Yuan, 2010), molecular imprinted polymers (Xu, Hu, Hu, Pan, & Li, 2012), C18 (Yu & Hu, 2012), and polyurethane (PU) (Rodriguez, Glories, Maujean, & Dubourdieu, 2012) have been successfully tested. In most cases, these polymers are not thermally stable and a liquid desorption process is required. Recently, new twisters coated with polyethyleneglycol-modified silicone (EG-Silicone) and a polyacrilate/polyethylenegly col phase (PA) have been commercialised. These new coatings offer the possibility of recovering compounds with higher polarity than PDMS (Gilart, Marcé, Borrull, & Fontanals, 2014). EG-Silicone and PA twisters have been already tested to determine the different volatile compounds in food matrices, such as scotch whisky, fruit juice, and white wine (Nie & Kleine-Benne, 2011), vegetable matrices (Sgorbini et al., 2012), and wine (Cacho, Campillo, Viñas, Her nández-Córdoba, 2014).

To improve the sensitivity of the extraction process, a good strategy is to increase the volume of the extraction phase. This volume increase can be achieved by increasing the number of twisters used for the extraction because it is possible to analyse the compounds retained in several twisters in a single chromatographic analysis. Moreover, the combination of twisters with different coatings may extend the range of polarity of the compounds to be determined, which increases the total number of determined compounds. In this sense, Ochiai, Sasamoto, Ieda, David, and Sandra (2013) obtained better recovery percentages with the combined use of PDMS and EG-Silicone twisters.

SBSE has been widely used for analysing volatile and semi-volatile compounds in wines (Zalacain et al., 2007), and HSSE has also been successfully applied for this purpose (Callejón et al., 2010; Weldegergis, Tredoux, & Crouch, 2007). An advantage of the HSSE method is an increase in the lifetime of the stir bar. The SBSE method extracts a large amount of aromatic compounds from samples, but HSSE has been shown to be more efficient in extracting compounds that are more volatile, such as methyl acetate, acetaldehyde diethylacetal, and ethyl 2-methylbutyrate among others (Callejón et al., 2010). Therefore, using both extraction methods, i.e., by immersion and in the headspace, to analyse the aroma may extend the volatility range of the extracted compounds.

The goal of this work is to determine for the first time the volatile composition of Chilean sparkling wines produced by the Charmat and traditional methods. For this purpose, a method for determining a large number of compounds is established by comparing the use of EG-Silicone and PDMS polymeric phases, both by immersion, as well as in the headspace, and by a simple and sequential extraction procedure combining both coatings.

2. Material and Methods

2.1. Reagents, materials and samples

Ethanol, methanol, and acetonitrile, which were used for the twister cleaning procedure, and 4-methyl-2-pentanol (internal standard) were purchased from Merck (Darmstadt, Germany). Sodium chloride was obtained from Sigma–Aldrich (Madrid, Spain).

The polymeric phases employed for this study were polyethyleneglycol-modified silicone (EG-Silicone) and polydimethylsiloxane (PDMS). These materials were obtained from Gerstel (Müllheim and der Ruhr, German). The length of EG-Silicone Twisters was 10 mm, and they had a 32 μL coating; the length of the PDMS Twisters was 10 mm, and they had a 24 μL (0.5 mm) coating.

Sixteen Chilean sparkling wines were analysed; eight were produced by the Charmat method and eight by the traditional method. These wines were donated by six main wineries producing Chilean sparkling wines. The Chilean wines came from four different production zones: Leyda, Casablanca, Curicó, and Maipo. Among the sparkling wines analysed were monovarietal wines (Pinot noir, Chardonnay) and varietals wines (Chardonnay/Pinot noir and Chardonnay/Pinot noir/Semillon).

In addition, to test different sampling procedures, a representative sparkling wine was used. This sample was a common sparkling wine made using Chardonnay and Pinot meunier grapes by the traditional method.

2.2. Sampling procedures

Two sampling procedures, i.e., headspace (HSSE) and immersion (SBSE), were tested. In these assays, two different polymeric phases, i.e., polydimethylsiloxane (PDMS) and Ethylene glycol (EG-Silicone), were used. Moreover, two types of sequential extraction methods were carried out using two twisters in each sample, i.e., first SBSE and then HSSE. In these methods, we combined the use of PDMS and EG-Silicone twisters in the following manner: SBSE-EG-Silicone/HSSE-PDMS and SBSE-PDMS/HSSE-EG-Silicone.

In all cases, 7.5 mL of the sample were placed in a 20 ml vial, and 2.25 g of NaCl (30%) plus 10 μL of the internal standard 4-methyl-2-pentanol (405 mg/L) were added. A special device made of stainless wire was designed to maintain the integrity and to extend the shelf life of the polymer as much as possible. This device was fixed to the septum of the stopper. The extraction by immersion was performed by placing the twister in the stainless wire device and stirring the sample with a conventional magnetic stir bar (non-coated stir bar) for one hour at 200 rpm at room temperature. The headspace extraction was performed by placing a new twister in an open glass insert inside the vial and heating the sample in a water bath at 62 °C for one hour (Callejón et al., 2010). In both cases, the vial was tightly capped and, after extraction, the stir bar was removed with tweezers, rinsed with Milli-Q water, and dried with a lint-free tissue paper. Then, it was thermally desorbed in a gas chromatograph/mass spectrometer (GC/MS).

2.3. Thermal desorption and GC–MS conditions

Gas chromatography analysis was carried out using a 6890 Agilent GC system coupled to an Agilent 5975 inert quadrupole mass spectrometer and equipped with a thermo desorption system (TDS2) and a cryo-focusing CIS-4 PTV injector (Gerstel). The thermal desorption was performed in splitless mode with a flow rate

of 70 mL/min. The desorption temperature program was the following: The temperature was held at 35 °C for 0.1 min, ramped at 60 °C/min to 210 °C, and then held for 5 min. The temperature of the CIS-4 PTV injector, with a Tenax TA inlet liner, was held at −35 °C using liquid nitrogen for the entire desorption time and was then raised at 10 °C/s to 260 °C and held for 4 min. The solvent vent mode was used to transfer the sample to the analytical column. A CPWax-57CB column with dimensions of 50 m × 0.25 mm and a film thickness of 0.20 μm (Varian, Middelburg, Netherlands) was used, and the carrier gas was He at a flow rate of 1 mL/min. The oven temperature program was the following: The temperature was held at 35 °C for 4 min and then raised to 220 °C at 2.5 °C/min (held for 15 min). The quadrupole, source, and transfer line temperatures were maintained at 150 °C, 230 °C, and 280 °C, respectively. Electron ionization mass spectra in the full-scan mode were recorded at 70 eV with a scan range from *m*/*z* 18 to 300 for the extraction assays and between *m*/*z* 29 and 300 amu for the samples.

