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Influence of the polysaccharides and the nitrogen compounds on foaming properties of sparkling wines

V. Moreno-Arribas, E. Pueyo, F.J. Nieto, P.J. Martín-Álvarez, M.C. Polo *

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

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Abstract

The increase in the height of the wine contained in a tube, when air is passed through a fritted plate placed at its bottom was measured and used to evaluate the foam characteristics of base wines and sparkling wines. The height was detected with an ultrasonic wave transmitter/receiver, using original equipment and software. The relationship between the foam characteristics of these wines and their content in neutral and acid polysaccharides, free amino-acids, peptides and protein nitrogen was studied. It was seen that there are significant differences in foam parameters according to grape variety and aging time. It was also seen that the height reached by the foam shows a positive correlation with most of the free amino-acids, with the polysaccharides and with the protein nitrogen. No relationship was found between foam characteristics and concentrations of wine peptides. Predictive models were obtained for the parameters defining foam, based on content in neutral polysaccharides, protein nitrogen and the free amino-acid phenylalanine.

Keywords: Sparkling wine; Cava; Foam; Nitrogen compounds; Polysaccharides

1. Introduction

The characteristics of foam are an extremely important property of sparkling wines. They are known to be influenced by grape variety, harvest and the technological processes undergone in the winery that affect the chemical composition of the wine (Andrés-Lacueva, López-Tamames, Lamuela-Raventós, Buxaderas & de la Torre-Boronat, 1996). Research has been conducted in recent years to ascertain which are the compounds in wine that affect the quality of foam, in order to improve it. Most of the studies have been carried out on base wines (Andrés-Lacueva, López-Tamames et al., 1996; Brissonet & Maujean, 1991, 1993; Malvy, Robillard & Duteurtre, 1994) but fewer studies have been conducted to ascertain which compounds of sparkling wines are those that influence the foam. Findings obtained with base wines cannot be directly extrapolated to sparkling wines, because important changes take place in the composition of the latter during their manufacture by the Champenoise method, due to the autolysis of yeasts during aging with lees. The main changes that occur are an increase in the concentration of amino-acids (Feuillat & Charpentier, 1982; Moreno-Arribas, Pueyo, Polo & Martín-Alvarez, 1998; Suarez, Polo & Llaguno, 1979), a reduction in the concentration of proteins and the appearance of peptides of different sizes (Luguera, Moreno-Arribas, Pueyo & Polo, 1997; Moreno-Arribas, Pueyo & Polo, 1996), an increase in the concentration of polysaccharides (Feuillat, Charpentier, Picca & Bernard, 1988; Llaubères, Dubourdieu & Villetaz, 1987; Pueyo, Olano & Polo, 1995). Pueyo, Martín-Álvarez and Polo (1995) and Andrés-Lacueva, Gallart, López-Tamames and Lamuela-Raventós (1996) found a relation between the concentration of proteins and polysaccharides and the quality of foam in sparkling wines. Free amino acids and small peptides have been described as good foaming agents in beer (Dale, 1986; Goristein et al., 1980), however, we have not found precedents, in the literature, of research into the influence of these compounds, in sparkling wines on the properties of their foam.

In previous studies conducted in our laboratory, designed to expand the knowledge of the nitrogenous fraction of wine, it was observed (Luguera et al. 1997; Moreno-Arribas et al., 1996) that during secondary fermentation and aging with yeasts, peptides are released into the wine and these later give rise to smaller-sized peptides and free amino-acids. Characterization of

* Corresponding author. Tel.: +349-1-5613481; fax: +349-1-5644853.
E-mail address: mcpolo@ifi.csic.es (M.C. Polo).

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these compounds showed (Bartolomé, Moreno-Arribas, Pueyo & Polo, 1997; Moreno-Arribas, Bartolomé, Pueyo & Polo, 1998) that they had a very similar amino-acid composition and that they probably came from the yeasts. Consequently, this study aims to evaluate the possible relationship between the composition of wines as regards the compounds that are most modified during aging with yeasts, free amino acids, polysaccharides, peptides and proteins, and their foam characteristics. The variables to be taken into account were considered to be the grape variety that the wines were made from and their aging time with yeasts. For this purpose four mono-varietal base wines and the sparkling wines made from them were manufactured, with aging times with yeasts from 9 to 24 months. Nine months is the minimum amount of time that a wine must age with yeasts in order to be called "cava" (sparkling wines made according to the traditional or Champenoise method in certain regions of Spain). The foaming capacity of these wines was measured with an apparatus especially developed for this research. The data on the composition of the wines was related to their foaming capacity using correlation and regression analysis.

