Impact of using Trepat and Monastrell red grape varieties on the volatile and nitrogen composition during the manufacture of rosé Cava sparkling wines

Pozo-Bayón, M.A*., Martín-Álvarez, P.J., Moreno-Arribas, M.V., Andujar-Ortiz, I., Pueyo, E.

Instituto de Fermentaciones Industriales (CSIC), c/ Juan de la Cierva, 3, 28006, Madrid, Spain

* corresponding author email: mdelpozo@ifi.csic.es; phone +34 91 562 2900 ext 388

Abstract

The impact of using Trepat and Monastrell red grape varieties during the manufacture of rosé sparkling Cava wines on the nitrogen and volatile composition compared to a white Cava manufactured with a blend of typical white grape varieties (Xarello: Macabeo: Parellada) has been investigated. The wines were industrially manufactured in a cellar, and the concentrations of outstanding nitrogen compounds and 23 target volatile compounds belonging to different chemical classes were determined in the base wines and in the corresponding Cava wines after 9, 12, 15 and 18 months of aging on lees. After the application of multivariate statistical analysis, the results showed the large effect of the variety employed in the manufacture of Cavas compared to the changes in wine composition due to the aging time. Depending on the composition, Trepat and White Cava wines were more similar than those manufactured with the variety Monastrell. However, the sensory study showed that the two rosé Cava wines had good sensory attributes and even slightly better foam characteristics than the white ones. The sensory study highlighted for the first time the adequacy of using Monastrell red grape variety to manufacture rosé sparkling wines.

Key Words: rosé Cava sparkling wines, red grape varieties, nitrogen compounds, volatile compounds

1. Introduction

In recent years, a new market strategy in the oenological industry based on the diversification of wine production and on the exploitation of the characteristics and peculiarities of autochthonous grape varieties is emerging. In this frame, sparkling wines produced by the traditional method (second fermentation and aging in the same bottle that reaches the consumers) and manufactured with red grape varieties may be considered as a good example of these types of new products. Although different factors such as variations in winemaking technology employed in their production and other viticultural characteristics (soil, vineyard yield, etc) can influence sparkling wine composition, the grape variety used in their manufacture can be considered one of the most important (Pozo-Bayón, Martínez-Rodríguez, Pueyo & Moreno-Arribas, 2009).

In the case of Cava wines (Spanish denomination of origin for sparkling wines manufactured by the traditional method), although they are mainly produced with white grape varieties, the red varieties Garnacha, Monastrell, Trepat and Pinot noir can be used to produce rosé Cava (BOE, 20 Noviembre de 1991). The Trepat variety is exclusively autochthonous from the Penedès region (Catalonia), where nearly 98 % of Cava is produced, therefore it is a variety of great interest in terms of maintaining the identity and idiosyncrasy of Cava (Girbau-Sola, Lopez-Barajas, Lopez-Tamames & Buxaderas, 2002a). In addition, Monastrell is one of the most appreciated red grape varieties by many worldwide producers.

However, few studies have been focused on sparkling wines made exclusively with red grape varieties. Some of them have aimed on the evolution of phenolic compounds (Pozo-Bayon, Hernandez, Martin-Alvarez & Polo, 2003a; Pozo-Bayon, Monagas, Polo & Gomez-Cordoves, 2004) because of their importance for the colour in rosé sparkling wines. In addition to these works, and due to the importance of foam characteristics in sparkling wines, Girbau-Solá and collaborators (Girbau-Sola et al., 2002a; Girbau-Sola, Lopez-Tamames, Bujan & Buxaderas, 2002b) performed outstanding studies in which they showed the foam aptitude of Trepat and Monastrell red varieties and their coupages with white varieties for rosé and *blanc de noir* Cava manufacture.

Aroma can be also considered one of the most decisive quality attributes in wines. In sparkling wines manufactured by the traditional method, it has been shown that the second fermentation and the aging of wine on lees can modify the volatile composition of sparkling wines (Francioli, Torrens, Riu-Aumatell, Lopes-Tamames & Buxaderas, 2003; Pozo-Bayon, Pueyo, Martin-Alvarez, Martinez-Rodriguez & Polo, 2003b). Nevertheless, most of these studies have been carried out on wines manufactured with white grapes varieties. To our knowledge, only two works have focused on sparkling wines exclusively produced with red grapes. In the first study, witch was based on sensory and analytical studies, Hidalgo and collaborators (Hidalgo, Pueyo, Pozo-Bayon, Martinez-Rodriguez, Martin-Alvarez & Polo, 2004), showed the adequacy of the Garnacha red variety for the manufacturing of rosé sparkling wines. In the second work, for the first time, Riu-Aumatell and co-workers (Riu-Aumatell, Bosch-Fuste, Lopez-Tamames & Buxaderas, 2006) analysed the volatile composition of a rosé Cava wine manufactured with the red Trepat variety and a white Cava by using two different SPME polymers. Although the work mainly focused on the performance of both types of fibres for volatile analysis, they noticed important differences in some volatile compounds between the two types of wines. As far we know, there are no others works in the literature focused on sparkling wines manufactured with the Monastrell variety.

