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Sensory and Analytical Study of Rosé Sparkling Wines Manufactured by Second Fermentation in the Bottle

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The sensory and analytical characteristics of five rosé sparkling wines manufactured by the traditional method have been determined. Moreover, the changes that take place in the nitrogen and volatile fraction of the wines during the second fermentation and the aging with the yeasts have been studied. Each of these wines was made from a single industrial rosé base wine of the Garnacha Tinta variety, with five selected yeasts strains. The base wine had a low content in free amino acids, 16 mg/L, and the yeast consumed more peptides than free amino acids during second fermentation. From the application of the two-way analysis of variance, yeast strain, and aging time factors to the data of volatile compounds, it has been found that most of the differences between these sparkling wines are due to the aging time. It has been verified that these rosé sparkling wines have foam of good quality and that the grape variety Garnacha Tinta is suitable for the production of rosé sparkling wines.

KEYWORDS: Rosé sparkling wines; sensory analysis; foam characteristics; nitrogen compounds; volatile compounds

INTRODUCTION

The manufacture of sparkling wines is done by second fermentation in the bottle, the champenoise or traditional method, or in large containers, the charmat method, of a so-called base wine to which a solution containing sugar and yeast is added. The wine obtained in this way is aged with the yeasts for a variable time period of several months for wines manufactured by the traditional method or days when made by the charmat method. The composition and the quality of sparkling wines, therefore, depend on several factors associated with the production process. These include the quality of the base wine, the transformations occurring during the second fermentation and aging with yeasts, the duration of the aging process, and the yeast strain used for the wine fermentation and aging processes (*1*−*3*).

Over the past few years, there has been a growing interest in diversifying production in response to market demand and the possibility of making sparkling wines with varieties of red grapes has been considered. In this case, the base wines are obtained by fermentation without the skins, blanc de noir sparkling wines, or by partial fermentation with the skins, rosé sparkling wines. The different types of grapes, white or red, and the different procedures used to make wines from the red grape varieties produce wines of different qualities.

The influence of many of the factors mentioned above on the quality and characteristics of sparkling wines made with white grape varieties, the majority, or with a mixture of red and white varieties has been the object of a number of studies (*4*−*8*). Nevertheless, there are few studies on the sparkling wines manufactured only with red varieties. The foam properties, the enological parameters, and the phenolic compositions of blanc de noir sparkling wines have been the objectives of recent studies (*9*−*12*). However, to our knowledge, only in two papers aimed at determining the phenolic fraction (*11*−*12*) have rosé sparkling wines been studied.

The present work was developed because of the lack of studies concerning rosé sparkling wines. The Garnacha Tinta variety, named Grenache in France, is the red variety that is most cultivated in the world (*13*). However, no previous studies about the manufacture of rosé sparkling wines with the Garnacha variety have been done. Thus, the main objective of this investigation is to carry out a sensorial and analytical study of rosé sparkling wines manufactured by the traditional method with the Garnacha Tinta variety. Moreover, the influence of five different yeast strains on the nitrogen and volatile fractions and on the sensory and foaming characteristics of these wines was also studied.

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MATERIALS AND METHODS

Wine Production and Sampling Times. The rosé base wine was manufactured industrially with the Garnacha Tinta grape variety in a cellar from the Denomination of Origin (D.O.) Vinos de Madrid. The Garnacha Tinta variety is a variety authorized by this D.O. for rosé sparkling wine production (*14*). Five sparkling wines were produced from this base wine in a pilot plant, using the traditional method. Bentonite was not added to the tirage solution to avoid interference with the nitrogen compounds released during yeast autolysis (*15*). Four of the sparkling wines were produced with four different yeast strains from the collection of the Instituto Madrileño de Investigación Agraria y Alimentaria (IMIA): *Saccharomyces cerevisiae* IMIA-2010, IMIA-2011, IMIA-2012, and IMIA-2013. These yeasts were selected for being autochthonous to the geographical area where the grapes were cultivated and for presenting appropriate characteristics for the production of sparkling wines. A fifth sparkling wine was produced with the commercial strain *Saccharomyces bayanus* EC-1118 (Lallemand Spain, SA) as the control. The second fermentation and aging with yeast took place at the cellar temperature, approximately 15−16 °C. Samples were taken from the base wine and then after 1, 4, 6, and 9 months of aging. Nine months is the minimum time established by the D.O. Vinos de Madrid regulations (*14*) for aging. For analytical determinations, six bottles at each sampling point were mixed and homogenized before the analysis. All of the determinations were performed in duplicate on wines previously centrifuged for 15 min at 5000*g*. For sensory analysis, the yeast lees were eliminated by hand from the bottles.

