Research Article

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Evaluation of grape ripeness, carbonic maceration and pectolytic enzymes to improve the chemical and sensory quality of red sparkling wines

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Abstract

BACKGROUND: Red sparkling wines are and innovative product for the oenology market, and oenologists are looking for technologies to improve their winemaking. The present study aimed to use both carbonic maceration and pectolytic enzymes applied to premature grapes during the winemaking of red sparkling wines. Both could modify the release of polyphenols, as well as improve the foaming, aroma and sensory properties of the wines.

RESULTS: Red sparkling wines made with mature grapes showed the highest content of polyphenols, ethyl esters, alcohol acetates, total volatile acids and foam stability time. They were characterised by a high foam collar and foam area, full-body, astringency, persistence, and olfactory intensity, and were the best evaluated with respect to global perception in the sensory analysis. Treatment with pectolytic enzymes was not effective with unripe grapes. These wines showed a high content of total ethyl esters and the highest content of lactones, producing wines with high olfactory intensity and fruity aromas. Red sparkling wines made by carbonic maceration showed the lowest content of total polyphenols, anthocyanins and proanthocyanidins, as well as high contents of C6 alcohols and total ethyl esters, and were characterised by vegetal aroma notes. Both treatments produced red sparkling wines with good foam characteristics.

CONCLUSION: Winemaking of red sparkling wines with premature grapes and pectinolytic enzymes or carbonic maceration did not achieve an improvement with respect to their chemical and sensory qualities. The use of mature grapes and traditional winemaking is the best option for elaborating red quality sparkling wines.

Keywords: red sparkling wines; carbonic maceration; pectolytic enzymes; chemical composition; instrumental foam parameters and sensory analysis

INTRODUCTION

Sparkling wines produced by the traditional method owe their peculiar characteristics to a double process of fermentation and to the ageing with yeast that takes place in the same bottle as that reaching the consumer.[1]

Sparkling wines present in the market are mainly white and rosés, whereas the presence of red sparkling wines is practically non-existent as a result of difficulties in their elaboration process. One of the main difficulties in the winemaking of red sparkling wines is the presence of phenolic compounds, which are predominant in red still wines.

The organoleptic quality of red sparkling wines depends not only on flavour and colour, but also on the capacity of the wine to create foam. When making a red sparkling wine, the amount of the different phenolics is essential because they are directly associated with important organoleptic characteristics, such as colour, body and taste sensations.[2,3] Moreover, phenolics have been shown to improve the foamability of rosé sparkling wines.[4]

Wine phenolic composition and quantity is strongly affected by grape maturity.[5,6] Indeed, the base wines for red sparkling wine elaboration must have a low alcohol content, of between 10% and 11.5% vol, as well as an adequate colour intensity and mouthfeel, which is difficult to achieve in grapes harvested at this

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prematurity stage that show low phenolic maturity. Moreover, some studies have shown that grapes picked at maturity provided sparkling wines with the highest concentration of volatiles, and that grapes picked earlier led to wines with more intense herbaceous notes.[7]

To minimize the inconvenience of harvesting too early, it is essential to look for oenological strategies to work with less mature grapes with the aim of enhancing the release of polyphenols, favouring the formation of stable pigments, and improving the foaming, aroma and sensory properties of red sparkling wines. In this context, our previous studies have evaluated the suitability of different techniques, with premature and mature grapes, aiming to produce adequate base wines for elaborating quality red sparkling wines.[8,9] On the one hand, pre-fermentative cold maceration with premature grapes produced wines with a volatile composition similar to that of red sparkling wines produced from mature grapes, as well as best valued with respect to the foam instrumental and sensory descriptors. On the other hand, treatments to reduce the alcoholic degree resulted in a remarkably high valuation for gustatory perception. The present aimed to investigate other oenological techniques for winemaking using premature grapes: carbonic maceration and traditional winemaking with the addition of pectolytic enzymes.

Carbonic maceration produces young fruity red wines, as well as aged wines with suitable balance and likewise sparkling wines.[10] The main feature that defines carbonic maceration is intracellular fermentation. Whole grape clusters are subjected to anaerobic atmosphere, rich in CO_2, and undergo intracellular fermentation carried out by endogenous enzymes, without yeast intervention. Therefore, carbonic maceration produces the transformation of a small quantity of sugar into alcohol, a decrease of the content of malic acid, a diffusion of phenolic and volatile compounds from the skin to the pulp, and an increase in the content of amino acids.[10,11] Several studies have reported that the carbonic maceration does not improve the extraction of anthocyanin compounds,[12,13] although the colour density of carbonic maceration wines appears to be more stable during storage, revealing that, in carbonic maceration wines, the reactions of polymerization and copigmentation prevailed over the degradation of phenolic compounds.[12] The carbonic macerated grapes are richer in volatile compounds than traditionally made wines.[14,15] Indeed, the development of anaerobic conditions produces significant changes in the profile of organic compounds. The wines produced by carbonic maceration are thus characterized by a distinctive aroma with red berry notes, although, recently, carbonic maceration also showed a higher dominance of woody, spicy, pungent and acid sensations.[16,17]

Pectolytic enzymes are used in the winemaking to degrade the polyosidic structure of the skin cell membranes, thereby increasing the extraction of polyphenols and varietal aromas (free and bounded fractions).[18] Commercial enzymatic preparations consist of a mixture of pectolytic enzymes with endo- and exo-polygalacturonase, pectinlyase and pectinmethylesterase, developed for improving polyphenol extraction during maceration. Nevertheless, the effect of pectolytic enzymes in wine remains unclear. Some studies have reported an increase of anthocyanins compounds or an improvement of colour,[19–22] whereas others have shown a decrease or an unclear effect.[23–25]

Therefore, the present study aimed to use both carbonic maceration and pectolytic enzymes applied to premature grapes in the winemaking of red sparkling wines. Both could modify the release of polyphenols, and improve the foaming, aroma and sensory properties of red sparkling wines. However, to our knowledge, there are no previous scientific studies that have analysed their advantages or disadvantages.

Four different winemaking experiences were thus carried out: traditional winemaking with mature grapes; traditional winemaking with premature grapes; carbonic maceration with premature grapes; and, finally, traditional winemaking with the addition of pectolytic enzymes to premature grapes. The effect of the winemaking elaboration process was studied in red sparkling wines aged on lees for 9 months. A detailed analysis of the chemical composition (polyphenol content, volatile composition and foam parameters) and sensory properties of the wines was carried out.

MATERIALS AND METHODS

Chemicals

All reagents were analytical grade unless otherwise stated. The volatile and phenolic compound standards were purchased from Extrasynthèse (Lyon, France), Sigma-Aldrich (Beerse, Belgium), Fluka (Buchs, Switzerland), Scharlab (Barcelona, Spain) and Alfa Aesar (Heysham, UK). High-performance liquid chromatography (HPLC) grade reagents and the remaining reagents were supplied by Carlo Erba (Rodano, Milan, Italy) and Panreac (Barcelona, Spain). Toyopearl gel HW-50F was obtained from Tosoh Corporation (Tokyo, Japan). Water Milli-Q was obtained via a Millipore system (Millipore, Bedford, MA, USA). Helium BIP (99.9997%), air zero (99.998%) and Premier plus hydrogen (99.9992%) were provided by Carburos Metálicos S.A. (Valladolid, Spain).