Therefore, the present work was mainly aimed to evaluate the effect of the use of red grape varieties (Trepat, Monastrell) during the industrial production of rosé Cava wines on the volatile profile, compared to a common white Cava produced from a blend of white grape varieties. Moreover, outstanding parameters such as the nitrogen composition and the sensory quality of the rosé Cava sparkling wines were also studied. All the Cava wines were produced on an industrial scale, in the same winery and from the same vintage.

2. Materials and Methods

2.1 Wine Samples

All the wines of this study were industrially manufactured from grapes from the 1997 harvest in a wine cellar from the Penedès (Catalonia, Spain). Firstly, the base wines were manufactured. The rosé base wines (Bw) were made from Trepat (T-Bw) and Monastrell (T-Bw) grape varieties. For each type of variety, two types of base wines were obtained from two lots of grapes that were pressed and fermented separately (A and B). Therefore two base wines from Trepat (TA-Bw and TB-Bw) and two from Monastrell (MA-Bw and MB-Bw) were made. In addition, a white blend base wine (WB-Bw) was made from Macabeo, Xarel.lo and Parellada (58:36:6) white grape varieties. All the wines were fermented without skins. The fermentation took place in tanks of 100000 L at 16 to 18 ºC, after the inoculation of the selected winery yeast

(*Saccharomyces cerevisiae*; 2 million cells/mL grape juice). Once fermentation was finished, wines were racked and settled three times, filtered and then passed through a 0.45 µm filter to obtain the sparkling base wines. Malolactic fermentation took place in base wines from Trepat and Monastrell before the second fermentation. Due to their high colour intensity M-Bw (A and B) were clarified before the blending with 75g/100 L of active carbon. From each of the five base wines a batch of sparkling wines was industrially manufactured by the traditional method in the same vinery. Second fermentation was performed when base wines were inoculated with the *tirage liquor* formed by sucrose (200 g/L), the yeast *Saccharomyces bayanus* (above 1.6 cellules/mL) and bentonite (10 g/L). Second fermentation and aging with yeast were carried out at approximately 15-16 ºC (cellar temperature). Disgorging was performed in the vinery after 9, 12, 15 and 18 months of aging with the. *Expedition liquor* (2 g sucrose/L) was added to all the sparkling wines immediately after disgorging. At each disgorging time, six bottles of the same wine were mixed and homogenized before sampling. Each analysis was conducted in duplicated on wines that had been previously centrifuged at 5ºC and 5000g for 15 minutes. Samples were kept in the freezer (-18 ºC) until their analysis.

2.2 Global composition

Alcoholic grade, total acidity, volatile acidity, pH, total SO_2, and reducing sugars were determined by the European Commission methods (European Community, 1990). Colour intensity was determined as the sum of the absorbance at 420 nm and 520 nm.

2.3 Determination of Nitrogen Compounds

Total nitrogen was determined using the Kjeldahl method with a Tecator Digestion System and a Kjeltec 1030 Auto Analyzer (Tecator AB, Höganäs, Sweden). Free amino acids were determined by the method described by Doi and collaborators (method 5) (Doi, Shibata & Matoba, 1981), based on the reaction of ninhydrin/Cd with the free amino group. The absorbance was determined at 507 nm and the results were expressed as mg of amino nitrogen /L. High molecular weight nitrogen (HMWN) was estimated as the difference between total nitrogen and the nitrogen from amino acids.

2.4 Volatile analysis

Analysis of the major volatile compounds was performed by direct injection into a gas chromatograph under the following conditions: Carbowax 20M fused-silica capillary column (30 m x 0.25 mm I.D), coated with a stationary phase of 0.25 μm thickness (Quadrex, New Haven, USA); split/splitless injector; FID detector; injector and detector temperature were 220 ºC. The initial oven temperature was 40 ºC (10 minutes hold). The temperature gradient was 7 ºC/min to 150 ºC, 30ºC/min to 210 º (2 minutes hold). The carrier gas was helium (12.5 psi, split 1/15). The compounds determined by this method were: acetaldehyde, ethyl acetate, methanol, 1-propanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol and ethyl lactate.

Minor volatile analysis was carried out by Gas Chromatography (GC) of the head space extract obtained with a 100 μm polydimethylsiloxane (PDMS) coated fused silica fiber (Supelco, Bellefonte, PA, USA), in the conditions described by Pozo-Bayón and co-workers (Pozo-Bayon, Pueyo, Martin-Alvarez & Polo, 2001). The compounds determined by this method were: 1-hexanol, *cis*-3-hexen-1-ol, isobutyl acetate, isopentyl acetate, hexyl acetate, butyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate, hexanoic acid, octanoic acid, decanoic acid and γ-butyrolactone.

The peak identities were assigned by the comparison of the relative retention times to the internal standards, 3-pentanol (60 mg/100 mL ethanol:water 10g/100mL) for the major volatile compounds, and methyl nonanoate (10 mg/100 mL absolute ethanol) for the minor volatile compounds, with those of the standards of analytical quality more than 99% purity from Sigma-Aldrich (St Louis, MO, USA) and Merck KGaA (Darsmstadt, Germany). For quantification purposes, calibration curves of each standard compound in synthetic wines were made under the same conditions as the samples.