Chemical Analysis of the Wines. Alcoholic grade, total acidity, volatile acidity, pH, free and total SO_2, and reducing sugars were determined by the European Commission methods (*16*).

Viable Cell Counts. Serial decimal dilutions were prepared in saline solution, and appropriate volumes were spread onto fresh plates of yeast extract−malt extract agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, and 2% agar, pH 5.5) for yeast counts. The number of colony forming units was assessed after the plates were incubated at 25 °C for 3 days.

Nitrogen Compounds Analysis. The total nitrogen was determined by the Kjeldahl method with a Tecator Digestion System and a Kjeltec 1030 Auto Analyzer (Tecator AB, Höganäs, Sweden). The high molecular weight nitrogen (HMWN) content was estimated by the Bradford dye-binding assay (*17*). Free amino nitrogen was quantified by the ninhydrin−cadmium method (*18*). Peptide nitrogen was estimated as the difference between the total nitrogen and the HMWN plus free amino nitrogen.

Volatile Compounds. Analysis of the major volatile compounds was performed by direct injection on a Hewlett-Packard (Palo Alto, CA) 5890 series II gas chromatograph equipped with a flame ionization detection and a split/splitless injector. Separations were carried out on a Carbowax 20M fused silica capillary column (30 m × 0.25 mm i.d.) coated with a stationary phase of 0.25 μm of thickness (Quadrex Co., New Haven, CT). The injector and detector temperatures were 220 °C. The temperature program was as follows: initial temperature 40 °C (10 min hold) and ramps of 7 °C/ min to 150 °C and 30 °C/min to 210 °C. The carrier gas was helium (12.5 psi). A total of 50 μL of 3-pentanol (6 mg/mL 10% ethanol) was added as internal standard to 10 mL of wine, and 2 μL of wine with the internal standard was injected in the split mode. A ChemStation data system (HP 3365 series II, v. A.03.21) was used for data acquisition and processing. The compounds determined by this method were ethyl acetate, ethyl lactate, methanol, propanol, isobutanol, 2- and 3-methyl-1-butanol, and acetaldehyde.

Minor volatile analysis was carried out by gas chromatography of the headspace extract obtained with a 100 μm poly-dimethylsiloxane coated fused silica fiber (Supelco, Bellefonte, PA), in the conditions described by Pozo-Bayón et al. (*19*) using methyl nonanoate as the internal standard. The compounds determined by this method were butyl acetate, isobutyl acetate, isopentyl acetate, hexyl acetate, ethyl butyrate, diethyl succinate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, hexanol, *cis*-3-hexen-1-ol, hexanoic, octanoic, and decanoic acids, and γ-butyrolactone.

Peak identities were assigned by comparison of the relative retention times to the internal standard, with those of the standards of analytical quality, more than 99% purity, from Sigma-Aldrich (St. Louis, MO) and Merck KGaA (Darmstadt, Germany).

Analysis of Foam Properties. For the analysis of the foam characteristics of the wines, a device developed in the Instituto de Fermentaciones Industriales was used, based on the measuring of the increase in height occurring in a liquid when air is passed through it. The change in the height of the liquid was quantified by means of an ultrasound wave emitter-detector. The parameters quantified were the maximum height reached by the wine with foam (peak H) and the height at which the foam stabilizes (plateau H). The methodology used has been described by Moreno-Arribas et al. (*20*).

