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Influence of the yeast strain on the changes of the amino acids, peptides and proteins during sparkling wine production by the traditional method

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The influence of five yeast strains on the nitrogen fractions, amino acids, peptides and proteins, during 12 months of aging of sparkling wines produced by the traditional or Champenoise method, was studied. High-performance liquid chromatography (HPLC) techniques were used for analysis of the amino acid and peptide fractions. Proteins plus polypeptides were determined by the colorimetric Bradford method. Four main stages were detected in the aging of wines with yeast. In the first stage, a second fermentation took place; amino acids and proteins plus polypeptides diminished, and peptides were liberated. In the second stage, there was a release of amino acids and proteins, and peptides were degraded. In the third stage, the release of proteins and peptides predominated. In the fourth stage, the amino acid concentration diminished. The yeast strain used influenced the content of free amino acids and peptides and the aging time in all the nitrogen fractions.
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Introduction

The traditional method of sparkling wine production requires addition of sucrose and yeast strains to the base wine for a second fermentation, which takes place in the characteristic sparkling wine bottles. Yeast strains that take part in the second fermentation are selected on the basis of different analytical and technological criteria, among them the resistance to ethanol concentration, pressure and temperature, high flocculation capacity and good autolytic and foam properties [2,3,16]. After the second fermentation, the wines are aged with yeast for several months. In the aging process, yeast autolysis takes place [6], and different compounds that could modify the sensorial properties of wine are released by the yeast into the wine. Nitrogen compounds are generally considered the major compounds released in wine during autolysis. In previous studies, it was determined that the amount of nitrogen compounds in sparkling wines, mainly amino acids and peptides, is influenced by the grape variety and aging time [19,20]. However, the real impact of the yeast strain used on the nitrogen composition of wine in the second fermentation is unclear. The total increase of the compounds coming from yeast autolysis amounts to only a few milligrams per liter. Sato *et al* [23] and Usseglio-Tomasset *et al* [27] consider that aging with yeast has only a minimum effect on the sensory quality of wines aged in such a way. However, Leroy *et al* [14] found differences between wines elaborated with two yeast strains traditionally used in sparkling wine vinification, and Martínez-Rodríguez *et al* [18] detected significant differences in the amounts of nitrogen compounds released into the medium by three strains of *Saccharomyces cerevisiae* autolysed in a model system. The present study was designed to determine the effect of the different yeast strains on the nitrogen fraction of the wines, especially on the free amino acids and the peptides. To this end, under industrial conditions and using the same base wine, five sparkling wines with five different yeast strains were produced and samples were taken during the second fermentation and during aging with the yeasts. Total nitrogen, protein and free amino acids were determined in the wines. The evolution of the peptide fraction determined by high-performance liquid chromatography (HPLC) was also studied.

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Materials and methods

Wine production and sampling times

Sparkling wines were produced with the same base wine using the traditional method. Bentonite was not added to the tirage solution to avoid interference with the nitrogen compounds released during yeast autolysis. Five different yeast strains previously selected because of their appropriate characteristics for the production of sparkling wines [17] were used to inoculate the base wines: *S. bayanus EC-1118*, a commercial strain supplied by Lallemand (Spain); *IFI-473* and *IFI-475*, two *S. cerevisiae* strains belonging to the collection of enological yeasts of the Instituto de Fermentaciones Industriales (IFI) of the CSIC (Madrid, Spain); *S. cerevisiae P-29*, a native strain from the Penedés region, from the Instituto Catalán de la Viña y el Vino (INCAVI; Spain), and *S. cerevisiae J*, a strain belonging to the collection of the company that produced the wines. Second fermentation and aging with yeast were carried out at approximately 15–16°C — the cellar temperature. Samples were taken from the base wine (time 0) and then after 20, 40, 90, 180, 270 and 365 days of aging with yeast. In order to carry out analytical determinations of the nitrogen compounds, samples from six bottles at each sampling point were mixed and homogenized before the analysis. All the analyses were performed in duplicate on wines that had been centrifuged for

15 min at 5000×*g*. After 40 days, fermentation was complete in all the wines. The wines had 10.6–10.7 degrees of alcohol, 0.17–0.28 g/l acetic acid of volatile acidity, 5.76–6.04 g/l tartaric acid of total acidity and a pH of 3.33–3.36. No differences were detected in these parameters due to yeast strain or aging time with the yeast (unpublished data).

Cell viability

Viability was determined by plate counting on Malt Extract Agar (ADSA Micro, Spain) for yeast counts and on Lafon–Lafourcade Agar [13], supplemented with actidione for lactic acid bacteria counts. Plates were incubated at 30°C for 3 days. These analyses also provided a check on culture purity.

Total nitrogen

Total nitrogen was determined by the Kjeldahl method with a Tecator Digestion System and a Kjeltec 1030 Auto Analyzer (Tecator, Höganäs, Sweden).

Soluble proteins plus polypeptides

Soluble proteins plus polypeptides were determined by the Bradford dye-binding assay [5].