All data were recorded using MS ChemStation. The samples were analysed in triplicate, and blank runs using an empty glass tube were performed before and after each analysis.

2.4. Compound identification and data processing

Compound identification was based on mass spectra matching using the standard NIST 98 library and the retention index (LRI) of authentic reference standards. The relative area was calculated by dividing the peak area of the target ion of each compound by the peak area of the target ion of the internal standard. To compare the different sampling modes, we normalized the relative area (NRA) of different compounds with respect to the mean values obtained using the HSSE-PDMS method (Table 1). When the peak areas resulting from the HSSE-PDMS method were below quantification or detection limits, we normalized the data with respect to the lowest relative area value for this compound.

2.5. Statistical analyses

Analysis of variance (ANOVA) and multivariate analysis of data including principal component analysis (PCA) and linear discriminant analysis (LDA) with leave-one-out cross-validation were performed using the Statistica (version 7.0) software package (Statsoft, Tulsa, USA).

3. Results and Discussion

First of all, several extraction procedures were tested to establish a method that allows for the determination of a large number of compounds. Then, the study of the volatile compositions of Chilean sparkling wines was performed.

3.1. Selection of the extraction method for sparkling wines

Currently, the routine sampling method used for analysis of volatile compounds in our lab is HSSE employing PDMS twisters, which has obtained successful results (Callejón et al., 2010). However, we proposed to verify if it was possible to improve the sensitivity of this method in determining compounds from the aroma of sparkling wines.

In comparing the different sampling methods, we have taken into account the total sum of the compounds determined (i.e., the number of compounds with areas greater than the quantification limits) and the values of the relative area because these are the parameters that we will use in the study of the volatile compounds in sparkling wines. Additionally, we also considered the amount of water in each analysis because the EG-Silicone twister retains water.

We compared the EG and PDMS polymeric phases using both immersion, as well as headspace techniques. The combined use of both coatings was also tested. These assays were conducted by sequential extractions by immersion and headspace.

3.1.1. Comparison of PDMS and EG-Silicone twisters

In the headspace, the results showed that by using a PDMS polymeric phase, 30 compounds were detected, and 28 compounds were detected by using the EG-Silicone phase (Table 1). These compounds consisted of aldehydes, alcohols, esters, ketones, lactones, and C_{13}-norisoprenoids. Additionally, the values of the relative area of different compounds obtained using HSSE-PDMS were greater compared with using HSSE-EG-Silicone. Therefore, for the extraction in the headspace, the PDMS polymeric phase turned out to be better than EG-Silicone.

Our results were opposite to those of Sgorbini et al. (2012), who obtained better results using the EG-Silicone polymeric phase compared with PDMS in different matrices. Conversely, our results showed that HSSE-EG-Silicone was only a better extraction technique compared with HSSE-PDMS for three alcohols (isobutanol, 1-butanol, and *cis*-3-hexenol).

However, when the extraction was carried out by immersion, the use of the EG-Silicone twister improved the sensitivity, in that 39 compounds were determined and only one was below the detection limit (acetoin). In contrast, with the PDMS twister, only 30 compounds had peak areas greater than the quantification limits (Table 1). The values of the relative areas of the alcohols and the volatile phenols were observed to be greater in the extraction using EG-Silicone, and esters were greater in the case of PDMS. Acetoin was not detected in either case. Our results were in agreement with that of Sgorbini et al. (2012) and Ochiai et al. (2013), except for 2-methylpyrazine, 2-furfuraldehyde, and 1-hexanol.

3.1.2. Comparison between HSSE and SBSE

Different phenomena are involved in these two extraction processes. In HSSE, the recovery of the analyte is conditioned by its volatility and distribution within the matrix, headspace, and sorbent polymer (Sgorbini et al., 2012). Conversely, in SBSE, the recovery depends on the sorption of the analyte onto the extraction polymeric phase and diffusion within the polymer (Baltussen, Sandra, David, & Cramers, 1999). In a simple extraction and independent of the type of polymeric phase used, we observed greater relative areas for most of the compounds when the extraction was performed by immersion as opposed in the headspace, especially in the cases of 2-phenylethanol, diethyl succinate, diethyl malate, ethyl-3-hydroxydodecanoate (tentatively identified), 2-phenylethyl acetate, isoamyl lactate (tentatively identified), and β-damascenone (Table 1).

HSSE was a better extraction technique for isobutanol, ethyl acetate, ethyl decanoate, and 5-hydroxymethylfurfuraldehyde. In the case of the first two compounds, the reason for the greater extraction might be due to the high volatility because these compounds are the most volatile in their corresponding chemical groups. However, we were surprised in the case of 5-hydroxymethylfurfuraldehyde due to its low volatility.

3.1.3. Comparison of different sequential extraction methods

In these extraction assays, the extractions by immersion and in the headspace were performed using two sequential steps and not simultaneously because several authors have observed that a high temperature may decrease the extraction efficiency and reproducibility of extraction by direct immersion (Prieto Basauri, Rodil, Usobiaga, Fernandez, Etxebarria, & Zuloaga, 2010).

The SBSE-EG-Silicone/HSSE-PDMS method was more sensitive than the other assayed methods because a larger number of compounds was determined (40). When using the SBSE-PDMS/

Table 1
Comparative of determination of volatile compounds by different extraction methods. Peak relative area normalized respect to HSSE-PDMS.

