JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Accelerated Autolysis of Yeast on the Composition and Foaming Properties of Sparkling Wines Elaborated by a Champenoise Method

YOLANDA P. NUNEZ, ALFONSO V. CARRASCOSA, RAMÓN GONZÁLEZ, MARÍA C. POLO, AND ADOLFO J. MARTÍNEZ-RODRÍGUEZ*

Instituto de Fermentaciones Industriales, Consejo Superior de Investigaciones Científicas, C/Juan de la Cierva 3, 28006 Madrid, Spain

Five mutants (obtained by UV mutagenesis) and the parent strain were selected to produce sparkling wines following the traditional or champenoise method. The wines were aged with the yeast for 9 months, with samples being taken each month for analytical and sensory determinations. The wines elaborated with mutant strain IFI473I demonstrated an accelerated release of protein, amino acids, and polysaccharides. An analysis of the secreted polysaccharides revealed that mannose was the major sugar present. The effects of the products released by yeasts on the foaming properties of the wines were determined by both sensory and instrumental analysis. In all cases, the wines elaborated with mutant strain IFI473I showed improved foaming properties as compared to wines fermented without this strain. Similar results were obtained at a decreased aging time of 6 months, thereby confirming the capacity of IFI473I strain to carry out an accelerated autolysis. These results demonstrate that mutant strain IFI473I can significantly reduce production times of high-quality sparkling wines.

KEYWORDS: Sparkling wines; foaming properties; yeast autolysis; autolytic mutant; sensory analysis

INTRODUCTION

In previous works, authors have stated that the yeast strain used to elaborate sparkling wines following the champenoise method could play an important role in the final quality of the wines (*1*, *2*). The autolysis process takes place during the aging with yeast, thereby releasing certain intracellular yeast compounds that modify the chemical composition and sensory properties of the wines (*3*, *4*). Autolysis is a process lasting from a few months to years (*5*, *6*), depending on various variables such as temperature, nutrient availability, grape variety, or yeast strain used. Thus, accelerating this process is highly desirable for wine producers as production costs can be significantly reduced.

Until now, various ways of accelerating yeast autolysis effects have been examined. Increasing the aging temperature (*7*), addition of yeast autolysate (*8*), or the use of mixed cultures containing killer resistant and sensitive yeast strains (*1*) have been used both successfully and less successfully, sometimes giving contradictory results. Besides this, some authors have reported good results adding yeast autolysates (*9*), while others considered that this procedure negatively affects the organoleptic properties of the wines (*10*). In other cases, as in the use of mixed cultures containing killer yeast, some promising results have been obtained but they correspond only to preliminary experiments carried out at laboratory scale (*1*).

* Corresponding author: fax (34) 91 564 48 53; phone (34) 91 562 29 00, ext. 216; e-mail amartinez@ifi.csic.es.

An interesting strategy for obtaining yeast strains carrying out accelerated autolysis could be by genetic modification of the yeast. This procedure could improve the final quality of the product without having to modify the elaboration process and avoiding sensory problems resulting from autolysate addition and increasing the aging temperature. Among the strategies that could be used to obtain genetically modified yeast are random mutagenesis and genetic engineering (*11*). Genetic engineering poses important advantages, such as the possibility to incorporate foreign genes with a high precision level (*12*). However, its application is restricted to simple and genetically well characterized traits. In addition, products obtained by genetic engineering are controlled through strict legislation (*13*) and may have poor consumer acceptance. For mutants obtained by random mutagenesis, it is not mandatory to possess extensive knowledge about the system in which the modification will be carried out. Mutagenesis methods are simple and the general acceptance, regulation, and commercial possibilities of products containing these mutants are higher than for genetically engineering products.

González et al. (*14*) obtained autolytic mutants from the industrial second fermentation strain *Saccharomyces cerevisiae* IFI473 using UV mutagenesis. The most promising strain (IFI473I) presented the highest release of nitrogen compounds in a model system at low temperatures. The main objective of this work is to study the behavior of this strain by a practical approach following the industrial elaboration protocol. Other similar mutants will also be investigated.

10.1021/jf050191v CCC: $30.25
Published on Web 08/06/2005

MATERIALS AND METHODS

Winemaking and Experimental Design. This investigation consisted of two experimental elaborations in which sparkling wines were prepared from one monovarietal base wine (Parellada white grape variety). For the first elaboration, six different wines were prepared, from six different strains. The base wine containing sucrose was inoculated with yeast (approximately 8% of inoculum). During the secondary fermentation in bottles and subsequent 9 months aging, samples were taken at 1, 3, 6, and 9 months. To carry out the analytical determinations, six bottles at each sampling point were mixed and homogenized. All the analyses were conducted in duplicate on wines that had been centrifuged for 10 min at 7600*g*.