Equipment and materials

HPLC was performed using a modular 1100 Agilent liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with one G1311A quaternary pump, an on-line G1379A degasser, a G1316A column oven, a G1313A automatic injector and a G1315B photodiode-array detector (DAD), controlled by the ChemStation software (Agilent). Gel permeation chromatography was carried out using a Gilson liquid chromatograph (Gilson SAS, Villiers-le-Bel, France) equipped with one 331 pump, one 332 pump, a 172 diode array detector, a 402 syringe pump and a GX-271 aspec with a direct injection module controlled by the Trilution LC software (Gilson SAS). Chromatography gas was performed using an Agilent 7890A gas chromatograph equipped with a flame ionization detector (FID) and with a HP-6890N gas chromatograph coupled to a HP-5973 inert MS detector. The internal pressure of the bottles was measured with an aphrometer (Ligapal, Cormontreuil, France) and the foaming properties of sparkling wines were evaluated using a classical sparging Mosalux apparatus.

Winemaking process

The red base and red sparkling wines were elaborated and aged in the experimental winery of the Oenological Station (ITACyL) sited in Rueda (Valladolid, Spain). All of the base wines were elaborated in stainless steel tanks of 150 L in duplicated.

Grapes from *Tempranillo Tinto* (VIVC 12350) were collected by hand from the Cigales Denomination of Origin (D.O.). The grapes were harvested in two maturity moments. Premature grapes (PM) were harvested when had achieved an acidity and sugar level suitable for sparkling wine production, and showed a maturity index (MI: sugars/total acidity) of 27.83. The grapes harvested at their optimum degree of phenolic maturity, mature grapes (M), showed a MI of 34.52.

The mature grapes were elaborated following the traditional red winemaking process and were used as control wines (M-T). Three winemaking techniques were carried out with premature grapes: traditional red winemaking process (PM-T); carbonic maceration (PM-CM); and traditional winemaking with addition of pectolytic enzymes (PM-E).

In the traditional winemaking (M-T and PM-T), red grapes were destemmed, crushed, and sulphited (0.05 g L^{-1}). Alcoholic fermentation was carried out with commercial *Saccharomyces cerevisiae* yeasts (FERM ES 488; Enartis, San Martino, Italy; 0.2 g L^{-1}) at a controlled temperature < 25 ± 2 °C. Once the alcoholic fermentation was over (reducing sugars < 2 g L^{-1}), the wines were gently pressed (C.E.P. vertical press SIRIO 60; Costruzione Enologiche Padovane, Curtarolo, Italy) and racked into new tanks. Wines were inoculated with commercial *Oenococcus oeni* lactic acid bacteria (Viniferm OE104; Agrovin, Alcazar de San Juan, Spain; 0.01 g L^{-1}) to carry out the malolactic fermentation, and the temperature was maintained at 18 ± 2 °C. The base wines were cold-stabilized at −5 °C and clarified with Gel-Red porcine gelatine (Enolviz; 0.25 mL L^{-1}).

In carbonic maceration (PM-CM), whole grape bunches were placed into the stainless-steel tanks and kept at controlled temperature of 20 °C. The tanks were filled with carbon dioxide to maintain anaerobic conditions. After 13 days of intracellular fermentation/maceration, the free run wine was eliminated, the mash was pressed and the wine obtained allowed to finish alcoholic fermentation. Thereafter, the elaboration process was performed as described above. The red base wines elaborated by traditional winemaking with addition of pectolytic enzymes (PM-E) followed the same process as the M-T and PM-T, but pectolytic enzymes (Vinozym vintage, Lamothe-Abiet, France; 0.04 g kg^{-1}) were added at the beginning of the alcoholic fermentation. This enzyme preparation showed only polygalacturonase activity, 7500 IU g^{-1}, in accordance with the manufacturer's instructions.

Red sparkling wines elaborated by the traditional method were obtained after a second fermentation in closed bottles in contact with lees for 9 months (EC Regulation N° 606/2009 for sparkling wines with a protected designation of origin). Therefore, after cold-stabilization and clarification of base wines (T0), the tirage liquor was added, the wines were bottled and were kept in a cellar at a temperature and relative humidity controlled for 9 months. The tirage liquor was formed by 0.30 g L^{-1} yeast *S. cerevisiae var. bayanus* (IOC 18-2007; Oenologique Institut de Champagne, Epernay, France), 22 g L^{-1} sugar and 0.03 g L^{-1} bentonite calcium activated (Laffort, Bordeaux, France). The bottles were loaded in a horizontal position in 'pupitres', riddled (i.e. given a sharp quarter-turn daily and gradually tilted upside-down) and the sediment worked its way to the bottle neck. The pressure and residual sugars were measured periodically to control the second fermentation. After 9 months of ageing on lees (T9), the sparkling wines were riddled and disgorged (no expedition liquor was added), and were maintained in the same cellar for 4 months before the sensory analysis. Because the second fermentation takes place in individual bottles, three bottles of each sparkling wine experience were analysed.

Analysis of oenological parameters

Standard general parameters were measured using official analysis methods.[26]

Analysis of phenolics

Anthocyanins, hydroxycinnamic acids and flavonols were analysed by HPLC-DAD in accordance with the methodology described previously.[27] The content of non-acylated anthocyanins (A), acetyl-glucoside anthocyanins (A-Ac), coumaryl-glucoside anthocyanins (A-Cm), total monomeric anthocyanins (T-A), total hydroxycinnamic acids (T-HA), total flavonols (T-Flavo) and total phenolic compounds (T-Phenolics) was calculated.[4]

For the analysis of proanthocyanidins, wines were firstly fractionated by gel permeation chromatography on a Toyopearl gel HP-50F column (particle size distribution, 30–60 μm; exclusion limit, 1.8 × 104 Da; resolution, 1.3 min; Tosoh Bioscence GmbH, Stuttgart, Germany) as described previously.[28] Phloroglucinol adducts were analyzed in the second fractions by reversed-phase HPLC.[29] Total proanthocyanidin content and the apparent mean degree of polymerization (mDP) were calculated.[4]

Analysis of volatile compounds

Major volatile compounds were identified and quantified by a gas chromatography-FID detector.[9] Minor volatile compounds were extracted and analysed using a gas chromatography-mass spectrometer.[9,30,31]

Thirty-six volatile compounds were identified and quantified in red sparkling wines that were classified into nine groups: ethyl esters, alcohol acetates, acids, C6 alcohols, higher alcohols, terpenes, lactones, vanillin derivates and volatile phenols.

