The influence of pre-fermentative maceration and ageing factors on ester profile and marker determination of Pedro Ximenez sparkling wines

María José Ruiz-Moreno, José Manuel Muñoz-Redondo, Francisco Julián Cuevas, Almudena Marrufo-Curtido, Juan Manuel Leon, Pilar Ramírez, José Manuel Moreno-Rojas

PII: S0308-8146(17)30424-7
DOI: http://dx.doi.org/10.1016/j.foodchem.2017.03.048
Reference: FOCH 20752

To appear in: *Food Chemistry*

Received Date: 17 October 2016
Revised Date: 3 March 2017
Accepted Date: 9 March 2017

Please cite this article as: Ruiz-Moreno, M.J., Muñoz-Redondo, J.M., Julián Cuevas, F., Marrufo-Curtido, A., Leon, J.M., Ramírez, P., Moreno-Rojas, J.M., The influence of pre-fermentative maceration and ageing factors on ester profile and marker determination of Pedro Ximenez sparkling wines, *Food Chemistry* (2017), doi: http://dx.doi.org/10.1016/j.foodchem.2017.03.048

The influence of pre-fermentative maceration and ageing factors on ester profile and marker determination of Pedro Ximenez sparkling wines

María José Ruiz-Moreno[a*], José Manuel Muñoz-Redondo[a], Francisco Julián Cuevas[a], Almudena Marrufo-Curtido[b], Juan Manuel Leon[c], Pilar Ramírez[c], José Manuel Moreno-Rojas[b*]

[a]Postharvest technology and food industry department. Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA). Centro Alameda del Obispo, Avda Menéndez Pidal, 14004 Córdoba, España.

[b]Laboratory for Aroma Analysis and Enology. Instituto Agroalimentario de Aragón (IA2). Associate unit to Instituto de las Ciencias de la Vid y el Vino (ICVV) (UR-CSIC-GR). Department of Analytical Chemistry, Faculty of Sciences, University of Zaragoza, 50009 Zaragoza, Spain

[c]Crop production department. Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA). Centro de Cabra, Ctra Cabra-Doña Mencía, km 2.5, 11940 Cabra, España.

Abstract

The influence of pre-fermentative maceration and ageing factors on the ester profiles of Pedro Ximenez sparkling wines was evaluated. The pre-fermentative maceration consisted of the skin-maceration of musts at 10 ºC for 6 hours. The sparkling wines were produced following the Champenoise method. Samples were monitored at 3, 6 and 9 months of ageing on lees. Sparkling wines with pre-fermentative maceration displayed higher contents of ethyl esters of branched acids and cinnamates. Meanwhile, those without maceration showed higher levels of ethyl esters of fatty acids and higher alcohol acetates. The study of

statistical interactions elucidated different hydrolytic kinetics and developments in higher alcohol acetates and ethyl esters of branched acids during ageing. The application of a dual criterion based on univariate (ANOVA) and multivariate analyses (OPLS-DA) allowed us to identify new potential volatile markers related to pre-fermentative maceration and ageing time, reported for the first time in sparkling wines.

Keywords: aroma, ester, volatile, chemometrics, multivariate analyses, sparkling wines, OPLS-DA, markers

*Corresponding authors

Tel.: +34 671 53 27 58; +34 671 53 27 16; Fax: +34 957 01 60 43

E-mail: josem.moreno.rojas@juntadeandalucia.es

E-mail: mariaj.ruiz.moreno@juntadeandalucia.es

1. Introduction

Recently, a new wine market paradigm based on product diversification has emerged for winemakers (Pozo-Bayón, Martínez-Rodríguez, Pueyo, & Moreno-Arribas, 2009). This has given consumers a large choice of typologies of wines, different qualities and prices. A good example of the diversification of wine types is sparkling wines. While the worldwide production of still wines has increased by 7% over the 10 last years, that of sparkling wines increased by more than 40% over the same period (OIV, 2014). In addition, although the production of sparkling wine is lower than other wines in terms of quantity, the economic impact of this product is very important, due to its high added value and increased production on a global scale (Caliari, Burin, Rosier, & BordignonLuiz, 2014; Torresi, Frangipane, & Anelli, 2011). This increasing interest in sparkling wines is bound to new market segments for sparkling wines, resulting in changes in the global market for this product. Thereby, cava exceeded the exports of champagne in volume terms during 2015 (Institut del Cava, 2015, Le Comité Champagne, 2015) and the production of sparkling wines from Russia, USA, Ukraine, Australia, Hungary or Brazil has rapidly increased in the last few years (OIV, 2014).

Winemakers and the scientific community are searching for new collaborative platforms to enhance the peculiarities and distinctive characteristics of their wines (Caliari et al., 2014; Pozo-Bayon et al., 2009). These distinctive characteristics of the wines are usually given by local or regional grape varieties. In this context, Pedro Ximenez is an autochthonous white grape variety traditionally used for the production of Sherry-type wines in the Montilla–Moriles designation of origin (Andalusia, Spain). The versatility and attitude of this variety have been well proven (comprising the organic production, sun-drying and oxidative and biological ageing) producing many different styles of wines. The grape variety and other

factors, such as crop management, ripeness, and base wine composition, have been well reported to impact on the quality of sparkling wine (Pozo-Bayón et al., 2009; Riu-Aumatell, Bosch-Fusté, López-Tamames, & Buxaderas, 2006). Skin maceration induces compositional modifications as well as the extraction of grape-derived components in grape juices. Furthermore, the traditional or Champenoise method, involving a second fermentation and ageing in contact with lees, also produces compositional changes impacting on the final quality of the product (Pozo-Bayón et al., 2009; Riu-Aumatell et al., 2006).

Skin maceration is usually performed in the production of rosé sparkling wines. Several authors concur with the use of this operation as an oenological practice to improve the quality of sparkling wines from red grape varieties (Martínez-Lapuente, Guadalupe, Ayestarán, & Pérez-Magariño, 2015; Pozo-Bayón et al., 2009). However, to our knowledge, comparative studies have not explored the influence of pre-fermentative strategies on the distinctive aroma styles of sparkling wines, and this requires investigation. In this sense, deepening our understanding of the aromatic impact of this pre-fermentative operation may offer a promising strategy for obtaining sparkling wines with differential aromatic characteristics.

Aroma is considered one of the most decisive quality attributes in wines and a key factor impacting on consumers' preferences and tasting experience (Antalick et al., 2015; Lockshin & Corsi, 2012; Sáenz-Navajas, Ballester, Pêcher, Peyron, & Valentin, 2013). In this sense, the role of ester compounds in wine aroma is a current topic of research. The growing interest in the characterization of wine esters is not only due to their direct sensory contribution but also to complex synergistic interactions affecting aroma perception (Escudero, Campo, Fariña, Cacho, & Ferreira, 2007; Lytra, Tempere, Le Floch, de Revel,

& Barbe, 2013). Esters are formed as a result of the reaction of an alcohol with a carboxylic acid functional group. These molecules are mainly synthesized by two mechanisms in wines: during alcoholic fermentation through enzymatic reactions produced by yeasts and during wine ageing by chemical esterification between alcohol and acid functional groups at low pH (Sumby, Grbin, & Jiranek, 2010). Besides the mechanisms of genesis, ester hydrolysis and ester oxidation by hydroxyl radical-related processes could modulate their contents over the winemaking process (Ramey & Ough, 1980). Recent studies have reported that the maturity of grapes, fermentation strategy and ageing factors greatly affect the ester profile of wine and consequently impact on its aroma. In the case of the Pedro Ximenez grapes, the impact of pre-fermentative maceration on cinnamates has not been reported. Moreover, studies focused on the role of ester compounds in sparkling wines modified by skin maceration and ageing are scarce.