For the second elaboration the same protocol was followed, but only three different wines were prepared (from parental strain IFI473 and mutants IFI473I and IFI473K). Furthermore, wines were aged with the yeast for 6 months instead of 9 months.

Yeast Strains and Culture Conditions. *Saccharomyces cerevisiae* IFI473, a yeast strain previously selected as starter for a second fermentation of sparkling wines (*15*), and the mutants strains derived from it, IFI473E, IFI473G, IFI473I, IFI473J and IFI473K (*14*), were used to inoculate the wines for conducting the second fermentation. The procedure to obtain the mutant strains from IFI473 has been extensively explained by González et al. (*14*) but can be summarized as follows: Cells from an overnight culture, grown in YPD (yeast extract 2%, peptone 4%, and dextrose 4%) at 30 °C and 150 rpm, were plated on YPD agar (YPD containing 2% agar) to give approximately 500 cells/plate. Plates were irradiated with a G8T5 germicidal UV tube, obtaining a survival rate of about 50%, and were then incubated at 20 °C for 48−72 h. The selection method for autolytic mutants is based on the release of active alkaline phosphatase into the medium. Yeast colonies were replica-plated on YPD or YPD supplemented with BCIP (5-bromo-4-chloro-3-indolyl phosphate, 40 mg/L), incubated at 20 °C for 1 day, and transferred to permissive (20 °C) or restrictive (37 °C) temperatures. Autolytic mutants stained blue after 18−24 h at 37 °C.

Cultures were prepared by the traditional protocol: a portion of an overnight yeast culture was inoculated into sterile diluted (1:5) must. This culture was incubated at 25 °C for 48 h until a cell concentration around $10^{8-}10^{9}$ cfu/mL was obtained. The culture was then added to a medium having the following composition: sucrose (200 g), yeast extract (0.8 g), tartaric acid (5 g), and base wine (1000 mL) (final volume was 3000 mL). This medium was incubated at 20 °C for 48 h. The yeasts obtained were inoculated in base wine containing sucrose until a density of 0.994−0.998 g/cm^3 was achieved (around 24 h of incubation at 20 °C). This procedure was used to prepare the inocula for each strain.

Viability Assay. Serial 10-fold dilutions of the samples employing saline solution were plated on YPD agar (YPD containing 2% agar). The number of colony-forming units (cfu) was assessed after plates had been incubated at 20° C for 1−2 days.

Analytical Determinations: *Nitrogen Compounds.* The concentration of soluble protein and polypeptides was determined following the Bradford method (*16*), based on the reaction of the protein with Coomassie blue G-250. Absorbance was determined at 595 nm 15 min after the addition of the reactant. The results were expressed in milligrams of bovine serum albumin (BSA)/liter.

Free amino acids were determined by the method of Doi et al. (*17*), based on the reaction of ninhydrin/cadmium with the amino group. Absorbance was determined at 507 nm. The results were expressed in milligrams of leucine/liter.

Isolation and Hydrolysis of Polysaccharides. The procedure described by Segarra et al. (*18*) was used for the isolation of polysaccharides. Ethanol (5 mL; 96% v/v) and HCl (50 μL, 1 N) were added to 1 mL of wine. After 18 h of incubation at 22 °C, the tubes were centrifuged (1800*g*, 20 min), after which the supernatant was discarded and the pellet was washed three times in ethanol (96% v/v). Samples obtained were hydrolyzed at 100 °C for 48 h in a closed vial containing 1 mL of 30% formic acid and 0.5 mL of *myo*-inositol (0.1% w/v; internal standard) solution (*19*). After hydrolysis, the mixture was evaporated to dryness under vacuum.

Silylation of Neutral Monosaccharides and Chromatographic Analysis. The dried hydrolyzed residue was silylated following the procedure of Troyano et al. (*20*). The sample was dissolved in 100 μL of anhydrous pyridine, and 100 μL of trimethylsilylimidazole, 100 μL of trimethylchlorosilane, 100 μL of *n*-hexane, and 200 μL of deionized water were sequentially added, with shaking during each step. The silylated derivatives present in the organic phase were immediately injected in the GC. Trimethyilsilyl derivatives were analyzed on a Hewlett-Packard 6890 chromatograph, equipped with a flame ionization detector (FID) and split/splitless injector. Samples were injected on a Carbowax 20M column (30 m × 0.25 mm) coated with a stationary phase of 0.25 μm thickness. Temperatures were as follows: injector and detector, 220 °C; oven, held at 40 °C for 10 min, then increasing 7 °C/min to 150 °C, and finally programmed at 30 °C/min to 210 °C. The carrier gas was helium (12.5 psi, split 1/15). Response factors were calculated with a series of pure standards at different concentrations with *myo*-inositol as internal standard. The response factors (RF) and the relative standard deviation (RSD) of the standard sugars relative to the *myo*-inositol were the following: arabinose (RF 1.87, RSD 2.69%), mannose (RF 1.4, RSD 1.17%), galactose (RF 1.25, RSD 2.14%), and glucose (RF 1.35, RSD 1.5%). The identification of the compounds present in the samples was carried out by comparing the retention time of the peaks with those of pure standards. When two peaks of different carbohydrates overlapped, the percentages of the individual carbohydrates were calculated from the area of the well-resolved anomer.