Foam parameters

Three foam parameters were measured using the Mosalux procedure.[32] This parameters were: (i) maximum height reached by foam after CO_2 injection (HM, expressed in mm), which represents the foamability; (ii) foam stability height during CO_2 injection (HS, expressed in mm), which represents the ability of wine to produce stable foam persistence of foam collar; and (iii) foam stability time (TS, expressed in seconds), evaluated as the time until all bubbles collapsed when CO_2 injection was interrupted, which could represent the foam stability time once effervescence has decreased.

Sensory analysis

The sensory analysis was carried out in a designed test room in accordance with ISO 8589 Standard (2010).

Panellists rated the sparkling wines for visual, gustatory, olfactory and foam quality conformance to sparkling wine typology. The sensory analysis was carried out by 12 expert tasters from the Regulatory Councils of various Spanish D.O. and wineries. Tasters defined the descriptors used in the sensory analysis as described elsewhere.[33] Two attributes were selected for the visual phase: visual colour intensity and red tone; six for the olfactory phase: olfactory intensity, fruity, yeasty aromas, oxidized, reduced notes and vegetal notes; and seven for the gustative analysis: freshness, acidity, astringency, bitterness, full-body, persistence and equilibrium. Finally, global perception also was evaluated. Similarly, four descriptors previously defined were selected to determine foam quality: initial foam, foam area, foam collar and bubble size.[34] Previous to the sensory analysis, panellists worked to stablish similar qualitative and quantitative criteria and to select a consensual group of descriptors. Then, tasters were trained to quantify these descriptors using structured numerical scales. The training was carried out in accordance with UNE-87-020-93 Norm, corresponding to ISO 4121:1987 Norm. The sparkling wines were evaluated in duplicate in two different sessions and the serving temperature was 8–10 °C. Samples were

presented in random order, and a structured numerical scale of ten points was used for the visual, olfactory and gustatory phase (where '1' represents no intensity and '10' represents the highest intensity), and a scale of three points to determine foam sensory quality. Wine samples of 50–60 mL were served in new standard wine-tasting glasses, with no faults or marks. Each bottle was opened slowly, with the cork held in the hand and without shaking the bottle. To avoid air bubble formation, the wine was poured slowly into the glass. The sparkling wines were tasted after 9 months of ageing on lees and 4 months in bottle ageing (T9).

Statistical analysis

An analysis of variance (ANOVA) was carried out to identify significant differences among wines. $P < 0.05$ was considered as statistically significant for all tests. Principal component analysis (PCA) and generalized Procrustes analysis (GPA) were applied for sensory attributes. SPSS, version 24.0 (IBM Corp., Armonk, NY, USA) was used to performed ANOVA evaluations. XLSTAT Premium software (2018.3) (Addinsoft, New York, NY, USA) was used for PCA analysis.

RESULTS AND DISCUSSION

General oenological parameters

Standard oenological parameters were determined in the red base wines (T0), as well as in the sparkling wines after 9 months of ageing on lees (T9) (Table 1).

As expected, the red base wines elaborated by traditional winemaking with mature grapes (M-T) showed a higher alcohol content (with a difference of 1.1% vol) and colour intensity (CI) compared to the red base wines elaborated with premature grapes. However, pH values did not show significant differences.

The red base wines elaborated by traditional winemaking with addition of pectolytic enzymes (PM-E) showed the highest titratable acidity. The red base wines elaborated by carbonic maceration (PM-CM) showed slightly lower CI (although this was not significant) compared to the wines elaborated traditionally with premature grapes, in agreement with previous studies because carbonic maceration wines are usually less coloured.[35] Surprisingly, the CI values of the PM-E red base wines were not significantly higher. This result is in contrast to previous studies reporting that the addition of pectolytic enzymes produces an increase of CI.[20–22] It should be noted that all these studies used mature grapes for the elaboration process. In studies by Romero-Cascales,[36] the effect of maceration enzymes was tested during the winemaking of grapes at different stages of maturity. It was concluded that the effect of the enzymes was much lower with less mature grapes, probably as a result of the rigidity of the cell middle lamella and primary wall when the grapes are less mature. During grape ripening, there is a softening of the grape as a result of the degradation of the polysaccharides of the cell middle lamella and primary wall. The cell wall structure weakens and the level of cell adhesion decreases. The main effect of the application of pectolytic enzymes is the degradation of the pectic fraction of the cell wall, although this effect mainly occurs when the grape is sufficiently mature.[36]

In relation to hue, the PM-CM and PM-E red base wines, showed significantly higher values compared to the wines elaborated by traditional winemaking, although all tonality values were those typical of young wines.

During ageing in the bottle, the general parameters evolved as expected. The second fermentation in bottle produced an increase of 1.0–1.3% vol in all red sparkling wines. The alcohol content reached in the sparkling wines made with premature grapes was suitable for red sparkling wines. The values of volatile acidity showed a good preservation state, with an absence of microbial alterations, and only the increase observed in the PM-T red sparkling wines was significant. The CI values were maintained during the second fermentation and ageing on lees. With exception of the PM-E red sparkling wines, hue values increased during ageing. All of the wines completed the second fermentation because all of them presented concentrations of reducing sugars lower than 1.5 g L^{-1}. The pressure values were all within the range of 5.2–5.6 bars, not being dependent on the alcoholic degree, in contrast to previous studies where sparkling wines with a greater alcohol content showed higher values of pressure.[37] The oenological techniques applied did not produce any change in the pressure of the sparkling wines obtained. The values of pressure were suitable for sparkling wines.

Table 1. General oenological parameters[a] of base (T0) and sparkling wines aged on lees for 9 months (T9)

Stage[b]	Technique[c]	pH	TA[d]	Alcohol[d]	VA[d]	C.I.[d]	Hue[d]	P[d]
T0	M-T	3.45 ± 0.1 a,α	5.5 ± 0.2 a,α	12.3 ± 0.2 b,α	0.55 ± 0.06 b,α	7.5 ± 0.2 b,α	0.50 ± 0.01 a,α	
	PM-T	3.57 ± 0.1 a,α	5.7 ± 0.2 a,α	11.2 ± 0.2 a,α	0.23 ± 0.03 a,α	5.7 ± 0.2 a,α	0.50 ± 0.01 a,α	
	PM-CM	3.53 ± 0.1 a,α	5.3 ± 0.2 a,α	11.2 ± 0.2 a,α	0.28 ± 0.03 a,α	5.2 ± 0.2 a,α	0.58 ± 0.01 b,α	
	PM-E	3.50 ± 0.1 a,α	6.2 ± 0.2 b,α	11.2 ± 0.2 a,α	0.57 ± 0.06 b,α	5.4 ± 0.2 a,α	0.60 ± 0.01 b,α	
T9	M-T	3.42 ± 0.1 a,α	5.6 ± 0.2 a,α	13.3 ± 0.2 b,β	0.52 ± 0.05 b,α	7.7 ± 0.2 b,α	0.56 ± 0.01 a,b,β	5.2 ± 0.3 a
	PM-T	3.43 ± 0.1 a,α	5.6 ± 0.2 a, α	12.4 ± 0.2 a,β	0.38 ± 0.05 a,β	5.6 ± 0.2 a,α	0.54 ± 0.01 a,β	5.6 ± 0.3 a
	PM-CM	3.53 ± 0.1 a,α	5.5 ± 0.2 a,α	12.4 ± 0.2 a,β	0.34 ± 0.04 a,α	5.1 ± 0.2 a,α	0.61 ± 0.01 c,β	5.3 ± 0.3 a
	PM-E	3.41 ± 0.1 a,α	6.3 ± 0.2 b,α	12.5 ± 0.2 a,β	0.53 ± 0.05 b,α	5.3 ± 0.2 a,α	0.58 ± 0.01 b,α	5.2 ± 0.3 a