Given the importance of ester compounds and their sensory impact, numerous approaches have been developed to characterize ester compounds in wines (Marquez, Serratosa, Merida, Zea, & Moyano, 2014; Ubeda, Callejón, Troncoso, Peña-Neira, & Morales, 2016). In this sense, headspace solid-phase microextraction (HS-SPME) is a suitable, quick, simple and solvent-free technique. HS-SPME coupled to gas chromatography with mass spectrometry detection (GC-MS) has been widely used for this purpose (Antalick, Perello, & de Revel, 2010; Perestrelo, Barros, Rocha, & Câmara, 2014). This technique can produce a large amount of data for each sample. Therefore, multivariate approaches are used to handle tangled data since the univariate analysis may ignore other interactions found in complex models (Cozzolino, Cynkar, Shah, Dambergs, & Smith, 2009).

The aim of this study was to examine the impact of pre-fermentative maceration and ageing factors on the ester composition of Pedro Ximenez sparkling wines. For that purpose, a

novel methodology based on HS-SPME-GC-MS and chemometrics was developed to highlight the potential volatile markers of both factors.

2. Materials and methods

2.1. Wine samples

Sparkling wines were elaborated at IFAPA, Cabra-Priego (37º 29' 53"N; 04º 25' 51" W) following the traditional or Champenoise method (consisting of a second fermentation of base wines in closed bottles and ageing on lees before disgorging). A 600-kg batch of Pedro Ximenez grapes from the 2014 campaign were harvested at 18.5–19.0 ºBrix and divided into two. The first 300-kg batch of grapes (NM samples) was destemmed, crushed and pressed. The grape juices were divided into stainless steel tanks of 50 L. They were corrected, sulfited (at 70 mg L^{-1}) and then alcoholic fermentation was carried out at a controlled temperature of 18 ºC, obtaining the NM base wines. The yeast and nutrients used were Pasionviniferm (Agrovin, Spain) at 20 g hL^{-1} and Actimax Bio at 10 g hL^{-1} (Agrovin, Spain), respectively. Next, the base wines were clarified, stabilized and filtered. In the second batch (M), 300 kg of grapes were destemmed, crushed and sulfited (at 50 mg kg^{-1}). Enozym AROME enzyme at a dose of 30 mg kg^{-1} (Agrovin, Spain) was added. Afterwards, a pre-fermentative maceration of the must in contact with the skins was performed for 6 hours at 10 ºC before pressing. Then, the grape juices were corrected and fermented following the same conditions described for the NM base wines. The tirage liquor consisted of 24 g L^{-1} of sucrose, yeast Viniferm PDM at 20 g hL^{-1} (Agrovin. Spain), Actimax Bio at 15 g hL^{-1} (Agrovin, Spain) and bentonite at 20 g hL^{-1} (Laffort, France). A second fermentation was performed at 15 ºC in closed bottles of 0.75 L. The pressure and residual

sugars were measured periodically. This fermentation was completed after 11–12 weeks. Then, the sparkling wines were kept at 12 ºC and collected at 0, 3, 6 and 9 months of ageing on lees, riddled, disgorged, corked and submitted to analysis. A total of 48 bottles were analyzed. The two treatments (N and NM) were sampled in duplicate at each time (beginning of ageing, 3, 6 and 9 months) for each of the three fermentative tanks.

2.2. Oenological parameters

Ethanol (% vol.), residual sugars, pH, total and volatile acidity were determined following the official analytical methods (OIV, 2014). Optical density at $\lambda = 420$ nm was determined using a spectrophotometer (Lambda 25; Perkin-Elmer, Waltham, MA). Total phenolic content was determined by photometric procedure (Folin-Ciocalteu). The results were expressed as mg L^{-1} of gallic acid.

2.3. Chemicals and reagents

HPLC-grade ethanol was obtained from J.T. Baker Chemicals B. V. (Deventer, Holland). Milli-Q water was obtained from a Milli-Q Plus water system (Millipore, Spain). Sigma Aldrich (Madrid, Spain) supplied the sodium chloride, ACS reagent grade (purity ≥ 99.8%) and standard compounds; ethyl butyrate (≥ 99%), ethyl hexanoate (≥ 99%), ethyl octanoate (≥ 99%), propyl acetate (≥ 99%), isobutyl acetate (≥ 99%), isoamyl acetate (≥ 99%), hexyl acetate (≥ 99%), phenylethyl acetate (≥ 99%), ethyl isobutyrate (98%), ethyl 2-methylbutyrate (≥ 99%), ethyl isovalerate (≥ 99%), ethyl phenylacetate (98%), ethyl dihydrocinnamate (98%), ethyl cinnamate (98%), methyl hexanoate (≥ 99%), methyl octanoate (≥ 99%), methyl decanoate (≥ 99%), isoamyl butyrate (98%), isoamyl hexanoate

(98%), isoamyl octanoate (98%), ethyl heptanoate (98%), ethyl nonanoate (98%), ethyl propanoate (≥ 99%), isobutyl hexanoate (≥ 99%).

2.4. Automated HS-SPME-GC-MS analysis

The sparkling wine samples (25 mL) were spiked with 20 µL of internal standard mix solution at 200 µg L^{-1} of isotopically labelled esters: [$^2H_3$]-ethyl butyrate, [$^2H_{11}$]-ethyl hexanoate, [$^2H_{15}$]-ethyl octanoate, and [$^2H_5$]-ethyl cinnamate, supplied by CDN isotopes (Pointe-Claire, Canada). Spiked samples (10 mL) diluted 1:3 with Mili-Q water were placed into a 20-mL SPME vial filled with 3.5 g of NaCl. The capped vials were homogenized for 30 seconds in a vortex shaker, placed in a Combipal autosampler tray (CTC Analytics, Zwingen, Switzerland) and analysed by HS-SPME-GC-MS. A previously conditioned 100 µm PDMS fibre (Supelco, Bellefonte, PA) was used. The vials were stirred at 500 rpm for 2 min at 40 ºC. Extraction was set at 40 ºC for 30 min and desorption was performed at 250 ºC for 15 min. The fibre was desorbed into a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific S.p.A., Rodano, Milan, Italy) coupled to an ISQ Single Quadrupole MS spectrometer (Thermo Fisher Scientific, Austin, TX). The injection mode was splitless for 0.75 min. The column was a BP21 of 50 m × 0.32 mm, 0.25 µm film thickness (SGE Analytical Science, UK). The carrier gas was helium at a column head pressure of 8.0 psi. The oven temperature was programmed at 40 ºC for 5 min, raised to 220 ºC at 3 ºC min^{-1}, and then held for 30 min. The MS transfer line and source temperature were 230 ºC and 200 ºC, respectively. The mass spectrometer operated in electron ionization mode at 70 eV using selected ion monitoring (SIM) mode. Identification was carried out by comparing retention times and mass spectra with those of pure standards. Calibration curves were built using a commercial sparkling wine spiked with a

mixture of the target compounds at nine concentrations levels and analyzed following the procedure described above. The method performance was evaluated in a sparkling wine matrix (Supplementary Table 1).