Analysis of the Foaming Properties. The foaming properties of the wines were analyzed by both sensory and instrumental analysis.

Instrumental Analysis of Foaming Properties. For the analysis of the foaming properties, an apparatus developed at the Instituto de Fermentaciones Industriales (IFI) was used to determine the height increase occurring in a liquid when air is passed through it. The change in the height of the liquid was quantified by an ultrasound wave emitter−detector following the methodology described by Moreno-Arribas et al. (*21*). Three parameters were measured:

(a) H_{peak} is the maximum height reached by the foam after air injection through a glass frit. H_{peak} has been related to the wine's ability for foaming.

(b) H_{plato} is the foam height stability during air injection. This has been related to the average bubble lifetime.

(c) Sd_{300} is the standard deviation of foam measures in the last 300 points. This has been related to the effervescence of the wine.

The relative standard deviations ($n = 6$) for the parameters studied were 6.1% for H_{peak} values, 3.6% for H_{plato}, and 2.9% for Sd_{300}.

Sensory Evaluation: *Sensory Analysis.* An expert panel consisting of 10 judges with extensive experience in tasting sparkling wines and their descriptive analysis conducted sensory analysis of the wines. The tasting cards used are among those recommended by the Office International de la Vigne et du Vin (OIV) for international wine competitions (*22*). The scores used establish that better quality wines receive a higher score. The different parameters carry different weights. Visual aspect carries a weight of 1 for limpidity (scale from 1 to 5), while for color and effervescence the weight is 2 (scale from 2 to 10). Intensity and genuineness of nose and taste also carries a weight of 1, but the scale is from 3 to 7. The same weight and scale is applied to taste persistence. Quality (nose and taste) carries a weight of 2 with a scale from 6 to 14. Finally, the overall judgment carries a scale from 8 to 12. The wines were tested individually and not comparatively. For the first elaboration, six wines in two different session were tested and the sensory analysis was developed on wines aged for 9 months. In the second elaboration were analyzed three wines after 6 months of aging.

Descriptive Analysis of Foaming Properties. Descriptive analysis of foam quality and effervescence was carried out for the wines of the second elaboration by the protocol described by Obiols et al. (*23*) and Martinez-Rodriguez and Polo (*24*). The attributes assessed and the characteristics of the descriptors used can be summarized as follows: initial foam formed (abundant, normal, poor); foam collar (total, partial, none); foam area (total, partial, none); bubble size (small, medium, large); and effervescence speed (fast, medium, slow). Foam and effervescence are considered to be of the best quality when abundant initial foam is formed, a total foam collar covers all the surface area, the bubbles are small, and effervescence is fast. The mode of the scores

Table 1. Changes in Yeast Viability in Wines during the Second Fermentation and Aging

		log cfu/mL[a]			
yeast strains	t_0[b]	1 month	3 months	6 months	9 months
IFI473	6.6	7.6	4.2	<10	<10
IFI473E	6.5	7.3	4.0	<10	<10
IFI473G	6.3	7.4	2.9	<10	<10
IFI473I	6.5	7.3	3.5	<10	<10
IFI473J	6.3	7.6	3.9	<10	<10
IFI473K	6.5	7.7	2.8	<10	<10

[a] Data are the average of triplicates of log cfu/mL. [b] t_0: viable cells after wine inoculation.

given by the 10 tasters was used to show the final score for each parameter corresponding to the foam characteristics.

Statistical Methods. The statistical method used for data analysis was a two- way analysis of variance (ANOVA) to test the effect of two factors studied (yeast strain and aging time), considering the main effects to analyze the first-order (noninteractive) effects on two categorical independent variables (factors). To process the data from sensory analysis, an ANOVA was used (two-factor design without interaction, repeated measures) for each judge and parameter analyzed. Data processing was conducted with statistical software (SPSS, version 9.0 for Windows) (*25*).

RESULTS

Behavior of Yeast Viability. Table 1 shows the results for yeast viability during the second fermentation and aging. During the first month an increase in yeast population (around 1 log) was observed in all wines, independently of the yeast strain used. Although it was possible to recover viable yeast from all wines after 3 months of aging, significant differences ($p < 0.05$) were found between the different yeast strains. IFI473, the parent strain, showed the highest viability at this point (1.6×10^4 cfu/mL), while mutants strains showed lower viability, especially strains IFI473G and IFI473K, where only 7.9×10^2 and 6.3×10^2 cfu/mL were recovered, respectively. This variability could be due to different growth kinetics observed for various strains used in this experiment, as also has been reported by Gonzalez et al. (*14*). From 6 months of aging onward, no viable cells were recovered from any wine.

