Influence of Aeration on the Physiological Activity of Flor Yeasts

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The effect of periodic aeration on the physiological activity of a strain of *Saccharomyces cerevisiae* yeast during development of velum (flor) and biological aging of Sherry wine of the Fino type was investigated. L-Proline amino acid was the main nitrogen source for yeasts cells during the biological aging, and its exhaustion may be the cause of the production and consumption of other compounds that are involved in the aroma of wines. Aeration was found to increase adenylate energy charge, growth, and viability of the yeast cells. Also, it affected the intracellular redox equilibrium and the consumption and production of compounds including acetoin, acetaldehyde, higher alcohols, ethanol, glycerol, and acetic acid. Acetaldehyde reached its highest level after the second aeration, which coincided with the exhaustion of the nitrogen source in the medium. The enzyme activity of alcohol dehydrogenases I and II decreased immediately after each aeration, subsequently increasing once all of the dissolved oxygen in the wine had been consumed by yeast cells. Aldehyde dehydrogenase activity was detected only after the first aeration, and it may be related to the production and consumption of acetic acid in the wine.

Keywords: *Aeration; biological aging; flor yeasts; Saccharomyces cerevisiae*

INTRODUCTION

Biological aging is the biochemical process by which Sherry Fino wine matures. It is effected by various types of facultative aerobic yeasts, most of which are strains of *Saccharomyces cerevisiae* displaying a great heterogeneity at the level of the nuclear and mitochondrial genome (*1*). Flor yeasts form a film over wine in the butts (*2*); their metabolism causes major changes in its sensory properties and leaves a reduced environment (*3*, *4*).

Biological aging takes place in oak barrels (500−600 L) through a dynamic process that involves a number of intermediate steps (or scales) and is called the "criaderas and solera" system (*5*). The system consists of a series of casks holding wine in the process of maturing; the casks are arranged in such away as to facilitate progressive, fractional blending. The scale containing the oldest wine is called "solera" and is followed by the first, second, and third "criadera" in a four-scale system (usually, the number of scales ranges from four to six). Sherry wine for marketing is collected from the solera and replaced with an identical amount of wine (one-fourth of the total volume) from the first criadera, which in turn is replenished with wine from the second criadera, and so on, young wine being added to the last scale to close the cycle. The solera system is a slow process, which substantially raises the production cost of these wines. In fact, the need to keep the wine over long time periods in vast cellars, invest in expensive wood casks, conduct periodic transfers from younger wines to older ones, and perform control analyses increases costs in proportion to the length of the aging period. The need thus exists for a way to expedite the process without altering the quality of the resulting wine. In this way, previous works have demonstrated that periodic and short aerations during the wine aging can accelerate the process (*6*, *7*).

Flor yeasts that grow in wine with a high ethanol concentration (15.5%) and a low fermentable sugar content adapt to these conditions by forming a flor film (velum) on the surface. As a result, yeast cells undergo changes in size, morphology, and hydrophobicity (*8*); also, their metabolism becomes purely oxidative (respiratory), albeit limited by the availability of dissolved oxygen (*9*). Aguilera et al. (*10*) found the formation of flor velum to be related to an increased proportion of unsaturated long-chain fatty acids in yeast cells. This probably increases the ethanol tolerance and hydrophobicity of flor yeast cells, decreases their density, and facilitates floating on the wine surface, as a result. Flor yeasts use and transform various substances such as ethanol and glycerol and produce acetaldehyde, acetic acid, acetoin, and intermediate compounds that are oxidized via reactions in which they act as electron donors.

In this work, metabolic changes during flor velum development were examined. Specifically, we studied the effect of two short aerations on the adenylate energy charge of the cells as a measure of metabolic activity, on oxidized and reduced pyridine nucleotides as a measure of the oxidation−reduction status of the cells, and on the specific activities of alcohol and aldehyde dehydrogenases in flor yeasts, which play an active role in the metabolism of ethanol, acetaldehyde, and acetic acid (*9*, *11*). The potential relationship of these factors to the production of acetaldehyde, acetoin, and higher alcohols, which affect the rate of aging of Fino wine, may be investigated in the future with a view to finding a way to expedite the process without decreasing the quality of the end product.