Compound	HSSE-PDMS	HSSE-EG-Silicone	SBSE-PDMS	SBSE-EG-Silicone	SBSE-EG-Silicone/HSSE-PDMS	SBSE-PDMS/HSSE-EG-Silicone
Aldehydes						
Benzaldehyde	1	0.24	1.06	0.54	0.36	0.50
2-Furfuraldehyde	1	0.14	0.49	0.43	1.03	0.26
5-Methyl-2-furfuraldehyde[1]	nq	nq	nq	1.83	3.45	1
5-Hydroxymethylfurfural	1	0.50	nq	0.30	8.58	0.49
Alcohols						
Isobutanol	1	1.92	0.43	1.46	0.82	2.34
1-Butanol	1	1.19	nq	1.48	1.16	1.99
3-Methyl-1-butanol	1	0.69	0.77	1.20	0.94	1.07
1-Hexanol	1	0.52	1.31	1.92	1.35	0.92
cis-3-Hexenol[2]	nq	1	nq	4.27	3.02	1.93
Furfuryl alcohol	1	0.16	0.51	0.66	0.85	0.33
Benzyl alcohol[3]	nd	nq	nq	1.43	1	nd
2-Phenylethanol	1	0.36	14.6	38.7	25.8	8.60
Ethyl Esters						
Ethyl acetate	1	0.21	0.47	0.12	0.23	0.81
Ethyl propanoate	1	0.19	0.67	0.20	0.17	0.38
Ethyl isobutyrate	1	0.16	1.05	0.22	0.23	0.41
Ethyl butyrate	1	0.19	1.15	0.28	0.27	0.48
Ethyl 2-methylbutyrate	1	0.19	1.38	0.26	0.25	0.40
Ethyl isovalerate	1	0.47	1.28	0.24	0.21	0.36
Ethyl hexanoate	1	0.15	1.56	0.31	0.19	0.38
Ethyl lactate	1	0.37	0.93	1.17	0.94	0.90
Ethyl octanoate	1	0.24	1.88	0.40	0.22	0.49
Ethyl furoate	1	nq	2.44	1.35	0.77	0.93
Ethyl decanoate	1	0.20	0.94	0.19	0.11	0.23
Diethyl succinate	1	0.08	9.96	4.07	2.59	3.59
Ethyl-9-decanoate	1	0.27	2.28	0.45	0.22	0.80
Ethyl phenylacetate[3]	nq	nq	7.24	1.61	1	2.36
Diethyl malate[4]	nd	nd	1	11.5	8.25	1.14
Ethyl-3-hydroxydodecanoate	1	nd	12.9	4.43	1.93	2.94
Acetic Esters						
Isoamyl acetate	1	0.18	1.53	0.31	0.21	0.49
Hexyl acetate	1	0.13	1.59	0.28	0.31	0.44
2-Phenylethyl acetate	1	0.18	9.17	2.41	1.42	3.78
Others Esters						
Isoamyl lactate	1	0.13	2.94	2.36	1.64	1.44
Ketones						
2-Nonanone	1	0.27	3.26	1.61	0.56	0.86
Acetoin[1]	np	np	np	Np	2.12	1
Lactones						
γ-butyrolactone	1	0.20	0.64	1.13	0.79	0.44
C_{13}-Norisoprenoinds						
β-Damascenone	1	np	3.94	1.18	0.65	2.08
Volatile Phenols						
Guaiacol	np	np	nq	1.18	1	np
4-Vinylphenol	np	np	nq	33.4	23.1	1
4-Vinylguaiacol	np	np	np	5.08	4.10	1
Others						
2-Methylpyrazine	nq	np	nq	4.45	1	nq
Total detected compounds	30	28	30	39	40	37
Water	1	1.46	0.70	1.67	1.82	1.43

Peak relative area normalized respect to the lowest relative area value for this compound: [1]SBSE-PDMS/HSSE-EG-Silicone; [2]HSSE-EG-Silicone; [3]SBSE-EG-Silicone/HSSE-PDMS; [4]SBSE-PDMS.
For each compound, the highest values are underlined.
np: no peak; nd: below detection limit (a signal-to-noise ratio higher than or equal to 3); nq: below quantification limit (a signal-to-noise ratio higher than or equal to 10).

HSSE-EG-Silicone method, we found 37 volatile compounds that were above the quantification limits (Table 1).

In the double extraction experiments, when the PDMS twisters were immersed into the sample, we obtained the greatest values of the relative area for 23 compounds, whereas we obtained the greatest values for only 17 when we used PDMS in the headspace and EG-Silicone by immersion. The first procedure was the best for esters, and the second procedure was the best for alcohols, volatile

phenols, and aldehydes of the furfural group. This observation was very interesting because the determination of esters can allow for easy differentiation of sparkling wines produced using the traditional method (higher quality) from sparkling wines produced using a faster method, such as the Charmat method (Caliari et al., 2015).

3.1.4. Comparison of simple and sequential extractions

In general, double extraction techniques were better compared with simple extraction techniques with respect to the number of determined compounds, with the exception of SBSE-EG-Silicone. Therefore, if we compared the best double (SBSE-EG-Silicone/HSSE-PDMS) extraction method and the simple SBSE-EG-Silicone extraction method, the only difference was in one compound, i.e., acetoin. This compound can only be determined by sequential extraction methods (Table 1). However, with respect to the values of the relative area, the simple method resulted in better results.

3.1.5. Extraction method for sparkling wines

The results above demonstrated that the best extraction method was SBSE-EG-Silicone/HSSE-PDMS. It is important to note that the use of EG-Silicone twisters has a disadvantage in the large amount of water it retains. When we monitored the water, we observed significant quantities in all of the extraction methods that used the EG-Silicone twisters. Therefore, the greatest amount of water was retained using the SBSE-EG-Silicone/HSSE-PDMS method, a little less with SBSE-EG-Silicone, d the lowest value with the SBSE-PDMS method.

We tried different manufacturer recommendations for water removal, but the results did not improve, and the content of water remained high.

Therefore, we had to select a sampling method that improved the sensitivity with a low water background. The method that fulfilled these requirements was SBSE-PDMS.