Amino acid analysis

Analyses in duplicate were carried out by HPLC using a Waters (Milford, MA) liquid chromatograph controlled by a Waters Maxima 820 chromatography workstation. Samples were submitted to an automatic precolumn double derivatization with *o*-phthaldialdehyde (OPA) to determine primary amino acids and with 9-fluorenylmethyl chloroformate (FMOC) to detect secondary amino acids [9]. All separations were performed on a Waters Novapak C-18, 60 Å, 4 μm column (3.9 cm×150 mm). Eluents and gradient conditions were those described by Gonzalez del Llano *et al* [11]. Detection was by fluorescence using wavelengths of excitation and emission at 340 and 425 nm, respectively, for OPA derivatives and at 250 and 335 nm, respectively, for FMOC derivatives. Samples were injected in duplicate into the column after being filtered through a 0.22-μm filter. All reagents used were HPLC grade. Variation coefficients of amino acid determination were lower than 6%.

Peptide analysis

The method described by Moreno-Arribas *et al* [19] was used for peptide analysis. The analysis includes the elimination of compounds with a high molecular weight by precipitation with ethanol in an acid medium and fractionation into two groups containing the compounds with molecular weight higher and lower than 700, respectively, by chromatography of molecular exclusion on Sephadex G-10 and the separation by HPLC of each of these fractions. The separation was carried out on a Waters Nova Pak C-18 column (150×3.9 mm i.d., 60 Å, 4μm). A Beckman (Beckman Instruments, Fullerton, CA, USA) M168 diode array detector was used. Eluent A was 0.1% trifluoroacetic acid in water and eluent B was 0.1% trifluoroacetic acid in acetonitrile. The gradient of B was increased from 0% to 40% over 70 min. The flow rate was 1 ml/min.

Statistical methods

Statistical methods used for the analysis were the following: a two-way analysis of variance was used to test the effects of strain and time factors; cluster analysis (Ward's method from standardized data) was used to discover natural groupings of the samples. The STATISTICA [26] program was used for data processing.

Results and discussion

Cellular viability

Figure 1 shows the number of viable yeast cells at each of the sampling times. The number of viable cells in all wines after inoculation of the yeasts (time 0) was around 10^6 cfu/ml. Twenty days after inoculation, the number of viable cells was only slightly higher in the wines produced with the *EC-1118*, *J* and *P-29* strains and lower in the wines produced with the *IFI-473* and *IFI-475* strains. Forty days after the inoculation, no viable cells were detected (<10 cfu/ml) in the wine produced with the *EC-1118* strain and, after 90 days, there was none in any of the wines analyzed.

Lactic bacteria did not exceed the detection limit of the counting method used on the plate (<10 cfu/ml) in any of the wines analyzed.

Effect of the yeast strain used in the tirage solution and of the aging time on the total nitrogen as well as on the protein plus polypeptide content of the wines

By applying the analysis of variance to the total nitrogen data of the wines, it was verified that there were significant differences ($P<0.05$) with regard to the time of aging, but not with regard to the yeast strain used in the second fermentation. Figure 2 shows the total nitrogen values of the base wine and the mean values ±SD of the total nitrogen of the sparkling wines produced with each of the yeast strains. The total nitrogen content of the wine after 20 days was lower than that of the base wine. At this stage, most of the second fermentation had taken place and the yeasts had used nitrogen compounds for their multiplication. Between 20 and 40 days after that, an increase of the total nitrogen content was detected. This increase continued during the 365 days the wine was aged with the yeasts. The release of nitrogen compounds toward the end of the fermentation and during the aging with the yeasts is a widely documented phenomenon associated with progressive loss of viability and later autolysis of the yeasts [8,21].

With the Bradford colorimetric method [5], polypeptides with a molecular weight higher than 3500 were quantified together with the proteins [24]. Therefore, the data that appear in Figure 3 correspond to the concentration of proteins together with

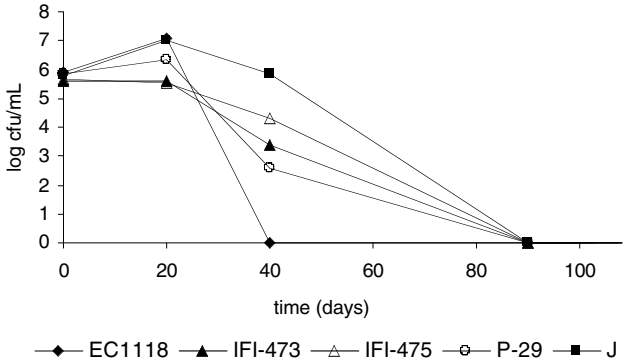

Figure 1 The yeast population (log cfu/ml) in the course of time.