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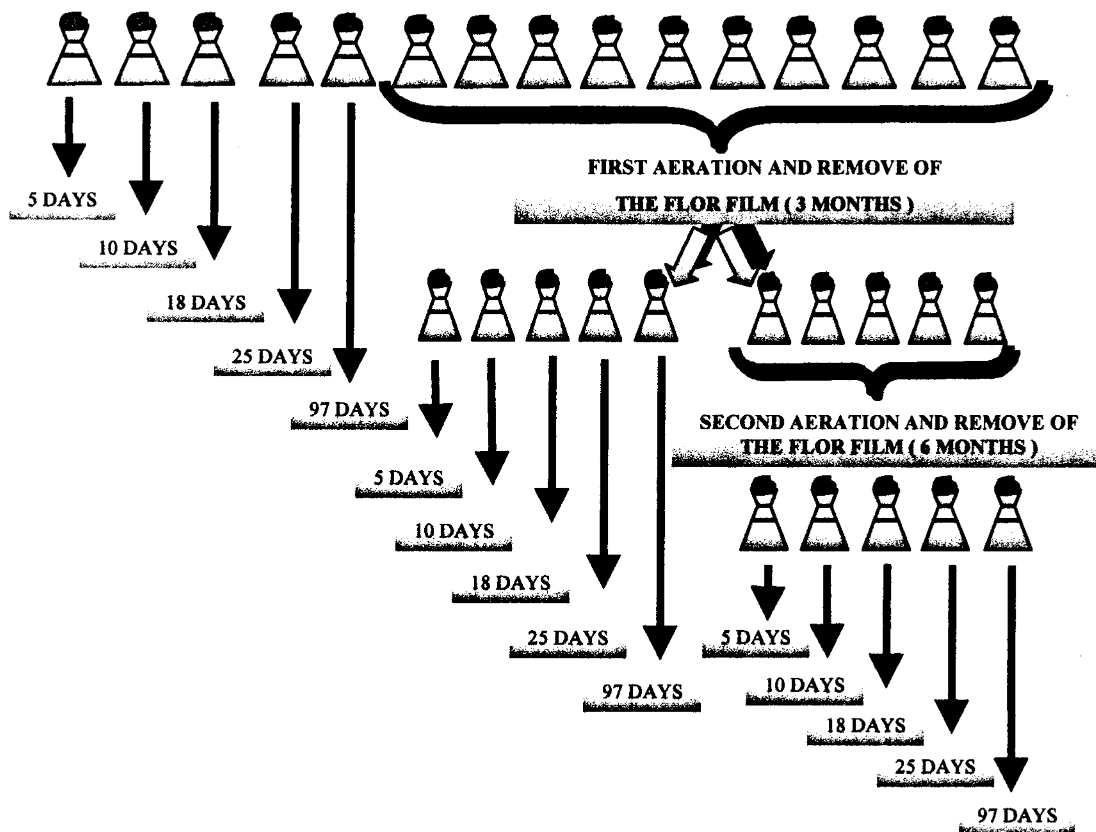

Figure 1. Scheme for the collection of samples during biological aging of Fino wine under *S. cerevisiae*.

MATERIALS AND METHODS

Yeast Strain. The yeast strain used in this study was capensis G-1. This yeast strain was isolated from a velum of wine produced in the Montilla-Moriles region (southern Spain) (*12*), cultured in YEPD agar (0.3% yeast extract, 0.5% peptone, 1% glucose, and 2.5% agar, pH 6.5) at 4 °C in our laboratory, and characterized as *S. cerevisiae* (*13*). The criteria and tests performed for its selection were reported in detail by us in previous papers (*6*, *9*, *12*, *14*).

Wine. The wine used was obtained from Bodegas Alvear in the Montilla-Moriles region. The wine, of dry nature, contained 15.8 ± 0.15% v/v ethanol, 4.15 ± 0.005 g/L reducing sugars, 67.9 ± 1.78 mg/L acetaldehyde, and 4.1 mequiv/L acetic acid. Its pH was 3.2 ± 0.00 and its dissolved oxygen content 7.9 mg/L. It was sterilized by filtration through a Supra EK filter from Seitz (D-6550, Bad Kreuznach, Germany).

Inoculation and Growing Conditions. Yeasts cells for inocula were grown in YM medium supplemented with 5% glucose at 28 °C for 48 h, collected by centrifugation at 5000*g* for 5 min, and washed twice with sterile distilled water. Cells were then resuspended in a known volume of sterile wine. The number of total and viable cells was determined by counting under a light microscope in a Thoma chamber following staining with Methylene Blue (*15*).

Wine under biological aging at the laboratory was split into 15 Erlenmeyer flasks (one per sample) containing 4950 mL of sterile wine at the same surface/volume ratio as in the cellar barrels (0.016 cm^{-1}) and inoculated with 1×10^6 viable cells/mL (Figure 1). The flasks were plugged with hydrophobic cotton and incubated at 18 ± 2 °C in the dark. Five Erlenmeyer flasks were analyzed at 5, 10, 18, 25, and 97 days after inoculation. At 98 days, the wine in the remaining Erlenmeyer flasks was subjected to a short aeration with 0.75 L air/L of wine/min for 10 min, during which the concentration of dissolved oxygen increased in the wine to 4 mg/L. The aeration system used preserved the integrity of the velum (it prevented breaking of the film). Five of the latter flasks were analyzed at 5, 10, 18, 25, and 97 days after aeration. The rest of the flasks were subjected to an additional short aeration that raised the dissolved oxygen content in the wine to 6.2 mg/L. The dissolved oxygen content was measured by using a special-purpose meter (Crison, model Oxy-92). The cell dry weight was obtained after drying at 105 °C for 24 h.

The last five Erlenmeyers flasks were analyzed at the same times as the previous ones, so the biological aging process spanned a 9 month period.