Finally, we carried out a comparison study testing the double extraction of the headspace and by immersion with two PDMS twisters. Here, the peak relative areas were normalized with respect to SBSE-PDMS. When we compared the simple extraction method using SBSE-PDMS with the sequential extraction method using SBSE-PDMS/HSSE-PDMS, we observed similar low quantities of water in both methods. In the former case, 5 compounds presented peak areas below the detection limit. Moreover, except for one compound (methyl decanoate, NRA = 1), we obtained higher relative area values with the sequential extraction method than with the simple extraction method (Fig. 1). The most remarkable result was that in the sequential sampling method, most of the compounds had peaks with double or higher values of the relative area (>65% of compounds). Therefore, the double extraction method was more sensitive than the SBSE-PDMS method, and it was selected to determine the volatile composition of sparkling wines. This selected extraction method was in house validated. Sensitivity, intermediate precision and recovery percentage were calculated for a number of compounds representatives of each different chemical group. Good recovery results were obtained ranged 94.6–110.9%. Intermediate precision was evaluated by analysing a sample six different days; RSD values were acceptable for most of the compounds. Quantification of limits (LOQ), calculated as signal-to-noise ratio higher than or equal to 10, were bellow 5 μg/L for most of the compounds. A summary with some representative results of method validation are included in Table S1.

3.2. Volatile composition of Chilean sparkling wines

In the general volatile profiles of Chilean sparkling wines, 130 compounds were determined. These compounds belonged to different chemical groups: Ethyl, acetic and other esters, alcohols,

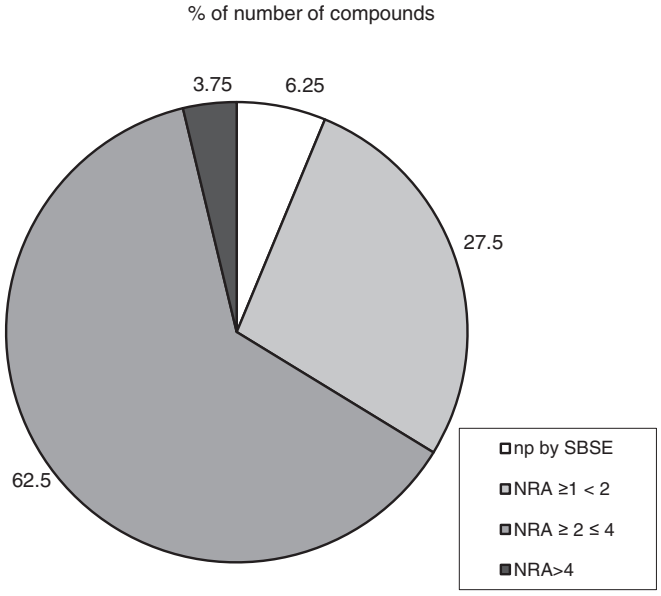

Fig. 1. Comparison of simple and sequential extraction methods with PDMS twisters.

acids, aldehydes, acetals, aldehydes, terpenes, C_{13}-norisopronoids, lactones, and volatile phenols (Table 2). 78 compounds were positively identified through the comparison of LRI and mass spectral data with those of authentic standards, and 19 compounds were tentatively identified through the comparison of mass spectral data with a database and LRI with the literature (Table 2). The chemical group of esters had the major number of compounds in both types of sparkling wines, followed by alcohols and acids. Within the esters, most of them were ethyl esters (31%). These compounds are mainly produced during alcoholic fermentation by yeasts, in reactions between alcohols and acetyl-CoA and contribute to the fruity and flowery character of wine (Mamede, Cardello, & Pastore, 2005). Among the ethyl esters, the major compounds determined were ethyl octanoate, diethyl succinate, ethyl hexanoate, ethyl butyrate, and ethyl acetate (in descending order of the relative area values).

The alcohols that exhibited higher relative areas were 3-methyl-1-butanol, 2-methyl-1-butanol, and 2-phenylethanol (Table 2). These alcohols are important products of alcoholic fermentation (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006).

With regards to the acids, octanoic, hexanoic, and decanoic acids were the major compounds determined. These compounds are responsible for the rancid and cheesy aromatic notes of wine (Caliari et al., 2015).

Minor compounds are also important contributors to wine aroma, as in the case of terpenes, which contribute to the diversity and complexity of wine and are also varietal aromas (Ganss et al., 2011). In the analysed wines, the main terpenes found were α-terpineol and geraniol.

In the aldehyde group, furfuraldehyde was the predominant compound, and 2-hydroxycyclopent-2-en-1-one and 2-nonanone were the main compounds in the ketone group (Table 2). We note that 2-hydroxycyclopent-2-en-1-one had been described before in wines (Welke, Manfroi, Zanus, Lazarotto, & Alcaraz Zini, 2012) but not in sparkling wines.

Others significant compounds found were cyclotene (3-methyl-2-cyclopenten-2-ol-l-one) and the furan derivative coumaran (2,3-dihydrobenzofuran). Cyclotene has a strong caramellic-maple aroma that is similar to furaneol. This volatile compound

Table 2
Ranges of peak relative areas of Chilean sparkling wines.