Global Sensory Quality and Visual Evaluation of Foam and Effervescence. Sensory evaluation of the wines was carried out by a panel of experts comprised of eight judges. The tasting card used was among those recommended by the OIV (*21*) for international wine competitions, partially modified by the Instituto Nacional de Denominaciones de Calidad, of the Spanish Ministry of Agriculture, Fisheries and Food. The scores used were penalizing scores so better quality wines receive a lower score. In visual aspect, special attention was paid not only to the color but also to the observation of foam characteristics, which many consumers consider to be one of the most important characteristics of a sparkling wine. The final scores were the average of the scores of each judge after eliminating those that differed by more than one standard deviation as compared to the sample's 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 et al. (*22*). 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-Blair et al. (*23*) 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.

Statistical Methods. The statistical methods used for the data analysis were two-way analysis of variance (ANOVA) to test the effect of two factors studied (yeast strains and aging time) and Student−Newman−Keuls test for means' comparisons. The STATISTICA program for Windows, version 5.1. (*24*), was used for data processing.

RESULTS AND DISCUSSION

Chemical Analysis and Yeast Viability. The rosé base wine of the Garnacha Tinta variety used in this study was selected from among those produced in a cellar from the Denomination of Origin Vinos de Madrid for being suitable to produce a sparkling wine (*25*). The base wine had an alcoholic grade of 10.7%, 0.2 g acetic acid/L of volatile acidity, 5.1 g tartaric acid/L total acidity, a pH of 3.1, and free and total sulfur dioxide contents of 20 and 60 mg/L, respectively. The wines were fermented to dryness obtaining a mean alcoholic grade of 11.6% with maximum and minimum values of 11.7 and 11.5%, respectively. Total acidity, volatile acidity, and pH were not modified during the second fermentation in any of the wines. The free and total sulfur dioxide values diminished during tirage so that the sparkling wines had 10 and 42 mg/L, respectively, at the end of the second fermentation (1 month old wines).

Figure 1 shows the viability of the yeasts in the different wines since inoculation in the base wine until 9 months of aging with yeasts. The initial inoculum was similar in the five wines, of the order of 10^6 ufc/mL. At the end of the second fermentation (1 month old wines), the number of viable cells was of the same order in all of the wines. After, small differences in viability were observed depending on the strain used. The wines elaborated with the yeast strains IMIA-2010, IMIA-2012, and EC1118 showed no viable cells, while in the wines elaborated

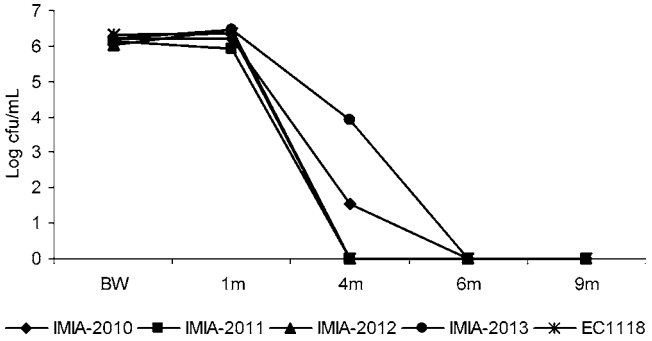

Figure 1. Cell viability (log cfu/mL) in the base wine and in the sparkling wines produced with different yeast strains (IMIA-2010, IMIA-2011, IMIA-2012, IMIA-2013, and EC1118) at different aging times.

with IMIA-2011 and IMIA 2013, some yeasts were still viable after 4 months of aging. After 6 months of aging, viable yeasts were not detected in any of the wines. As yeast autolysis is induced by cell death (*3*), these results could indicate that from this time the yeast autolysis could be triggered in all of the wines.