2.5. Statistical analysis

Univariate analysis was performed using Statistix (v 9.0, Analytical Software, Tallahassee, FL). The data were subjected to analysis of variance using Shapiro Wilk's and Lenève's tests for normality and homoscedasticity requirements. Differences at $p < 0.05$ were considered to be statistically significant. A comparison of means based on least significant differences (LSD, Fisher's test) was performed. Multivariate analysis (OPLS-DA, orthogonal-partial least squares discriminant analysis) was performed using PLS toolbox (v. 5.5.1, Eigenvector Research Inc., Manson, WA) under MATLAB 2008R (v. 7.6.0; Mathworks, Natick, MA) workspace.

3. Results and discussion

3.1. Oenological parameters

The oenological parameters of the sparkling wines are shown in Table 1. Regarding the pre-fermentative strategy, higher values for pH, volatile acidity, absorbance at $\lambda = 420$ nm and total phenolic content were found in the M sparkling wines. Meanwhile, ageing factor only affected the pH of the sparkling wines, a slight decrease being observed at 3 months of ageing. Despite the differences found, the oenological parameters confirmed that the sparkling wines fulfilled the legal and quality standards.

3.2. Aroma composition

The volatile compounds were classified into different groups according to their chemical structure and origin (Table 2). The chemical groups included: ethyl esters of fatty acids (EEFAs), higher alcohol acetates (HAAs), ethyl esters of branched acids (EEBAs), cinnamates, methyl esters of fatty acids (MEFAs), isoamyl esters of fatty acids (IEFAs), ethyl esters of 'odd carbon number' fatty acids (EEOCNFA) and compounds grouped in a miscellaneous group (MEs). Concerning their relative composition (Supplementary Table 2 and Supplementary Table 3), EEFAs were the major group (with concentration ranges between 855–1537 μg L^{-1}), followed by HAAs (239–771 μg L^{-1}), EEBAs (96–284 μg L^{-1}) and miscellaneous compounds (98–209 μg L^{-1}). Meanwhile, IEFAs (1.7–3.7 μg L^{-1}), EEOCNFA (1.6–3.0 μg L^{-1}), MEFAs (0.9–1.5 μg L^{-1}) and cinnamates (0.19–0.3 μg L^{-1}) showed the lowest quantitative contribution. A two-way analysis of variance (ANOVA) was carried out. The factors studied were pre-fermentative strategy and ageing. The results are shown in Table 2.

3.3. Univariate analysis

3.3.1. Pre-fermentative maceration factor

Regarding the pre-fermentative maceration, significant differences between the aroma profiles were observed. The sparkling wines from pre-fermentative maceration (M) showed a lower content of total esters (Table 2). This behaviour is mainly due to a decrease in the EEFA and HAA groups in the M sparkling wines. These results are in agreement with the literature (Herraiz, Martin-Alvarez, Reglero, Herraiz, & Cabezudo, 1990). Piñeiro et al. (2006) also found a similar behaviour in EEFA contents in wines produced under pre-fermentative maceration, due to a decrease in ethyl hexanoate and octanoate compounds. However, the mechanisms involved in the reduction of EEFAs in wines from

pre-fermentative maceration still remain unclear. EEFAs are generally produced during alcoholic fermentation by yeasts (Antalick et al., 2014). Winemaking conditions such as temperature, aeration, skin contact and yeast strain have been described as the main factors affecting their EEFA concentrations (Antalick et al., 2014; Sumby et al., 2010). Moreover, the differential composition of the fermentative medium (lipids, amino acids, phenolic compounds) has been reported to modulate the ester profile of wines (Antalick et al., 2015, 2014).

Higher levels of phenolic compounds limit lipoxygenase activity (Yu et al., 2013). The repression of the lipoxygenases limits the degradation of unsaturated fatty acids that are related to the production of EEFAs. Moreover, the substrate of this reaction has been described as the major factor limiting the production of EEFAs (Robinson et al., 2014). Thus, higher levels of phenolic compounds (Table 1) could be related to the lower EEFA contents. Meanwhile, HAAs are formed from an alcohol and acetyl-CoA during alcoholic fermentation (Saerens, Delvaux, Verstrepen, & Thevelein, 2010) and are catalysed by alcohol acetyltransferases I and II (ATF1, ATF2). The increasing amount of unsaturated fatty acids in the medium (derived from a lower lipoxygenase activity) repress the enzymatic activity and, subsequently, the production of HAAs during fermentation (Robinson et al., 2014). In this sense, the lower HAA concentration found in the M sparkling wines could also be due to higher phenolic content (Table 1), as suggested in the literature (Antalick et al., 2014; Ribéreau-Gayon, 2000). Therefore, the higher levels of phenolic compounds obtained in the M sparkling wines could be involved in the reduction of HAAs and EEFAs in a different manner, limiting the substrate of the reaction (EEFAs) or limiting the kinetics of the reaction (HAAs).

On the other hand, the M sparkling wines presented higher levels of EEBAs, cinnamates, MEFAs, EEOCNFAs and miscellaneous compounds, compared to the NM sparkling wines. EEBAs derive from the metabolism of amino acids. Recently, variations in EEBAs related to grape maturity have been reported (Antalick et al., 2015). However, our samples did not present significant differences in their degree of ripeness, emphasizing, therefore, the impact of the pre-fermentative maceration. Increases in this group have been postulated in rosé and red wines to be related to a higher extraction of the corresponding amino acids during the winemaking stages in the presence of skins (Antalick et al., 2014). Nonetheless, to our knowledge, no data concerning the impact of pre-fermentative strategies on EEBAs have been described in sparkling wines. In this sense, our results advocate the modulation of EEBA contents by pre-fermentative maceration, as has been suggested in the literature for rosé and red wines.

Concerning cinnamates, the M sparkling wines presented higher concentrations of ethyl dihydrocinnamate (20% approx.) than the NM sparkling wines. The varietal contribution to concentrations of ethyl dihydrocinnamate is well known. It could also be modulated during the winemaking process. Noble rot or sun-drying dehydration processes have been correlated with increases in this compound by still unknown mechanism(s) (Antalick et al., 2014; Campo, Cacho, & Ferreira, 2008).

Regarding MEFAs, higher levels of methyl hexanoate and octanoate were found in the M sparkling wines and lower levels of methyl decanoate. The EEOCNFA group was also affected by the maceration process, its concentration increasing (1.9 $\mu g\ L^{-1}$ in NM sparkling wines compared to 3.0 $\mu g\ L^{-1}$ in M ones), ethyl heptanoate standing out as the main contributor to this group. Concerning the miscellaneous compounds, ethyl propanoate was affected by the maceration procedure, its concentration increasing in M sparkling

wines. The higher concentrations of ethyl propanoate in the M sparkling wines may derive from enriched macerated juices in amino acids, as suggested in the literature (Antalick et al., 2014).