2.5 Global sensory quality and visual evaluation of foam and effervescence

Sensory evaluation of the wines was carried out by a panel of experts (8 judges, 5 males and 3 females). A tasting card recommended by OIV (OIV, 1994) and modified by the Instituto Nacional de Denominaciones de Calidad of the former Spanish Ministry of Agriculture, Fisheries and Food was used. The scores used were penalizing meaning that better quality wines received a lower score. In this tasting card, each of the sensory attributes is evaluated and rated independently. The sensory attributes evaluated were visual aspect (0-9), aroma intensity (0-18), aroma quality (0-18), taste intensity (0-18), taste quality (0-27) and harmony (0-27). The sum of all the individual attributes allows to classify the wines as excellent (0-7), very good (8-23), good (24-44), correct (45-62), regular (63-78); inadequate (79-90) and >90 eliminated. In terms of visual aspect, special attention was not only paid to the colour but also to the observation of foam characteristics, which is of special importance in sparkling wines. The final scores were the average of the score of each judge after eliminating those that differed by more than one standard deviation when compared to the samples mean value. The wines were tested individually and not comparatively.

Visual evaluation of foam quality and effervescence was carried out by a panel of eight trained tasters, who used the protocol described by Obiols and co-workers (Obiols, De la Presa-Owens, Buxaderas, Bori & De la Torre-Boronat, 1998). The attributes assessed were the initial quantity of foam formed, whether the foam covered the whole surface of the wine, the presence of a foam collar on the surface of the wine, the size of the bubbles and the effervescence. The origin of the bubbles and the number of nucleation sites were not considered since the results obtained by Liger-Belair and collaborators (Liger-Belair, 2002) indicate that the bubble production depends on several kinds of particles present in the wine. The mode of the scores given by the eight tasters was used to reach the final score for each parameter.

2.5 Statistical Analysis

Two-way ANOVA was used to test the effect of the two factors studied (type of wine and aging time) and Student Newman-Keuls test for mean comparisons. Cluster analysis was used in order to obtain a preliminary view of the main causes for the changes in the analysed variables (the squared Euclidean distance was taken as a measure of proximity between two samples and Ward's method was used as a linkage rule). Principal Component Analysis (PCA) was used to examine the relationship among the variables (nitrogen compounds, volatile compounds, type of wine and aging time). STATISTICA for Windows (Version 7.1) was used for data processing (StatSoft, Inc., 2005, www.statsoft.com). This program was run on a personal computer.

3- Results and Discussion

3.1 Global composition of base wines and Cava wines

Table 1 shows the global composition of the base wines and Cava wines. In the table, the values corresponding to the base wines of two different lots but from the same variety have been averaged. In addition, the values of the different parameters determined in Cavas from the same variety but with different aging time (9, 12, 15 and 18 months) have also been reported as averaged values. The alcohol content in the base wines ranged between 10.98 g/100 mL in the Monastrell wines and 9.84 g/ 100 mL in the Trepat wines. The rosé base wines showed higher pH values (above 3.2) when compared to the white base wine (2.9). In addition, the rosé base wines showed higher values of volatile acidity. The concentration of SO_2 in Monastrell base wine was much higher (above 200 mg/L) than those determined for the other two base wines (above 80 mg/L). However, the values of total acidity were close in the three base wines. Regarding the colour intensity, the Trepat base wines showed intermediate values (0.19) with the lowest values corresponding to the white base wine (0.10) and the highest calculated for the Monastrell base wines (0.28).

Concerning Cava sparkling wines, in every case there was a general increase of the alcoholic degree (above 1.5 g/100 mL) because of the second fermentation. The pH of the wine however remained unchanged, similarly to what happened to the volatile acidity. However, the total acidity decreased in the three types of wines. In addition, there was a general reduction (14-21 g/100 mL) in the total SO_2 content. The colour intensity also slightly decreased in the sparkling wines compared to the base wines. However, the decrease in colour intensity was more significant in the Monastrell sparkling wines than in the Trepat wines, which exhibited values very similar to those of the base wines. The lower amount of anthocyanin content in the sparkling wines manufactured with this variety and the scarce formation of new pigments during the aging of the wines with lees may explain the differences in colour intensity between Monastrell base wines and sparkling wines (Pozo-Bayon et al., 2004).

3.2 Changes in volatile and nitrogen compounds during the manufacture of Cavas depending on the type of wine

From the 23 target volatile compounds studied, three of them (isoamyl acetate, butyl acetate and γ-butyrolactone) were not found in either the white and rosé base wines or the Cava sparkling wines. The data corresponding to the rest of volatile and nitrogen compounds determined in the wines (variables of the study) were submitted to a cluster analysis in order to obtain a preliminary view of the main causes for the change in these variables. **Figure 1** shows the dendrogram obtained. The squared Euclidean distance was taken as a measure of proximity between two samples and Ward's method was used as a linkage rule. In the figure, two large sample groups can be distinguished, one of them consisting of wines manufactured with the Trepat variety and white wines manufactured with the blend of white grape varieties. The second group clearly distinguishes the wines manufactured with the Monastrell variety. In both groups, it was also possible to distinguish a clear separation between the base and sparkling wines, confirming the large effect of the second fermentation on these groups of compounds

(Girbau-Sola et al., 2002b; Martinez-Rodriguez & Polo, 2000a; Martinez-Rodriguez & Polo, 2000b; Moreno-Arribas, Bartolome, Pueyo & Polo, 1998). However, the dendrogram did not show a clear grouping accordingly to the age of the sparkling wine. Therefore, the cluster revealed a greater similarity between the wines manufactured with the Trepat red variety and the wines manufactured with the white grape varieties than with those manufactured with the Monastrell variety in spite that both of them were manufactured with red varieties.