Changes in Nitrogen Compound. Figure 1a represents protein and polypeptide concentrations during the second fermentation and aging. The pattern observed for the first 3 months shows an increase of protein and polypeptide content during fermentation in all wines. This is followed by a decrease attributed to the rise of alcohol concentration of the wines (*26*) and the protease activity (*27*). Samples taken at 6 months of aging demonstrated an increase in protein and polypeptide content in the wines elaborated with IFI473I strain, while for the rest of the wines, a decrease (or a slight increase for IFI473E strain) was seen. After 9 months of aging, protein and polypeptide concentrations increased again in all wines. Similar behavior was observed in the second experiment. Protein and polypeptide changes observed in the wines during aging were significantly correlated ($p < 0.05$) with the time of aging but not with the yeast strain used.

Figure 1b shows the variation in amino acid content of the wines during the second fermentation and aging. During the first month of aging, a decrease in amino acid content was observed, caused by the amino acid consumption of the yeast. This period was followed by a slight increase in concentration. This is related to a physiological process called exorption (*28*) in which amino acids are passively released into the wine. After

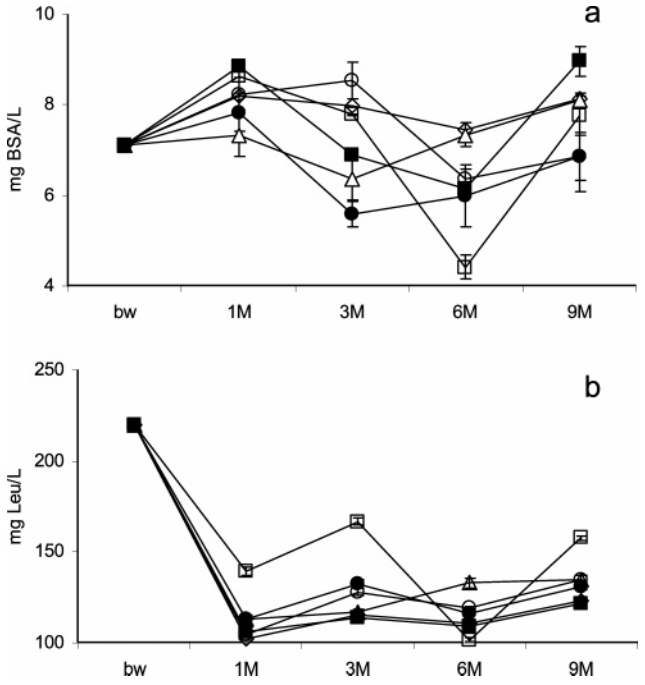

Figure 1. (**a**) Protein (milligrams of BSA/liter) and (**b**) amino acids (milligrams of Leu/liter) in the base wine (bw) and sparkling wines after 1, 3, 6, and 9 months of aging. Parent strain (◇), IFI473E (●), IFI473G (○), IFI473I (△), IFI473J (□) e IFI473K (■).

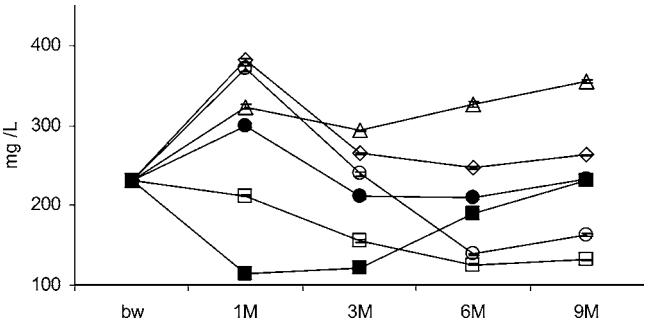

Figure 2. Polysaccharide content (milligrams/liter) in the base wine (bw) and sparkling wines after 1, 3, 6, and 9 months of aging. Parent strain (◇), IFI473E (●), IFI473G (○), IFI473I (△), IFI473J (□) e IFI473K (■).

6 months of aging, the wine elaborated with IFI473I showed an increase in the amino acid concentration, while others showed a reduction. This result indicated the presence of accelerated autolysis by this strain throughout the early release of amino acids into the wine. After 9 months of aging, a somewhat similar pattern was seen for all wines, except for the wine elaborated with strain IFI473J, which presented the highest amino acid content. The general analysis of variance indicated that there were significant differences ($p < 0.05$) between the free amino acids contents in terms of aging time and the yeast strains used.