Nitrogen Compounds. The base wine had 147 mg/L of total nitrogen, 16 mg/L of amino nitrogen, 120 mg/L of peptide nitrogen, and 11.2 mg/L of HMWN. **Figure 2** shows the evolution of the nitrogen composition of the wines during the second fermentation and subsequent aging. The total nitrogen content decreased during the second fermentation (**Figure 2a**), and the yeasts consumed more peptides (between 16 and 28 mg/L, **Figure 2b**) than free amino acids (between 4 and 5 mg/L, **Figure 2c**). These results confirm that when the yeasts are in an amino acid deficient medium they can consume peptides directly without first hydrolyzing them to amino acids since they have their own peptide transport system (*26*, *27*). The total nitrogen content of most wines (**Figure 2a**) and of peptide (**Figure 2b**) and amino nitrogen (**Figure 2c**) of all of the wines after 4 months of aging with yeasts was greater than that of the wines at the end of the second fermentation. Enrichment of the wines with nitrogen compounds when there are viable cells in the medium can be due to a physiological response of yeasts to insufficient nutrients (*15*, *28*−*30*). Between the fourth and the ninth months of aging with yeasts, there were small quantitative changes in the contents of these nitrogen fractions. The amino nitrogen content diminished from 4 to 6 months in the wine made with the IMIA-2013 strain. This result could be attributed to the presence of viable cells in this wine during this period of time (see **Figure 1**). The HMWN (**Figure 2d**) rose slightly in the first month of the production process to subsequently decrease. All of these results can be related with the autolytic process of yeasts that takes place in these wines due to the special conditions of their production that can extend over a considerable time, even for years (*1*, *2*). In this process, there is a simultaneous release of active enzymes and other products and degradation of the released products (*1*, *27*, *31*).

Volatile Compounds. Table 1 shows the contents of volatile compounds in the base wine and the mean values ± standard deviation of the different sparkling wines at each sampling point. The results obtained by applying two-way ANOVA to test the significant main effects of the yeast strains and the aging time factors (the interaction and the within error terms were pooled) are also included in the table. In the second fermentation, there is an increase in esters, mainly ethyl lactate and diethyl succinate, that rise by 70 and 17%, respectively. There is also an overall increase in the alcohols concentration and a decrease in the fatty acids content. The aging time with the yeasts influenced significantly the increase of most of the ethyl esters (**Table 1**) and the decrease in the acetaldehyde concentration and that of the alcohols methanol, propanol, and isobutanol. The yeast strain only significantly influenced the propanol content of the wines. All of these changes were quantitatively less important than those produced during the second fermenta-