3.3.2. Ageing factor

The ester profile was significantly influenced by the ageing on lees (Table 2). The highest value for the total esters parameter was found at the beginning of the ageing (0 months of ageing, 2072 $\mu g\ L^{-1}$) while, afterwards, it decreased (at 3 months) maintaining those values until the end of the ageing period (9 months of ageing). Nonetheless, not all the ester groups were influenced in the same way. The EEFA, cinnamate, IEFA, EEOCNFA and the miscellaneous groups were not affected by the ageing factor, as was demonstrated in previous research (Antalick et al., 2014). Concerning the EEFA group, the explanation was related to the equilibrium between the corresponding acids and ethanol for the medium-chain ethyl fatty acids (Antalick et al., 2014).

The ageing process significantly affected the concentrations of the HAA, EEBA and MEFA groups. However, the impact of ageing on those groups was different. HAAs decreased by more than 50% during the ageing, reaching the lowest concentrations in the sparkling wines at the end of this period (after 9 months). This trend was in accordance with the literature (Francioli, Torrens, Riu-Aumatell, López-Tamames, & Buxaderas, 2003; Riu-Aumatell et al., 2006), phenylethyl, hexyl and isoamyl acetates being the main contributors to this decrease (around 60%, 55% and 50% respectively). The results were in agreement with another study (Antalick et al., 2014) in which it was hypothesised that longer carbon chain acetates suffered faster hydrolysis compared to the shorter ones (propyl acetate and isobutyl acetate).

The EEBA group presented increased concentrations with ageing (from 119 µg L^{-1} to 230 µg L^{-1}), according to results reported in the literature (Antalick et al., 2014; Díaz-Maroto, Schneider, & Baumes, 2005; Rodríguez-Bencomo, Ortega-Heras, & Pérez-Magariño, 2010). The main EEBA compounds contributing to this trend were ethyl isobutyrate, ethyl 2-methylbutyrate and ethyl isovalerate, which showed a 1.9-fold increase between sparkling wines from 0 to 9 months of ageing. Meanwhile, ethyl phenylacetate was the least affected compound during this period. A lower synthesis ratio has also been described in the literature for this compound (Antalick et al., 2014), supporting our results. The MEFA group showed the highest concentrations at 0 months of ageing, decreasing by about 15% in sparkling wines at 3 months and remaining constant during the remaining ageing period. MEFAs have not been studied in depth in the literature. Only a few studies reported lower contents of this group in aged red wines than in young ones (Antalick et al., 2014; Gammacurta, Marchand, Albertin, Moine, & de Revel, 2014) and no references concerning ageing on lees have been found to date.

3.3.3. Interaction effects

The total content of esters displayed significant interactions (Table 2). It meant that ageing factor affected the aroma profile of the M and NM sparkling wines in a different way. Total esters decreased during ageing in the NM (from 2480 to 2150 µg L^{-1}) but they remained constant in the M wines (around 1600 µg L^{-1}, Supplementary Table 4). The HAA and EEBA groups also showed significant interactions. Decreases in HAA in both types of sparkling wines were observed once 3 months in contact with lees was reached. After this period, different trends were found between the NM and M sparkling wines (Supplementary Table 4). In the NM sparkling wines, HAAs decreased by around 16%,

from 3 to 9 months of ageing. Meanwhile, the M wines displayed non-significant differences. Moreover, the hydrolysis phenomena described for the HAA group in the last paragraph was significantly less marked in the M sparkling wines. Lower variations in hexyl and isobutyl acetates were found in the M sparkling wines (decreases of 2.0 and 1.0-fold for hexyl and isobutyl acetates after 9 months of ageing in M sparkling wines, compared to the 2.5-fold and 1.3-fold decreases in the NM ones), supporting the less important hydrolysis phenomena in M sparkling wines. The impact of different chemical compositions of wines on ester hydrolysis has been suggested (Makhotkina & Kilmartin, 2012; Ribéreau-Gayon, 2000), which might help to understand the different trends observed.

EEBAs increased during the first 3 months of ageing, remained at constant levels until 6 months and increased again in the last period. However, the interaction effect revealed that the behaviour of the NM and M sparkling wines differed, ethyl 2-methylbutyrate standing out as the most affected compound. A higher synthesis of this compound was found in the M sparkling wines compared to the NM ones. The synthesis of EEBAs during ageing has been widely demonstrated. However, to the authors' knowledge this is the first study to report that the use of pre-fermentative maceration in sparkling wines seems to induce a higher genesis of EEBAs over ageing. EEBA volatilization was less altered by the non-volatile matrix, as reported in the literature (Lorrain et al., 2013). This fact might help to explain the higher synthesis of EEBAs observed in the M sparkling wines. Further studies to elucidate the mechanisms involved in this issue should be conducted.

3.4. Multivariate analysis

A multivariate analysis approach was performed to establish how the factors under study affected the ester profiles. The supervised chemometric techniques are able to match the analytical data obtained to a label or class. The most widely used of the supervised techniques is the partial least squares discriminant analysis (PLS-DA). PLS-DA is a multivariate method that has been referenced in the analysis of volatile compounds in wine for this purpose (Cynkar, Dambergs, Smith, & Cozzolino, 2010; Perestrelo et al., 2014). This technique combines principal component analysis with multiple regression features. It is based on a co-variance algorithm mixing two matrices, the explanatory matrix (*X*) that represents the rows dataset and the explicative matrix (*Y*) as a vector in accordance with the different classes studied. In addition, orthogonal signal correction can be used alongside the PLS-DA model as a way to remove variation in the irrelevant dataset to predict the dependent variable, and this resultant model (OPLS-DA) can be easier to interpret.

Two OPLS-DA models were performed (Fig. 1). The matrix used was built with 24 rows × 24 columns. The rows represented the samples and the columns the volatile compounds. The first model was based on the pre-fermentative strategy, labelling the NM sparkling wines as class 1 and the M ones as class 2. The second OPLS-DA model was built based on ageing effect, labelling sparkling wines without ageing (0 months) as class 1 and aged sparkling wines (3, 6 and 9 months of ageing) as class 2. In both statistical methods, only two latent variables were selected due to the percentage of covariance captured in the *X*-matrix and the lowest root mean squares error in cross validation (RMSECV). The scores and loadings were plotted in a plane defined by these two latent variables (LV1 and LV2) in each case. The models achieved high sensitivity and specificity rates (100%, Table 3). Hotelling-s T^2 *versus* Q^2 residuals plot was selected as the outlier detection technique and it has been referenced in multivariate studies (Lindon, Nicholson,

& Holmes, 2011). Therefore, each volatile compound was weighted according to their value, to improve the model prediction (Berrueta, Alonso-Salces, & Héberger, 2007). Several pre-processing techniques were evaluated (log transformation, mean centring, pareto scaling and autoscaling), 'auto-scaling' achieving the highest prediction ability. The re-sampling methodology used in this study was leave-one-out cross validation (LOOCV). Moreover, two data subsets were randomly obtained, the training set (66% of the samples) and the test set (33% of the samples for the external validation).