In a second step, two-way ANOVA was applied to understand the effect of the two factors (variety and aging time) in the data from all the studied variables (nitrogen and volatile compounds). The results corroborated a large influence on the first factor (variety) on most of the variables, while only the aging time was an important factor to explain the differences between the base wines and the sparkling wines (data not shown), but not between Cava wines of different age. In this last case, when we only consider the effect of aging time on the volatile and nitrogen composition, only six variables: ethyl acetate, 1-propanol, isobutanol, 3-methyl-1-butanol, total nitrogen and high molecular weight nitrogen (HMWN), were significantly influenced ($p < 0.05$) by the aging time. **Figure 2** shows an example of the evolution of two of these (total nitrogen and HMWN) during the aging of Cava wines. As can be seen, there was a general trend showing an increase in the concentration of these compounds between 9 and 18 months that was more evident for Trepat and white sparkling wines. This increase might be explained because of the release of nitrogen compounds and enzymes from yeast autolysis into the wines that could modify the original nitrogen composition (Alcaide-Hidalgo, Martinez-Rodriguez, Martin-Alvarez & Pueyo, 2008; Feuillat & Charpentier, 1982; Martinez-Rodriguez, Carrascosa, Martin-Alvarez, Moreno-Arribas & Polo, 2002). In fact, the release of proteins into wines has been associated to final steps of the autolysis process when bigger pores in the yeast wall can be formed (Martinez-Rodriguez, Carrascosa & Polo, 2001). The observed changes in the nitrogen composition during the aging of Cavas may be responsible for the increase in the concentration of some higher alcohols, since their content in wine is closely linked to the nitrogen composition of the wines (Escudero, Charpentier & Etievant, 2000).

To summarise the results obtained from the individual analysis of the volatile and nitrogen compounds and because after the application of ANOVA analysis we found that variety was the main factor influencing most of the studied variables, **Table 2** shows the average values ± the standard deviation of all the compounds analysed in the wines grouped accordingly to the grape variety used in their manufacture. The results of the application of the Student-Newman-Keuls test to compare the means of the Cava sparkling wines for each variety are also included in the table. As commented before, there was a clear influence of the second fermentation on the composition of the wines. Therefore, in **table 2** quantitative differences in the concentration of most of the volatile and nitrogen compounds between base and Cava wines can be seen. In general, Cava wines showed a lower concentration in most of the ethyl esters and acetates. These results are in agreement with previous works performed in white sparkling wines (Cavazza, Versini, Grando & Romano, 1990; Pueyo, Martin-Alvarez & Polo, 1995). However, Hidalgo and co-workers (Hidalgo et al., 2004) showed an increase in some ethyl esters during the second fermentation and aging of Garnacha sparkling wines. This could be explained by the longer maceration time with the grape skins of the wines from their study compared to our wines, which could have favoured a higher extraction of amino acids that could act as a reservoir to be transformed into volatile compounds during yeast autolysis. In this study only the esters ethyl acetate, ethyl lactate and diethyl succinate showed higher values in the sparkling wines compared to the base wines. The increase in diethyl succinate during the aging of wines on lees is one of the main conclusions of most of the studies performed on sparkling wines (Hidalgo et al., 2004; Pozo-Bayon et al., 2003b; Pueyo et al., 1995; Riu-Aumatell et al., 2006). In addition, we observed a general decreased in the fatty acid content in the sparkling wines compared to the base wines that could be due to the adsorption phenomena of these compounds by the yeast lees (Gallardo-Chacon, Vichi, Lopez-Tamames & Buxaderas, 2009; Lubbers, Charpentier, Feuillat & Voilley, 1994). Moreover, there was a slight but a general increase in the concentration of some higher alcohols (1-propanol, isobutanol, 2-methyl-1-butanol), although this fact was more dependent on the type of wine, that might be related to the initial nitrogen content of the grape variety employed in their manufacture. Hidalgo and collaborators (Hidalgo et al., 2004), observed however, a general decrease in propanol and isobutanol during the aging of sparkling wines manufactured with the red Garnacha variety. Some possible reasons for the lack of agreement between the different studies, could have been due to differences in the experimental conditions, in the grape varieties, but also, because of the simultaneous degradation and synthesis of volatile compounds that occurs over the course of aging with yeast, resulting that at any given time either of these processes can predominate (Pozo-Bayon et al., 2003b). In addition to the volatile compounds, the nitrogen compounds were in general lower in the sparkling wines compared to the base wines that can be a consequence of the important consumption of these compounds during the second fermentation (Martinez-Rodriguez & Polo, 2000b).