2. Materials and methods

2.1. Manufacture of wines

Four varietal base wines and the sparkling wines made from them (*Champenoise* method) were industrially manufactured, from Macabeo, Xarel.lo, Parellada and Chardonnay white grape varieties from the Penedès region (Spain). The base wines were made from sulfited must (80 mg of SO_2/l) in 100,000 l tanks at 16–18°C, after the addition of a selected winery yeast strain (*Saccharomyces cerevisiae*). The wines were clarified (20 g of bentonite/hl and 1 g of gelatin/hl) and tartrate stabilized.

A total of 120 bottles of sparkling wines, 30 of each variety, were obtained by inoculation of the varietal base wines with a yeast culture from the winery collection (*Saccharomyces bayanus*). Degorging was performed after 9, 12, 15, 18 and 24 months of aging with yeast. Since secondary fermentation and aging with yeast take place in individual bottles, six bottles at each degorging time were mixed and homogenized before sampling. All the analyses were conducted in duplicate on wines after centrifugation for 15 min at 5000 g.

2.2. Determination of foam characteristics

Bikerman's method (Bikerman, 1938) was adapted to the determination of foam characteristics in wines. A diagram of the equipment used is shown in Fig. 1. The method is based on the measurement of the increase in height of the wine contained in a 30×3 cm glass tube [1], when air is passed through a fritted glass plate placed at its bottom. The glass tube has a jacket through which passes the liquid from a thermostatic bath [2]. The height of the wine is detected by means of an ultrasonic wave transmitter/receiver [3] (Baumer Electric Mod. Unam 3019103), fed by a 24 V supply [4], and at the end of which is placed a wave guide [5]. A mass flow controller [6] (Hitec, Mod. F201c-Fa) regulates the flow of pressured air, regulated in turn by a pressure reducer [7] through a regulation valve [8]. The wine was degassed before being introduced in the tube. Experimental conditions were the following: volume of degassed wine in the tube, 50 ml; temperature 20°C; air flow rate, 125 ml/min; measurement time 1200 s. The valve for air feeding to the measurement tube was controlled and the data gathered, by a personal computer [9] using original software that displays on screen the variations in foam height during experimentation and stores the data in files for later analysis. To clean the equipment between measurements, the tube was filled successively with Milli-Q water (Millipore Corp., Milford, Ma.) and with the sample to be analyzed, and air was passed through the fritted plate.

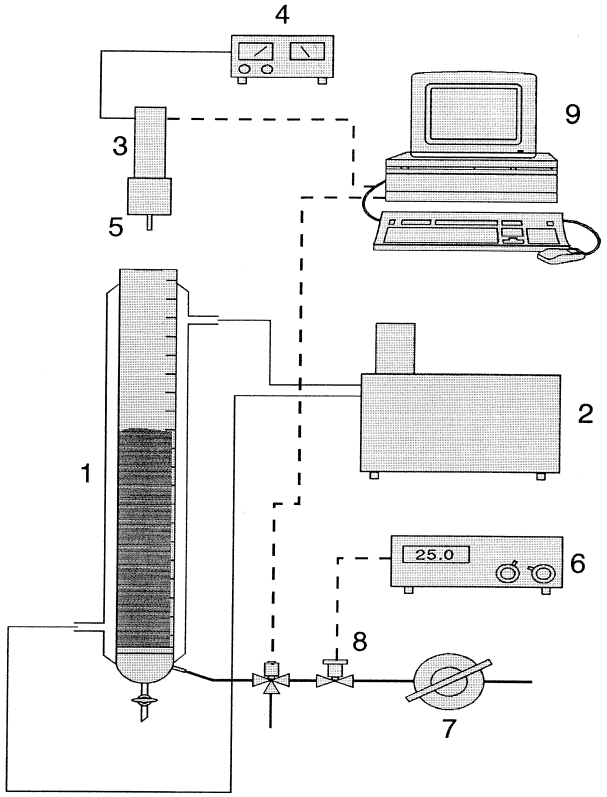

Fig. 1. Diagram of the equipment used for the measurement of the foam. [1] glass tube, [2] thermostatic bath, [3] ultrasonic wave transmitter/receiver, [4] 24 V supply, [5] wave guide, [6] mass flow controller, [7] pressure reducer, [8] regulation valve, [9] personal computer.

2.3. Polysaccharides analysis

These were determined in the ethanol-insoluble fraction obtained as indicated by Usseglio-Tomasset and Castino (1975). The phenol-sulphuric and carbazol-sulphuric methods of Dubordieu, Hadjinicolaou and Ribéreau-Gayon (1981) were used. These methods determine neutral and acid polysaccharides. The sum of both groups are considered as total polysaccharides.