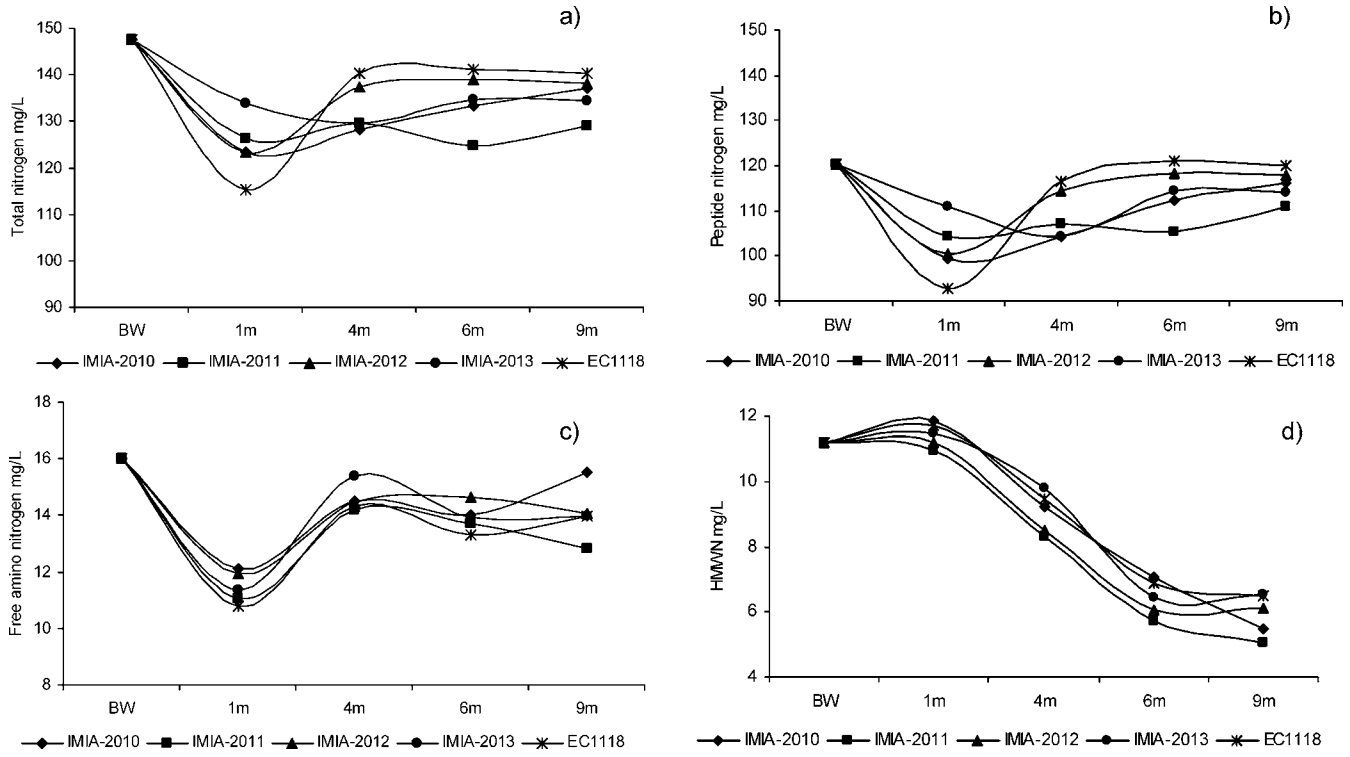

Figure 2. Total nitrogen (**a**), peptide nitrogen (**b**), free amino nitrogen (**c**), and HMWN (**d**) in the base wine and in sparkling wines produced with different yeast strains (IMIA-2010, IMIA-2011, IMIA-2012, IMIA-2013, and EC1118) at different aging times.

Table 1. Volatile Compounds Content (mg/L) in the Base Wine and Mean Values ± Standard Deviation of the Volatile Compounds (mg/L) in the Sparkling Wines ($n = 5$) and Results of the Two-Way ANOVA When the Interaction and the within Error Terms Were Pooled

		factors effect		sparkling wines (time 1–9 months)			
	base wine	time	strain	1 month	4 months	6 months	9 months
ethyl acetate	32.4	*		$36.1^{b} \pm 1.92$	$31.2^{a} \pm 4.10$	$36.3^{b} \pm 3.35$	$36.9^{b} \pm 0.72$
butyl acetate	<0.1			<0.1	<0.1	<0.1	<0.1
isobutyl acetate	<0.1			<0.1	<0.1	<0.1	<0.1
isopentyl acetate	2.8			3.0 ± 0.56	2.4 ± 0.24	2.4 ± 0.35	2.4 ± 0.09
hexyl acetate	0.9			0.9 ± 0.00	0.9 ± 0.05	0.9 ± 0.04	0.9 ± 0.00
ethyl butyrate	1.9			1.9 ± 0.04	1.9 ± 0.04	1.9 ± 0.05	1.9 ± 0.00
ethyl lactate	12.9	*		$21.6^{a} \pm 1.88$	$24.3^{ab} \pm 2.13$	$28.8^{b} \pm 5.01$	$25.8^{ab} \pm 3.69$
diethyl succinate	2.7	*		$3.5^{a} \pm 0.52$	$3.7^{a} \pm 0.27$	$4.3^{b} \pm 0.19$	$4.7^{b} \pm 0.60$
ethyl hexanoate	1.8	*		$1.9^{b} \pm 0.20$	$1.6^{a} \pm 0.04$	$1.8^{b} \pm 0.08$	$1.9^{b} \pm 0.05$
ethyl octanoate	0.6	*		$0.6^{b} \pm 0.19$	$0.2^{a} \pm 0.05$	$0.5^{b} \pm 0.13$	$0.5^{b} \pm 0.09$
ethyl decanoate	0.1			0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
methanol	15.3	*		$30.3^{ab} \pm 13.22$	$51.5^{b} \pm 28.17$	$29.2^{ab} \pm 8.02$	$17.1^{a} \pm 2.52$
propanol	16.9	*	*	24.9 ± 1.75	23.8 ± 1.66	25.1 ± 1.47	22.7 ± 2.23
isobutanol	17.0	*		$20.3^{ab} \pm 0.86$	$19.7^{ab} \pm 0.78$	$20.8^{b} \pm 1.13$	$19.2^{a} \pm 0.63$
2+3 methyl-1-butanol	122.4			145.4 ± 4.02	144.2 ± 3.09	147.8 ± 3.41	142.9 ± 6.26
hexanol	2.2			2.2 ± 0.05	2.2 ± 0.04	2.2 ± 0.00	2.2 ± 0.04
cis-3-hexen-1-ol	<0.5			<0.5	<0.5	<0.5	<0.5
hexanoic acid	5.3			4.8 ± 0.16	4.6 ± 0.07	4.7 ± 0.14	4.5 ± 0.18
octanoic acid	6.2			5.5 ± 0.41	5.1 ± 0.32	5.6 ± 0.43	5.7 ± 0.34
decanoic acid	1.1	*		$0.8^{a} \pm 0.13$	$0.8^{a} \pm 0.11$	$1.7^{b} \pm 0.50$	$0.7^{a} \pm 0.15$
acetaldehyde	52.0	*		$21.0^{b} \pm 6.91$	$14.3^{a} \pm 2.08$	$11.1^{a} \pm 3.14$	$9.0^{a} \pm 4.24$
γ-butyrolactone	<1.4			<1.4	<1.4	<1.4	<1.4