Volatile Compounds	ID	LRI	TRADITIONAL	CHARMAT
Ethyl esters				
Ethyl propionate	A	927	0.08–0.17	0.10–0.16
Ethyl 2-methylpropanoate	A	938	0.02–0.24	0.01–0.12
Ethyl butyrate	A	1003	1.05–1.87	1.03–1.45
Ethyl 2-methylbutyrate	A	1016	0.10–0.33	0.04–0.07*
Ethyl isovalerate	A	1031	0.19–0.41	0.08–0.13*
Ethyl valerate	A	1096	0.003–0.006	0.003–0.004
Ethyl 2-butenoate	B[1]	1127	0.03–0.07	0.03–0.05
Ethyl hexanoate	A	1207	3.8–9.5	5.6–8.9
Ethyl 3-hexanoate	A	1289	0.002–0.009	0.001–0.005
Ethyl 3-ethoxypropionate	C	1316	0.002–0.007	nd-0.008
Ethyl heptanoate	A	1318	0.004–0.018	0.003–0.007
Ethyl lactate	A	1349	0.53–5.20	0.66–3.93
Ethyl octanoate	A	1418	8.2–18.6	6.8–12.0
Ethyl nonanoate	A	1521	nd-0.007	0.001–0.004
Ethyl 2-hydroxy-4-methylpentanoate	B[2]	1537	0.05–0.08	0.03–0.29
Ethyl 3-(methylthio)propionate	C	1551	0.003–0.007	0.002–0.004*
Ethyl furoate	A	1605	0.08–0.23	0.05–0.15*
Ethyl decanoate	A	1632	0.28–1.50	0.64–1.29
Diethyl fumarate	C	1634	0.003–0.026	0.001–0.005*
Ethyl benzoate	A	1649	0.008–0.019	0.004–0.009*
Diethyl succinate	A	1676	6.8–21.7	2.75–5.04*
Ethyl 9-decenoate	B[2,3]	1683	0.01–0.23	0.02–0.13
Diethyl glutarate	B[4]	1777	0.06–0.12	0.03–0.06*
Ethyl benzeneacetate	A	1778	0.05–0.13	0.04–0.08*
Ethyl dodecanoate	A	1841	nd-0.04	0.006–0.019
Diethyl 2-hydroxy-3-methylbutanedioate	C	1858	0.02–0.10	0.003–0.008*
Diethyl malate	B[5]	2056	0.44–1.00	0.10–0.42*
Ethyl-3-hydroxydodecanoate	C	2116	0.13–0.30	0.07–0.15*
Ethyl cinnamate	A	2141	0.002–0.026	0.005–0.012
Ethyl hexadecanoate	A	2266	0.005–0.553	0.01–0.05
Total sum of ethyl esters			21.9–29.3	25.5–47.1
Acetic esters				
Ethyl acetate	A	873	1.38–2.17	1.6–2.8*
Propyl acetate	A	942	nd	nd-3.2*
Isobutyl acetate	A	955	0.01–0.02	0.04–0.07*
Isoamyl acetate	A	1089	0.15–1.18	2.6–5.3*
Hexyl acetate	A	1259	nd-0.30	0.45–1.18*
cis-3-Hexenyl acetate	A	1289	nd-0.01	0.01–0.05*
Phenylmethyl acetate	A	1718	nd-0.002	nd-0.004*
2-Phenylethyl acetate	A	1811	0.05–0.88	0.97–3.00*
Total sum of acetic esters			6.3–10.9	1.8–3.9
Methyl esters				
Methyl hexanoate	A	1147	nd-0.02	0.005–0.018
Methyl octanoate	C	1418	0.01–0.02	0.01–0.02
Methyl decanoate	A	1584	nd-0.005	0.002–0.013
Methyl salicylate	C	1761	0.002–0.022	0.003–0.011
Total sum of methyl esters			0.02–0.05	0.03–0.06
Isoamyl esters				
Isoamyl butanoate	C	1240	0.005–0.012	0.006–0.013
Isoamyl hexanoate	C	1458	0.01–0.03	0.007–0.018*
Isoamyl lactate	B[2]	1566	0.005–0.081	0.01–0.06
Isoamyl octanoate	A	1653	0.01–0.02	nd-0.033
Total sum of isoamyl esters			0.03–0.11	0.04–0.12
Other esters				
Furfuryl formate	C	1261	0.009–0.150	0.005–0.011*
Propyl hexanoate	C	1288	0.003–0.008	0.002–0.005
(4E)-4-Hexenyl hexanoate	C	1297	nd-0.02	0.01–0.08*
Vinyl octanoate	C	1501	0.01–0.18	0.02–0.05
Vinyl decanoate	C	1718	0.001–0.051	0.005–0.013
Hexyl salicylate	C	2212	0.007–0.017	0.009–0.017
Total sum of other esters			0.07–0.16	0.06–0.41
Alcohols				
1-Propanol	A	1019	0.37–0.92	0.04–0.85
Isobutanol	A	1081	0.06–0.13	0.08–0.25*
1-Butanol	A	1165	0.04–0.08	0.05–0.09
2-Methyl-1-butanol	A	1221	0.85–1.33	1.1–1.7*
3-Methyl-1-butanol	A	1240	7.6–13.5	6.9–13.0
3-Methyl-1-pentanol	C	1335	0.02–0.06	0.03–0.05
1-Hexanol	A	1362	0.59–1.25	0.56–1.30
cis-3-Hexen-1-ol	A	1387	0.01–0.04	0.01–0.04
Heptanol	A	1458	0.007–0.079	0.007–0.019

Table 2 (*continued*)