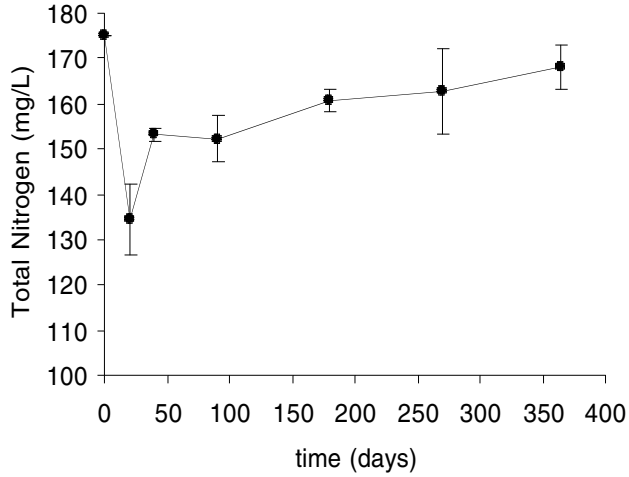

Figure 2 Total nitrogen concentration (mg/l) in the base wine (time 0) and mean±SD of total nitrogen in sparkling wines produced with different yeast strains (*EC-1118*, *IFI-473*, *IFI-475*, *P-29* and *J*) at different aging times.

polypeptides. After applying the analysis of variance to the concentration data of polypeptides plus proteins of each of the wines, it was verified that it was influenced significantly ($P<0.05$) by the time of aging, but not by the yeast strain used during the second fermentation. Therefore, Figure 3 represents the mean values ±SD of the data obtained for the five sparkling wines at each aging interval, together with those of the base wine (time 0). The content of these nitrogen compounds 20 days after the *tirage* was slightly higher than that of the base wine. Between 20 and 180 days, a decrease of these polymerized compounds was observed. This may be partly due to insolubilisation because of the increase of the degree of alcohol by 1.2 and 1.3 degrees during the second fermentation. Between 180 and 270 days after the *tirage*, there was an increase of the protein plus polypeptide content.

Effect of the yeast strain used in the tirage solution and of the time of aging on the free amino acid content of the wines

The individual values of the free amino acid content are shown in Table 1. Most of the amino acids in the base wine were proline (271.5 mg/l), which made up 58% of the total content of free amino acids. In order of concentration, but with much lower values, this amino acid was followed by glutamic acid, lysine and arginine, with 24.6, 21.7 and 15.8 mg/l, respectively.

After applying the analysis of variance to the individual data of the free amino acids and to those of their sum, it was verified that the concentration of all amino acids and of their sum was significantly influenced ($P<0.05$) by the yeast strain used in the *tirage* and that all of them, except gamma aminobutyric acid, were influenced by the aging time with the yeasts.

Figure 4 represents the sum of free amino acids, except proline, of each wine. Proline was not taken into account so that the changes that occur in this amino acid, which on average make up 59% of the total of the free amino acids in the wines, do not disguise those that occur in the remaining amino acids.

The concentration of free amino acids in the different wines 20 days after inoculation with the yeasts was lower than that of the base wine (time 0). This decrease is due to their assimilation by the yeasts, whose population is on the increase or maintains a high viability during this stage (Figure 1). The decrease of free amino acids by yeasts is ascribed to the use of amino acids for the synthesis of proteins, RNA and DNA and for their accumulation as a reserve in vacuoles [25]. Between 20 and 90 days after inoculation, a stage in which there were still viable cells (Figure 1), a release of amino acids was observed in all the wines. This may be due to a physiological response of the yeasts to the lack of nutrients [4]. After 90 days, the increase of amino acids was slower. Between 270 and 365 days, the sum of the amino acids varies in a different way in the different wines. While they continue to increase in the wines produced with the *EC-1118* strain, a decrease was observed in wines produced with the *IFI-475*, *P-29* and *J* strains and there were no variations in the amount of amino acids in the wine manufactured with the *IFI-473* strain. This may point to the fact that the yeasts of these wines had reached a more advanced autolysis stage. As a cause for the decrease of amino acids, different authors have pointed to deamination reactions and to their participation in the formation of different compounds [10,12].

The amino acids that decreased most during the first 20 days after the inoculation of the yeasts were proline, aspartic acid, glutamic acid and leucine. After 20 days and especially between 20 and 40 days, most amino acids increased, especially proline, with values approaching those of the base wine. Probably due to release by dead yeasts, there was a remarkable reduction of viable yeast cells (Figure 1).

Proline, which is not assimilated by yeasts, is accumulated in the vacuoles and later released into the medium [25]. After 40 days, the increase of amino acids became general, perhaps because some increase more than the others. Release of threonine and serine from the manoproteins of the yeast wall, observed by Alexandre *et al* [1] was not detected in these wines.