The first OPLS-DA model (Fig. 1.a) established the impact of the pre-fermentation strategy on the ester composition of the sparkling wines. LV1 and LV2 explained 81.83% of the total variance in the *X*-matrix and 99.10% of the variance found in *Y*-matrix. LV1 held up to 66.64% of the data variability and separated the M sparkling wines from the NM ones. The main characteristic compounds of the M sparkling wines were those with the highest positive coefficients of LV1 and separated them from NM ones. Likewise, compounds distributed on the highest negative values of LV1 were the most characteristic of NM sparkling wines. Additionally, the second multivariate analysis performed allowed us to establish the impact of ageing on the volatile profiles of the sparkling wines (Fig. 1b). The results obtained explained 44.30% of the total variance in the *X*-matrix, responding to 95.70% of the variance found in the *Y*-matrix. LV1 explained 33.55% of the data variability and separated young sparkling wines (0 months of ageing) from the rest (sparkling wines with 3, 6 and 9 ageing period). Compounds distributed on the highest positive coefficients of LV1 separated aged sparkling wines from the young ones. Meanwhile, compounds situated on the highest negative values differentiated the young sparkling wines. Both models were internally and externally validated and successful results were achieved (Table 3).

3.5. Potential volatile markers

A novel approach was followed to detect volatile marker compounds (Cuevas, Moreno-Rojas, Arroyo, Daza, & Ruiz-Moreno, 2016). The methodology was based on two criteria: variable importance projection (VIP) values (calculated from the OPLS-DA models in the multivariate analysis) and the category of the factorial analysis of variance (univariate analysis) used under low dimensional datasets. Potential volatile markers were selected in compounds with a VIP $\geq$ 1.5 and category ***a*** (non-shared with other compounds) in the univariate analysis with a Fisher's least significance level of 0.01 (Table 2). This methodology allowed us to identify potential markers linked to the pre-fermentative strategy and ageing factors.

Regarding the pre-fermentative maceration, ethyl heptanoate was the only compound selected as a candidate marker of the M sparkling wines (Table 2). Likewise, ethyl octanoate was selected as candidate marker of the NM sparkling wines. Nonetheless, other compounds such as ethyl phenylacetate, methyl hexanoate and ethyl propanoate for the M sparkling wines, and ethyl hexanoate, isoamyl octanoate and isobutyl hexanoate for the NM sparkling wines displayed VIP coefficients close to 1.5 and the non-shared category ***a*** in the univariate analysis. Thus, the above compounds may also be considered as potential contributors linked to each named factor.

With regard to ageing marker compounds, three EEBAs (ethyl isovalerate, ethyl isobutyrate and ethyl 2-methylbutyrate) were obtained as candidate markers of aged sparkling wines (Table 2). However, in the literature we can find that ethyl isobutyrate was reported as a marker of aged Fino wines (Moreno, Zea, Moyano, & Medina, 2005). On the other hand, the HAAs (phenylethyl acetate, isoamyl acetate, propyl acetate and hexyl acetate) were the

potential volatile markers of young sparkling wines. These results are in agreement with the literature, where it was found that isoamyl acetate, phenylethyl acetate and hexyl acetate were characteristic compounds of young sparkling wines (Francioli et al., 2003). Additionally, as described above for the pre-fermentative maceration factor, methyl octanoate displayed a VIP value close to 1.5 and it may also be considered as a potential aromatic contributor linked to young sparkling wines.

Among the volatile markers identified above, only a few may be considered as the main contributors to the sensory differences of the different sparkling wines. To identify them, the potential markers were related with their odour activity (Supplementary Table 1). Thus, ethyl octanoate (odour threshold 580 $\mu g\ L^{-1}$) presented concentrations above the threshold in all the NM sparkling wines. Meanwhile, ethyl hexanoate (odour threshold 14 $\mu g\ L^{-1}$) and isoamyl acetate (threshold 30 $\mu g\ L^{-1}$) were odorants, regardless of the pre-fermentative strategy. However, their concentrations were significantly higher in the NM sparkling wines than in the M ones. Ethyl isobutyrate (odour threshold 15 $\mu g\ L^{-1}$) and ethyl isovalerate (odour threshold 3 $\mu g\ L^{-1}$) presented concentrations above the threshold. However, the levels were significantly higher in the M sparkling wines. Ethyl 2-methylbutyrate (threshold 18 $\mu g\ L^{-1}$) showed a remarkably different behaviour. In the NM sparkling wines, it displayed levels above the threshold only at 9 months of ageing, while it was an odorant compound in all the M sparkling wines regardless of the ageing period.

4. Conclusions

The results highlighted the impact of pre-fermentative maceration and ageing on the aroma profile of sparkling wines. The pre-fermentative maceration significantly affected the ester profile of sparkling wines. Higher levels of EEBAs, cinnamates, MEFAs, EEOCNFAs and

miscellaneous compounds were found in the sparkling wines obtained from a pre-fermentative maceration and higher levels of ethyl esters of fatty acids (EEFAs) and higher alcohol acetates (HAAs) were found in those without pre-fermentative maceration. Ethyl heptanoate, ethyl phenylacetate, methyl hexanoate and ethyl propanoate stood out as potential volatile markers of the pre-fermentative maceration, while ethyl isovalerate, ethyl isobutyrate and ethyl 2-methylbutyrate were identified as ageing markers. The ageing factor affected the ester profile of the wines with and without maceration in different ways, highlighting the importance of additional research focused on the contribution of EEBAs to wine aroma during ageing. Further studies focusing on monitoring the sensory impact of the reported markers should also be performed to verify the impact of this differential ester profile resulting from the pre-fermentative maceration and ageing in sparkling wines.

Acknowledgments

This work was funded by the internal funds of the Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA) through the Project 'AVA complementario al Transforma “Vid y Vino” (PP.AVA.AVA201301.3), the European Social Fund (ESF), the European Rural Development Fund (ERDF) and the National Youth Guarantee System.

José Manuel Muñoz Redondo was granted a research contract funded by the Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), the European Social Fund (ESF) and the Youth Employment Initiative (YEI). Maria José Ruiz Moreno was granted a postdoctoral research contract funded by the Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA) and the European Social Fund (ESF).

References

Antalick, G., Perello, M.-C., & de Revel, G. (2010). Development, validation and application of a specific method for the quantitative determination of wine esters by headspace-solid-phase microextraction-gas chromatography–mass spectrometry. *Food Chemistry*, *121*(4), 1236–1245.

Antalick, G., Perello, M.-C., & de Revel, G. (2014). Esters in wines: New Insight through the Establishment of a Database of French Wines. *American Journal of Enology and Viticulture*, *65*(3), 293–304.

Antalick, G., Šuklje, K., Blackman, J. W., Meeks, C., Deloire, A., & Schmidtke, L. M. (2015). Influence of grape composition on red wine ester profile: comparison between Cabernet Sauvignon and Shiraz cultivars from Australian warm climate. *Journal of Agricultural and Food Chemistry*, *63*(18), 4664–4672.

Berrueta, L. A., Alonso-Salces, R. M., & Héberger, K. (2007). Supervised pattern recognition in food analysis. *Journal of Chromatography A*, *1158*(1), 196–214.

Caliari, V., Burin, V. M., Rosier, J. P., & BordignonLuiz, M. T. (2014). Aromatic profile of Brazilian sparkling wines produced with classical and innovative grape varieties. *Food Research International*, *62*, 965–973.