Volatile Compounds	ID	LRI	TRADITIONAL	CHARMAT
2-Ethyl-1-hexanol	A	1488	0.03–0.06	0.02–0.08
1-Octanol	A	1559	0.02–0.05	0.02–0.04
Furfuryl alcohol	A	1664	0.05–0.11	0.07–0.11
3-(Methylthio)-1-propanol	B[6]	1728	0.003–0.009	0.002–0.034
1-Decanol	A	1769	0.01–0.04	0.008–0.023
1-Undecanol	B[4]	1875	0.04–0.49	0.03–0.07*
Benzyl alcohol	A	1886	0.006–0.016	0.004–0.018
2-Phenylethanol	A	1926	3.0–4.3	3.2–4.9
1-Dodecanol	B[7]	1989	0.03–0.05	0.02–0.04
Total sum of alcohols			16.4–35.8	25.2–35.9
Acids				
Acetic acid	A	1452	0.16–0.38	0.23–0.55
Formic acid	C	1505	0.02–0.06	0.02–0.06
Propanoic acid	A	1540	0.01–0.03	0.02–0.03
Isobutyric acid	A	1569	nd-0.06	0.02–0.13
Pentanoic acid	A	1742	0.005–0.008	0.004–0.011
Hexanoic acid	A	1853	1.4–2.3	1.5–2.7
2-Ethylhexanoic acid	C	1956	nd-0.02	0.009–0.066
Heptanoic acid	A	1960	0.01–0.02	0.008–0.040
Octanoic acid	A	2086	5.3–12.0	8.3–16.4
Sorbic acid	C	2151	nd-0.06	nd-4.9
Nonanoic acid	A	2180	0.06–0.11	0.04–0.15
Decanoic acid	A	2299	0.89–4.06	2.4–7.9*
9-Decenoic acid	B[7]	2353	0.05–0.71	0.07–0.42
Undecanoic acid	C	2392	0.007–0.061	0.006–0.011
Ethoxy-4-oxobutanoic acid	C	2405	0.09–0.28	0.05–0.12*
Dodecanoic acid	B[4]	2466	0.05–0.09	0.03–0.16
Tetradecanoic acid	C	2604	0.05–0.12	0.03–0.13
Pentadecanoic acid	C	2699	0.008–0.056	0.006–0.064
Hexadecanoic acid	C	2817	0.02–0.19	0.01–0.22
Total sum of acids			15.2–33.6	8.4–19.8
Aldehydes				
Hexanal	A	1043	nd-0.002	nd-0.004
Furfuraldehyde	A	1449	0.07–0.16	0.09–0.14
Benzaldehyde	A	1505	0.006–0.024	0.003–0.018
5-Methylfurfural	A	1565	0.006–0.013	0.005–0.0013
Hexylcinnamaldehyde	C	2368	0.003–0.008	0.003–0.006
5-Hydroxymethylfurfural	A	2492	0.002–0.011	0.002–0.009
Total sum of aldehydes			0.13–0.18	0.10–0.20
Acetals				
Acetaldehyde diethylacetal	A	878	0.07–1.17	0.37–1.46
2,4,5-Trimethyl-1,3-dioxolane	C	916	0.008–0.143	0.03–0.13
Acetaldehyde ethyl amyl acetal	C	1074	0.01–0.18	0.003–0.178
Total sum of acetals			0.46–1.72	0.09–0.15
Ketones				
4-Methyl-2-pentanone	C	971	0.004–0.005	0.004–0.005
2,6-Dimethyl-4-heptanone	A	1137	0.007–0.011	0.007–0.011
2-Heptanone	C	1151	0.003–0.025	0.01–0.02
Acetoin	A	1280	0.005–0.014	0.01–0.04*
Acetol	A	1289	0.03–0.09	0.04–0.07
6-Methyl-5-hepten-2-one	A	1316	0.006–0.016	nd-0.01
2-Nonanone	A	1365	nd-0.154	0.05–0.13
2-Acetylfuran	A	1496	0.008–0.020	nd-0.198
Acetophenone	A	1640	0.007–0.025	0.005–0.009*
2-Hydroxycyclopent-2-en-1-one	C	1779	0.04–0.09	0.05–0.12
Total sum of ketones			0.26–0.46	0.12–0.35
Terpenes				
Limonene	A	1142	0.005–0.055	0.003–0.028
cis-Linalool oxide	B[8]	1442	0.002–0.033	0.002–0.010*
trans-Linalool oxide	B[8]	1469	0.009–0.014	0.004–0.008*
Linalool	A	1540	nd-0.01	0.009–0.105
Hotrienol	C	1603	0.003–0.016	0.004–0.050
α-Terpineol	A	1704	0.006–0.038	0.01–0.14
Geraniol	A	1855	0.01–0.02	0.01–0.03
γ-Eudesmol	B[4]	2184	0.007–0.030	0.004–0.011*
Total sum of terpenes			0.07–0.36	0.09–0.18
C_{13}-norisoprenoids				
TDN	C	1721	0.01–0.03	0.007–0.021*
β-Damascenone	A	1817	0.003–0.089	0.002–0.099
β-Ionone	A	1945	nd-0.05	0.002–0.008
Total sum of C_{13}-norisoprenoids			0.04–0.12	0.01–0.16

(*continued on next page*)

Table 2 (*continued*)

Volatile Compounds	ID	LRI	TRADITIONAL	CHARMAT
Lactones				
Cyclotene	B[4]	1844	0.006–0.013	0.007–0.015
γ-Decalactone	A	2151	0.008–0.149	0.009–0.025
Total sum of lactones			0.02–0.04	0.02–0.04
Volatile phenols				
Guaiacol	A	1855	nd-0.004	0.001–0.003
4-Ethylguaiacol	A	2034	nd-0.25	nd-0.009
Eugenol	A	2175	0.001–0.009	nd-0.003
4-Ethylphenol	A	2184	nd-0.216	0.002–0.009
4-vinylguaiacol	B[4]	2208	0.008–0.024	0.01–0.03*
Coumaran	C	2408	0.01–0.02	0.01–0.05*
Total sum of volatile phenols			0.04–0.11	0.05–0.49
Miscellaneous				
Methylpyrazine	B[4]	1260	0.003–0.009	0.004–0.010
Pyrrole	B[4]	1500	0.01–0.02	0.01–1.19
2-Methyltetrahydrothiophen-3-one	B[4]	1521	0.009–0.020	0.009–0.032

nd: values under detection limits.
ID: reliability of identification: A, mass spectrum and LRI agreed with standards; B, mass spectrum agreed with mass spectral data base and LRI agreed with the literature data: Hwan and Chou (1999), Pino and Queris (2011), Bosch-Fusté et al. (2007), National Center for Biotechnology Information (2005), Lee and Noble (2003), Miranda-Lopez, Libbey, Watson, and McDaniel (1992), Li et al. (2008) and Loscos, Hernandez-Orte, Cacho, & Ferreira (2007); C, mass spectrum agreed with mass spectral data base.
LRI: Linear Retention Index.
* There is significant difference ($P = 0.05$) with the Traditional Chilean sparkling wines.

was determined for first time in these types of wines probably due to the increased sensitivity of the sequential extraction procedure. Coumaran, as far as we know, has never been described before in these sparkling wines but has been described, for example, in South African red wines (Weldegergis, Crouch, Górecki, & De Villiers, 2011) and more recently, in Verdejo white wines (Sánchez-Palomo, Alonso-Villegas, & González-Viñas, 2015).

3.2.1. Comparison of the volatile profiles of Chilean sparkling wines produced by traditional and Charmat methods

The different production methods, i.e., traditional and Charmat, led to several differences in the obtained products that can affect the aroma profile. In terms of the total sum of the relative area of each chemical group, ethyl esters were significantly higher in the wines produced by the traditional method, while acetic esters and ketones were predominant in those made by the Charmat method. In particular, 100% of the determined acetic esters presented relative area values significantly higher in the Charmat sparkling wines (Table 2). This was in agreement with the previous results of Riu-Aumatell et al. (2006), where the acetate concentration decreased along the ageing time of cava in contact with lees. Among the acetates, isoamyl, hexyl, isobutyl, and 2-phenylethyl acetates doubled their values of the relative area in some cases. These compounds give fruity nuances to wines, except for the last one which gives a rose odour (Caliari et al. 2015; Li, Tao, Wang, & Zhang, 2008).