2.4. Nitrogen compounds analysis

Amino-acid analysis was carried out by reversed phase HPLC of the *o*-phtaldialdehide and 9-fluorenylmethylchloroformate derivatives as described in Moreno-Arribas, Pueyo et al. (1998). Derivative reactions were performed automatically, and separations were carried out on a Nova-Pak C_{18} column (150×3.9 mm i.d., 60Å, 4 μm). Detection was by fluorescence. Total nitrogen was determined by the Kjeldahl method with a Buchi 425 digestor and a Buchi 315 distillation unit. Protein nitrogen was calculated by dividing the protein content, determined by the Bradford dye-binding assay (Bradford, 1976) by 6.25. Peptide analysis was carried out in the ethanol-soluble fraction, following the methodology described by Moreno-Arribas et al. (1996). The analysis includes the fractionation of the ethanol soluble fraction by molecular exclusion chromatography (Sephadex G-10) to give rise to two peptide subfractions containing the compounds with molecular weight lower and higher than 700, respectively, and the separation of peptides from both subfractions by reversed-phase HPLC. In each peptide subfraction, the sum of the areas and the area of hydrophilic and hydrophobic peptides were calculated.

2.5. Statistical methods

Correlation analysis was used to examine the linear relationships between chemical composition and foam characteristics. Stepwise regression was used to model the quantitative relationships. The calculations were carried out by means of the STATISTICA program (Statsoft, 1996). This program was run on a personal computer.

3. Results and discussion

3.1. Foam characteristics of wines

Fig. 2 shows, by way of example, the plot of the height of foam versus time during gasification of the Chardonnay variety base wine. The valve for air feeding to the measurement tube was open at 60 s and closed at 1000 s. As can be observed, in the first moments when the air enters, the height of the wine reaches its maximum and then descends and remains stable while the air is passing through the wine. The parameters quantified are the maximum height reached by the wine with foam and the height at which the foam stabilizes, which has been calculated as the mean value of the last 300 points previous to the instant in which the air passage was closed. These two parameters were named Peak

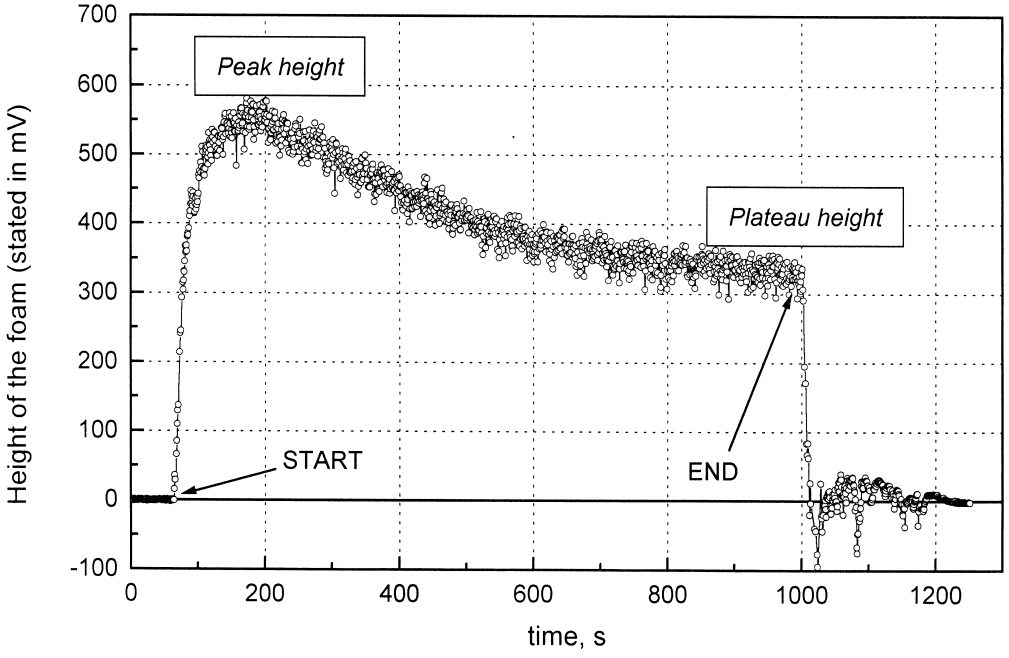

Fig. 2. Foam profile of the base wine from the Chardonnay variety.

Height (*Peak H*) and Plateau Height (*Plateau H*), respectively. Gasification was repeated twice and the values of the second gasification were those considered.

The relative standard deviations ($n=6$) were 6.1% for the *Peak H* values and 3.6% for the *Plateau H* values. It was observed that there is a positive correlation ($r=0.83$) between these two parameters, as was also observed by Andrés-Lacueva et al. (1996) in base wines and by Andrés-Lacueva, López-Tamames et al. (1996), in sparkling wines using the Mosalux® (Maujean, Poinsaut, Dantan, Brissonnet & Cossiez, 1990) equipment.

The values of the *Peak H* and *Plateau H* foam parameters in the base wines and their evolution during manufacture and aging of the sparkling wines with yeasts are shown in Figs. 3a and 3b respectively.