Campo, E., Cacho, J., & Ferreira, V. (2008). The chemical characterization of the aroma of dessert and sparkling white wines (Pedro Ximenez, Fino, Sauternes, and Cava) by gas chromatography- olfactometry and chemical quantitative analysis. *Journal of Agricultural and Food Chemistry*, *56*(7), 2477–2484.

Cozzolino, D., Cynkar, W. U., Shah, N., Dambergs, R. G., & Smith, P. A. (2009). A brief introduction to multivariate methods in grape and wine analysis. *International Journal of Wine Research*, *1*(1), 123–130.

Cuevas, F. J., Moreno-Rojas, J. M., Arroyo, F., Daza, A., & Ruiz-Moreno, M. J. (2016). Effect of management (organic vs conventional) on volatile profiles of six plum cultivars (Prunus salicina Lindl.). A chemometric approach for varietal classification and determination of potential markers. *Food Chemistry*, *199*, 479–484.

Cynkar, W., Dambergs, R., Smith, P., & Cozzolino, D. (2010). Classification of Tempranillo wines according to geographic origin: Combination of mass spectrometry based electronic nose and chemometrics. *Analytica Chimica Acta*, *660*(1), 227–231.

Díaz-Maroto, M. C., Schneider, R., & Baumes, R. (2005). Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging. *Journal of Agricultural and Food Chemistry*, *53*(9), 3503–3509.

Escudero, A., Campo, E., Fariña, L., Cacho, J., & Ferreira, V. (2007). Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry*, *55*(11), 4501–4510.

Francioli, S., Torrens, J., Riu-Aumatell, M., López-Tamames, E., & Buxaderas, S. (2003). Volatile compounds by SPME-GC as age markers of sparkling wines. *American Journal of Enology and Viticulture*, *54*(3), 158–162.

Gammacurta, M., Marchand, S., Albertin, W., Moine, V., & de Revel, G. (2014). Impact of yeast strain on ester levels and fruity aroma persistence during aging of bordeaux red wines. *Journal of Agricultural and Food Chemistry*, *62*(23), 5378–5389.

Herraiz, T., Martin-Alvarez, P. J., Reglero, G., Herraiz, M., & Cabezudo, M. D. (1990). Effects of the presence of skins during alcoholic fermentation on the composition of wine volatiles. *VITIS-Journal of Grapevine Research*, *29*(4), 239–249.

Institut del Cava. (2015). <http://www.institutdelcava.com/identidad/estadisticas-del-cava> Accessed October 10, 2016

Le Comité Champagne. (2015). <http://www.champagne.fr/fr/economie/expeditions-de-vins-de-champagne> Accessed October 10, 2016

Lindon, J. C., Nicholson, J. K., & Holmes, E. (2011). *The Handbook of Metabonomics and Metabolomics*. Elsevier.

Lockshin, L., & Corsi, A. M. (2012). Consumer behaviour for wine 2.0: A review since 2003 and future directions. *Wine Economics and Policy*, *1*(1), 2–23.

Lorrain, B., Tempere, S., Iturmendi, N., Moine, V., de Revel, G., & Teissedre, P.-L. (2013). Influence of phenolic compounds on the sensorial perception and volatility of red wine esters in model solution: An insight at the molecular level. *Food Chemistry*, *140*(1), 76–82.

Lytra, G., Tempere, S., Le Floch, A., de Revel, G., & Barbe, J.-C. (2013). Study of sensory interactions among red wine fruity esters in a model solution. *Journal of Agricultural and Food Chemistry*, *61*(36), 8504–8513.

Makhotkina, O., & Kilmartin, P. A. (2012). Hydrolysis and formation of volatile esters in New Zealand Sauvignon blanc wine. *Food Chemistry*, *135*(2), 486–493.

Marquez, A., Serratosa, M. P., Merida, J., Zea, L., & Moyano, L. (2014). Optimization and validation of an automated DHS–TD–GC–MS method for the determination of aromatic esters in sweet wines. *Talanta*, *123*, 32–38.

Martínez-Lapuente, L., Guadalupe, Z., Ayestarán, B., & Pérez-Magariño, S. (2015). Role of major wine constituents in the foam properties of white and rosé sparkling wines. *Food Chemistry*, *174*, 330–338.

Moreno, J. A., Zea, L., Moyano, L., & Medina, M. (2005). Aroma compounds as markers of the changes in sherry wines subjected to biological ageing. *Food Control*, *16*(4), 333–338.

Perestrelo, R., Barros, A. S., Rocha, S. M., & Câmara, J. S. (2014). Establishment of the varietal profile of Vitis vinifera L. grape varieties from different geographical regions based on HS-SPME/GC–qMS combined with chemometric tools. *Microchemical Journal*, *116*, 107–117.

Piñeiro, Z., Natera, R., Castro, R., Palma, M., Puertas, B., & Barroso, C. G. (2006). Characterisation of volatile fraction of monovarietal wines: Influence of winemaking practices. *Analytica Chimica Acta*, *563*(1), 165–172.

Pozo-Bayón, M. Á., Martínez-Rodríguez, A., Pueyo, E., & Moreno-Arribas, M. V. (2009). Chemical and biochemical features involved in sparkling wine production: from a traditional to an improved winemaking technology. *Trends in Food Science & Technology*, *20*(6), 289–299.

Ramey, D. D., & Ough, C. S. (1980). Volatile ester hydrolysis or formation during storage of model solutions and wines. *Journal of Agricultural and Food Chemistry*, *28*(5), 928–934.

Ribéreau-Gayon, P. (2000). *The chemistry of wine stabilization and treatments*. Wiley.

Riu-Aumatell, M., Bosch-Fusté, J., López-Tamames, E., & Buxaderas, S. (2006). Development of volatile compounds of cava (Spanish sparkling wine) during long ageing time in contact with lees. *Food Chemistry*, *95*(2), 237–242.

Robinson, A. L., Boss, P. K., Solomon, P. S., Trengove, R. D., Heymann, H., & Ebeler, S. E. (2014). Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *American Journal of Enology and Viticulture*, *65*(1), 1–24.

Rodríguez-Bencomo, J. J., Ortega-Heras, M., & Pérez-Magariño, S. (2010). Effect of alternative techniques to ageing on lees and use of non-toasted oak chips in alcoholic fermentation on the aromatic composition of red wine. *European Food Research and Technology*, *230*(3), 485–496.

Sáenz-Navajas, M.-P., Ballester, J., Pêcher, C., Peyron, D., & Valentin, D. (2013). Sensory drivers of intrinsic quality of red wines: effect of culture and level of expertise. *Food Research International*, *54*(2), 1506–1518.

Saerens, S. M., Delvaux, F. R., Verstrepen, K. J., & Thevelein, J. M. (2010). Production and biological function of volatile esters in Saccharomyces cerevisiae. *Microbial Biotechnology*, *3*(2), 165–177.

Sumby, K. M., Grbin, P. R., & Jiranek, V. (2010). Microbial modulation of aromatic esters in wine: current knowledge and future prospects. *Food Chemistry*, *121*(1), 1–16.