The sparkling wines manufactured with different grape varieties showed in general very different volatile and nitrogen composition (**Table 2**). As it has been previously noticed, this factor largely influences the nitrogen and volatile composition of Cava wines. In general, Cavas manufactured with the Monastrell variety showed significantly lower concentrations of fatty acids ethyl esters (ethyl octanoate and decanoate and ethyl lactate) and octanoic acid, compared to the sparkling wines manufactured with the other two varieties. For example, the concentration of the first two above mentioned ethyl esters was between 6 and 10 times higher in Trepat and white blend Cava sparkling wines than in the Cavas manufactured with Monastrell (**Table 2**). One explanation could be the possible removal of these compounds from the Monastrell wines because of the active carbon used for clarification during manufacture. The same idea could also be used to explain the lowest content in nitrogen compounds determined in these Cavas compared to the Trepat and the white blend Cavas. However, Monastrell Cava wines showed the highest concentration of some major volatile compounds such as acetaldehyde, ethyl acetate and methanol. Compared to sparkling Monastrell Cava wines, Trepat sparkling wines showed the highest concentration of some minor acetates, such as isobutyl and hexyl acetates, linked to fruity and floral aromatic nuances in wines (Lilly, Bauer, Lambrechts, Swiegers, Cozolino & Pretorius, 2006). White sparkling wines showed significant and higher concentration of some alcohols such as cis-3-hexen-1-ol and propanol. However, they showed the lowest values for the higher alcohols, 2 and 3-methyl-butanol that have been considered as negative compounds for wine aroma when their concentration exceeds 300 mg/L (Polaskova, Herszage & Ebeler, 2008) that is below the values determined in the studied Cava sparkling wines.

In order to obtain more information on the causes of the variability in the values found in the analysis of the volatile and nitrogen compounds in the wines, principal component analysis, from the correlation matrix, was performed. It was observed that about 63% of the variation in these values could be explained by the first two principal components. The first principal component (PC1) explained more than 44% of data variability and was strongly correlated with ethyl hexanoate (-0.908), ethyl octanoate (-

0.927), octanoic acid (-0.894), ethyl decanoate (-0.873), hexanoic acid (-0.863) and nitrogen from amino acids (-0.818) among others, while isobutyl acetate (0.744), diethyl succinate (0.712) and 3-methyl-1-butanol (0.71) contribute more strongly to the second principal component (PC2). The plot of the 25 wines on the plane defined by these first two principal components is shown in **Figure 3**. Here, the base wines of the red Trepat variety and from the blend of white grape varieties (TB-Bw, TA-Bw and WB-W) appear on the left side of the plane, showing higher and negative values for the PC1 and were located in the plane a large distance compared to their corresponding sparkling wines. On the right side of the plane, the wines manufactured with the Monastrell variety exhibiting positive and high values for PC1 were situated. These wines formed a very homogeneous group and there were not any clear differences between base and Cava sparkling wines and neither between Cavas of different age. The second group of wines corresponded to the sparkling wines manufactured with the Trepat variety and showed very similar values for PC1 to those exhibited by white sparkling wines but the PC2 values were higher. These sparkling wines showed some more similar characteristics to white sparkling wines than to the sparkling wines manufactured with the red Monastrell variety. In addition, they showed greater differences between sparkling wines of different age that were not evident in the Monastrell cava sparkling wines. Finally, white sparkling wines were perfectly differentiated from Monastrell and Trepat sparkling wines and they showed negative and high PC2 values. Therefore, the figure clearly shows that the greatest cause of variation among the samples was due to the factor variety, followed by the aging factor.

3.3. Global sensory analysis

To better know the sensory characteristics of the Cava wines manufactured with Trepat and Monastrell red varieties compared to the more conventional Cava manufactured with a typical blend of white grapes, a global sensory analysis and a visual analysis was performed in the nine month sparkling wines, since 9 months is the minimum aging time established by the Cava regulation. These results are shown in **Table 3.** In this table, the scores from the two batches of Cava wines (TA-Sw, TB-Sw and MA-Sw, MB-Sw) were averaged. As can be seen, in general, the three types of wines were qualified as correct and showed very similar scores, between 47.7 for the white Cava and 49.8 for the Trepat Cavas. Monastrell Cavas showed a score between the two of them. Hidalgo and collaborators (Hidalgo et al., 2004) found very similar scores for rosé sparkling wines manufactured with the Garnacha variety and different yeast strains. As shown in **Table 3,** the aroma intensity, had a slightly better evaluation in the two rosé sparkling wines, although the aroma quality was quite similar between them. In addition, the three types of wines presented good foam characteristics, although Monastrell sparkling wines showed more abundant foam that the white and Trepat Cavas. In addition, the rosé Cavas showed small sized bubbles, and in the case of Monastrell Cavas, they showed a fast effervescence. Both attributes are considered good quality attributes of sparkling wines (Gallart, Tomas, Suberbiola, Lopez-Tamames & Buxaderas, 2004; Hidalgo et al., 2004; Liger-Belair, Marchal & Jeandet, 2002). These results are in agreement with the results reported by Girbau-Sola and co-workers (Girbau-Sola et al., 2002b) who instrumentally measured the foam characteristics of rosé Cavas showing that those manufactured with the Trepat variety, exhibited very good foam characteristics, such as higher bikermann coefficient ($\sum$) (average bubble lifetime) and foam stability time (TS) compared to white Cava wines.

4. Conclusions

In this study, it has been shown that there is a large effect of the grape variety employed in the manufacture of Cava wines (at least on the 23 target volatile compounds followed in this study), compared to the effect produced by the aging time on lees. However, it is important to notice that in older Cava wines (more than 21 months), other minor volatile compounds could appear due to the slow hydrolysis of precursors because of yeast autolysis (Riu-Aumatell et al., 2006), which could greatly influence the aroma of these types of wine and that were not determined in the present study. In addition, the results of this study confirm the good aptitude of Monastrell grapes to make rosé sparkling wines, increasing the potential of this variety largely cultivated in many geographical zones around the world.