Significant differences ($P<0.05$), due to variety and aging time factors, were observed for *Peak H* and *Plateau H* values when two-way analysis of variance was applied and the interaction and within-error terms were

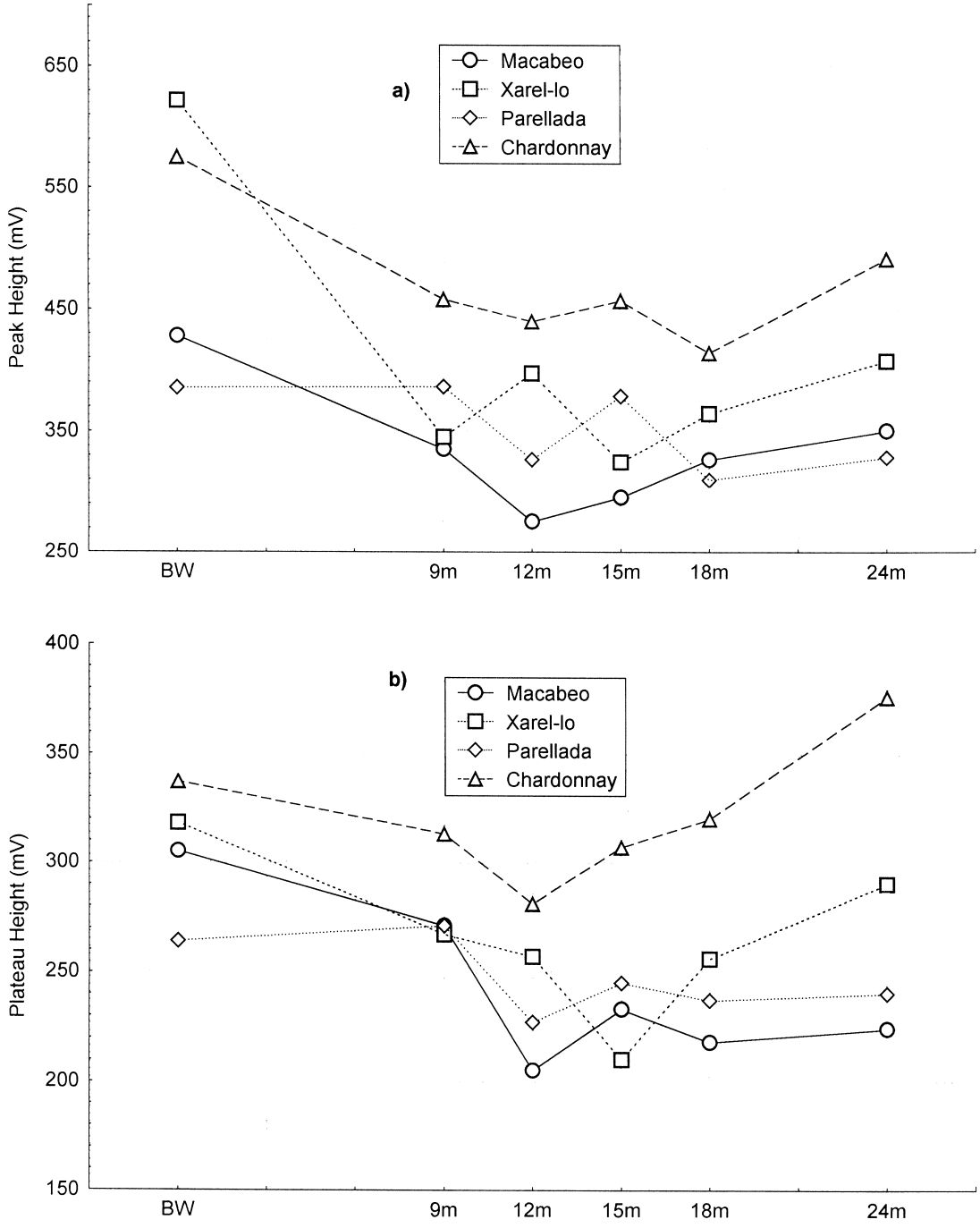

Fig. 3. *Peak H* (a) and *Plateau H* (b) values in the base wines (BW) and in the sparkling wines after 9, 12, 15, 18 and 24 months of aging with yeast.

pooled. No significant differences were observed for *Peak H* and *Plateau H* values when only the wines of the Macabeo, Xarel.lo and Parellada varieties, after 9 months of aging with yeast, were considered. *Peak H* and *Plateau H* values of the base wine were higher than those of the sparkling wines except for those of the Parellada variety.

