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

Ubeda, C.[1,*]; Callejón, R.M.[2]; Troncoso, A.M.[2]; Peña-Neira, A.[3]; Morales, M.L.[2]

[1] Universidad Autónoma de Chile, Chile.

[2]Área de Nutrición y Bromatología. Facultad de Farmacia. Universidad de Sevilla.

C/ P. García González nº 2, E- 41012. Sevilla. Spain.

[3]Departamento de Agroindustria y Enología, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Santiago, Chile.

[*]Corresponding author: c_ubeda@us.es. Tel.: 56-02-23036022

ABSTRACT

The volatile compositions of Charmat and traditional Chilean sparkling wines were studied for the first time. For this purpose, an extraction method was established comparing the use of EG-Silicone and PDMS polymeric phases. 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 high, 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). The difference observed in the volatile composition of País variety sparkling wine showed its utility in the production of these wines to extend the commodities range.

***Keywords*:** Chilean sparkling wine; volatile compounds; SBSE; HSSE; polydimethylsiloxane; polyethyleneglycol-modified silicone.

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 this increased 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. This would allow for the differentiation of quality between these wines and those from other countries and will bring out a further increase in the consumption of this type of wine within and outside the country.

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 (Stefenon, Bonesi, Marzarotto, Barnabé, Spinelli, Webber, & Vanderlinde, 2014; Caliari, Panceri, Rosier, & Bordignon, 2015).

Aroma is one of the most important indicators of sparkling wine quality (Kempt Alexandre, Robillard, & Marchal, 2015). Therefore, due to this and 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, where terpenes, such as limonene, linalool, or lilial have an important role in the overall aroma (Coelho, Coimbra, Nogueira, & Rocha, 2009; Riu-Aumatell, Bosch-Fusté, López-Tamames, & Buxaderas, 2006; Bosch-Fusté, Riu-Aumatell, Guadayol, Caixach, López-Tamames, & Buxaderas, 2007). 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 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 (SBSE) (Zalacain, Marin, Alonso, & Salinas, 2007) or placing the stir bar into the headspace (HSSE) (Callejón, Clavijo, Ortigueira, Troncoso, Paneque, Morales, 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/polyethyleneglycol 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, Cagliero, Cordero, Liberto, Rubiolo, Ruosi, & Bicchi, 2012), and wine (Cacho, Campillo, Viñas, Hernández-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, & 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

(Weldegergis, Tredoux, & Crouch, 2007; Callejón et al., 2010). 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 País) 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 gr 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 62ºC 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 ºC for 0.1 min, ramped at 60 ºC/min to 210 ºC, and then held for 5 min. The temperature of the CIS-4 PTV injector, with a Tenax TA inlet liner, was held at –35 ºC using liquid nitrogen for the entire desorption time and was then raised at 10 ºC/s to 260 ºC 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 x 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 ºC for 4 min and then raised to 220 ºC at 2.5 ºC/min (held for 15 min). The quadrupole, source, and transfer line temperatures were maintained at 150 ºC, 230 ºC, and 280 ºC, 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 29-300 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 el 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/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 (Figure 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 (data not shown).

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, 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 of unknown compounds 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 et al., 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 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 (Li, Tao, Wang, & Zhang, 2008; Caliari et al. 2015).

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 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 the very low percentage of the cumulative variance (49.2%), and in Figure 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 Figure S1.

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. In this multivariate analysis, the sample from the País grape variety was not included. LDA was performed using the "leave one out" method to check the utility of the discriminate 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. First, we applied the LDA standard to the samples considering the method of

production as a grouping criterion, and we obtained 100% of the correct classification of all samples in all check processes by the “leave one out” method.

3.2.2. Comparison of traditional Chilean sparkling wines produced from classical grape varieties versus recently used País grape variety

The traditional sparkling wines analysed in this study have been produced from different varieties of grapes and locations. It is very well known that there are several factors that can influence the volatile profile composition of a sparkling wine, such as variations in the winemaking technology as discussed above, soil, vineyard yield, etc. Nevertheless, the grape employed to produce the wine is one of the most important factors (Pozo-Bayón, Martínez-Rodríguez, Pueyo, & Moreno-Arribas, 2009). The classical grape varieties used to obtain sparkling wines are Chardonnay, Pinot noir, and Riesling; however these days, innovative varieties are being used to produce sparkling wines (Caliari et al., 2014), and this is the case for the País grape variety. This grape is estimated to have arrived to Chile almost 500 years ago and was the first strain grown in this country. Traditionally, this grape has been mixed with other varieties to produce poor quality wines, but today it is beginning to be used in the production of high quality wines. In fact, there is already a commercial sparkling wine made from País grapes produced by the traditional method. This wine was analysed, and it presented huge differences when compared to the other traditional Chilean sparkling wines produced by Chardonnay and Pinot noir (Table 2). Except for one sample, the total sums of the relative areas of the esters, alcohols, and acids were more than double in the wine made from País grapes.

Among the esters, compounds that contributed to wine aroma were ethyl hexanoate and octanoate (fruity, pineapple/apple), isobutyl, isoamyl, and 2-phenylethanol acetates (banana, fruity/flowery). 3-Methyl-1-butanol, 1-hexanol, and 2-phenylethanol were the

alcohols which exhibited the highest relative areas compared to the other traditional wines (Table 2). Sparkling wines produced using the País variety seemed to have more acidity due to the higher values of some acids, specifically octanoic, decanoic, and hexanoic acids. A possible consequence of high amounts of octanoic acid could be the higher values of different esters of this acid (ethyl, isoamyl, or methyl octanoate) in wines made from the País grape variety.