Acknowledgments

The authors are grateful to CAM and CSIC for their respective contracts.

References

Boletin Oficial del Estado 20 de Noviembre de 1991. Reglamentación de la denominación Cava y de su Consejo Regulador. Orden 14 de Noviembre de 1991. BOE no. 189278:37587-93.

Alcaide-Hidalgo, J. M., Martinez-Rodriguez, A. J., Martin-Alvarez, P. J., & Pueyo, E. (2008). Influence of the elaboration process on the peptide fraction with angiotensin I-converting enzyme inhibitor activity in sparkling wines and red wines aged on lees. *Food Chemistry*, *111*(4), 965-969.

Cavazza, A., Versini, G., Grando, M. S., & Romano, F. (1990). Variabilita indotta dai ceppi di lievito nella rifermentazione dei vini spumanti. *Industria della bevande*, *19*, 225-228.

Doi, E., Shibata, D., & Matoba, T. (1981). Modified colorimetric ninhydrin methods for peptidase assay. *Analytical Biochemistry*, *118*(1), 173-184.

Escudero, A., Charpentier, M., & Etievant, P. (2000). Characterization of aged champagne wine aroma by GC-O and descriptive profile analyses. *Sciences Des Aliments*, *20*(3), 331-346.

European Community (1990). Community methods for the analysis of wine. Commission Regulation (EEC) No. 2676/90 of 17 September 1990. . vol. 33 (L272) (pp. 1-191): Official Journal of European Community

Feuillat, M., & Charpentier, C. (1982). Autolysis of Yeasts in Champagne. *American Journal of Enology and Viticulture*, *33*(1), 6-13.

Francioli, S., Torrens, J., Riu-Aumatell, M., Lopes-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.

Gallardo-Chacon, J., Vichi, S., Lopez-Tamames, E., & Buxaderas, S. (2009). Analysis of Sparkling Wine Lees Surface Volatiles by Optimized Headspace Solid-Phase Microextraction. *Journal of Agricultural and Food Chemistry*, *57*(8), 3279-3285.

Gallart, M., Tomas, X., Suberbiola, G., Lopez-Tamames, E., & Buxaderas, S. (2004). Relationship between foam parameters obtained by the gas-sparging method and sensory evaluation of sparkling wines. *Journal of the Science of Food and Agriculture*, *84*(2), 127-133.

Girbau-Sola, T., Lopez-Barajas, M., Lopez-Tamames, E., & Buxaderas, S. (2002a). Foam aptitude of Trepat and Monastrell red varieties in Cava elaboration 2 second fermentation and aging. *Journal of Agricultural and Food Chemistry*, *50*(20), 5600-5604.

Girbau-Sola, T., Lopez-Tamames, E., Bujan, J., & Buxaderas, S. (2002b). Foam aptitude of Trepat and Monastrell red varieties in Cava elaboration 1 base wine characteristics. *Journal of Agricultural and Food Chemistry*, *50*(20), 5596-5599.

Hidalgo, P., Pueyo, E., Pozo-Bayon, M. A., Martinez-Rodriguez, A. J., Martin-Alvarez, P., & Polo, M. C. (2004). Sensory and analytical study of rose sparkling wines manufactured by second fermentation in the bottle. *Journal of Agricultural and Food Chemistry*, *52*(21), 6640-6645.

Liger-Belair, G. (2002). Physicochemical approach to the effervescence in Champagne wines. *Annales De Physique*, *27*(4), 1-+.

Liger-Belair, G., Marchal, R., & Jeandet, P. (2002). Close-up on bubble nucleation in a glass of champagne. *American Journal of Enology and Viticulture*, *53*(2), 151-153.

Lilly, M., Bauer, F.F., Lambrechts, M.G., Swiegers, J.H., Cozzolino, D., Pretorius, I.S. (2006). The effect of increased yeast alcohol acetyltransferase and esterase activity on the flavour profiles of wine and distillates. *Yeast*, *23*(9), 541-654.

Lubbers, S., Charpentier, C., Feuillat, M., & Voilley, A. (1994). Influence of Yeast Walls on the Behavior of Aroma Compounds in a Model Wine. *American Journal of Enology and Viticulture*, *45*(1), 29-33.

Martinez-Rodriguez, A., & Polo, M. C. (2000a). Enological aspects of yeast autolysis. In: S. G. Pandalay, *Recent Research Developments in Microbiology*, vol. 4 (pp. 285-301): Trivandrum:Research Singpost.

Martinez-Rodriguez, A., & Polo, M. C. (2000b). Characterization of the nitrogen compounds released during yeast autolysis in a model wine system. *Journal of Agricultural and Food Chemistry*, *48* (4), 1081-1085.

Martinez-Rodriguez, A. J., Carrascosa, A. V., Martin-Alvarez, P. J., Moreno-Arribas, V., & Polo, M. C. (2002). Influence of the yeast strain on the changes of the amino acids, peptides and proteins during sparkling wine production by the traditional method. *Journal of Industrial Microbiology & Biotechnology*, *29*(6), 314-322.