In order to verify whether there was a grouping of the wines on the basis of the data for free amino acids, cluster analysis was applied (Figure 5). Two main groups were obtained: the wines produced with the *EC-1118*, *IFI-473* and *IFI-475* strains corresponding to the production of 20, 40 and 90 days appear in one group and the remaining wines appear in the other group. The second group was divided into four subgroups. The influence of the yeast strain used can be observed because the wines produced

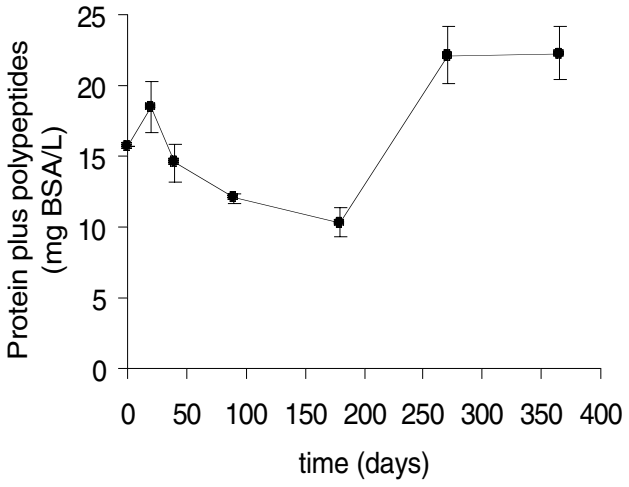

Figure 3 Proteins plus polypeptide concentration (mg/l BSA) in the base wine (time 0) and mean±SD of total nitrogen in the sparkling wines produced with different yeast strains (*EC-1118*, *IFI-473*, *IFI-475*, *P-29* and *J*) at different aging times.

Table 1 Free amino acids content (mg/l) in the base wine (time 0) and in sparkling wines (time 20–365 days)

	Strain	Time (days)					
		Base wine	Sparkling wines				
		0	20	40	90	180	270	365
Asp		15.2						
	EC1118		5.2	6.2	6.6	8.7	11.8	11.0
	IFI-473		6.3	7.8	6.4	9.7	13.7	12.5
	IFI-475		6.1	6.7	6.3	8.7	13.1	11.5
	P-29		11.5	12.1	11.1	13.4	19.1	15.2
	J		9.5	10.5	10.0	12.3	15.8	14.2
Glu		24.6						
	EC1118		10.8	11.9	12.5	13.4	16.1	14.9
	IFI-473		12.1	13.9	12.9	15.2	19.2	17.7
	IFI-475		11.9	12.9	11.9	13.5	17.8	16.4
	P-29		20.6	20.5	20.1	21.0	25.5	23.3
	J		19.7	19.0	19.4	20.1	23.9	22.9
Asn		10.4						
	EC1118		7.2	4.0	7.9	8.3	9.7	6.7
	IFI-473		3.2	3.5	5.6	6.8	7.7	6.5
	IFI-475		3.2	3.5	5.3	6.1	6.5	6.8
	P-29		4.9	6.6	10.6	10.8	11.8	6.8
	J		4.8	4.4	7.4	7.7	7.4	6.8
Ser		6.1						
	EC1118		1.4	1.6	2.5	5.4	3.6	5.4
	IFI-473		2.4	2.1	2.3	5.3	4.1	5.8
	IFI-475		2.4	1.7	1.8	5.3	3.5	5.6
	P-29		5.0	4.6	4.3	7.5	6.0	7.3
	J		2.9	4.0	4.1	7.2	4.9	6.6
Gln		8.3						
	EC1118		1.7	1.7	1.6	5.8	5.0	7.8
	IFI-473		3.6	1.8	1.6	7.4	7.1	9.4
	IFI-475		3.0	2.2	1.7	6.5	5.7	8.6
	P-29		2.8	2.3	1.8	7.1	7.9	8.2
	J		4.0	1.8	1.5	6.3	5.8	7.6
Hys		1.3						
	EC1118		5.9	6.4	6.2	2.6	2.2	1.6
	IFI-473		7.8	7.4	6.4	2.8	2.1	2.0
	IFI-475		8.4	7.1	6.5	2.4	2.7	1.5
	P-29		7.9	7.6	7.5	3.5	2.8	3.1
	J		5.8	7.4	6.7	3.2	2.9	2.2
Gly		4.3						
	EC1118		7.3	6.5	8.3	8.3	7.0	4.9
	IFI-473		8.6	8.9	7.9	10.5	9.5	5.3
	IFI-475		11.2	9.0	8.1	10.3	9.4	5.9
	P-29		12.1	12.0	12.5	13.9	13.0	7.4
	J		11.6	11.3	11.2	12.3	12.0	6.5
Thr		4.8						
	EC1118		1.7	1.5	2.4	2.6	3.9	3.2
	IFI-473		1.7	2.3	2.0	3.0	4.3	3.4
	IFI-475		1.9	2.0	2.3	3.2	3.6	3.4
	P-29		3.0	3.1	3.6	4.3	6.0	4.3
	J		2.6	3.4	3.4	4.4	4.9	3.9
Arg		15.8						
	EC1118		7.7	9.4	10.0	10.1	9.2	12.5
	IFI-473		6.3	8.2	7.2	8.7	9.6	11.4
	IFI-475		6.4	6.5	8.0	8.3	10.0	10.5
	P-29		14.2	16.3	15.6	16.1	19.4	16.9
	J		15.5	15.3	16.2	16.5	19.0	17.1
Ala		12.6						
	EC1118		9.9	11.3	12.7	12.3	11.3	12.4
	IFI-473		12.1	13.2	12.0	13.5	14.2	13.5
	IFI-475		12.7	11.9	11.9	12.6	13.1	12.8
	P-29		19.0	20.3	20.5	20.3	22.5	18.4
	J		18.2	18.8	19.9	20.3	21.1	18.5
GABA*		5.3						
	EC1118		5.2	6.1	7.7	5.6	3.2	7.5
	IFI-473		7.0	7.1	6.4	6.3	7.8	7.3
	IFI-475		8.6	7.1	7.0	6.5	7.2	7.3
	P-29		7.2	8.8	10.0	8.3	10.8	8.1
	J		6.6	6.5	8.1	7.1	8.6	7.4