[a] Mean values obtained from three bottles and two elaborations/deposits by elaboration ($n = 6$). Values with different lowercase letters in each parameter indicate statistically significant differences at $P < 0.05$. Latin letters (a, b, c) are used to compare techniques in the same stage. Greek letters (α, β) are used to indicate statistically significant differences among stages.
[b] T0, red base wines; T9, red sparkling wine aged on lees for 9 months.
[c] M-T, wines elaborated by traditional winemaking with mature grapes; PM-T, wines elaborated by traditional winemaking with premature grapes; PM-CM, wines elaborated by carbonic maceration with premature grapes; PM-E, wines elaborated by traditional winemaking and pectolytic enzymes with premature grapes.
[d] TA, titratable acidity (g L^{-1} tartaric acid); alcohol (% v/v: mL ethanol 100 mL^{-1} wine); VA, volatile acidity (g L^{-1} acetic acid); CI, colour intensity as sum of absorbances at 420, 520 and 620 nm; hue, A_{420}/A_{520}; P, pressure (bars).

Phenolic compounds

Table 2 shows the total concentration of anthocyanins, proanthocyanidins, mDP, flavonols, hydroxycinnamic acids and phenolic compounds of red sparkling wines aged on lees for 9 months (T9).

As expected, the wines made with premature grapes showed significantly lower contents of total anthocyanins than wines made with mature grapes,[5] also in agreement with the lower values of CI obtained in all these wines.

The use of pectolytic enzymes in the winemaking of premature grapes did not increase the content of phenolics, and the PM-T and the PM-E wines showed no significant differences in the content of total anthocyanins, also in good agreement with the similar values for their CI values. As explained above, these results confirmed that the use of pectolytic enzymes was not effective for degrading the cell walls of the premature grapes, and thus it did not improve the polyphenol extraction.

Regarding red sparkling wines elaborated with premature grapes and carbonic maceration (PM-CM), they showed slightly lower values of total anthocyanins (Table 2) and CI values (Table 1) (although this was not significant) compared to the other wines elaborated with premature grapes, in agreement with previous studies in that carbonic maceration wines are usually less coloured.[13,35,38] The lower content of anthocyanins in the PM-CM red sparkling wines could be explained because the maceration with intracellular fermentation did not favour the extraction of monoglucoside anthocyanins. As expected, non-acylated anthocyanins were the main fraction of the total anthocyanins in all wines (the concentration ranged from 90 to 156 mg L^{-1}), followed by coumarylated (from 9.4 to 14.5 mg L^{-1}) and acetylated anthocyanins (from 3.9 to 5.2 mg L^{-1}).

Red sparkling wines elaborated with mature grapes showed a significant higher content of proanthocyanidins compared to red sparkling wines elaborated with premature grapes. This result is in contrast to the literature, where a previous study[5] reported that proanthocyanidin content is highest at veraison and, subsequently, it decreases until complete ripeness is achieved, when the proanthocyanidin content remains relatively constant. However, other studies,[39] suggested that factors other than grape proanthocyanidin content, such as the physiological integrity of the fruit, can also influence wine proanthocyanidin concentration. The degradation of the cell walls increases with maturity, increasing the release of proanthocyanidins. As in the case of anthocyanins, when premature grapes were used, pectinolytic enzymes did not increase the content of proanthocyanidins, and the PM-T and the PM-E wines did not show significant differences in their content, confirming that enzymes were not able to degrade the cell middle lamella and primary wall of grapes with low maturity.

Red sparkling wines elaborated with premature grapes and carbonic maceration showed the lowest content of proanthocyanidins. This result is in agreement with previous studies where higher levels of flavan-3-ols were detected in red still wines elaborated by traditional winemaking compared to carbonic macerated wines.[12,35] However, it should be noted that this will be dependent on the variety, the concentration of ethanol reached or the maturity of the grapes employed in the elaboration process. In this sense, the literature also describes higher concentrations of proanthocyanidins in wines elaborated by carbonic maceration compared to in those made by traditional winemaking.[38]

With regard to the mDP of proanthocyanidin compounds, the oenological techniques produced statistically significant differences among the red sparkling wines. All of the values were in agreement with the values described in the literature for Tempranillo wines.[40] Red sparkling wines made with mature grapes (M-T) showed higher proanthocyanidin mDP than wines made with premature grapes, in agreement with the literature reporting that riper grapes release a higher proportion of proanthocyanidins from skins than from seeds, and the mDP of proanthocyanidin from its skins is higher than seed proanthocyanidins.[41] The mDP value was similar in the wines made with premature grapes and

Table 2. Total concentrations of anthocyanins, proanthocyanidins, mDP, flavonols, hydroxycinnamic acids and phenolic compounds (mg L^{-1}) in sparkling wines aged on lees for 9 months (T9)[a]

	Treatment[b]			
Chemical compound	M-T	PM-T	PM-CM	PM-E
A[c]	156.21 ± 5.34 c	107.94 ± 3.76 b	90.09 ± 3.28 a	105.21 ± 3.73 b, a
A-Ac[c]	5.20 ± 0.14 b	4.06 ± 0.11 a	3.90 ± 0.10 a	5.73 ± 0.15 b
A-Cm[c]	14.50 ± 0.44 b	9.82 ± 0.26 a	11.45 ± 0.33 b	9.42 ± 0.29 a
T-A[c]	175.91 ± 5.36 b	121.82 ± 3.77 a	105.44 ± 3.30 a	120.36 ± 3.75 a
PA[c]	472 ± 5 c	455 ± 7 b	373 ± 5 a	436 ± 12 b
mDP[c]	12.83 ± 0.72 c	11.75 ± 0.19 b	11.17 ± 0.21 b	9.72 ± 0.18 a
T-Flavo[c]	26.82 ± 0.87 b	25.98 ± 0.84 b	17.82 ± 0.53 a	17.75 ± 0.58 a
Free acids	2.39 ± 0.11 c	1.97 ± 0.08 b	2.50 ± 0.11 c	1.07 ± 0.05 a
Esterified acids	66.27 ± 2.12 c	54.72 ± 1.74 b	62.78 ± 2.07 c	43.82 ± 1.44 a
T-HA[c]	68.66 ± 2.12 c	56.69 ± 1.74 b	65.27 ± 2.07 c	44.89 ± 1.44 a
T-Phenolics[c]	743.39 ± 4.52 d	659.49 ± 9.02 c	558.53 ± 4.75 a	619 ± 4.72 b

[a] Mean values of three bottles and two elaborations/tanks by treatment ($n = 6$). Values with different lowercase letters in each compound indicate statistically significant differences at $P < 0.05$ between treatments.
[b] M-T, wines elaborated by traditional winemaking with mature grapes; PM-T, wines elaborated by traditional winemaking with premature grapes; PM-CM, wines elaborated by carbonic maceration with premature grapes; PM-E, wines elaborated by traditional winemaking and pectolytic enzymes with premature grapes.
[c] A, non-acylated anthocyanins; A-Ac, acetyl-glucoside anthocyanins; A-Cm, coumaryl-glucoside anthocyanins; T-A, total anthocyanins; PA, total proanthocyanidins; mDP, mean degree of polymerization; T-Flavo, total flavonols; T-HA, total hydroxycinnamic acids; T-Phenolics, total phenolics.

traditional winemaking (PM-T) and premature grapes and carbonic maceration (PM-MC). Red sparkling wines elaborated with addition of pectolytic enzymes showed the lowest mDP.