3.2. Chemical composition of wines

Fig. 4 shows the concentration of neutral and acid polysaccharides (mg/l) in the base wines and sparkling wines of the four varieties studied. In all the wines, the neutral polysaccharide content is higher than that of acid polysaccharides. The values found range from 736.3 to 107.5 mg/l for neutral polysaccharides and from 91.6 mg/l to not detected amounts for acid polysaccharides. There is no agreement among the different authors on the polysaccharide concentration in wines since the values reported in the literature depend to a large extent on the method used for their extraction and quantification. Andrés-Lacueva, Lamuela-Raventos, Buxaderas and de la Torre-Boronat (1997), using precipitation with ethanol in an acid medium as extraction method, and double reaction with phenol-sulphuric and hydroxyphenyl-sulphuric as quantification method, give values for sparkling wine of the same origin as those studied here of from 280.1 to 202.1 mg/l for neutral polysaccharides and from 58.7 to 42.2 mg/l for acid polysaccharides.

Significant differences ($P < 0.05$) between neutral polysaccharide content values due to variety and aging time factors were observed when two-way analysis of variance was applied and the interaction and within-error terms were pooled. Significant differences due to aging time only were observed from the application of variance analysis to the acid polysaccharide values. The Chardonnay variety wines have a higher neutral polysaccharide content than those of the Macabeo, Xarel.lo and Parellada varieties. During secondary fermentation there is a fall in the concentration of polysaccharides, except for the neutral polysaccharides in the Macabeo variety wine that had been aged for 24 months and the acid polysaccharides in the Chardonnay variety wine that had been aged 15 months, so that the sparkling wines had lower concentrations of acid and neutral polysaccharides than their corresponding base wines. From 18 months of aging with yeasts, an important increase was observed in neutral polysaccharides. This increase in neutral polysaccharides seems to indicate that mannans and glucomannans have been released from the yeast walls. The increase in polysaccharides during the aging of wine with yeasts has been indicated by several different authors (Andrés-Lacueva et al., 1997; Feuillat et al., 1988; Llaubéres et al., 1987; Pueyo, Martín-Álvarez et al., 1995). According to Llaubères et al. (1987) the release of yeast exocellular polysaccharides varies according to yeast strain, temperature and duration of storage over lees. In spite of the differences between the values found here and those found in

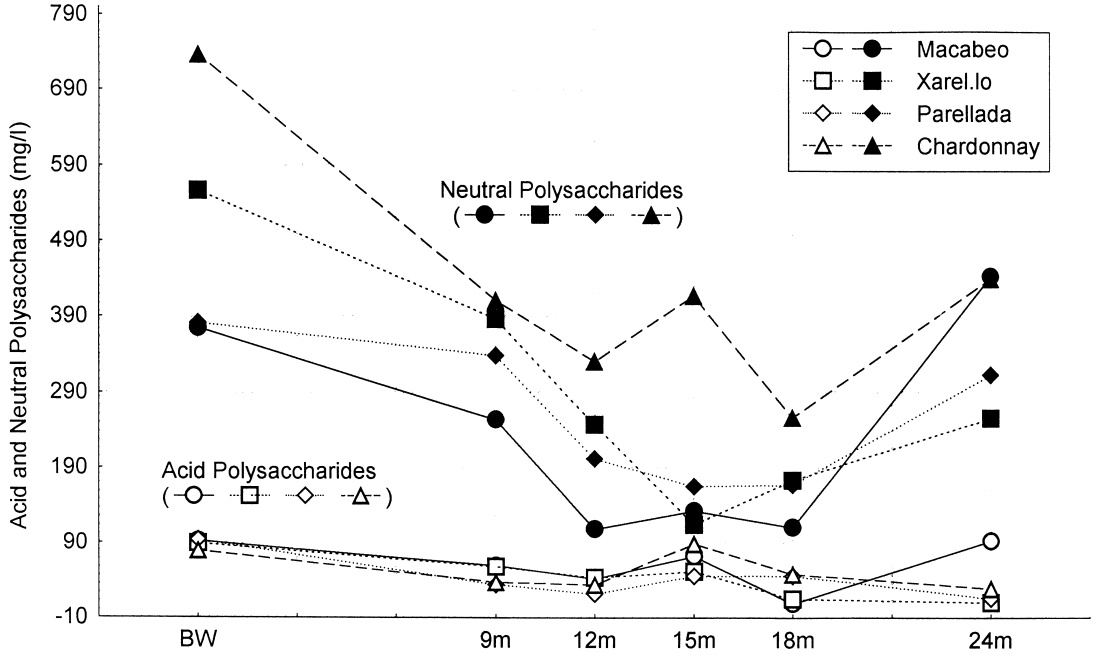

Fig. 4. Acid (empty symbols) and neutral (filled symbols) polysaccharides content (mg/l) in the base wines (BW) and in the sparkling wines after 9, 12, 15, 18 and 24 months of aging with yeast.