(*continued on next page*)

Table 1 (*continued*)

	Strain	Time (days)					
		Base wine	Sparkling wines				
		0	20	40	90	180	270	365
Tyr		8.3						
	EC1118		7.8	7.8	9.1	9.7	8.4	10.5
	IFI-473		8.2	9.0	7.9	10.6	10.0	11.7
	IFI-475		8.1	8.0	9.3	10.3	10.3	11.4
	P-29		9.8	9.8	11.3	11.7	12.2	10.8
	J		10.5	9.2	10.6	11.1	10.7	10.5
Met		5.1						
	EC1118		0.5	0.7	1.1	1.1	1.1	2.5
	IFI-473		0.0	0.9	1.2	1.3	1.5	2.6
	IFI-475		0.9	0.8	1.0	1.1	1.4	2.5
	P-29		1.6	1.8	2.2	2.1	1.9	2.9
	J		1.1	1.4	2.0	1.9	2.2	2.7
Val		8.2						
	EC1118		4.9	5.3	6.1	6.8	6.3	7.0
	IFI-473		5.4	6.0	5.8	7.3	7.1	7.4
	IFI-475		5.2	5.1	5.2	6.6	7.5	6.8
	P-29		8.5	8.3	8.5	9.6	10.4	8.8
	J		8.4	8.5	8.1	8.6	9.7	8.5
Trp		2.6						
	EC1118		0.0	0.0	1.4	1.0	1.5	2.0
	IFI-473		0.6	0.0	1.3	0.6	1.4	1.0
	IFI-475		0.5	0.0	1.2	0.9	1.8	1.0
	P-29		0.0	0.0	1.3	1.3	1.0	0.0
	J		0.0	0.0	1.3	1.4	1.9	0.0
Phe		13.2	2.0					
	EC1118		7.2	8.0	9.1	9.8	9.2	11.0
	IFI-473		7.9	9.4	8.9	10.7	11.0	12.3
	IFI-475		8.2	8.4	8.4	9.8	10.0	11.6
	P-29		12.4	12.7	13.6	13.9	15.1	14.3
	J		11.3	10.4	12.0	12.7	12.3	14.1
Ile		6.1						
	EC1118		1.6	1.9	2.2	2.4	2.4	3.2
	IFI-473		1.6	2.0	2.1	2.6	2.6	3.4
	IFI-475		1.5	1.7	2.0	2.4	2.6	3.2
	P-29		3.9	4.1	4.2	4.5	5.0	4.9
	J		3.4	3.7	3.9	4.3	4.3	4.7
Leu		18.0						
	EC1118		6.0	7.3	8.1	9.8	9.8	10.1
	IFI-473		7.6	9.5	9.4	11.7	12.5	12.4
	IFI-475		7.1	7.8	8.4	10.5	11.9	10.8
	P-29		15.1	15.3	17.0	17.9	21.0	16.7
	J		12.7	13.2	14.9	16.1	17.3	15.3
Orn		3.2						
	EC1118		1.3	1.3	2.2	5.5	3.2	2.0
	IFI-473		3.0	2.5	1.3	6.3	4.0	3.4
	IFI-475		3.3	2.4	1.7	6.4	4.2	2.9
	P-29		2.2	1.8	2.3	6.3	2.5	1.5
	J		1.1	1.9	1.4	5.7	3.3	0.6
Lys		21.7						
	EC1118		22.4	20.0	29.1	26.9	26.7	29.3
	IFI-473		21.0	23.0	22.8	32.1	28.4	28.3
	IFI-475		27.0	21.0	22.5	30.5	32.9	27.0
	P-29		26.8	27.8	37.3	38.8	42.9	30.1
	J		27.3	27.8	34.0	35.9	39.6	29.2
Pro		271.5						
	EC1118		203.1	247.3	332.3	310.7	305.9	234.8
	IFI-473		201.0	214.6	263.7	277.7	249.6	246.3
	IFI-475		212.7	213.3	279.8	257.6	234.9	237.4
	P-29		233.2	224.8	262.1	249.5	241.9	239.0
	J		196.8	234.8	248.1	228.1	239.2	251.6
Sum		468.2						
	EC1118		318.9	366.8	480.0	466.8	457.8	404.6
	IFI-473		327.7	353.6	395.4	400.2	427.5	427.9
	IFI-475		350.7	339.4	410.8	419.7	410.8	409.6
	P-29		421.8	421.2	477.8	481.7	499.9	452.6
	J		374.3	413.4	444.5	443.2	468.7	455.7

*GABA=gamma aminobutyric acid.