Not statistically significant differences were found in the total flavonol content between the red sparkling wines made by traditional winemaking with mature or premature grapes (M-T and PM-T) (Table 2). The wines made with pectinolytic enzymes and carbonic maceration showed lower contents of total flavonols, although it is important to take into account that the amounts of flavonols were low in all of the wines investigated.[42] In a previous study, the effect of carbonic maceration on the flavonol content of red still wines was analysed[43] and a lower content of flavonols reported in the carbonic maceration wines compared to the control wines.

The M-T red sparkling wines showed higher content of total hydroxycinnamic acids (T-HA) compared to the PM-T red sparkling wines (Table 2). It should noted that the effect of carbonic maceration and the addition of pectolytic enzymes on the content of total hydroxycinnamic acids was the opposite. The carbonic maceration wines showed a higher content of T-HA compared to the PM-T wines, reaching values similar to those observed in wines made with mature grapes. By contrast, wines made with premature grapes and pectolytic enzymes had the lowest content of T-HA. Similar results were also obtained in other studies.[24] There are not previous studies in the literature describing the effect of carbonic maceration on the hydroxycinnamic acids in red wines.

Regarding total phenolic compounds, the sparkling wines elaborated with mature grapes (M-T) showed the highest content because these wines showed the highest content of anthocyanins, proanthocyanidins, flavonols and hydroxycinnamic acids. The M-T wines were followed by the wines elaborated by traditional winemaking with premature grapes, the wines elaborated with premature grapes and pectinolytic enzymes, and, finally by the carbonic macerated wines made with premature grapes. All of the wines showed statistically significant differences as a result of the winemaking employed for the elaboration process.

Volatile compounds

Thirty-six volatile compounds were identified and quantified in the red sparkling wines and were classified into nine groups (Table 3).

No significant differences were found in the content of higher alcohols, γ-lactones, terpenes, vanillin derivatives and volatile phenols between the wines obtained from premature (PM-T) and mature grapes (M-T). Therefore, only the volatile compounds with statistically significant differences are considered here. The sparkling wines elaborated with mature grapes (M-T) presented a higher concentration of total ethyl esters, total alcohol acetates and total acids compared to the red sparkling wines elaborated with premature grapes, with the content of these compounds the highest in all red sparkling wines studied. These results agree with other studies,[44] although there is no clear tendency by grape maturity, with grape variety also being an important factor.[45] The formation of ethyl esters depends on grape composition[44,45] and yeast metabolism.[46] Taking into account that the yeast used and the fermentation conditions were the same, the differences in the concentration of these compounds found in the present study are a result of the grape composition (i.e. concentration of their precursors) or the effect of the oenological treatments applied. These compounds contribute to the fruity aroma of young wines, and play a significant role in wine aroma perception,[47] even at values lower than threshold levels due to synergistic effects.[48] Regarding C6 alcohols, the M-T sparkling wines showed a lower content compared to the PM-T sparkling wines, which is in agreement with previous studies reporting that wines elaborated with grapes at an early maturity stage produce wines with more herbaceous notes.[7]

The winemaking techniques carried out with premature grapes modified the volatile composition of the sparkling wines. The PM-CM wines showed a higher concentration of ethyl esters compared to the PM-T wines, which is in agreement with the results found in other studies using different grape varieties, such as Cariñena, Garnacha and Fer Servadou[49] or Gamay.[50] The anaerobic conditions in the carbonic maceration restrict oxygen levels in the first days of winemaking, which can favor the production of ethyl esters.[50,51] The PM-CM also had the highest concentration of ethyl cinnamate because its synthesis (esterification between trans-cinnamic acid and ethanol) is favored in carbonic maceration conditions.[49,50,52] The concentration of ethyl cinnamate in the PM-CM wines is above its odor threshold value (1.1 $\mu g\ L^{-1}$)[48] and can contribute to sweet and floral notes. On the other hand, these wines presented a lower content of alcohol acetates and total higher alcohols compared to the PM-T wines, probably as a result of the anaerobic conditions of this winemaking process. Oxygen is an important factor that influences the formation of volatile compounds during fermentation and, in a previous study, it was found that the oxygen supply favours the synthesis of higher alcohols and acetate esters.[50] Therefore, under restrictive oxygen conditions, such as the intracellular fermentation of carbonic maceration, the formation of these compounds is inhibited. The carbonic maceration also reduced the content of fatty acids, with the exception of hexanoic acid, comprising compounds that are produced in the lipid metabolism of yeast and are related to fatty, cheese and rancid attributes. The concentration of γ-lactones in the sparkling wines elaborated by carbonic maceration was the lowest, which could be a result of a lower concentration of their hydroxyl acid precursors. These wines had the highest C6 alcohol concentration.

The addition of pectolytic enzymes also modified the volatile composition of the red sparkling wines (PM-E), with these wines having a higher concentration of total ethyl esters compared to the wines made without enzymes (PM-T). These results agree with those found in studies of red or rosé wines.[18,53] However, other studies had indicated that pre-fermentative enzyme maceration did not favour the formation of ethyl esters.[54,55] Among the wines made with premature grapes, the sparkling wines obtained with enzyme addition showed the highest content of lactones, compounds related to fruity aromas.[56] The effect of enzyme treatment on the content of higher alcohols, alcohol acetates, terpenes and volatile phenols depended on each individual compound but, in general, no remarkable differences were found. Considering the results found in the literature and in the present study, a clear effect of the enzymatic treatment on the volatile composition of wines cannot be established, and will depend on several factors, such as grape maturity stage, vintage and grape variety.[18,55]

Foam and sensory properties

Figure 1 shows the foaming instrumental parameters, HM, HS and TS of the red sparkling wines. Figure 2 shows the PCA consensus configuration of the wines as determined by the instrumental and sensory foam quality attributes.