With respect to the compounds that contribute to the varietal aromas of the wine, i.e., in the cases of terpenes and C13-norisoprenoids, limonene, trans-linalool oxide, and linalool exhibited large relative areas in wine from País grape, contributing to the characteristic citrus and flowery aromas of the sparkling wine. Additionally, in the case of β-damascenona, with baked apple aroma, the relative area was superior to that of the other wine samples. Concerning the ketones, the main differences were found for the compounds 6-methyl-5-hepten-2-one (herbaceous/pungent odour) and 2-nonanone (fruity/floral odour).

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. The use of the País grape variety produced a sparkling wine with a different volatile profile.

Acknowledgments

The authors are grateful for the funding provided by CONICYT (Project Fondecyt 11140275 and Project Fondequip EQM130129) and the Universidad Autónoma de Chile.

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Figure captions

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

Figure 2. Data scores plot on the plan made up of the first two principal components (PC1 against PC2) for Chilean sparkling wine (without País variety sparkling wine).

Figure 1.

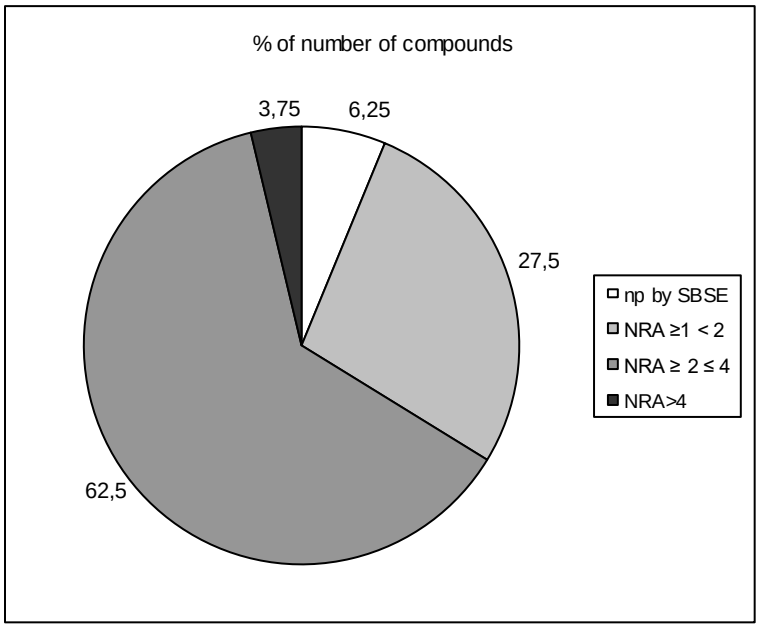
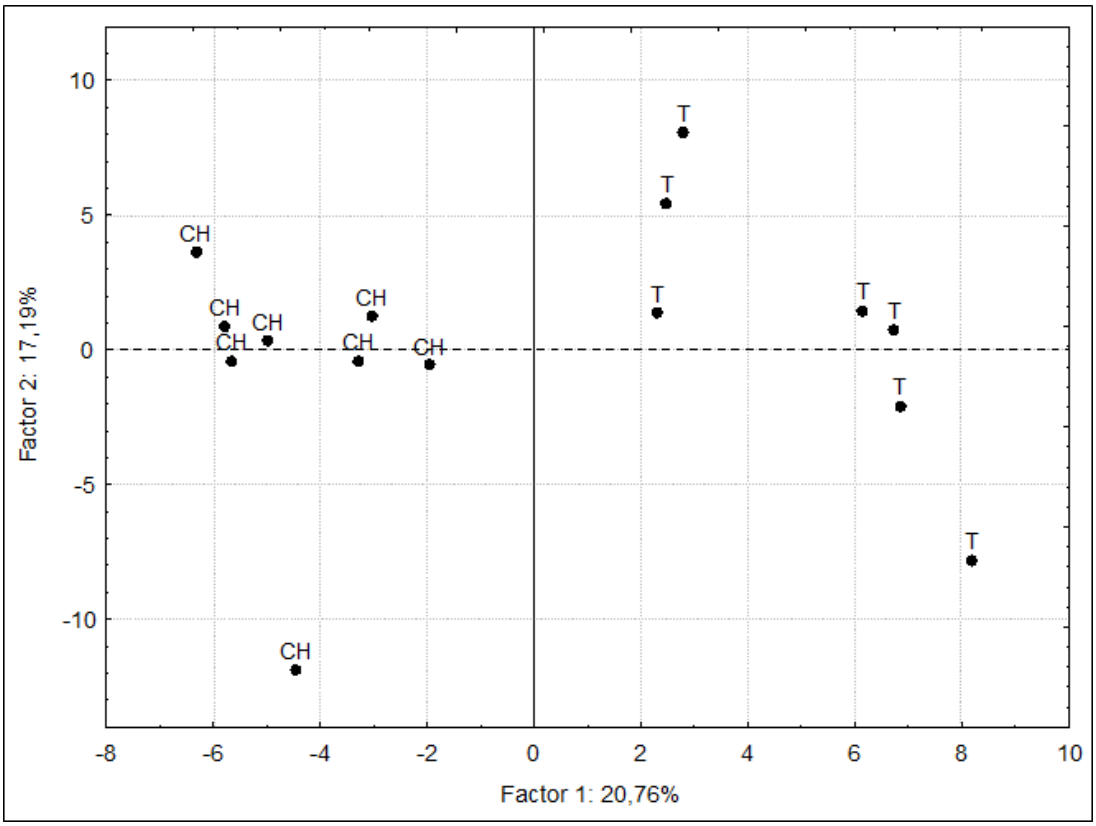


Figure 2.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0
-5
-10
-8
-6
-4
-2
0
2
4
6
8
10
Factor 2: 17,19%
Factor 1: 20,76%
CH
CH
CH
CH
CH
CH
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Figure S1. Variable loading plots on the planes made up of the first two principal components (PC1 against PC2).