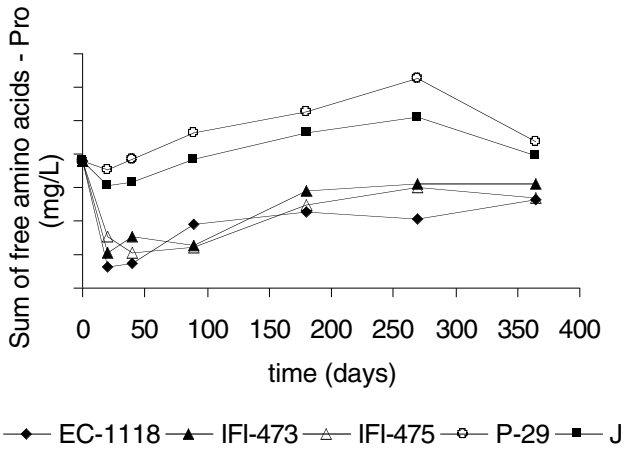

Figure 4 Sum of the free amino acids minus proline (mg/l) of the base wine (time 0) and of the sparkling wines produced with different yeast strains (*EC-1118*, *IFI-473*, *IFI-475*, *P-29* and *J*) at different aging times.

with the *EC-1118*, *IFI-473* and *IFI-475* strains were different from the wines produced with the *P-29* and *J* strains. Aging time also had an influence. The wines of 20, 40 and 90 days were different from the wines of 180 and 270 days and from the wines of 365 days, which present a very similar amino acid concentration to that of the base wine.

Effect of the yeast strain used in the tirage solution and of the aging time on the peptide content of the wines

The peptide fraction of the wine is the least known despite the fact that it makes up the majority of the nitrogen fraction [7,19,22]. There are no data in the literature either on the changes that occur in the peptide fraction during fermentation or on the influence of the yeast strain on changes that occur in aging. Therefore, the peptide fractions of the wines with molecular weight higher and lower than 700 were studied by HPLC during the second fermentation and the aging with five different yeast strains. The data that are given here are the first reported in the literature on changes that occur in fermentation under industrial conditions as well as on the influence of the yeast strain. By way of example, Figure 6 shows some of the chromatograms obtained for the wines produced with the *P-29* strain. The chromatograms for the wines obtained with other strains were similar. Both the number of peptides with the fractions of molecular weight higher (Figure 6A) and lower (Figure 6B) than 700 and their concentration increased. During aging, peptides increased and decreased. This points to the fact that yeasts autolysis is a continuous process that extends over at least several months.

Figure 7 shows the evolution of the peptide fraction of molecular weight higher (Figure 7A) and lower (Figure 7B) than 700 during the second fermentation and the aging in the presence of the yeasts in the wines produced with the five strains. Due to the absence of standards to quantify the peptides separated by HPLC, the results are expressed as a relative area. After applying analysis of variance to these data, it was verified that the relative amount of peptides was significantly influenced ($P<0.05$) by the yeast strain and the aging time.

In Figure 7A and B, it can be observed that during the second fermentation, there was an increase of peptides with molecular weight both higher and lower than 700 in all the wines. A maximum was reached in the 40-day wines, with the exception of the peptides with molecular weight higher than 700 of the wine produced with the *P-29* strain, where the maximum value was reached after 20 days. Peptide release may be due to the action of intracellular protease A of the yeasts, which develop their greatest activity during fermentation [1]. This produces peptides that enter the extracellular space, i.e., the wine. After 40 days, a decrease in peptide content was also observed in all the wines. Proteins (Figure 3) also decreased in this stage, while amino acids increased (Figure 4 and Table 1). The fact that these changes occurred when there were still viable cells (Figure 1) suggests that there is a proteasic and peptidasic exocellular activity by viable cells, which lack nutrients [15].

Between 90 and 180 days, the changes that took place in the peptide fraction were somewhat different between some of the wines. While the concentrations of peptides with molecular weight higher than 700 after 180 days in the wines produced with the *EC-1118*, *IFI-473* and *IFI-475* strains were about the same as after 90 days of aging with yeasts, the concentration was higher in wines produced with the *P-29* and *J* strains. The content of peptides with a