In the PCA space, the first two principal components explained 95.83% of the accumulative variance. The consensus plot showed

Table 3. Volatile compounds of red sparkling wines aged on lees for 9 months (T9)[a]

	M-T	PM-T[b]	PM-CM	PM-E
Ethyl esters				
Ethyl butyrate	150 b	103 a	179 c	112 a
Ethyl 2-methylbutyrate	17 b	9 a	8 a	18 b
Ethyl isovalerate	23 b	12 a	11 a	26 c
Ethyl hexanoate	310 c	146 a	305 c	225 b
Ethyl octanoate	270 c	60 a	117 b	119 b
Ethyl decanoate	54 d	23 a	31 b	38 c
Ethyl cinnamate	ND	< 1.0	5	nd
Total ethyl esters	*824 d*	*353 a*	*651 c*	*538 b*
Ethyl lactate*	166 a	292 c	246 b	306 c
Alcohol acetates				
Isoamyl acetate	935 b	654 a	616 a	634 a
Hexyl acetate	10.4 d	4.8 b	< 2.0 a	6.2 c
2-Phenylethyl acetate	215 d	182 c	119 a	131 b
Total alcohol acetates	1160 c	841 b	735 a	771 a
Acids				
Isovaleric acid	1313 c	1024 b	711 a	1304 c
Hexanoic acid	2999 c	2442 b	3050 c	2106 a
Octanoic acid	4648 c	4798 c	3357 a	3644 b
Decanoic acid	497 c	497 c	320 a	425 b
Total acids	9457 c	8761 b	7438 a	7479 a
C6 alcohols				
1-Hexanol	413 a	1075 b	1590 d	1293 c
trans-3-hexen-1-ol	11 a	43 b	89 d	69 c
cis-3-hexen-1-ol	116 a	274 b	264 b	340 c
Total C6 alcohols	540 a	1392 b	1943 d	1702 c
Higher alcohols				
Benzyl alcohol	83 a	97 b	169 c	88 a
2-Phenylethanol*	63 b	60 b	34 a	61 b
1-Propanol*	14 a	13 a	18 b	16 b
Isobutanol*	57 b	60 c	47 a	57 b
Isoamyl alcohols*	296 b	294 b	236 a	290 b
Total higher alcohols	430 083 b	427 097 b	335 169 a	424 088 b
Terpenes				
Linalool	4.5 c	3.7 ab	4.1 bc	3.3 a
α-Terpineol	4.2 b	5.3 c	2.1 a	1.6 a
Citronellol	6.3	6.1	6.4	6.7
Geraniol	2.3 b	2.1 a	2.4 b	1.9 a
Total terpenes	17.3 b	17.2 b	15 a	13.5 a
Lactones				
γ-Butyrolactone*	15 b	16 b	11 a	18 c
γ-Nonalactone	2.9 c	2.7 c	1.4 a	2.4 b
Total lactones	15 003 b	16 003 b	11 001 a	18 002 c
Vanillin derivates				
Methyl vanillate	< 2.0	< 2.0	< 2.0	< 2.0
Ethyl vanillate	15 b	15 b	21 c	13 a
Acetovanillone	22 b	21 ab	20 a	20 a
Total vanillin derivates	38.5 b	37.3 b	43.0 c	34.2 a
Volatile phenols				
4-Ethylguaiacol	< 1.0	< 1.0	< 1.0	< 1.0
4-Ethylphenol[c]	ND	ND	ND	ND
4-Vinylguaiacol	4.2 b	4.7 c	3.8 a	3.6 a
4-Vinylphenol	38 a	39 a	38 a	47 b
Guaiacol	< 2.0	< 2.0	< 2.0	< 2.0
Eugenol	ND	ND	ND	ND
Total volatile phenols	44.2 a	45.5 a	44.2 a	52.5 b

[a] Mean value of three bottles and two elaborations/tanks by treatment ($n = 6$) in μg L^{-1} except those marked with an asterisk (*) that are expressed in mg L^{-1}. Values with different lowercase letters in each compound indicate statistically significant differences at $P < 0.05$.
[b] M-T, wines elaborated by traditional winemaking with mature grapes; PM-T, wines elaborated by traditional winemaking with premature grapes; PM-CM, wines elaborated by carbonic maceration with premature grapes; PM-E, wines elaborated by traditional winemaking and pectolytic enzymes with premature grapes.
[c] ND, not detected.

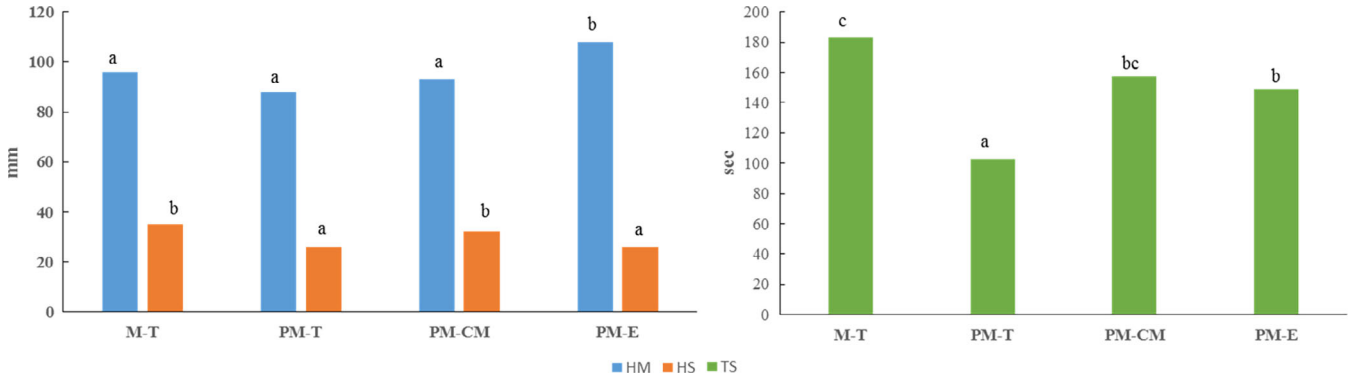

Figure 1. Mosalux foam parameters of sparkling wines aged on lees for 9 months (T9). Mean values of three bottles and two elaborations/tanks by treatment ($n = 6$) are shown. Different lowercase letters for each parameter indicate statistically significant differences at $P < 0.05$. M-T, sparkling wine elaborated by traditional winemaking with mature grapes; PM-T, sparkling wine elaborated by traditional winemaking with premature grapes; PM-CM, sparkling wine elaborated by carbonic maceration; PM-E, sparkling wine elaborated by traditional wine making with addition of pectolytic enzymes. HM, foam maximum height (mm); HS, foam stability height (mm); TS, foam stability time until all bubbles collapse (s).