Figure S1a.

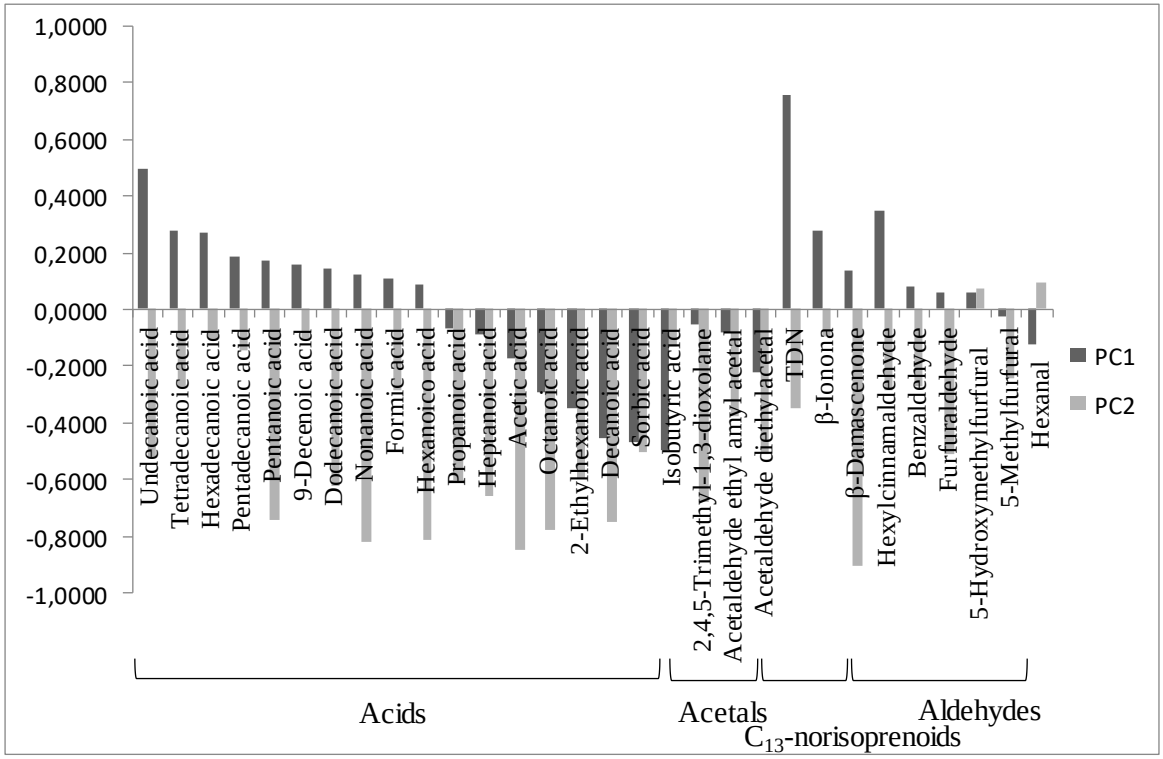

Figure S1b.