Regarding the ethyl esters, ethyl 2-methyl-butyrate, ethyl isovalerate, diethyl succinate, diethyl 2-hydroxy-3-methylbutanedioate, and diethyl malate exhibited the lowest values in traditional sparkling wines but were clearly superior to the highest values in the Charmat wines (Table 2). Diethyl succinate is one of the widely reported fermentative volatile

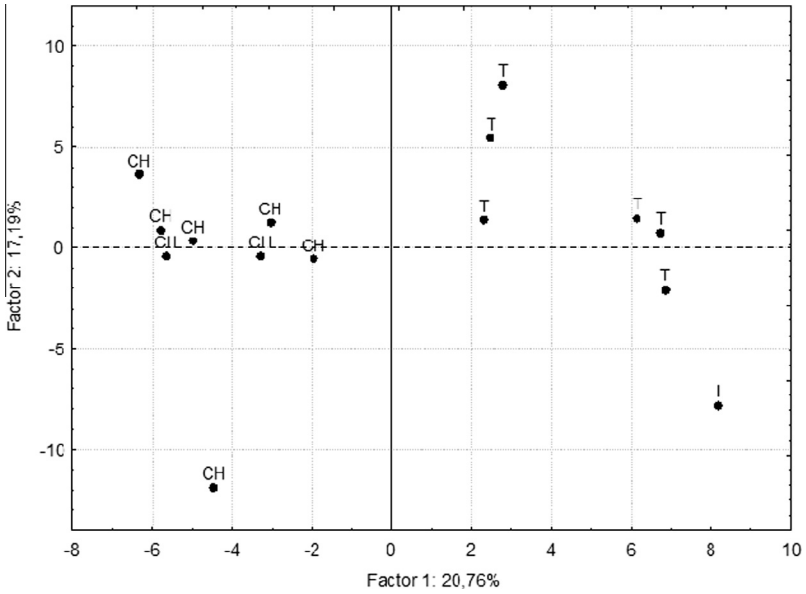

Fig. 2. Data scores plot on the plan made up of the first two principal components (PC1 against PC2) for Chilean sparkling wine.

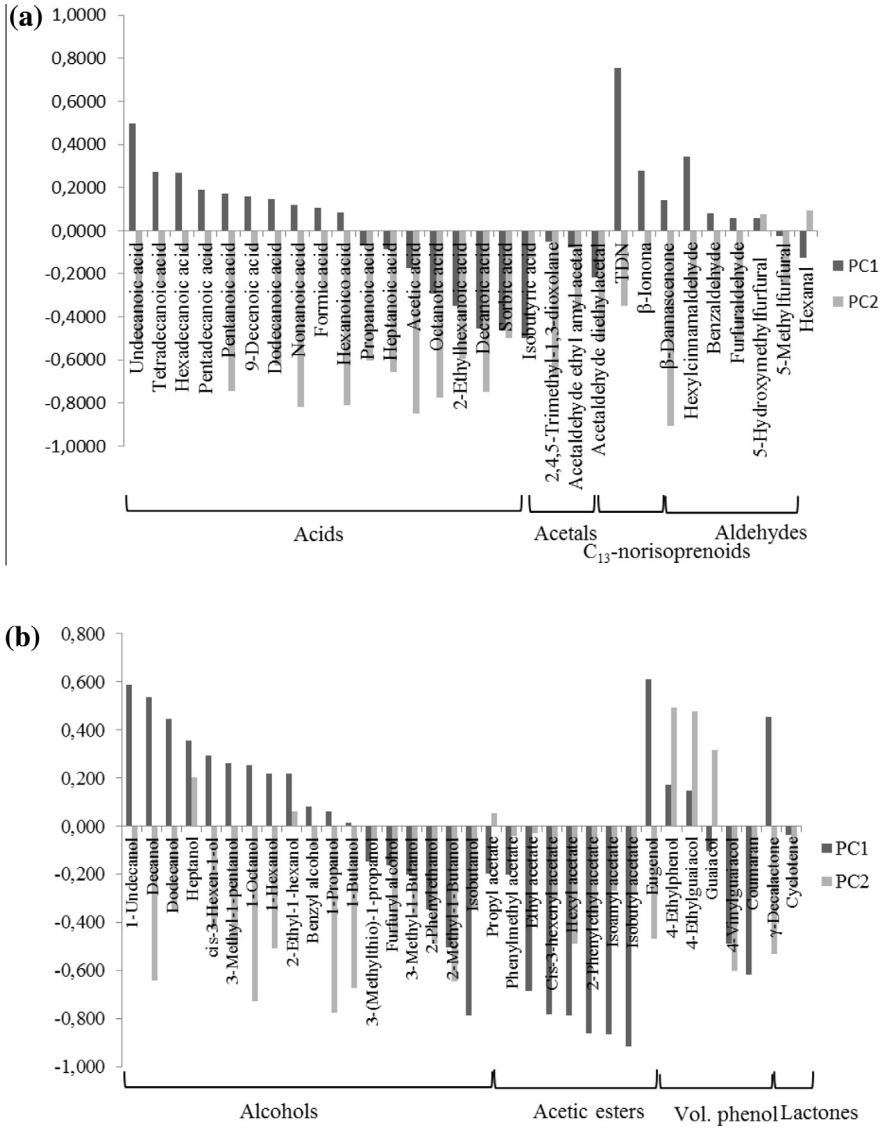

Fig. 3. Variable loading plots on the planes made up of the first two principal components (PC1 against PC2).

compounds formed during the ageing of cava in contact with lees (Riu-Aumatell et al., 2006).

The alcohols, i.e., isobutanol, 2-methyl-1-butanol, and 1-undecanol, showed significant differences between the two types of production methods. Isobutanol and 2-methyl-1-butanol had higher relative areas in sparkling wines produced by the Charmat method, and 1-undecanol exhibited a higher value in wines produced by the traditional method.

With respect to the ketones, the values of acetoin were remarkably superior when the second fermentation was carried out in hermetically sealed tanks. A contrary trend was observed for acetophenone.

Terpenes, i.e., varietal volatiles, have been previously reported to be released during ageing (Gallardo-Chacón et al., 2009). In this study, it was found that *cis* and *trans*-linalool oxides and γ-eudesmol reached higher values in the traditional wines (Table 2). *Cis* and *trans*-linalool oxides are associated with the aroma of flowers, and Caliari et al. (2015) observed a similar trend. Another volatile compound with higher relative areas in the traditional sparkling wines was TDN. This is a varietal C_{13}-norisoprenoid which increases during the ageing of cava (Riu-Aumatell et al., 2006).

The last observed significant difference was the large area exhibited by 4-vinylguaiacol and coumaran in the Charmat wines.