Table 1
Mean ± standard deviation values of free amino acid content (mg/l) in the base wines and sparkling wines (n = 4)

Amino acids	Base wine	9 Months	12 Months	15 Months	18 Months	24 Months
Asp	15.00 ± 6.45	12.6 ± 12.8	13.8 ± 8.67	13.5 ± 8.64	13.8 ± 7.20	13.0 ± 7.51
Glu	34.4 ± 19.6	19.5 ± 15.8	23.8 ± 18.0	24.1 ± 16.0	17.5 ± 10.8	17.9 ± 13.8
Asn	15.7 ± 10.0	25.2 ± 20.8	19.5 ± 13.7	24.1 ± 16.9	16.3 ± 12.8	14.4 ± 10.4
Ser	8.45 ± 6.52	7.74 ± 10.0	6.00 ± 6.74	7.00 ± 5.35	7.00 ± 6.71	6.35 ± 5.24
Gln	4.56 ± 3.95	0.66 ± 0.60	0.22 ± 0.19	0.00 ± 0.00	6.35 ± 5.94	5.59 ± 2.94
Hys	13.8 ± 6.27	12.0 ± 11.6	7.89 ± 8.22	15.5 ± 7.84	3.06 ± 0.92	7.61 ± 2.28
Gly	9.72 ± 4.11	11.6 ± 7.17	10.1 ± 4.29	8.36 ± 5.65	10.2 ± 3.50	7.33 ± 2.97
Thr	5.21 ± 3.94	8.68 ± 8.42	6.74 ± 5.48	13.0 ± 8.04	6.37 ± 4.32	7.16 ± 5.05
Arg	38.2 ± 41.4	49.3 ± 72.4	41.9 ± 55.5	47.6 ± 49.7	38.9 ± 50.1	29.0 ± 37.2
β-Ala	3.30 ± 2.58	3.76 ± 4.30	2.51 ± 2.57	7.71 ± 5.41	15.4 ± 7.84	4.07 ± 2.72
α-Ala	47.3 ± 58.5	52.8 ± 80.6	45.3 ± 62.1	57.1 ± 66.9	42.0 ± 53.0	34.2 ± 45.6
GABA[a]	40.0 ± 44.7	45.0 ± 64.0	37.3 ± 47.3	44.6 ± 50.6	33.4 ± 42.2	21.2 ± 25.9
Tyr	12.3 ± 6.27	12.1 ± 10.7	11.2 ± 7.06	13.3 ± 7.28	10.9 ± 5.97	10.2 ± 5.38
Met	3.44 ± 1.45	1.41 ± 1.76	0.24 ± 0.28	3.20 ± 1.56	2.30 ± 1.57	3.56 ± 1.46
Val	6.60 ± 4.47	6.83 ± 7.42	5.43 ± 5.40	8.53 ± 6.44	6.09 ± 5.08	6.67 ± 4.75
Trp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.73 ± 1.45	8.06 ± 3.19	0.00 ± 0.00
Phe	11.2 ± 4.72	9.29 ± 7.28	5.99 ± 3.02	11.3 ± 5.17	14.1 ± 6.11	9.31 ± 4.01
Ile	3.06 ± 1.55	2.09 ± 2.28	0.72 ± 0.80	11.3 ± 7.68	11.0 ± 5.31	2.88 ± 1.54
Leu	14.7 ± 6.46	12.2 ± 9.05	11.9 ± 5.48	13.9 ± 6.74	11.2 ± 4.71	10.1 ± 4.42
Orn	11.4 ± 6.14	9.74 ± 0.74	7.41 ± 2.49	9.44 ± 1.84	25.4 ± 7.64	10.0 ± 1.45
Lys	17.3 ± 7.29	23.3 ± 12.6	20.5 ± 7.65	18.6 ± 9.18	23.1 ± 8.58	14.8 ± 5.09
Pro	389 ± 306	324 ± 250	362 ± 272	314 ± 211	418 ± 311	306 ± 226

[a] GABA, γ-aminobutiric acid.

Andrés-Lacueva et al. (1997), the trend observed during the aging of wine with yeasts is analogous.

The data on both polysaccharides and on nitrogenous composition were used as the chemical variables for the study of the correlation between foam characteristics and wine composition. Table 1 shows a summary of the free amino-acid composition (mg/l) of the wines, grouped by type of wine. These values expressed as molar distribution (%) are to be found in Moreno-Arribas , Pueyo et al. (1998). Likewise, Table 2 shows a summary of the data on other nitrogenous fractions, the original values of which for base wines and sparkling wines aged 9, 12, 15 and 18 months are to be found in Moreno-Arribas et al. (1996). Both Tables are included here in order to facilitate the understanding of the results.