Analysis of the Polysaccharide Content. Figure 2 demonstrates the polysaccharide concentration over aging time. The polysaccharide content increases during the fermentation (in the first month) in the majority of wines, except for the ones elaborated with mutant strains IFI473J and IFI473K. The latter can be explained by different growth kinetics and fermentation power as compared to the parental strain that has been reported by González et al. (*14*). Although some mutant strains presented delayed fermentation power in a model wine, this fact does not eliminate their ability to perform the second fermentation process. After 3 months of aging, polysaccharide concentrations decreased in all wines except for the ones elaborated with

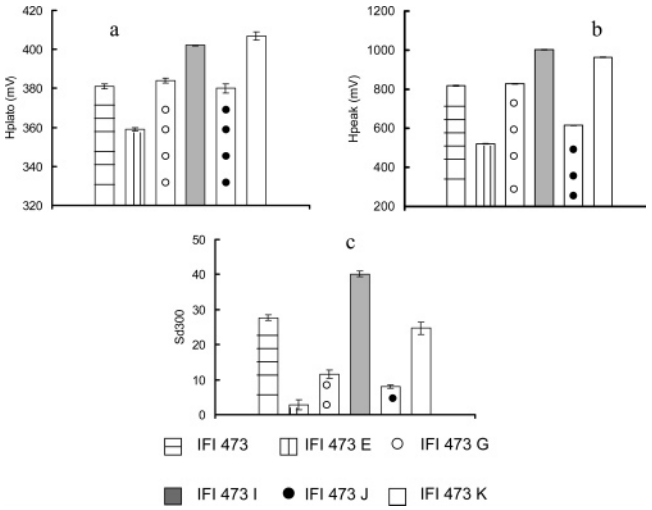

Figure 3. Behavior of the foam properties from the wines aged for 9 months. The parameters represented are H_{plato} (a), H_{peak} (b), and Sd_{300} (c).

Table 2. Mannose Composition of Wine Polysaccharides after 6 and 9 Months of Aging

	mannose concn[a] (mg/L)	
yeast strains	6 months of aging	9 months of aging
IFI473	100.7 (1.15)	102.6 (2.46)
IFI473E	79.2 (0.60)	94.2 (1.22)
IFI473G	52.8 (3.12)	62.7 (2.23)
IFI473I	126.4 (1.56)	136.6 (1.49)
IFI473J	52 (1.22)	52 (3.55)
IFI473K	70.5 (1.67)	96.1 (1.76)

[a] Data are the average of triplicate analyses; relative standard deviation is listed in parentheses.

IFI473K. This can be explained by the hydrolysis and consumption of polysaccharides by viable yeast during the early stages of aging, as previously reported by Moreno-Arribas et al. (*21*), Llauberes et al. (*29*), and Colagrande and Ottina (*30*). From 6 months of aging, a significant increase in polysaccharide concentration is observed in wines elaborated with strain IFI473I. This increase continued until 9 months of aging. Similar results were obtained in the second experiment, for wines aged 6 months. A similar pattern is observed for the wine elaborated with strain IFI473K, although the final polysaccharide concentration is lower as compared to strain IFI473I. Trends in polysaccharide concentration were similar for all other wines. The analysis of variance indicated there were significant differences ($p < 0.05$) between the polysaccharide content in terms of aging time and type of yeast strain.

The sugars identified in the wines were glucose, mannose, arabinose, and galactose, with mannose being the main sugar present (around 40% of the total sugar presented in the polysaccharides). The rest of the sugars were found in the following proportions: galactose and glucose, around 25%; and arabinose, 10%. **Table 2** presents the mannose concentrations found in each wine after 6 and 9 months of aging. For both aging times, wines elaborated with strain IFI473I yielded the highest mannose concentration.

Instrumental Analysis of the Foaming Properties. Figure 3 presents the behavior of foaming properties from wines aged 9 months. The parameters shown are H_{plato} (a), H_{peak} (b), and Sd_{300} (c). Wines elaborated with strains IFI473I and IFI473K gave higher values as compared to the other wines for all parameters analyzed. The high scores for H_{peak} and H_{plato} have been associated with high-quality foams (*21*, *24*). The Sd_{300} value (40.2) belonging to the wine elaborated with strain IFI473I was approximately 1.5 times higher than the Sd_{300} value (27.7) of the wine produced with strain IFI473, placed in second position. The variable sd300 has been related to wine effervescence (*31*). We had previously found that sparkling wines with good effervescence (evaluated for sensory analysis) present higher values for Sd_{300} (39.45 ± 3.06) than wines with poor effervescence (7.01 ± 1.23). It can therefore be concluded that the wine elaborated with strain IFI473I demonstrated the best effervescence. The second experiment (developed with yeast strains IFI473, IFI473I, and IFI473K and aged for 6 months) gave similar results.

Sensory Evaluation. A sensory analysis of sparkling wines aged 9 months was developed. The scoring system used gives higher scores for superior wines. The color for all the wines analyzed was considered as characteristic, and they presented the traditional fragrance and flavor with good intensity and character. The judges found that the overall quality (visual perceptions, olfactory sensations, and taste) was good for all wines and gave them a similar score (around 80 points from a maximum of 100) (data not shown). This was also the case for the wines of the second experiment aged for 6 months.