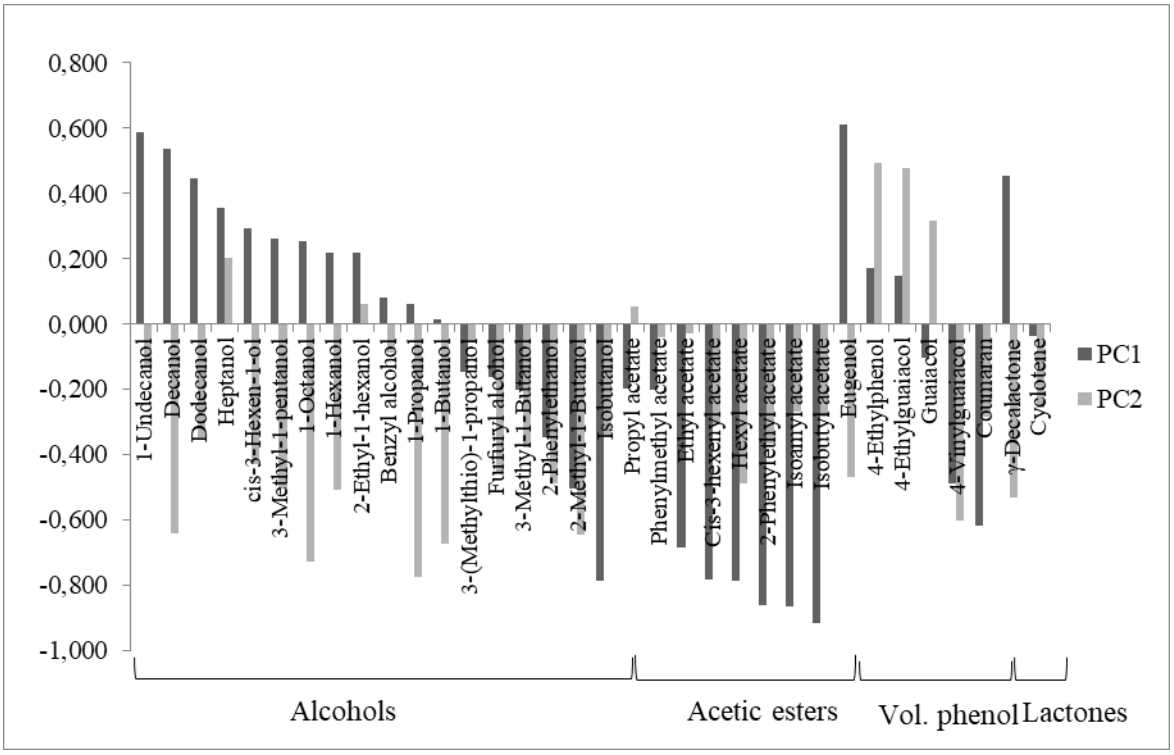
0,800
0,600
0,400
0,200
0,000
-0,200
-0,400
-0,600
-0,800
-1,000
1-Undecanol
Decanol
Dodecanol
Heptanol
cis-3-Hexen-1-ol
3-Methyl-1-pentanol
1-Octanol
1-Hexanol
2-Ethyl-1-hexanol
Benzyl alcohol
1-Propanol
1-Butanol
3-(Methylthio)-1-propanol
Furfuryl alcohol
3-Methyl-1-Butanol
2-Phenylethanol
2-Methyl-1-Butanol
Isobutanol
Propyl acetate
Phenylmethyl acetate
Ethyl acetate
Cis-3-hexenyl acetate
Hexyl acetate
2-Phenylethyl acetate
Isoamyl acetate
Isobutyl acetate
Eugenol
4-Ethylphenol
4-Ethylguaiacol
Guaiacol
4-Vinylguaiacol
Coumaran
γ-Decalactone
Cyclotene
PC1
PC2
Alcohols
Acetic esters
Vol. phenol
Lactones

Figure S1c.

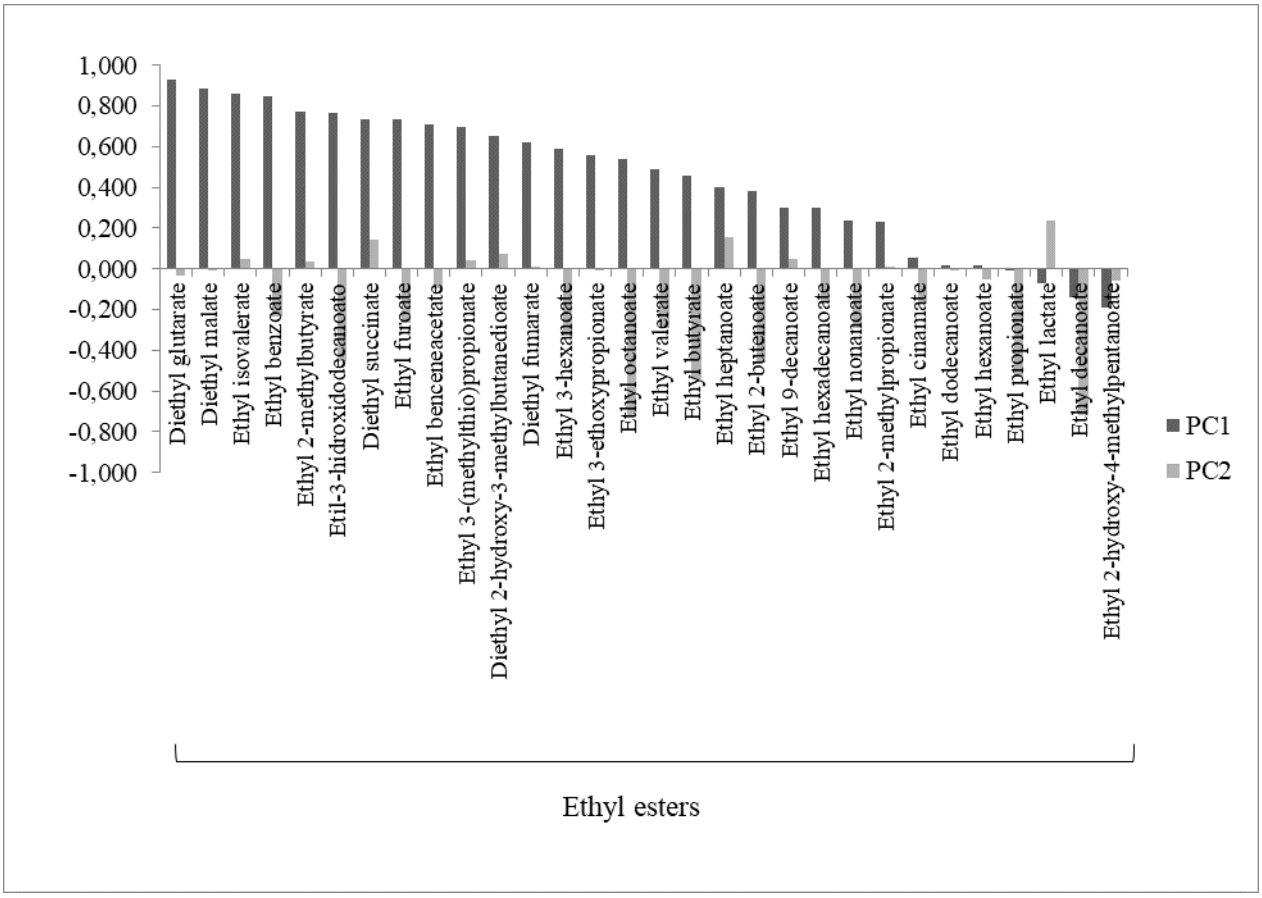
1,000
0,800
0,600
0,400
0,200
0,000
-0,200
-0,400
-0,600
-0,800
-1,000
Diethyl glutarate
Diethyl malate
Ethyl isovalerate
Ethyl benzoate
Ethyl 2-methylbutyrate
Etil-3-hidroxidodecanoato
Diethyl succinate
Ethyl furoate
Ethyl benceneacetate
Ethyl 3-(methylthio)propionate
Diethyl 2-hydroxy-3-methylbutanedioate
Diethyl fumarate
Ethyl 3-hexanoate
Ethyl 3-ethoxypropionate
Ethyl octanoate
Ethyl valerate
Ethyl butyrate
Ethyl heptanoate
Ethyl 2-butenoate
Ethyl 9-decanoate
Ethyl hexadecanoate
Ethyl nonanoate
Ethyl 2-methylpropionate
Ethyl cinamate
Ethyl dodecanoate
Ethyl hexanoate
Ethyl propionate
Ethyl lactate
Ethyl decanoate
Ethyl 2-hydroxy-4-methylpentanoate
PC1
PC2
Ethyl esters