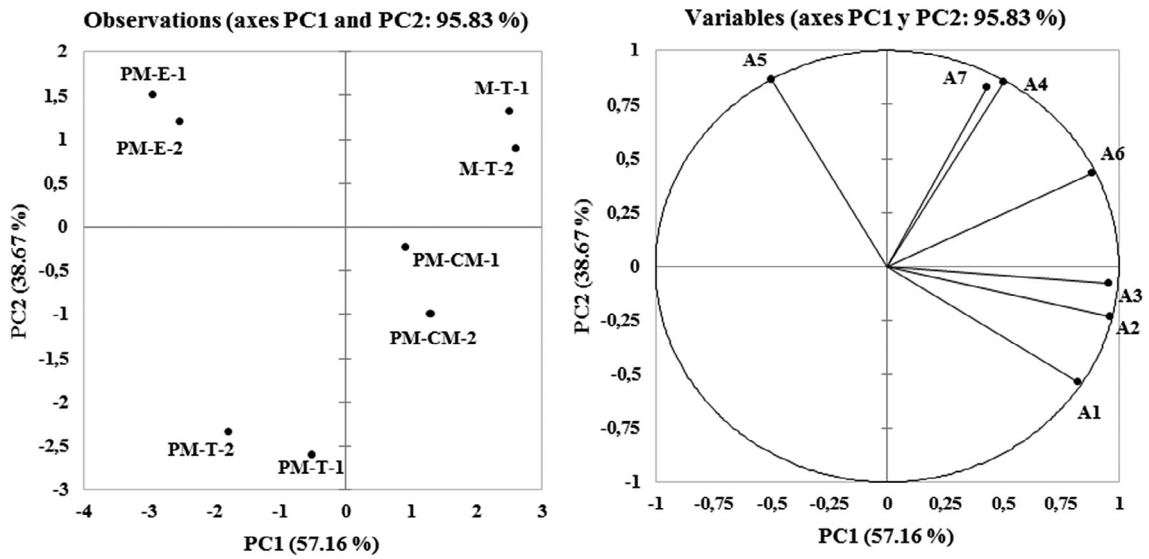

Figure 2. PCA for Mosalux foam parameters (HM, HS and TS) and sensory foam descriptors in the different red sparkling wines. M-T, sparkling wine elaborated by traditional winemaking with mature grapes; PM-T, sparkling wine elaborated by traditional winemaking with premature grapes; PM-CM, sparkling wine elaborated by carbonic maceration; PM-E, sparkling wine elaborated by traditional wine making with addition of pectolytic enzymes. Attributes: A1, initial foam; A2, foam area; A3, foam collar; A4, bubble size; A5, HM; A6, HS; A7, TS.

the red sparkling wines were quite spread regardless of the grape maturity. Principal component 1 (PC1) explained 57.16% of the variance and the second principal component (PC2) explained 38.67%. PC1 was correlated with foam collar (A3), foam area (A2), HS parameter (A6) and initial foam (A1) on the positive side, whereas PC2 was correlated with HM (A5) and TS (A7) parameters and bubble size (A4).

The PM-T red sparkling wines were located at negative values for PC1 and PC2, and did not show a direct correlation with any instrumental descriptor because they were characterized by the lowest values of the instrumental parameters (Fig. 1).

The PM-E red sparkling wines were located at negative values for PC1 and positive values for PC2, and they showed a high correlation with HM (A5) because these wines showed the highest HM value (Fig. 1). The PM-CM red sparkling wines were located at positive values for PC1 and negative for PC2, showing high correlations with initial foam (A1), foam area (A2) and foam collar

Table 4. Sensory evaluation of colour intensity and red tone of sparkling wines[a]

Stage	Wine[b]	Colour intensity	Red tone
T9	M-T	6.75 ± 0.70 a	6.75 ± 1.16 a
	PM-T	6.13 ± 0.83 a	6.13 ± 1.36 a
	PM-MC	6.25 ± 0.89 a	6.25 ± 1.03 a
	PM-E	5.50 ± 0.93 a	5.50 ± 2 a

[a] Values are the mean ± SD. Different lowercase letters in the same column indicate that means significantly differ at $P < 0.05$ on each vintage.
[b] M-T, wines elaborated by traditional winemaking with mature grapes; PM-T, wines elaborated by traditional winemaking with premature grapes; PM-CM, wines elaborated by carbonic maceration with premature grapes; PM-E, wines elaborated by traditional winemaking and pectolytic enzymes with premature grapes.

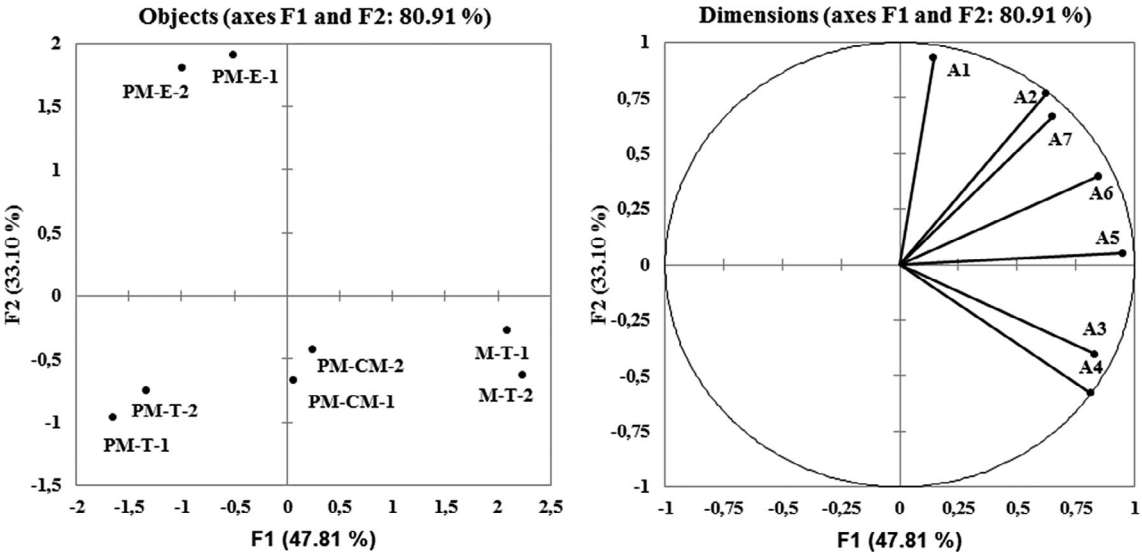

Figure 3. GPA of the mean ratings for gustatory phase in the different red sparkling wines. M-T, sparkling wine elaborated by traditional winemaking with mature grapes; PM-T, sparkling wine elaborated by traditional winemaking with premature grapes; PM-CM, sparkling wine elaborated by carbonic maceration; PM-E, sparkling wine elaborated by traditional wine making with addition of pectolytic enzymes. Attributes: A1, freshness; A2, acidity; A3, astringency; A4, bitterness; A5, full-body; A6, persistence; A7, equilibrium.

(A3). The sparkling wines made with mature grapes (M-T) were located at positive values for PC1 and PC2. They had the greater valuation on foam quality, showing high correlations with HS (A6), bubble size (A4), TS (A7), foam collar (A3) and foam area (A2), as well as higher values of TS and HS compared to the rest of the sparkling wines (Fig. 1).

Table 4 shows the results obtained for the visual phase of the sensory evaluation. The sparkling wines did not show any significant difference in the value of red tonality or colour intensity.