Martinez-Rodriguez, A. J., Carrascosa, A. V., & Polo, M. C. (2001). Release of nitrogen compounds to the extracellular medium by three strains of Saccharomyces cerevisiae during induced autolysis in a model wine system. *International Journal of Food Microbiology*, *68*(1-2), 155-160.

Martinez-Rodriguez, A. J., & Polo, M. C. (2000b). Characterization of the nitrogen compounds released during yeast autolysis in a model wine system. *Journal of Agricultural and Food Chemistry*, *48*(4), 1081-1085.

Moreno-Arribas, M. V., Bartolome, B., Pueyo, E., & Polo, M. C. (1998). Isolation and characterization of individual peptides from wine. *Journal of Agricultural and Food Chemistry*, *46*(9), 3422-3425.

Obiols, J. M., De la Presa-Owens, C., Buxaderas, S., Bori, J. L., & De la Torre-Boronat, C. (1998). Protocolo de evaluación de la formación de la efervescencia y espuma en un vino espumoso. *ACE Revista de Enología*, *15*, 3-15.

Office International de la Vigne et du Vin (1994). Resolution ENO 2/94. OIV standard for the international wine competition. *Bulletin de l'O.I.V,*(67), 551-597.

Polaskova, P., Herszage, J., & Ebeler, S. E. (2008). Wine flavor: chemistry in a glass. *Chemical Society Reviews*, *37*(11), 2478-2489.

Pozo-Bayon, M. A., Hernandez, M. T., Martin-Alvarez, P. J., & Polo, M. C. (2003a). Study of low molecular weight phenolic compounds during the aging of sparkling wines manufactured with red and white grape varieties. *Journal of Agricultural and Food Chemistry*, *51*(7), 2089-2095.

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*(6-7), 289-299.

Pozo-Bayon, M. A., Monagas, M., Polo, M. C., & Gomez-Cordoves, C. (2004). Occurrence of pyranoanthocyanins in sparkling wines manufactured with red grape varieties. *Journal of Agricultural and Food Chemistry*, *52*(5), 1300-1306.

Pozo-Bayon, M. A., Pueyo, E., Martin-Alvarez, P. J., Martinez-Rodriguez, A. J., & Polo, M. C. (2003b). Influence of yeast strain, bentonite addition, and aging time on volatile compounds of sparkling wines. *American Journal of Enology and Viticulture*, *54*(4), 273-278.

Pozo-Bayon, M. A., Pueyo, E., Martin-Alvarez, P. J., & Polo, M. C. (2001). Polydimethylsiloxane solid-phase microextraction-gas chromatography method for the analysis of volatile compounds in wines - Its application to the characterization of varietal wines. *Journal of Chromatography A, 922*(1-2), 267-275.

Pueyo, E., MartinAlvarez, P. J., & Polo, M. C. (1995). Relationship between foam characteristics and chemical composition in wines and cavas (sparkling wines). *American Journal of Enology and Viticulture, 46*(4), 518-524.

Riu-Aumatell, M., Bosch-Fuste, J., Lopez-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.

FIGURE LEGENDS:

Figure 1. Dendrogram resulting from applying cluster analysis to the data corresponding to the concentration of volatile and nitrogen compounds determined in the base wines and in the sparkling Cava wines. Wines are identified by a code formed by the first letter of the variety (M, T or W, for Monastrell, Trepat, White blend),

followed by the type of wine (Bw=base wine, Sw=sparkling wines), the lot of wine (A,B) and the aging time (00, 09, 12, 15, 18 months; 00 corresponds to the base wine)

Figure 2. Evolution of the total nitrogen and high molecular weight nitrogen (HMWN) during the aging of sparkling Cava wines (9m, 12m, 15m, 18m = months of aging on lees). Trepat wines (o); Monastrell wines (□); White blend wines (◊).

Figure 3. Representation of the base wines and Cavas manufactured with different grape varieties on the plane defined by the first two principal components obtained from the PCA with the data from the nitrogen and volatile compounds. (0= base wines; 9, 12, 15, 18 aging time of Cava wines in months. Trepat wines (o); Monastrell wines (□); White blend wines (◊).

Table 1. Global composition (average values) of base wine (Bw)[a] and Cava sparkling wines (Sw) [b].

	Rosé wines			
	Trepat		Monastrell	
	Bw	Sw	Bw	Sw
Alcohol content (% v/v)	9.84 ± 0.04	11.5 ± 0.07	10.9 ± 0.02	12.4 ± 0.0
pH	3.24 ± 0.01	3.29 ± 0.03	3.29 ± 0.10	3.29 ± 0.0
Volatile acidity (g/L acetic acid)	0.43 ± 0.01	0.47 ± 0.03	0.62 ± 0.18	0.59 ± 0.1
Total acidity (g/L tartaric acid)	3.66 ± 0.01	3.18 ± 0.02	3.47 ± 0.43	3.42 ± 0.2
Total SO_2 (mg/L)	85.2 ± 1.06	67.2 ± 2.99	205 ± 2.90	173 ± 3.3
Colour Intensity (Abs 420nm + Abs 520 nm)	0.19 ± 0.01	0.14 ± 0.01	0.28 ± 0.02	0.17 ± 0.0

[a] The results show the average values of two lots of the same base wine (A and B).
[b] The results show the average values of the sparkling wines manufactured from two different lots of base wines (A and B) and taking all the studied aging times (9, 12, 15 and 18 months) into consideration.