3.3. Correlation analysis

Table 3 shows the correlation coefficients of the variables corresponding to the foam characteristics, *Peak H* and *Plateau H*, and those corresponding to the chemical composition of the wines studied. It also indicates whether these coefficients are significant or not at $P < 0.05$. It can be observed that maximum height (*Peak H*) is significantly correlated with most of the amino-acids, with total and protein nitrogen and with polysaccharide content. Coefficients of over 0.60 were found for glutamic acid (0.66), for protein nitrogen content (0.62) and for neutral and total polysaccharide content (0.82 y 0.80, respectively). *Plateau H* is highly correlated with total nitrogen (0.71), with neutral polysaccharides (0.71)

Table 2
Mean ± standard deviation values of nitrogen compounds in the base wines and sparkling wines ($n = 4$)

	Base wines	9 Months	12 Months	15 Months	18 Months	24 Months
Total nitrogen (mg/l)	176 ± 115	168 ± 97.2	168 ± 97.1	172 ± 98.3	169 ± 96.3	177 ± 99.9
Protein nitrogen	1.76 ± 0.58	0.83 ± 0.49	1.46 ± 0.35	1.46 ± 0.32	1.59 ± 0.30	1.43 ± 0.29
Peptides < 700a[a]						
Hydrophobic	26.6 ± 14.5	18.3 ± 7.36	54.1 ± 46.7	63.6 ± 24.4	30.5 ± 17.4	16.2 ± 9.23
Hydrophilic	46.6 ± 24.6	38.9 ± 24.2	66.4 ± 20.1	80.6 ± 14.9	17.1 ± 15.0	15.4 ± 7.37
Total	73.2 ± 35.2	57.2 ± 30.5	120 ± 44.6	153 ± 11.6	38.2 ± 25.19	31.6 ± 15.9
Peptides > 700a[a]						
Hyrophobic	84.8 ± 39.1	31.8 ± 5.30	41.7 ± 23.1	24.3 ± 22.4	67.5 ± 42.5	22.9 ± 17.5
Hydrophilic	13.6 ± 14.3	16.9 ± 16.3	17.7 ± 16.9	37.7 ± 20.1	19..1 ± 14.7	8.13 ± 13.7
Total	99.5 ± 38.8	48.7 ± 20.7	58.4 ± 34.0	61.3 ± 41.8	86.6 ± 46.4	31.0 ± 31.1

[a] Sum of the choromatographics peaks areas.

and with glutamic acid (0.71). All coefficients significantly different from zero, for both variables *Peak H* and *Plateau H*, are positive. That is to say, high values of nitrogenous compounds and of polysaccharides in wine improve the quantity of foam formed by the wine.

3.4. Regression analysis

Stepwise multiple linear regression was used to select an equation for predicting the foam characteristics of wines, using only the variables correlated with these foam parameters (Table 3). Values of 4 and 3.99 were used for F-to-enter and F-to-remove limits. Neutral polysaccharides and protein nitrogen make a positive contribution to the prediction of maximum foam height (*Peak H*) (Fig. 5a). The model explains 76% of *Peak H* variation and the standard error of estimate was 43.1 mV. Similarly, 70% of the variation of the height at which the foam stabilizes (*Plateau H*) can be explained by the variables, neutral polysaccharides and phenyla-