Descriptive Analysis of Foam Quality and Effervescence. Table 3 shows the foam quality and effervescence results for wines of the second experiment (aged 6 months). The judges found differences between the wines produced with strain IFI473I and wines produced with other strains. They described the foam produced for the IFI473I wine as normal but totally covering the wine surface area. However, for all other wines investigated, the surface was only partially covered with bubbles. The judges also considered the size of the bubble of the IFI473I wine as small, while the bubble size of the IFI473 and IFI473K wines were regarded as medium or medium-large, respectively. Small bubbles, which often remain longer at the wine's surface, indicate high-quality wines (*24*). The judges evaluated the effervescence of the wines between medium and fast and gave the highest score to the wine elaborated with strain IFI473I.

DISCUSSION

The possibility of using autolytic mutants obtained by UV mutagenesis to elaborate sparkling wines was first reported by González et al. (*14*). These authors suggested that strain IFI473I showed the strongest temperature-sensitive and autolytic phenotype and therefore appeared to be the most promising strain to use for sparkling wine production. In this work, we conducted

Table 3. Results[a] Obtained from the Descriptive Analysis of Foam Quality and Effervescence of Sparkling Wines Aged for 6 Months

wines	initial foam formed	foam area	foam collar	bubble size	effervescence
IFI473	normal	partial	partial	medium	medium
IFI473I	normal	total	total	small	medium-fast
IFI473K	normal	partial–total	partial–total	medium-large	fast

[a] Values represent the mode of the scores given by the 10 tasters.

two different long-term storage trials of sparkling wines. During the first experiment, the wines were aged with strain IFI473 and five mutant strains for 9 months. This is the minimum time that sparkling wines must be in contact with yeast to be awarded the Designation of Quality cava, the highest quality sparkling wine produced in Spain.

Others authors have pointed out that cellular death is essential to trigger the autolysis process (*9*, *32*). In our experiment, no viable cells were detected after 6 months of aging, thereby indicating that the autolysis process could have started between 3 and 6 months. From 6 months of aging an increase in both nitrogen compounds (proteins, polypeptides, and amino acids) and polysaccharides was found in the IFI473I wine. In the other wines this rise was not detected until 9 months of aging (except for the IFI473K wine, which also demonstrated an increase in this period). Mannose was the main sugar present in the polysaccharides released by the yeast. This sugar is one of the main constituents of the yeast cell wall. An increase of polysaccharides rich in mannose is a result of the fermentation and/or aging process indicating the occurrence of yeast autolysis, as reported by Doco et al. (*33*) and others. In this study, the highest concentration of mannose was detected in the IFI473I wine. Our results suggest that the IFI473I strain possesses a great ability to accelerate autolysis, which was expressed in the fastest release of intracellular compounds into the wine.

An instrumental analysis of the foaming properties was conducted to elucidate whether the released compounds could affect the properties of the wines. Some authors reported elsewhere that the products released by the yeast during autolysis could modify the organoleptic properties of the wines, among them, foam properties (*19*, *21*, *34*). The latter are the most characteristic features observed in sparkling wines by consumers (*35*). In these experiments, we had obtained the best results for the parameters analyzed for the IFI473I wine. In other works carried out in our group (*24*), we had found similar behavior for wines with high-quality foam. However, no significant differences were detected in the general sensory analysis as carried out twice by an expert panel of judges. This can be explained by two factors. First, the products released by IFI473I during accelerated autolysis did not cause sensory changes detectable by the judges. Second, the method used for sensory analysis was not appropriate to detect the differences observed by the instrumental analysis. In other works, Martinez- Rodriguez et al. (*24*, *31*) had found that many tasting cards are not completely satisfactory since the descriptors on the card are mainly focused on other aspects such as aroma and taste.

Therefore, we decided to carry out another experimental elaboration with the parent strain and the mutants with the best behavior in the first experiment (IFI473I and IFI473K). With the results of the first elaboration taken into account, it was decided to age the wines for 6 months instead of 9 months. Additionally, we included a descriptive analysis of the foam quality and effervescence in the sensory evaluation, which had previously been used (*23*, *24*). The analytical results obtained were very similar to those obtained in the first elaboration and confirmed that strain IFI473I was capable of accelerating autolysis. Also, the instrumental analysis of foam properties gave better results for the IFI473I wine, but the general sensory analysis showed similar results for all the wines. However, the descriptive analysis of foaming properties indicated clearly that the wine elaborated with IFI473I strain presented the best foam quality.