[a,b] Mean values in the same row with the same letter indicate that there are no significant differences between them ($p < 0.05$).

Table 2. Sensory Attributes and Foam Characteristics of the Sparkling Wines at 9 Months of Aging with Yeast

	IMIA-2010	IMIA-2011	IMIA-2012	IMIA-2013	EC1118
		sensory attributes			
visual aspect (0–9)	3.3	4.0	2.6	3.5	3.0
aroma intensity (0–18)	7.0	6.3	7.3	8.0	7.0
aroma quality (0–18)	7.3	8.3	7.7	6.7	7.6
taste intensity (0–18)	7.0	6.4	6.6	6.7	7.1
taste quality (0–27)	10.7	10.5	9.5	10.5	10.2
harmony (0–27)	11.1	12.0	10.7	10.3	10.3
total[a]	46.4	47.5	44.4	45.7	45.2
		foam characteristics			
foam (abundant, normal, little)	abundant	normal	abundant	abundant	abundant
surface (full, partial)	full	full	full	full	full
foam collar (total, partial)	total	total	total	total	total
bubbles size (small, medium, large)	medium	small	medium	medium	small
effervescence (fast, normal, slow)	normal	fast	normal	normal	fast

[a] 0–7 = excellent; 8–23 = very good; 24–44 = good; 45–62 = correct; 63–78 = regular; 79–90 = inadequate; and >90 = eliminated.

tion. The change in the contents of volatile compounds that was observed in these rosé wines during aging with yeasts was similar to that obtained on analyzing sparkling wines made from white grapes (*8*) over a similar time period. In the different works published in the literature aimed at studying the changes in the volatile fraction during sparkling wine production (*4*, *5*, *32*), the results obtained on the changes occurring during the second fermentation are similar. However, the results obtained by the different authors over the aging process are variable in the different studies since, as mentioned previously for the nitrogen fraction, the autolysis process is continuous and prolongs over some time and the results obtained depend on the period of time studied.