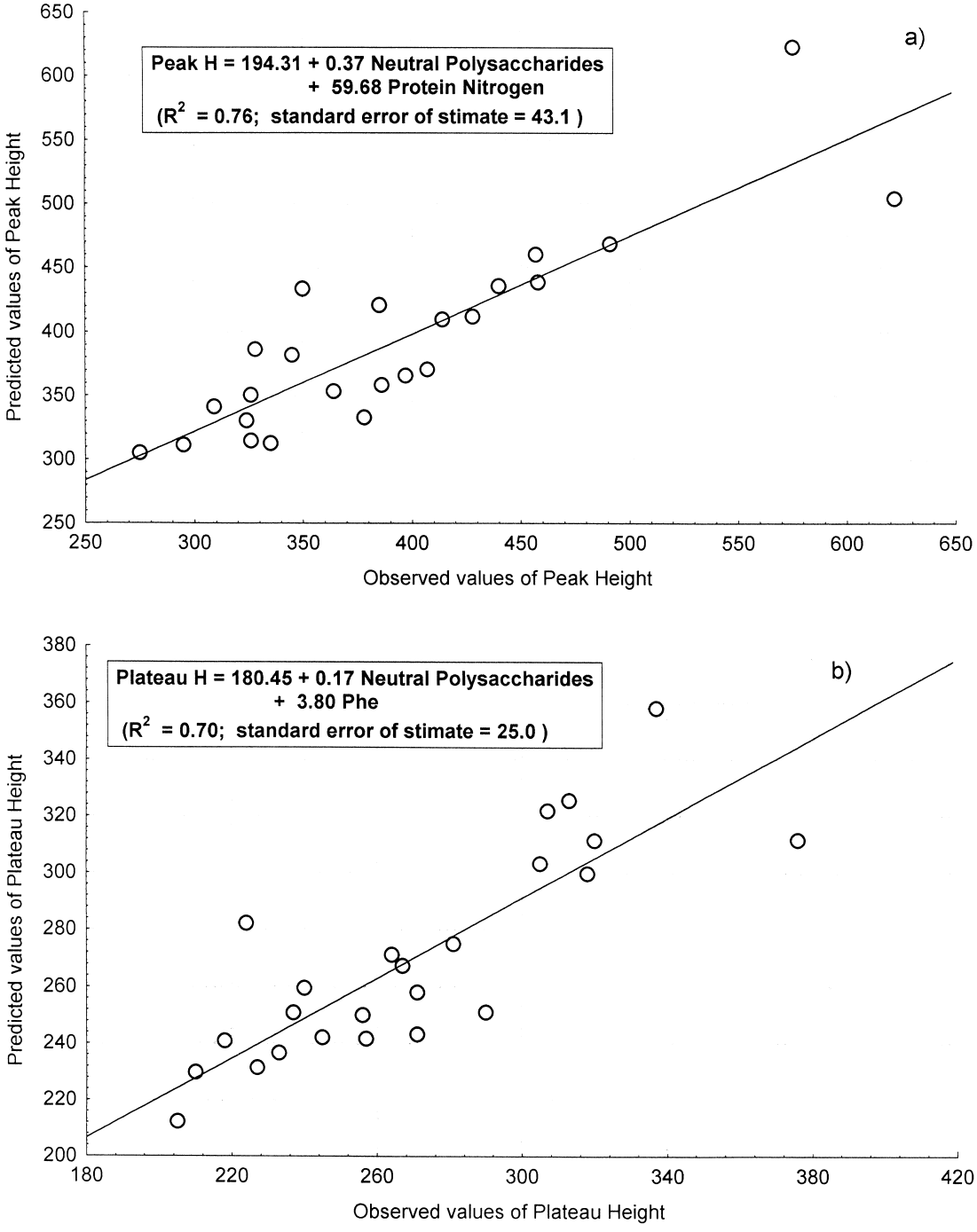

Fig. 5. Predicted values of *Peak H* (a) and *Plateau H* (b) versus observed values for base wines and sparkling wines.

Table 3
Correlation coefficients between foam parameters (*Peak H* and *Plateau H*) and chemical variables ($n = 24$)

Compounds	*Peak H* (mV)[a]	*Plateau H* (mV)[a]
Amino acids		
Asp	0.52*	0.67*
Glu	0.66*	0.71*
Asn	0.41*	0.57*
Ser	0.56*	0.68*
Gln	0.36	0.53*
Hys	0.50*	0.48*
Gly	0.41*	0.57*
Thr	0.19	0.32
Arg	0.50*	0.62*
β-Ala	0.12	0.30
α-Ala	0.53*	0.63*
GABA	0.52*	0.60*
Tyr	0.49*	0.63*
Met	0.51*	0.63*
Val	0.52*	0.67*
Trp	−0.17	−0.05
Phe	0.42*	0.62*
Ile	0.08	0.22
Leu	0.51*	0.64*
Orn	−0.17	0.03
Lys	0.36	0.52*
Pro	0.58*	0.69*
Total nitrogen	0.56*	0.71*
Protein nitrogen	0.62*	0.49*
Neutral polysaccharides	0.82*	0.71*
Acid polysaccharides	0.33	0.19
Total polysaccharides	0.80*	0.68*
Peptides > 700Da		
Total	−0.07	−0.08
Hydrophobic	0.03	−0.02
Hydrophilic	−0.25	−0.16
Peptides < 700Da		
Total	0.01	−0.18
Hydrophobic	−0.14	−0.29
Hydrophilic	0.17	0.01

[a] * Marked correlations are significant at $P < 0.05$.

lanine (Fig. 5b), which also make a positive contribution, with a standard error of estimate of 25 mV.

4. Conclusions

A positive relationship was found between content in free amino-acids, polysaccharides and proteins and values of *Peak H* and *Plateau H*. However, in spite of what could have been expected from studies carried out on beer foam (Dale, 1986; Goristein et al., 1980), no relationship was found between foam characteristics and concentration of wine peptides. Cava manufacturers have verified experimentally that wines manufactured from the Chardonnay variety give better quality foam than those manufactured from the Macabeo, Xarel.lo and Parellada varieties. The greater concentration of free amino-acids and proteins (Moreno-Arribas, Bartolomé et al., 1998; Moreno-Arribas, Pueyo et al., 1998) and polysaccharides in the Chardonnay variety wines could, at least partly, explain this fact. All the parameters showing a positive correlation with foam characteristics fall in advanced stages of aging (Andrés-Lacueva et al., 1997; Luguera et al., 1997; Moreno-Arribas, Bartolomé et al., 1998; Moreno-Arribas, Pueyo et al., 1998), and, therefore, it must be assumed that each sparkling wine has an optimum aging time from the point of view of foam quality, after which its quality diminishes.

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