Figure
S1d.

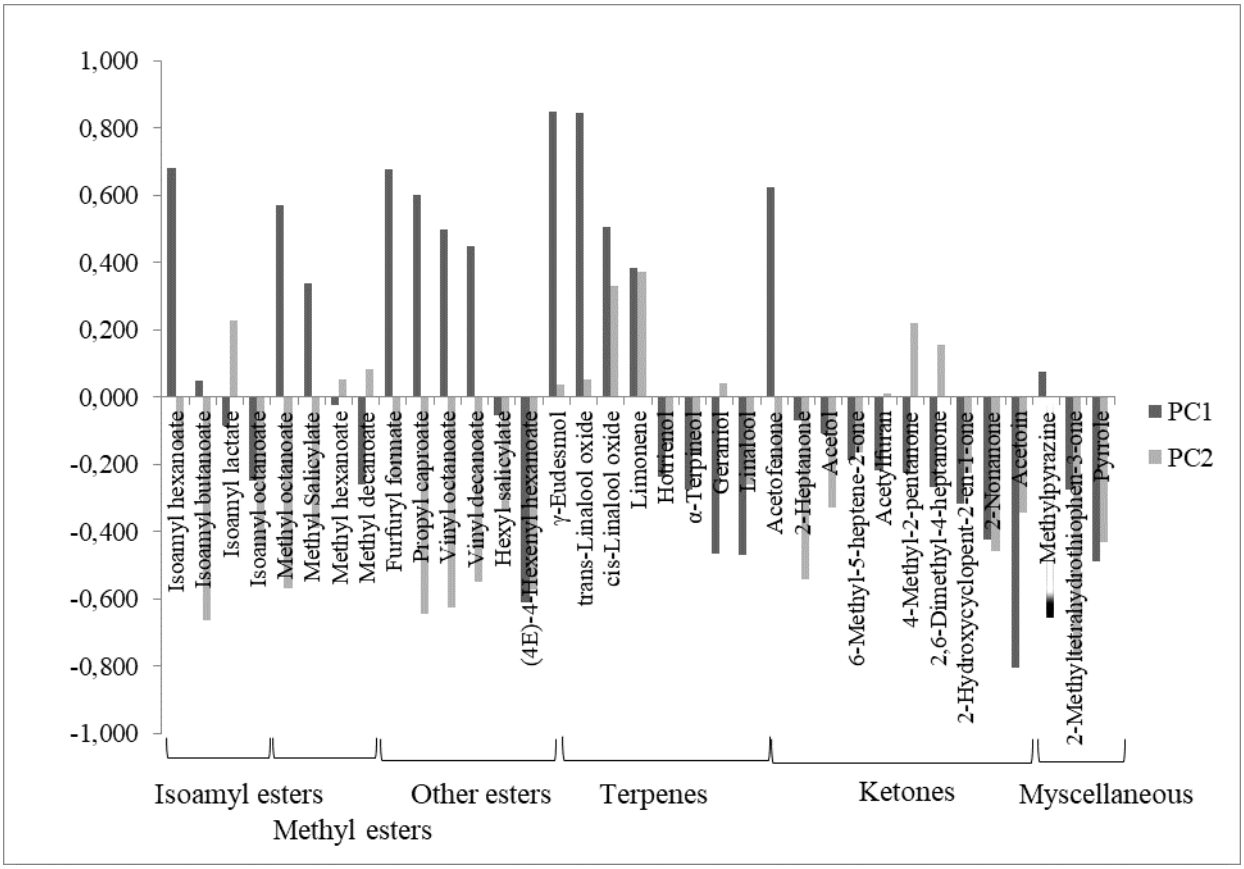
1,000
0,800
0,600
0,400
0,200
0,000
-0,200
-0,400
-0,600
-0,800
-1,000
PC1
PC2
Isoamyl hexanoate
Isoamyl butanoate
Isoamyl lactate
Isoamyl octanoate
Methyl octanoate
Methyl Salicylate
Methyl hexanoate
Methyl decanoate
Furfuryl formate
Propyl caproate
Vinyl octanoate
Vinyl decanoate
Hexyl salicylate
(4E)-4-Hexenyl hexanoate
γ-Eudesmol
trans-Linalool oxide
cis-Linalool oxide
Limonene
Hotrienol
α-Terpineol
Geraniol
Linalool
Acetofenone
2-Heptanone
Acetol
6-Methyl-5-heptene-2-one
Acetylfuran
4-Methyl-2-pentanone
2,6-Dimethyl-4-heptanone
2-Hydroxycyclopent-2-en-1-one
2-Nonanone
Acetoin
Methylpyrazine
2-Methyltetrahydrothiophen-3-one
Pyrrole
Isoamyl esters
Methyl esters
Other esters
Terpenes
Ketones
Myscellaneous