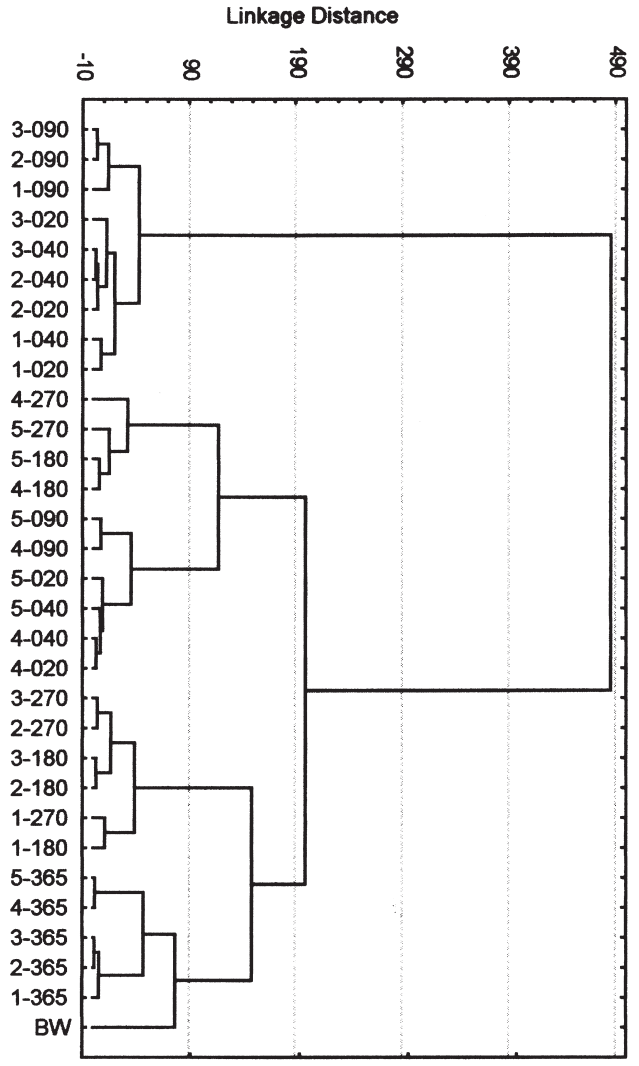

Figure 5 Dendrogram resulting from applying cluster analysis to the free amino acid data of base wine (BW) and of the sparkling wines (yeast strain-days) produced with different yeast strains (Table 1) at different aging times. (1) Strain *EC-1118*; (2) strain *IFI-473*; (3) strain *IFI-475*; (4) strain *P-29*; (5) strain *J*; 0–365 days.