In the gustatory GPA space for red sparkling wines (Fig. 3), the wines were properly located in the vectorial dimension defined by the first two factors, which accounted for 80.91% of the total variance. GPA was applied to the gustatory data to determine consistency among tasters (73.8%) and to provide information on the relationship between wines and attributes. The consensus plot showed the red sparkling wines were quite spread. The dimension F1 explained 47.81% of the variance and the second dimension (F2) explained 33.1%. The F1 was correlated with full

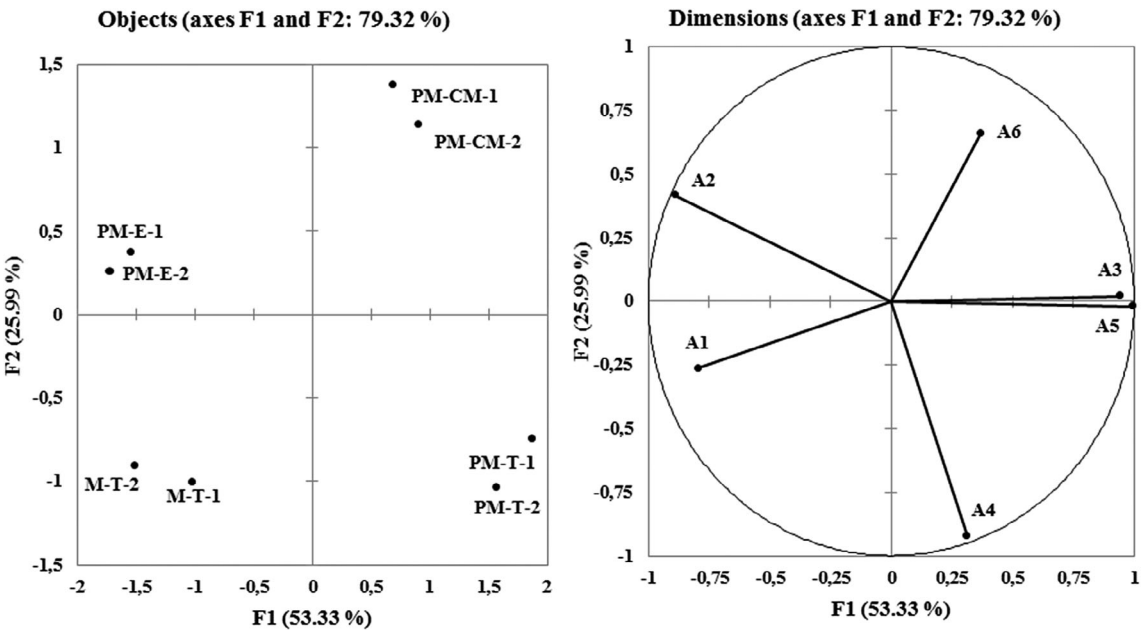

Figure 4. GPA of the mean ratings for olfactory phase in the different red sparkling wines. M-T: sparkling wine elaborated by traditional winemaking with mature grapes; PM-T, sparkling wine elaborated by traditional winemaking with premature grapes; PM-CM, sparkling wine elaborated by carbonic maceration; PM-E, sparkling wine elaborated by traditional wine making with addition of pectolytic enzymes. Attributes: A1, olfactory intensity; A2, fruity; A3, yeasty aromas; A4, oxidized; A5, reduced notes; A6, vegetal notes.

body (A5), persistence (A6), bitterness (A4) and astringency (A3) on the positive side, whereas F2 was positively correlated with freshness (A1) and acidity (A2). The red sparkling wines elaborated by traditional winemaking with addition of pectolytic enzymes showed a higher correlation with freshness (A1) and acidity (A2). The PM-E red sparkling wines scored the highest with respect to freshness (5.6), equilibrium (5.6) and acidity (4.8). This result could be explained by the highest acidity observed in the PM-E red sparkling wines in the standard general parameters (Table 1). The M-T red sparkling wines were characterized by full-body (A5), astringency (A3), bitterness (A4) and persistence (A6), which are attributes for which the M-T sparkling wines scored the highest punctuations. The correlation with astringency and bitterness is in agreement with the analytical data because the M-T sparkling wines showed the highest content of proanthocyanidins (Table 2). The PM-T and the PM-CM red sparkling wines did not emphasize any particular gustatory descriptor because they did not stand out in the gustatory phase. Figure 4 shows the wine and attribute average space obtained from the olfactory space where F1 explained 55.33% of the total variance and F2 accounted for 25.99%. Once again, the GPA was applied to the olfactory data to determine consistency among tasters (84.67%). The consensus plot showed the wines were quite spread, thus indicating a marked difference among wines. F1 was correlated with yeasty aromas (A3) and reduced notes (A5) on the positive side and with fruity (A2) and olfactory intensity (A1) on the negative side, whereas F2 was correlated with vegetal notes (A6) on the positive side and with oxidized (A4) on the negative side. The M-T red sparkling wines were located on the negative side for both dimensions, and showed a high correlation with olfactory intensity (A1), demonstrating the highest score for this attribute (6.8). This was in agreement with the analytical data because the M-T red sparkling wines showed the highest content on total ethyl esters, total alcohol acetates and total fatty acids. The PM-E red sparkling wines were characterized by fruity aromas (A2) and high olfactory intensity (A1), in agreement with the chemical data, because they showed a high content of ethyl esters and the highest content of lactones, both related to fruity aroma. As expected, the PM-CM red sparkling wines were correlated with vegetal notes (A6), also in agreement with the analytical results (Table 3), because the PM-CM red sparkling wines showed the highest content of C6 alcohols. The PM-T red sparkling wines were correlated with yeasty aromas (A3) and reduced notes (A5) because these wines obtained the highest punctuation on these attributes, although the scores were very low (≤ 3). Regarding the global perception of the sensory evaluation, the M-T red sparkling wines obtained the highest score (4.25) followed by the PM-E (4.0), PM-CM (3.62) and PM-T (3.19) red sparkling wines. These results are in agreement with the sensory punctuations obtained for each wine because the M-T wines generally showed the best valuation on the foam, gustatory and olfactory sensory parameters, whereas the PM-T red sparkling wines did not show a direct correlation with the foam and gustatory analysis.

CONCLUSIONS

The present study aimed to determine a good winemaking technology with respect to elaborating adequate red base wines for producing quality red sparkling wines. In this sense, the present study used both carbonic maceration and pectolytic enzymes applied to premature grapes in the winemaking of the red sparkling wines, aiming to modify the release of polyphenols and improve the foaming, aroma and sensory properties of the red sparkling wines.

The carbonic maceration produced red sparkling wines characterized by vegetal aroma notes. These wines were worse when evaluated with respect to global perception compared to the red sparkling wines elaborated with pectolytic enzymes. However, both treatments produced red sparkling wines with good foam characteristics. Treatment with pectolytic enzymes was not effective with unripe grapes.

In conclusion, the red sparkling wines elaborated with mature grapes were the best as evaluated on the sensory analysis. Therefore, the use of mature grapes and traditional winemaking appeared to be the best option for elaborating red quality sparkling wines, and, as observed in our previous studies, a treatment to partially reduce the alcoholic degree could be applied.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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