Table 2. Mean values ± standard deviation of the volatile and nitrogen compounds (mg/L) in the base wines (Bw) and 9 months Cava sparkling wines (Sw) manufactured with different grape varieties.

	Trepat		Monastrell		
	T-Bw	T-Sw	M-Bw	M-Sw	W-Bv
Ethyl butanoate	0.20 ± 0.12	0.02[a] ± 0.03	0.19 ± 0.05	0.02[a] ± 0.03	0.31
Isobutyl acetate	1.55 ± 0.19	0.35[b] ± 0.25	0.25 ± 0.03	0.15[a] ± 0.03	1.73
Ethyl hexanoate	1.07 ± 0.12	0.69[a] ± 0.47	0.27 ± 0.07	0.26[a] ± 0.03	1.56
Hexyl acetate	0.11 ± 0.05	0.01[b] ± 0.01	0.00 ± 0.00	0.00[a] ± 0.00	0.14
1-Hexanol	1.40 ± 0.44	1.04[a] ± 0.62	0.71 ± 0.18	0.59[a] ± 0.28	1.02
cis-3-Hexen-1-ol	1.21 ± 0.42	0.40[ab] ± 0.12	0.41 ± 0.53	0.29[a] ± 0.07	0.39
Ethyl octanoate	2.76 ± 0.66	0.52[b] ± 0.39	0.27 ± 0.03	0.08[a] ± 0.02	2.24
Ethyl decanoate	1.15 ± 0.09	0.16[b] ± 0.15	0.05 ± 0.00	0.02[a] ± 0.00	1.29
Diethyl succinate	2.07 ± 0.55	4.06[a] ± 2.77	3.34 ± 0.16	4.05[a] ± 1.37	0.93
Hexanoic acid	7.63 ± 1.15	3.94[a] ± 2.85	4.12 ± 1.24	1.11[a] ± 0.37	10.16

Octanoic acid	9.89 ± 4.35	6.07^{b} ± 5.01	1.44 ± 0.41	0.72^{a} ± 0.19	17.51
Decanoic acid	2.36 ± 0.33	0.94^{a} ± 0.47	1.99 ± 0.43	0.59^{a} ± 0.31	2.54
Acetaldehyde	27.26 ± 4.54	38.14^{a} ± 5.00	113.89 ± 42.59	86.12^{b} ± 13.09	43.73
Ethyl acetate	51.08 ± 1.85	53.77^{b} ± 12.12	41.62 ± 11.72	69.50^{c} ± 13.35	42.39
Methanol	44.83 ± 1.09	36.19^{a} ± 4.21	50.33 ± 3.88	48.59^{b} ± 9.04	36.49
1-Propanol	13.33 ± 1.87	14.37^{a} ± 3.53	18.61 ± 0.68	20.47^{b} ± 2.62	23.88
Isobutanol	46.60 ± 0.84	40.09^{c} ± 3.78	27.98 ± 6.12	31.04^{b} ± 3.95	16.24
2-Methyl-1-Butanol	39.51 ± 0.12	36.46^{b} ± 5.45	40.94 ± 9.77	41.26^{b} ± 8.33	22.71
3-Methyl-1-Butanol	201.26 ± 7.41	167.65^{b} ± 14.29	178.42 ± 8.19	169.27^{b} ± 14.80	115.3
Ethyl Lactate	35.63 ± 0.50	142.08^{b} ± 21.87	18.52 ± 11.33	56.99^{a} ± 41.17	73.69
Total Nitrogen	140.56 ± 1.58	134.93^{b} ± 8.42	71.19 ± 15.34	73.68^{a} ± 9.58	164.5
Amino Nitrogen	29.59 ± 0.32	24.47^{b} ± 5.63	16.28 ± 4.25	14.30^{a} ± 3.45	36.55
HMWN	110.88 ± 1.77	109.46^{b} ± 14.36	54.91 ± 11.09	59.38^{a} ± 9.84	127.0

$^{a-c}$ Mean values of Sw. in the three varieties, without a common superscript are significantly different (P<0.05). n=2

Table 3. Sensory attributes and foam characteristics of 9 months Trepat and Monastrell rosé and white Cava wines.

	Rosé Cavas		White Cava
	Trepat	Monastrell	White blend
Sensory attributes			
Visual aspect (0-9)	3.8	2.0	3.1
Aroma intensity (0-18)	6.8	6.2	7.0
Aroma quality (0-18)	7.1	8.01	7.3
Taste intensity (0-18)	7.4	7.12	7.3
Taste quality (0-27)	12.9	13.2	11.0
Harmony (0-27)	11.8	12.2	12.0
Total	49.8	48.8	47.7
Foam characteristics			
Foam (abundant, normal, little)	normal	abundant	normal
Surface (full, parcial)	full	full	full
Foam collar (total, parcial)	total	total	total
Bubbles size (small, medium, large)	small	small	medium
Effervescence (fast, normal, slow)	slow	fast	fast

0-7 = excellent; 8-23=very good, 24-44= good;; 45-62=correct; 63-78; regular; 79-90=inadequate; and >90 = eliminated

The final evaluation corresponds to the average value of the scores of two wines of each type tasted by the 8 panellists (n=16).