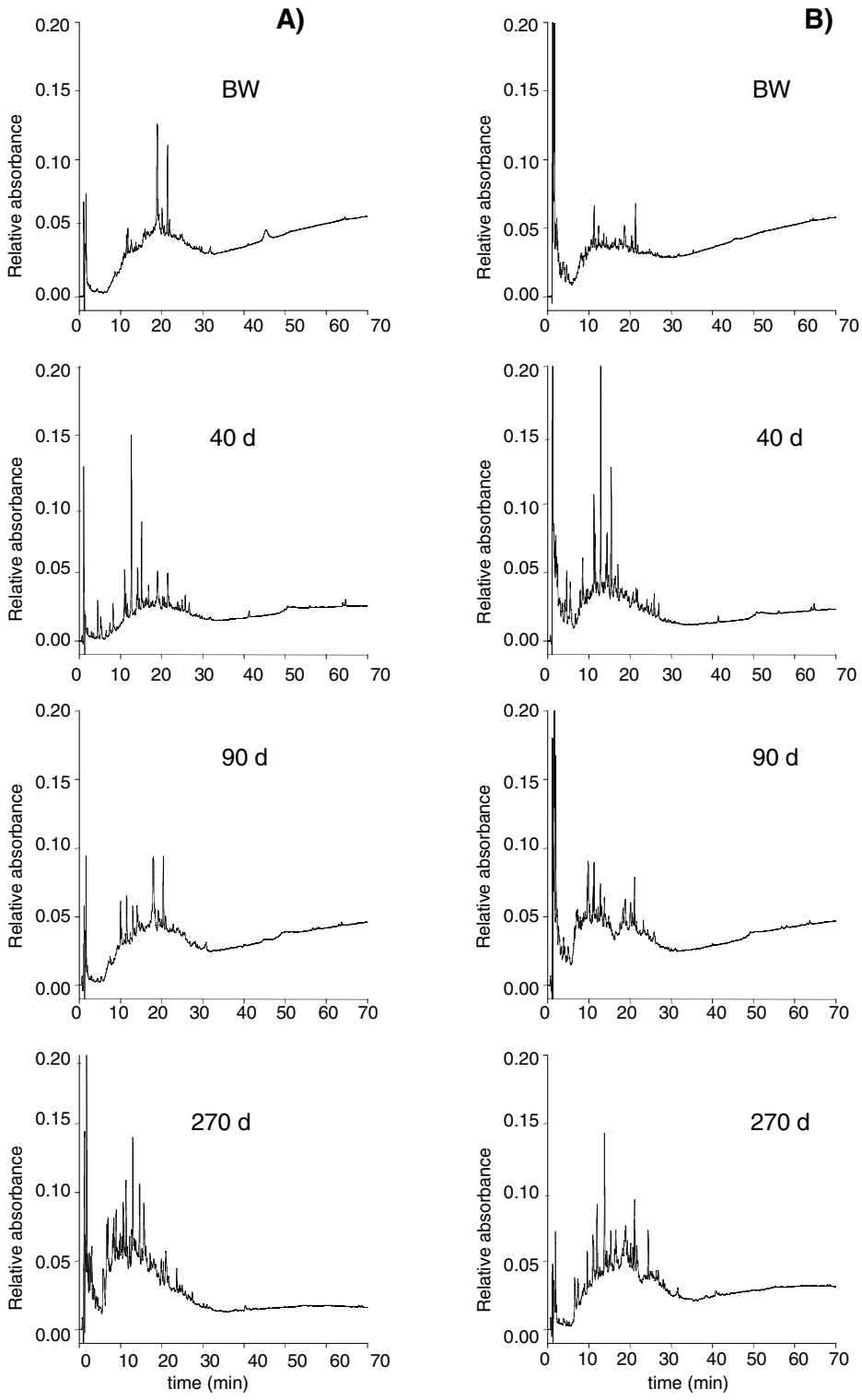

Figure 6 HPLC profiles of peptides with molecular weight higher (A) and lower (B) than 700, respectively, of the base wine and the sparkling wines produced with the *P-29* strain at different aging times.

molecular weight lower than 700 was lower after 180 days than after 90 days in the wines produced with the *EC-1118*, *IFI-473* and *P-29* strains and higher in the wines produced with the *IFI-475* and *J* strains. This decrease coincided with the protein decrease (Figure 3) and the amino acids increase (Figure 4 and Table 1). Between 180 and 270 days, the peptide content of all the wines increased again. Proteins (Figure 3) and free amino acids of the wines (Figure 4) also increased. Between 270 and 365 days, all the peptides with molecular weight higher than 700 increased in all wines, except those produced with the *P-29* strain, and all peptides

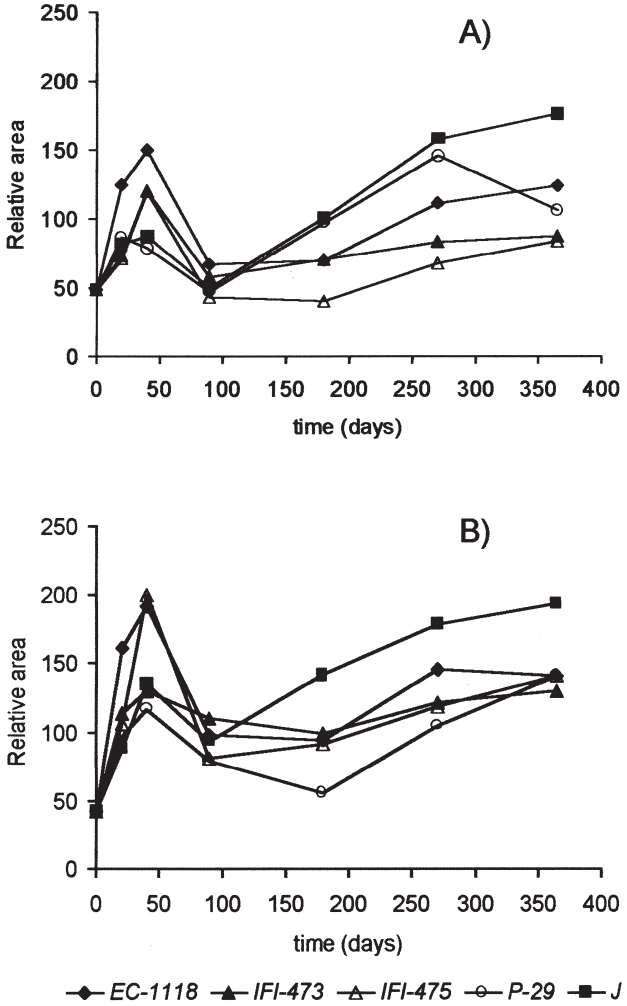

Figure 7 Relative area of the peptides with molecular weight higher (A) and lower (B) than 700, separated by HPLC, of the base wine (0 time) and of the sparkling wines produced with different yeast strains at different sampling times.

with molecular weight lower than 700 increased, except those of the wine produced with the *EC-1118* strain.

The differences found in the peptide fractions as well as in free amino acids among the wines produced with the different strains reveal that the speed at which autolysis takes place varies from one yeast strain to the next.

Conclusions

From the study of cellular viability and nitrogen composition data of wines produced with different yeast strains, it can be deduced that the changes that take place in the industrial production process of sparkling wines by the traditional method occur in at least four clearly differentiated stages. In the first stage, approximately between 0 and 40 days after *tirage*, a second fermentation takes place. Amino acids and proteins diminish and peptides are liberated. In the second stage, approximately between 40 and 90 days, viable and dead cells coexist. There is a release of nitrogen compounds used as nutrients for the viable cells. There is an intra- and extracellular protease activity and proteins are degraded to peptides, which in turn are converted to amino acids. In the third stage, approximately between 90 and 270 days after *tirage*, no viable cells are detected. Enzymatic action continues and the release of proteins and peptides predominates. In the fourth stage, after approximately 270 days of aging, the amino acids of some wines decrease.

The yeast strain influences the content of free amino acids and peptides and the aging time for all nitrogen fractions.

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