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).

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

Volatile Compounds	ID	LRI	CHILEAN SPARKLING WINES		
			CHARMAT	TRADITIONAL	
				Classical *var.*	País *var.*
Ethyl esters					
Ethyl propionate	A	927	0.10-0.16	0.08-0.17	0.23±0.03
Ethyl 2-methylpropanoate	A	938	0.01-0.12	0.02-0.24	0.22±0.03
Ethyl butyrate	A	1003	1.03-1.45	1.05-1.87	3.3±0.4
Ethyl 2-methylbutyrate	A	1016	0.04-0.07*	0.10-0.33	0.24±0.04
Ethyl isovalerate	A	1031	0.08-0.13*	0.19-0.41	0.40±0.01
Ethyl valerate	A	1096	0.003-0.004	0.003-0.006	0.012±0.002
Ethyl 2-butenoate	B[1]	1127	0.03-0.05	0.03-0.07	0.11±0.01
Ethyl hexanoate	A	1207	5.6-8.9	3.8-9.5	22±3
Ethyl 3-hexanoate	A	1289	0.001-0.005	0.002-0.009	nd
Ethyl 3-ethoxypropionate	C	1316	nd-0.008	0.002-0.007	nd
Ethyl heptanoate	A	1318	0.003-0.007	0.004-0.018	0.0030±0.0001
Ethyl lactate	A	1349	0.66-3.93	0.53-5.20	1.57±0.16
Ethyl octanoate	A	1418	6.8-12.0	8.2-18.6	28±2
Ethyl nonanoate	A	1521	0.001-0.004	nd-0.007	nd
Ethyl 2-hydroxy-4-methylpentanoate	B[2]	1537	0.03-0.29	0.05-0.08	0.124±0.007
Ethyl 3-(methylthio)propionate	C	1551	0.002-0.004*	0.003-0.007	0.0070±0.0004
Ethyl furoate	A	1605	0.05-0.15*	0.08-0.23	0.207±0.012
Ethyl decanoate	A	1632	0.64-1.29	0.28-1.50	2.1±0.3
Diethyl fumarate	C	1634	0.001-0.005*	0.003-0.026	0.0080±0.0003
Ethyl benzoate	A	1649	0.004-0.009*	0.008-0.019	0.021±0.002
Diethyl succinate	A	1676	2.75-5.04*	6.8-21.7	14±1
Ethyl 9-decenoate	B[2,3]	1683	0.02-0.13	0.01-0.23	0.180±0.022
Diethyl glutarate	B[4]	1777	0.03-0.06*	0.06-0.12	0.157±0.015

Ethyl benzeneacetate	A	1778	0.04-0.08*	0.05-0.13	0.407±0.008
Ethyl dodecanoate	A	1841	0.006-0.019	nd-0.04	0.029±0.002
Diethyl 2-hydroxy-3-methylbutanedioate	C	1858	0.003-0.008*	0.02-0.10	0.017±0.001
Diethyl malate	B[5]	2056	0.10-0.42*	0.44-1.00	0.578±0.010
Ethyl-3-hydroxydodecanoate	C	2116	0.07-0.15*	0.13-0.30	0.52±0.03
Ethyl cinnamate	A	2141	0.005-0.012	0.002-0.026	0.010±0.001
Ethyl hexadecanoate	A	2266	0.01-0.05	0.005-0.553	0.23±0.03
Total sum of ethyl esters			25.5-47.1	21.9-29.3	75.1
Acetic esters					
Ethyl acetate	A	873	1.6-2.8*	1.38-2.17	2.03±0.26
Propyl acetate	A	942	nd-3.2*	nd	nd
Isobutyl acetate	A	955	0.04-0.07*	0.01-0.02	0.047±0.002
Isoamyl acetate	A	1089	2.6-5.3*	0.15-1.18	4.4±0.5
Hexyl acetate	A	1259	0.45-1.18*	nd-0.30	0.007±0.001
cis-3-Hexenyl acetate	A	1289	0.01-0.05*	nd-0.01	0.078±0.007
Phenylmethyl acetate	A	1718	nd-0.004*	nd-0.002	0.005±0.0002
2-Phenylethyl acetate	A	1811	0.97-3.00*	0.05-0.88	3.9±0.3
Total sum of acetic esters			1.8-3.9	6.3-10.9	10.6
Methyl esters					
Methyl hexanoate	A	1147	0.005-0.018	nd-0.02	0.0241±0.002
Methyl octanoate	C	1418	0.01-0.02	0.01-0.02	0.87±0.08
Methyl decanoate	A	1584	0.002-0.013	nd-0.005	nd
Methyl salicylate	C	1761	0.003-0.011	0.002-0.022	0.014±0.002
Total sum of methyl esters			0.03-0.06	0.02-0.05	0.91
Isoamyl esters					