OIV. The International Organisation of Vine and Wine. (2014). <http://www.oiv.int/en/the-international-organisation-of-vine-and-wine> Accessed October 10, 2016

Torresi, S., Frangipane, M. T., & Anelli, G. (2011). Biotechnologies in sparkling wine production. Interesting approaches for quality improvement: A review. *Food Chemistry*, *129*(3), 1232–1241.

Ubeda, C., Callejón, R. M., Troncoso, A. M., Peña-Neira, A., & Morales, M. L. (2016). Volatile profile characterisation of Chilean sparkling wines produced by traditional and Charmat methods via sequential stir bar sorptive extraction. *Food Chemistry*, *207*, 261–271.

Yu, S.-H., Hsieh, H.-Y., Pang, J.-C., Tang, D.-W., Shih, C.-M., Tsai, M.-L., … Mi, F.-L. (2013). Active films from water-soluble chitosan/cellulose composites incorporating releasable caffeic acid for inhibition of lipid oxidation in fish oil emulsions. *Food Hydrocolloids*, *32*(1), 9–19.

Tables

Table 1. Sparkling wine oenological parameters. Two-way ANOVA for pre-fermentative maceration and ageing factors and interaction (pre-fermentative maceration × ageing) effect

Table 2. Concentrations of ester compounds (µg L^{-1}) in sparkling wines. Two-way ANOVA for pre-fermentative maceration and ageing factors and interaction (pre-fermentative maceration × ageing) effect

Table 3. Statistics results of the OPLS-DA models performed according to the pre-fermentative maceration and ageing factors

Supplementary Table 1. Ions, internal standards, linearity, repeatability, reproducibility, detection and quantification limits, and recoveries at two concentration levels in sparkling wines

Supplementary Table 2. Mean concentrations with standard deviation (µg L^{-1}) of ester compounds for NM sparkling wines at 0, 3, 6 and 9 months of ageing

Supplementary Table 3. Mean concentrations with standard deviation (µg L^{-1}) of ester compounds for M sparkling wines at 0, 3, 6 and 9 months of ageing

Supplementary Table 4. Two-way ANOVA for interaction effects of pre-fermentative maceration and ageing factors on ester compounds (µg L^{-1})

Figure caption

Figure 1. Orthogonal partial least squares discriminant analysis (OPLS-DA) performed on (a) pre-fermentative maceration and (b) ageing classes of sparkling wines. Scores and loadings are shown in the two first latent variables.

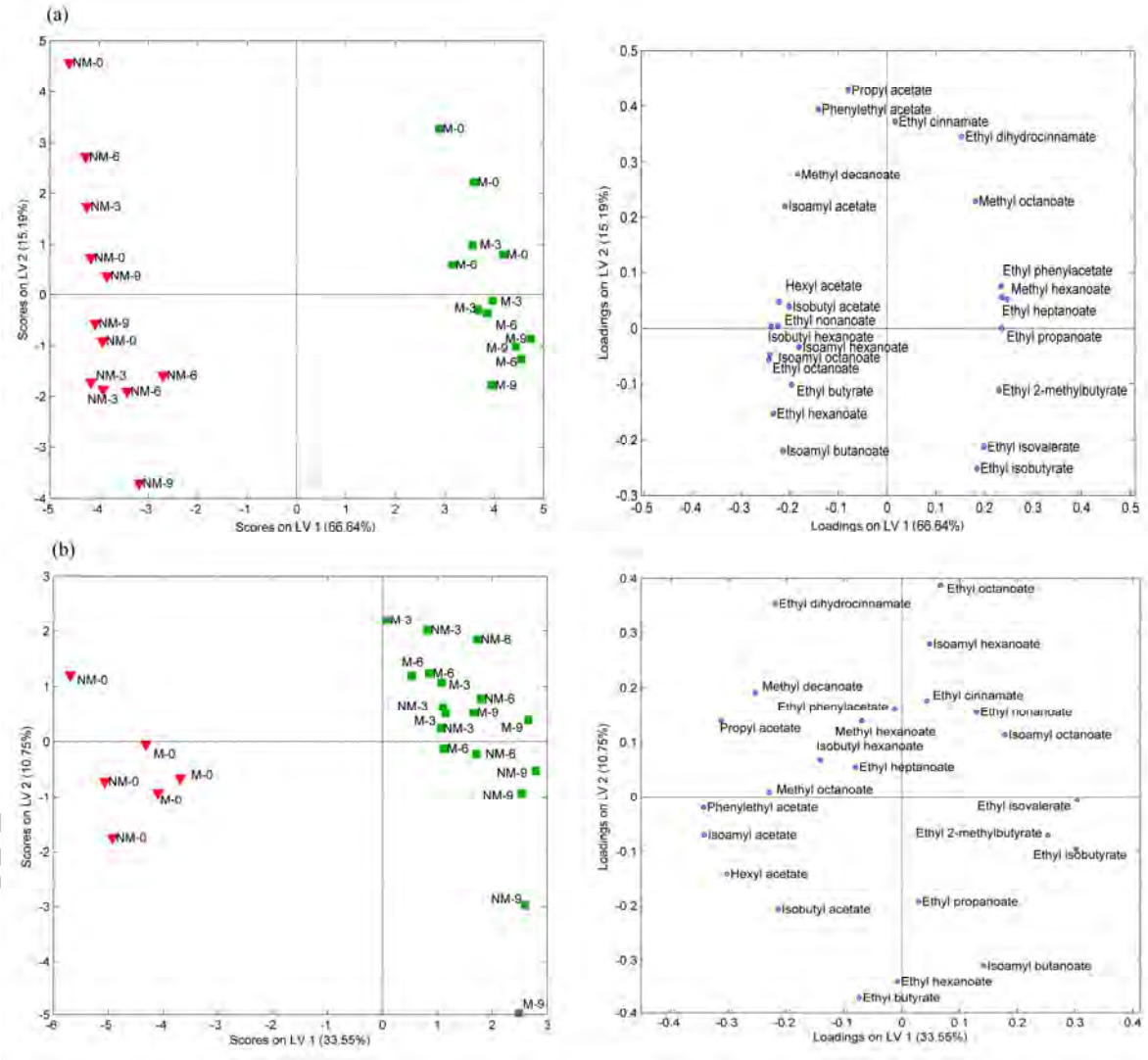

Table 1. Sparkling wine oenological parameters. Two-way ANOVA for pre-fermentative maceration and ageing factors and interaction (pre-fermentative maceration × ageing) effect

	pre-fermentative maceration			ageing (months)					interaction
	NM[a]	M[b]	*p*-value[c]	0	3	6	9	*p*-value[c]	*p*-value[c]
Ethanol (vol %)	12.6	12.4	ns	12.6	12.4	12.5	12.6	ns	ns
Residual sugars (g L^{-1})	2.3	2.4	ns	2.3	2.2	2.5	2.4	ns	ns
pH (20 ºC)	3.18b	3.24a	***	3.20ab	3.17b	3.24a	3.22a	**	ns
Total acidity[d] (g L^{-1})	5.58	5.67	ns	5.74	5.65	5.51	5.59	ns	ns
Volatile acidity[e] (g L^{-1})	0.33b	0.39a	***	0.37	0.39	0.37	0.35	ns	ns
Absorbance at 420 nm	0.04b	0.06a	***	0.05	0.05	0.05	0.05	ns	ns
Total phenolic content[f] (mg L^{-1})	116b	162a	***	139	141	138	137	ns	ns

[a]NM: Sparkling wines elaborated without skin maceration. [b]M: Sparkling wines elaborated from skin maceration. [c]Significance level: ns = non-significant. * = $p < 0.05$. ** = $p < 0.01$. *** = $p < 0.001$. [d]calculated as tartaric acid. [e]calculated as acetic acid. [f]Gallic acid equivalents.