This result does not ignore the first general sensory analysis for sparkling wines but clearly demonstrates that it is not sufficient to study the foaming properties of sparkling wines. Accelerated autolysis compounds released into the wine for IFI473I mutant modified the sensory properties, thereby having a positive effect on the foaming properties of the wine. The relationship between different compounds released by yeast during autolysis and the foaming properties of the wines has been fully documented in the literature. In this work, it is confirmed that IFI473I strain can be very effective to elaborate sparkling wines by the champenoise method. Quality wines with improved foam properties were obtained in a short time (6 months) by use of a yeast strain carrying out accelerated autolysis. This result is regarded as very important for producers that intend to reduce production times to improve economic benefits. In addition, the method of obtaining mutants (induced mutagenesis) is regarded as advantageous over genetic engineering because it is simple and more widely accepted than recombinant yeast (*14*). Although the mutation or mutations produced here are unknown, we can speculate that similar phenotypes have been reported for genes involved in the cell integrity−signaling pathway (*36*), indicating that mutations in this group of genes could be involved in the phenotype obtained. The characterization of the gene or genes involved in the expression of this autolytic phenotype could be very interesting in the genetic engineering experiments designed to obtain autolytic yeast strains to be used in enology.

ACKNOWLEDGMENT

We are grateful to J. M. Barcenilla for technical assistance.

LITERATURE CITED

(1) Todd, B. E. N.; Fleet, G. H.; Henschke, P. A. Promotion of autolysis through the interaction of killer and sensitive yeasts: potential application in sparkling wine production. *Am. J. Enol. Vitic.* **2000**, *51*, 65−72.

(2) Martínez-Rodríguez, A.; Carrascosa, A. V.; Martin-Alvarez, P. J.; Moreno-Arribas, V.; Polo, M. C. Influence of the yeast strain on the changes of the amino acids, peptides and proteins during sparkling wine production by the traditional method. *J. Ind. Microbiol. Biotechnol.* **2002**, *29*, 314−322.

(3) Martínez-Rodríguez, A.; Polo M. C. Characterization of the nitrogen compounds released during yeast autolysis in a model wine system. *J. Agric. Food Chem.* **2000**, *48*, 1081−1085.

(4) Escot, S.; Feuillat, M.; Dulau, L.; Charpentier, C. Release of polysaccharides by yeast and the influence of released polysaccharides on colour stability and wine astringency. *Aust. J. Grape Wine Res.* **2001**, *7*, 153−159.

(5) Leroy, M. J.; Charpentier, M.; Duteurtre, B.; Feuillat, M.; Charpentier, C. Yeast autolysis during champagne aging. *Am. J. Enol. Vitic.* **1990**, *41*, 21−28.

(6) Andrés-Lacueva, C.; Lamuela-Raventós, R. M.; Buxaderas, S.; De la Torre-Boronat, M. C. Influence of variety and aging on foaming properties of cava (sparkling wine) 2. *J. Agric. Food Chem.* **1997**, *45*, 2520−2525.

(7) Feuillat, M.; Charpentier, C. Autolysis of yeast in champagne. *Am. J. Enol. Vitic.* **1982**, *3*, 6−13.

(8) Feuillat, M. Préparation d'autolysats de levures et addition dans les vins effervescents élaborés selon la méthode champenoise. *Rev. Fr. Oenol.* **1987**, *109*, 17−27.

(9) Charpentier, C.; Feuillat, M. Yeast autolysis. In *Wine Microbiology and Biotechnology*; Fleet, G. H., Ed.; Harwood Academic Publishers: Lausanne, Switzerland, 1993; pp 225−242.

(10) Peppler, H. J. Yeast extracts. In *Economic Microbiology, Volume 7, Fermented Foods*; Rose, A. H., Ed.; Academic Press: London, 1982; pp 293−312.

(11) Pretorius, I. S. Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. *Yeast* **2000**, *16* (8), 675−729.

(12) Ramon, D.; González-Candelas, l.; Pérez-González, J. A.; González, R.; Ventura, l.; Sánchez-Torres, P.; Vallés, S.; Piñaga, F.; Gallego, M. V.; Férnandez-Espinar, M. T.; Querol, A. Aplicaciones de la biología molecular en enología. *Microbiología (Madrid)* **1995**, 67−74.

(13) European Commission, Regulation (EC) 1830/2003 of the European Parliament and of the Council, 22 September 2003. *Off. J. Eur. Union* **2003**, *L268*, 24.

(14) González, R.; Martínez-Rodríguez, A. J.; Carrascosa, A. V. Yeast autolytic mutants potentially useful for sparkling wine production. *Int. J. Food Microbiol.* **2003**, *84*, 21−26.

(15) Martínez-Rodríguez, A.; Carrascosa, A. V.; Barcenilla, J. M.; Pozo-Bayón, M. A.; Polo, M. C. Autolytic capacity and foam analysis as additional criteria for the selection of yeast strains for sparkling wine production. *Food Microbiol*. **2001**, *18*, 183−191.