Principal component analysis (PCA) was applied to check if the volatile compounds could group the samples according to their production methods. The first three principal components explained a very low percentage of the cumulative variance (49.2%), and in Fig. 2, it can be seen how the samples are separated by PC1 depending on the production method. In this case, the variables more positively correlated with PC1 and therefore, with traditional sparkling wines, were primary ethyl esters, γ-eudesmol, trans-linalool oxide and TDN, among others. Conversely, the variables more negatively correlated with PC1 and associated with the Charmat production method were acetates, isobutanol, and acetoin. Variable loadings are showed in Fig. 3.

Therefore, PCA confirmed the differences in the volatile profile between traditional and Charmat sparkling wines, as above mentioned. This was probably due to the contact with lees during ageing in the bottle; however, in the case of Charmat, this type of ageing did not exist.

LDA was conducted using the total sum of relative area of the different chemical classes as variables. LDA was performed using the "leave one out" method to check the utility of the discriminate

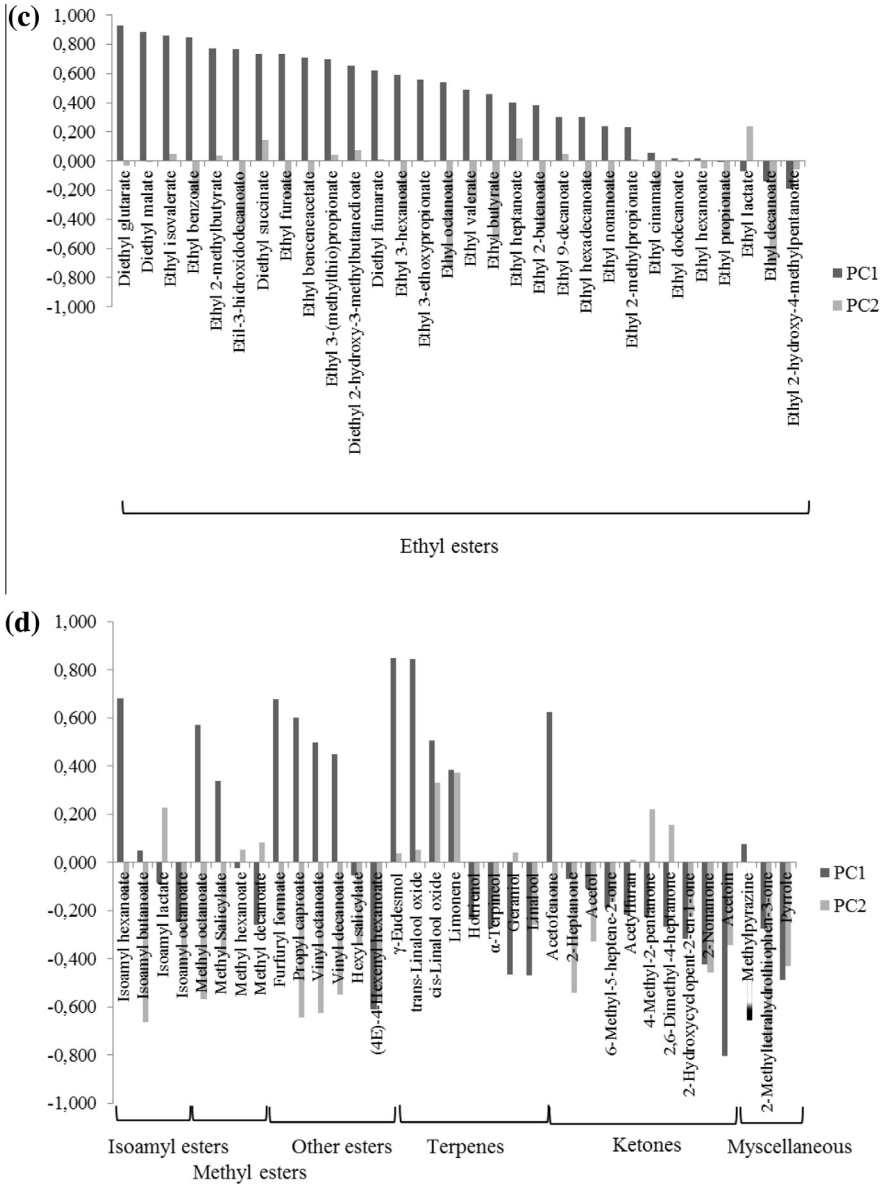

Fig. 3 (*continued*)

Table 3
Discriminant Function Analysis Summary.

Variable	Wilks'λ	Partial λ	F-remove	p-level	Tolerance	1-Tolerance
Total of acetic esters	0.659306	0.116370	75.93315	0.000006	0.676784	0.323216
Total of C_{13}-norisoprenoids	0.106958	0.717319	3.94079	0.075226	0.534704	0.465296
Total of isoamyl esters	0.103172	0.743647	3.44724	0.093021	0.674991	0.325009
Total of alcohols	0.084700	0.905821	1.03970	0.331925	0.578658	0.421341

function to correctly classify new samples. This way, the whole set of samples is divided into two groups: A training set holding all of the samples except for one, which is subsequently used as the test set. Thus, LDA was applied as many times as the number of samples. We applied the LDA forward to the samples considering the method of production as a grouping criterion, variables enclosed in the model by their discriminating power in accordance with Wilks'λ criterion are given in Table 3. We obtained 100% of the correct classification of all samples in all check processes by the "leave one out" method.

4. Conclusions

The comparison of different techniques for the extraction of volatile compounds in sparkling wine demonstrated that the SBSE-EG-Silicone/HSSE-PDMS method was the most sensitive regarding the number of compounds determined. However, due to the problem of the significant amount of water, the use of EG-Silicone twister was not advised. Based on the least amount of water retained and the trade-off between the quantity of compounds determined and their peak relative areas, the chosen

method for the extraction of volatile compounds from sparkling wines was SBSE-PDMS/HSSE-PDMS. In general, esters, alcohols, and acids stand out in the volatile profile of Chilean sparkling wines. The primary difference between the production methods of Chilean sparkling wines were the high presence of ethyl esters in the traditional wines and high amounts of acetic esters and ketones in the Charmat wines. PCA and LDA were able to group and classify the samples according to the production method by considering volatile compounds as variables.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.03.117.

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