Table 2. Concentrations of ester compounds (μg L^{-1}) in sparkling wines. Two-way ANOVA for pre-fermentative maceration and ageing factors and interaction (pre-fermentative maceration × ageing) effect

group[d]	compound	pre-fermentative maceration				ageing (months)						interaction
		NM[a]	M[b]	p-value[c]	VIP	0	3	6	9	p-value[c]	VIP	p-value[c]
EEFAs	ethyl butyrate	144a	102b	***	0.9	130	118	112	131	ns	0.2	ns
	ethyl hexanoate	571a	312b	***	1.4	449	451	407	459	ns	0.1	ns
	ethyl octanoate	**769a**	459b	***	**1.5**	606	626	628	594	ns	0.2	ns
	ΣEEFAs	1484a	872b	***	-	1186	1196	1147	1185	ns	-	ns
HAAs	propyl acetate	3.2	3.0	ns	0.1	**3.9a**	2.8b	3.0b	2.6b	***	**2.2**	ns
	isobutyl acetate	16a	12b	***	1.0	16a	13c	13c	14b	***	1.3	***
	isoamyl acetate	407a	251b	***	1.0	**502a**	290b	275bc	249c	***	**2.8**	***
	hexyl acetate	16a	7b	***	1.1	**18a**	10b	10b	8b	***	**2.2**	**
	phenylethylacetate	52a	37b	***	0.4	**76a**	40b	33c	30c	***	**2.9**	*
	ΣHAAs	495a	310b	***	-	617a	355b	333bc	304c	***	-	***
EEBAs	ethyl isobutyrate	97b	135a	***	0.7	78c	119b	118b	**149a**	***	**2.1**	**
	ethyl 2-methylbutyrate	15b	39a	***	1.2	17c	27b	29b	**36a**	***	**1.6**	***
	ethyl isovalerate	27b	40a	***	0.9	22c	35b	34b	**43a**	***	**2.2**	*
	ethyl phenylacetate	1.3b	2.8a	***	1.4	2.0ab	2.2a	1.8b	2.1a	*	0.0	ns
	ΣEEBAs	140b	217a	***	-	119c	183b	183b	230a	***	-	***
Cinnamates	ethyl dihydrocinnamate	0.18b	0.22a	***	0.7	0.2	0.2	0.2	0.2	ns	0.9	ns
	ethyl cinnamate	0.027	0.028	ns	0.0	0.027	0.026	0.027	0.031	ns	0.0	ns
	Σcinnamates	0.21b	0.25a	**	-	0.25	0.23	0.22	0.22	ns	-	ns
MEFAs	methyl hexanoate	0.36b	0.66a	***	1.3	0.52	0.48	0.52	0.51	ns	0.1	ns
	methyl octanoate	0.51b	0.64a	***	0.9	0.65a	0.56b	0.53b	0.57b	**	1.4	ns
	methyl decanoate	0.14a	0.07b	***	0.7	0.16a	0.12b	0.09bc	0.08c	**	1.2	ns
	ΣMEFAs	1.0b	1.4a	***	-	1.3a	1.1b	1.1b	1.2b	*	-	ns
IEFAs	isoamyl butanoate	0.32a	0.19b	***	1.2	0.24	0.25	0.24	0.28	ns	0.4	ns
	isoamyl hexanoate	1.32a	1.03b	***	0.8	1.13	1.24	1.17	1.16	ns	0.2	ns
	isoamyl octanoate	1.66a	0.57b	***	1.4	0.95b	1.19a	1.10ab	1.23a	*	0.7	ns
	ΣIEFAs	3.3a	1.8b	***	-	2.3	2.7	2.5	2.7	ns	-	*
EEOCNFAs	ethyl heptanoate	0.4b	**2.2a**	***	**1.5**	1.4	1.3	1.3	1.3	ns	0.2	ns
	ethyl nonanoate	1.42a	0.78b	***	1.2	0.98	1.12	1.09	1.22	ns	0.4	ns
	ΣEEOCNFAs	1.9b	3.0a	***	-	2.4	2.4	2.3	2.5	ns	-	ns
Miscellaneous	ethyl propanoate	103b	189a	***	1.3	144	137	146	158	ns	0.0	ns
	isobutyl hexanoate	0.22a	0.12b	***	1.3	0.19a	0.18a	0.16b	0.17ab	*	0.4	ns
	Σmiscellaneous	103b	189a	***	-	144	137	146	158	ns	-	ns
	total esters	2229a	1595b	***	-	2072a	1877b	1815b	1883b	***	-	*

[a]NM: Sparkling wines elaborated without skin maceration. [b]M: Sparkling wines elaborated from skin maceration. [c]Significance level: ns = non-significant. * = $p < 0.05$. ** = $p < 0.01$. *** = $p < 0.001$. [d]EEFAs: Ethyl esters of fatty acids; HAAs: Higher alcohol acetates; EEBAs: Ethyl esters of branched acids; MEFAs:

Methyl esters of fatty acids; IEFAs: Isoamyl esters of fatty acids; EEOCNFAs: Ethyl esters of odd carbon number fatty acids. Concentrations and VIP values considered for potential markers are highlighted in bold type letter.

Table 3. Statistics results of the OPLS-DA models performed according to the pre-fermentative maceration and ageing factors

classes[a]	pre-fermentative maceration[b]		ageing	
	NM	M	young wines	aged wines
calibration step				
sensitivity (Cal)	100	100	100	100
specificity (Cal)	100	100	100	100
RMSEC:	0.056	0.056	0.090	0.090
R^2	0.987	0.987	0.947	0.947
cross-validation step				
sensitivity (CV)	100	100	100	100
specificity (CV)	100	100	100	100
RMSECV	0.089	0.089	0.179	0.179
R^2	0.969	0.969	0.835	0.835
external validation				
sensitivity (CV)	100	100	100	100
specificity (CV)	100	100	100	100
RMSEP	0.09	0.09	0.136	0.136
R^2	0.975	0.975	0.921	0.921

[a]Sensitivity: Proportion of positives that are correctly identified. Specificity: Proportion of negatives that are correctly identified. RMSEC: Root mean squares error at the calibration step. RMSECV: Root mean squares error at the cross validation step. RMSEP: Root mean squares error at the external validation R^2: Regression coefficient. [b]NM: Sparkling wines elaborated without skin maceration; M: Sparkling wines elaborated from skin maceration

Highlights

Skin maceration and ageing factors modulated the ester profile of sparkling wines

A different development on sparkling wine ester contents during ageing was found

The aroma differentiation of sparkling wines was confirmed by chemometrics

Esters as markers of skin maceration and ageing were reported for the first time

EBBAs highlighted as ageing-markers and sensory contributors of sparkling wines