Foam Properties and Sensory Analysis. Peak H and plateau H values of the base wine and sparkling wines at each sampling point are shown in **Figure 3a**. Peak H and plateau H values for the base wine were 1545 and 563 mV, respectively, reflecting a good foaming capacity since wines with the best foam properties are those with the highest values of these parameters. Using the same measuring technique, Martínez-Rodríguez and Polo (*15*) obtained a value of 500 mV for the plateau H in a white base wine made with a blend of wines from the Macabeo, Xarello, and Parellada varieties and Moreno-Arribas et al. (*20*) obtained less than 650 and 350 mV for peak H and plateau H, respectively, in four varietal white wines made with the same varieties and with the Chardonnay variety. During the second fermentation, both foaming parameter values decreased, which was mainly attributed to an increase in the alcoholic grade (*33*). ANOVA revealed that there were not significant differences in the peak H or plateau H values either due to the yeast strain or to the aging time. There was a greater dispersion in the values of the wines 1, 4, and 6 months old as compared to those aged for 9 months. In other words, as aging time increases, the differences in the foam characteristic of the wines become smaller. The mean peak H value obtained for the 9 month old sparkling wine was 876 mV, and the mean plateau H was 417 mV, indicating a superior foam capacity than that obtained with this method in white sparkling wines (*15*, *20*, *34*). Peak H is correlated positively with the foaming characteristics evaluated by sensory analysis of the sparkling wines (*35*). Therefore, these rosé sparkling wines have better foaming properties than the whites sparkling wines previously studied.

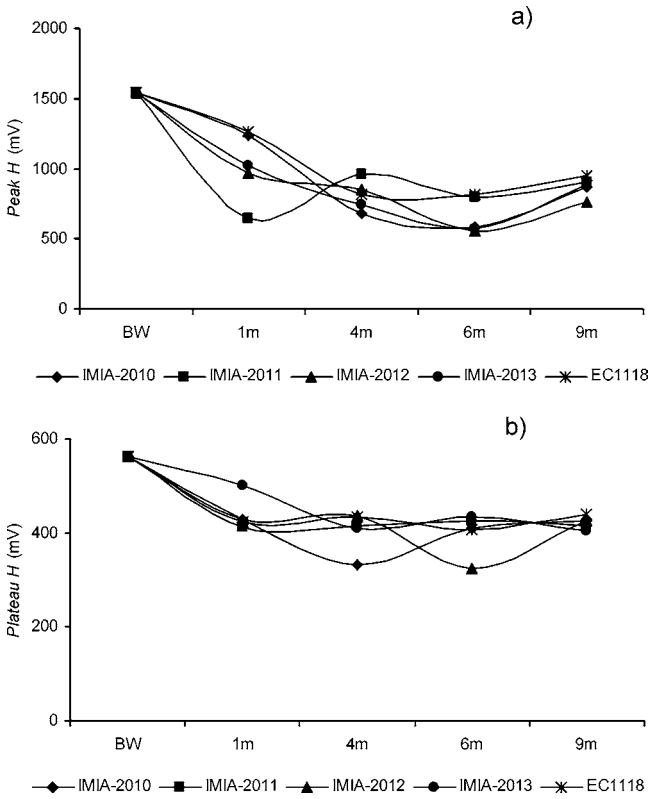

Figure 3. Height H (**a**) and plateau H (**b**) values of base wine and the sparkling wines produced with different yeast strains (IMIA-2010, IMIA-2011, IMIA-2012, IMIA-2013, and EC1118) at different aging times.

The results from the global sensory analysis and the visual analysis of the foam characteristics of the wines are shown in **Table 2**. All of the wines were qualified between correct and good and had very similar scores, ranging between 44.4 and 47.5 points. The wine made with the IMIA-2012 strain had a higher score, 44.4, than the wine made with the commercial strain, 45.2 points. In a detailed analysis of the foam characteristics of each of the wines by the tasters (**Table 2**), they considered the five wines to have a good foam characteristic, especially those produced with strains EC1118 (control) and IMIA-2011 owing to their small bubbles and rapid effervescence. Foam and effervescence are considered of the best quality when abundant foam is formed and covers the whole surface of the wine; when a foam collar appears on the surface of the wine, the bubbles are small and effervescence is fast (*22*, *35*, *36*).

The results obtained for the nitrogen and volatile fraction of the rosé sparkling wines are similar to those obtained previously in a study on white sparkling wines. However, in the instrumental and sensory analysis of the foam characteristics, the rosé wines were found to have a higher quality to that reported in previous works for sparkling wines made with white grapes.

The results obtained in the five wines produced were similar although these had been made with five different strains of yeast, indicating that the properties observed are mainly due to the grape variety, Garnacha Tinta, and to the production method, rosé.

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