Isoamyl butanoate	C	1240	0.006-0.013	0.005-0.012	1.83±0.20
Isoamyl hexanoate	C	1458	0.007-0.018*	0.01-0.03	0.023±0.001
Isoamyl lactate	B[2]	1566	0.01-0.06	0.005-0.081	0.032±0.003
Isoamyl octanoate	A	1653	nd-0.033	0.01-0.02	0.040±0.006
Total sum of isoamyl esters			0.04-0.12	0.03-0.11	0.10
Other esters					
Furfuryl formate	C	1261	0.005-0.011*	0.009-0.150	0.040±0.005
Propyl hexanoate	C	1288	0.002-0.005	0.003-0.008	0.0140±0.0001
(4E)-4-Hexenyl hexanoate	C	1297	0.01-0.08*	nd-0.02	0.077±0.007
Vinyl octanoate	C	1501	0.02-0.05	0.01-0.18	0.224±0.006
Vinyl decanoate	C	1718	0.005-0.013	0.001-0.051	0.100±0.0003
Hexyl salicylate	C	2212	0.009-0.017	0.007-0.017	0.042±0.001
Total sum of other esters			0.06-0.41	0.07-0.16	0.50
Alcohols					
1-Propanol	A	1019	0.04-0.85	0.37-0.92	0.89±0.07
Isobutanol	A	1081	0.08-0.25*	0.06-0.13	0.144±0.016
1-Butanol	A	1165	0.05-0.09	0.04-0.08	0.069±0.006
2-Methyl-1-butanol	A	1221	1.1-1.7*	0.85-1.33	1.68±0.13
3-Methyl-1-butanol	A	1240	6.9-13.0	7.6-13.5	22.7±0.6
3-Methyl-1-pentanol	C	1335	0.03-0.05	0.02-0.06	0.1060±0.0004
1-Hexanol	A	1362	0.56-1.30	0.59-1.25	3.17±0.15
cis-3-Hexen-1-ol	A	1387	0.01-0.04	0.01-0.04	0.108±0.004
Heptanol	A	1458	0.007-0.019	0.007-0.079	0.024±0.0002
2-Ethyl-1-hexanol	A	1488	0.02-0.08	0.03-0.06	0.038±0.001
1-Octanol	A	1559	0.02-0.04	0.02-0.05	0.159±0.015
Furfuryl alcohol	A	1664	0.07-0.11	0.05-0.11	0.10±0.03

3-(Methylthio)-1-propanol	B[6]	1728	0.002-0.034	0.003-0.009	0.006±0.001
1-Decanol	A	1769	0.008-0.023	0.01-0.04	0.082±0.003
1-Undecanol	B[4]	1875	0.03-0.07*	0.04-0.49	0.089±0.006
Benzyl alcohol	A	1886	0.004-0.018	0.006-0.016	0.019±0.001
2-Phenylethanol	A	1926	3.2-4.9	3.0-4.3	13±1
1-Dodecanol	B[7]	1989	0.02-0.04	0.03-0.05	0.081±0.012
Total sum of alcohols			25.2-35.9	16.4-35.8	68.6
Acids					
Acetic acid	A	1452	0.23-0.55	0.16-0.38	0.60±0.04
Formic acid	C	1505	0.02-0.06	0.02-0.06	0.09±0.03
Propanoic acid	A	1540	0.02-0.03	0.01-0.03	0.034±0.008
Isobutyric acid	A	1569	0.02-0.13	nd-0.06	0.036±0.005
Pentanoic acid	A	1742	0.004-0.011	0.005-0.008	0.009±0.001
Hexanoic acid	A	1853	1.5-2.7	1.4-2.3	4.5±0.3
2-Ethylhexanoic acid	C	1956	0.009-0.066	nd-0.02	0.043±0.003
Heptanoic acid	A	1960	0.008-0.040	0.01-0.02	0.032±0.001
Octanoic acid	A	2086	8.3-16.4	5.3-12.0	32±3
Sorbic acid	C	2151	nd-4.9	nd-0.06	0.089±0.024
Nonanoic acid	A	2180	0.04-0.15	0.06-0.11	0.232±0.013
Decanoic acid	A	2299	2.4-7.9*	0.89-4.06	10.7±0.9
9-Decenoic acid	B[7]	2353	0.07-0.42	0.05-0.71	0.92±0.05
Undecanoic acid	C	2392	0.006-0.011	0.007-0.061	0.021±0.001
Ethoxy-4-oxobutanoic acid	C	2405	0.05-0.12*	0.09-0.28	0.38±0.13
Dodecanoic acid	B[4]	2466	0.03-0.16	0.05-0.09	0.239±0.009
Tetradecanoic acid	C	2604	0.03-0.13	0.05-0.12	0.17±0.06
Pentadecanoic acid	C	2699	0.006-0.064	0.008-0.056	0.055±0.015
Hexadecanoic acid	C	2817	0.01-0.22	0.02-0.19	0.19±0.08
Total sum of acids			8.4-19.8	15.2-33.6	49.9