(16) Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein−dye binding. *Anal. Biochem*. **1976**, *72*, 248−255.

(17) Doi, E.; Shibata, D.; Matoba, T. Modified colorimetric ninhydrin methods for peptidase assay. *Anal. Biochem*. **1981**, *118*, 173−184.

(18) Segarra, I.; Lao, C.; López-Tamames, E.; Torre-Boronat de la, M. C. Spectrophotometric methods for the analysis of polysaccharides levels in winemaking products. *Am. J. Enol. Vitic*. **1995**, *46*, 564−570.

(19) Pueyo, E.; Olano, A.; Polo, M. C. Neutral monosaccharides composition of the polysaccharides from must, wines and cava wines. *Rev. Esp. Cienc. Tecnol. Aliment.* **1995**, *35*, 191−201.

(20) Troyano, E.; Olano, A.; Fernandez-Diaz, M. Gas-chromatographic analysis of free monosaccharides in milk. *Chromatographia* **1991**, *32*, 379−382.

(21) Moreno-Arribas, V.; Pueyo, E.; Nieto, F. J.; Martín-Álvarez, P. J.; Polo, M. C. Influence of the polysaccharides and the nitrogen compounds on foaming properties of sparkling wines. *Food Chem.* **2000**, *70*, 309−317.

(22) Office International de la Vigne et du Vin: Resolution ENO 2/94. OIV standard for the international wine competitions. *Bull. O.I.V*. **1994**, *67*, 551−597.

(23) Obiols, J. M.; De La Presa-Owens, C.; Buxaderas, S.; Bori, J. L.; De La Torre-Boronat, C. Protocolo de evaluación de la formación de la efervecencia y espuma en un vino espumoso. *ACE Rev. Enol.* **1998**, *44*, 3−8.

(24) Martínez-Rodríguez, A.; Polo, M. C. Effect of the addition of bentonite to the tirage solution on the nitrogen composition and sensory quality of sparkling wines. *Food Chem*. **2003**, *81*, 383−388.

(25) SPSS Inc. SPSS for Windows, version 9.0; SPSS: Chicago, IL, 1998.

(26) Dizy, M.; Polo, M. C. Changes of the concentration of nitrogenous compounds during fermentation of must at pilot-plant scale. *Food Sci. Technol. Int.* **1996**, *2*, 87−93.

(27) Kelly-Treadwell, P. H. Protease activity in yeast: Its relationship to autolysis and champagne character. *Aust. Grapegrowers Winemakers* **1988**, *4*, 58−66.

(28) Bidan, P.; Feuillat, M.; Moulin, J. P. Les vins mousseux. Rapport de la France. 65 iéme Assemblée Générale de I'OIV. *Bull. O.I.V*. **1986**, *59*, 563−626.

(29) Llauberes, R. M.; Dubourdieu, D.; Villettaz, J. C. Exocellular polysaccharides from *Saccharomyces* in wine. *J. Sci. Food Agric*. **1987**, *41*, 277−286.

(30) Colagrande, O.; Ottina, R. Lo spumante Classico italiano. Metodo Champenois. *L'Enotecnico* **1990**, *3*, 65−78.

(31) Martínez-Rodríguez, A. Estudio de la autolisis de las levaduras durante la elaboración de vinos de cava. Thesis, Universidad Autónoma de Madrid, Spain, 2000.

(32) Martínez-Rodríguez, A.; González, R.; Carrascosa, A. V. Morphological changes in autolytic wine yeast during aging in two model systems. *J. Food Sci*. **2004**, *69*, M233−M239.

(33) Doco, T.; Vuchot, P.; Cheynier, V.; Moutounet, M. Structural modification of wine arabinogalactans during aging on lees. *Am. J. Enol. Vitic*. **2003**, *54* (3), 150−157.

(34) Senee, J.; Robillard, B.; Vignes-Adler, M. Films and foam of Champagne wines. *Food Hydrocolloids* **1999**, *13*, 15−26.

(35) Gallart, M.; Tomas, X.; Suberbiola, G.; López-Tamames, E.; Buxaderas, S. Relationship between foam parameters obtained by the gas-sparging method and sensory evaluation of sparkling wines. *J. Agric. Food Chem*. **2004**, *84*, 127−133.

(36) Martin, H.; Arroyo, J.; Sanchez, M.; Molina, M.; Nombela, C. Activity of the yeast map kinase homologue SLT2 is critically required for cell integrity at 37-degrees-C. *Mol. Gen. Genet.* **1993**, 177−184.

Received for review January 27, 2005. Revised manuscript received June 6, 2005. Accepted June 16, 2005. This work was funded through Projects AGL2003-01762 and AGL2004-06933 from the Spanish Ministry of Science and Education (MEC) and MERG-CT-2004-513472 from the European Union.

JF050191V