Aldehydes					
Hexanal	A	1043	nd-0.004	nd-0.002	nd
Furfuraldehyde	A	1449	0.09-0.14	0.07-0.16	0.19±0.06
Benzaldehyde	A	1505	0.003-0.018	0.006-0.024	0.033±0.001
5-Methylfurfural	A	1565	0.005-0.0013	0.006-0.013	0.015±0.002
Hexylcinnamaldehyde	C	2368	0.003-0.006	0.003-0.008	0.014±0.002
5-Hydroxymethylfurfural	A	2492	0.002-0.009	0.002-0.011	nd
Total sum of aldehydes			0.10-0.20	0.13-0.18	0.25
Acetals					
Acetaldehyde diethylacetal	A	878	0.37-1.46	0.07-1.17	0.99±0.11
2,4,5-Trimethyl-1,3-dioxolane	C	916	0.03-0.13	0.008-0.143	0.107±0.007
Acetaldehyde ethyl amyl acetal	C	1074	0.003-0.178	0.01-0.18	0.149±0.004
Total sum of acetals			0.09-0.15	0.46-1.72	1.14
Ketones					
4-Methyl-2-pentanone	C	971	0.004-0.005	0.004-0.005	0.0080±0.0003
2,6-Dimethyl-4-heptanone	A	1137	0.007-0.011	0.007-0.011	0.017±0.001
2-Heptanone	C	1151	0.01-0.02	0.003-0.025	0.018±0.001
Acetoin	A	1280	0.01-0.04*	0.005-0.014	nd
Acetol	A	1289	0.04-0.07	0.03-0.09	nd
6-Methyl-5-hepten-2-one	A	1316	nd-0.01	0.006-0.016	0.709±0.200
2-Nonanone	A	1365	0.05-0.13	nd-0.154	0.148±0.011
2-Acetylfuran	A	1496	nd-0.198	0.008-0.020	0.019±0.003
Acetophenone	A	1640	0.005-0.009*	0.007-0.025	0.0180±0.0001
2-Hydroxycyclopent-2-en-1-one	C	1779	0.05-0.12	0.04-0.09	0.080±0.012

Total sum of ketones			0.12-0.35	0.26-0.46	1.02
Terpenes					
Limonene	A	1142	0.003-0.028	0.005-0.055	0.0870±0.0003
cis-Linalool oxide	B[8]	1442	0.002-0.010*	0.002-0.033	0.0070±0.0001
trans-Linalool oxide	B[8]	1469	0.004-0.008*	0.009-0.014	0.018±0.002
Linalool	A	1540	0.009-0.105	nd-0.01	0.029±0.001
Hotrienol	C	1603	0.004-0.050	0.003-0.016	0.017±0.001
α-Terpineol	A	1704	0.01-0.14	0.006-0.038	0.054±0.004
Geraniol	A	1855	0.01-0.03	0.01-0.02	0.035±0.005
γ-Eudesmol	B[4]	2184	0.004-0.011*	0.007-0.030	0.030±0.002
Total sum of terpenes			0.09-0.18	0.07-0.36	0.28
C_{13}-norisoprenoids					
TDN	C	1721	0.007-0.021*	0.01-0.03	0.060±0.002
β-Damascenone	A	1817	0.002-0.099	0.003-0.089	0.164±0.017
β-Ionone	A	1945	0.002-0.008	nd-0.05	0.0080±0.0004
Total sum* of *C_{13}-norisoprenoids			0.01-0.16	0.04-0.12	0.23
Lactones					
Cyclotene	B[4]	1844	0.007-0.015	0.006-0.013	0.019±0.002
γ-Decalactone	A	2151	0.009-0.025	0.008-0.149	0.17±0.03
Total sum of lactones			0.02-0.04	0.02-0.04	0.19
Volatile phenols					
Guaiacol	A	1855	0.001-0.003	nd-0.004	0.004±0.001
4-Ethylguaiacol	A	2034	nd-0.009	nd-0.25	0.0070±0.0001

Eugenol	A	2175	nd-0.003	0.001-0.009	0.005±0.001
4-Ethylphenol	A	2184	0.002-0.009	nd-0.216	0.0100±0.0003
4-vinylguaiacol	B[4]	2208	0.01-0.03*	0.008-0.024	0.036±0.004
Coumaran	C	2408	0.01-0.05*	0.01-0.02	0.053±0.007
Total sum of volatile phenols			0.05-0.49	0.04-0.11	0.12
Myscellaneous					
Methylpyrazine	B[4]	1260	0.004-0.010	0.003-0.009	nd
Pyrrole	B[4]	1500	0.01-1.19	0.01-0.02	nd
2-Methyltetrahydrothiophen-3-one	B[4]	1521	0.009-0.032	0.009-0.020	0.0050±0.0003

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: 1) Hwan & Chou (1999), 2) Pino & Queris (2011), 3) Bosch-Fusté et al. (2007), 4) National Center for Biotechnology Information (2015), 5) Lee & Noble (2003), 6) Miranda-Lopez, Libbey, Watson, & McDaniel (1992), 7) Li et al. (2008), 8) 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.