Experimental Analyses. *Nicotinamide Adenine Dinucleotides, Adenosine Phosphates, and Glucose 6-Phosphate.* The wine in each sample was homogenized, the flor velum being broken with the aid of a magnetic stirrer and a glass rod. Then, a volume of 2 L of wine was collected in two Erlenmeyer flasks filled with nitrogen. Cells were then collected by filtration through a 0.45 μm pore size Millipore filter. Oxidized pyridine nucleotides, glucose 6-phosphate, and adenosine phosphates were extracted in 0.5 M perchloric acid, whereas reduced pyridine nucleotides were extracted in 0.25 M NaOH (*16*). Pyridine nucleotides were then determined using the method of Slater and Swayer (*17*), and adenosine phosphates and glucose 6-phosphate were determined using the methods of Chaparro et al. (*16*). The adenylate energy charge (EC) was calculated from the equation EC = (ATP + $^1/_2$ADP)/(ATP + ADP + AMP).

Enzyme Activities. The remaining cells were collected by filtration through a 0.45 μm pore size Millipore filter and resuspended in cold distilled water containing 20 μg/mL chloramphenicol and 20 μg/mL cycloheximide. Cells were then collected by centrifugation (5000*g*, 5 min) and washed once in 0.1 M phosphate buffer, pH 7, containing 0.01 M EDTA. Cell pellets were resuspended in the same buffer. Aliquots of 0.5−1 mL containing 0.5×10^9 cells each were vortexed for 30 s with 1 g of glass beads (Sigma; 0.5 mm diameter) and cooled on ice−water for 30 s. This procedure was always repeated seven times. Cell debris and glass beads were removed by centrifugation (5000*g*, 5 min), and the supernatant was analyzed for enzyme activities. The protein concentration in the supernatant was determined according to the method of Bradford

(*18*), using bovine γ-globulin (Sigma) as standard. Alcohol and aldehyde dehydrogenase (ADH and ALDH) activities were determined spectrophotometrically according to the procedure of Mauricio et al. (*9*).

Nitrogen Compounds. Urea and ammonium ion in the wine were determined using the Boehringer Mannhein (Germany) enzymatic method (urea/ammonia UV method). Free amino acids were determined essentially according to the method of Botella et al. (*19*). Amino acids were quantified from the absorbance at 254 nm of their dansyl derivatives (*20*), which were previously separated by high-performance liquid chromatography (HPLC), using a 15 × 0.4 cm reversed-phase column packed with Spherisorb ODS2 resin of 5 μm particle size from Tracer Analitica (Barcelona, Spain).

Quantification of Ethanol, Acetaldehyde, Glycerol, and Acetic Acid in the Wine. Ethanol was determined according to the method of Crowell and Ough (*21*), and acetaldehyde and glycerol were quantified by using the enzymatic methods of Boehringer Mannhein. Volatile acidity was determined according to the EEC official method (*22*).

Determination of Aroma Compounds. Samples of 100 mL of wine were adjusted to pH 3.5, supplied with 2-octanol as internal standard, and extracted with 100 mL of Freon 11 in a continuous extractor for 24 h. Aroma compounds were quantified by gas chromatography (GC) (Hewlett-Packard 5890 series II) in an SP-1000 capillary column of 60 m × 0.32 mm i.d. (Supelco Inc., Bellefonte, PA) following concentration of the Freon extracts to 0.2 mL. A volume of 3 μL was injected into a chromatograph equipped with a split/splitless injector and a flame ionization detector (FID). The oven temperature program was as follows: 5 min at 45 °C, increased at 1 °C/min to 195 °C, and held for 90 min at 195 °C. The injector and detector temperatures were 275 °C. The carrier gas was helium, used at 9 psi and a split ratio of 1:100.

RESULTS AND DISCUSSION

In this study, during the biological aging process, flor yeast cells undergo a series of physiological changes at three distinct stages.

First Stage. The first stage starts at inoculation and lasts for 3 months. During this period, yeast cells are adapting to the wine (5 days), which experiences slight changes: the ethanol concentration does not change, that of acetaldehyde increases slightly (probably through decarboxylation of piruvic acid or some other metabolic intermediate, but certainly not through ethanol oxidation), and that of acetic acid decreases slightly (Figure 2).

Yeast cells from the inocula used most of the dissolved oxygen in the wine; thus, after only 5 days, the oxygen concentration in the wine was already very low (~1 mg/L). Subsequently, flor yeast cells in the wine started to proliferate and their viability to increase (Figure 3); cell compounds were actively biosynthesized, as reflected in the high concentration of NADH observed (Table 1) (*23*). Thus, a thin film was already present on the wine surface after 18 days. Wine under flor film is subject to some special conditions as a consequence of the oxidative metabolism of the yeast cells and the reducing environment they establish (*24*). Because of the limited amount of oxygen available, the reoxidation of nicotinamide adenine dinucleotides was effected by mechanisms other than the respiratory chain pathway as shown below. The NAD^+/NADH ratio increased during this step; it was very high at 97 days, the time by which cell biosynthesis processes and formation of the flor film must thus have finished (Table 1; Figure 3). Some of the amino acids in the wine, the concentration of which decreased in this stage (Table 2), may not only have been used as a nitrogen source but also transformed by

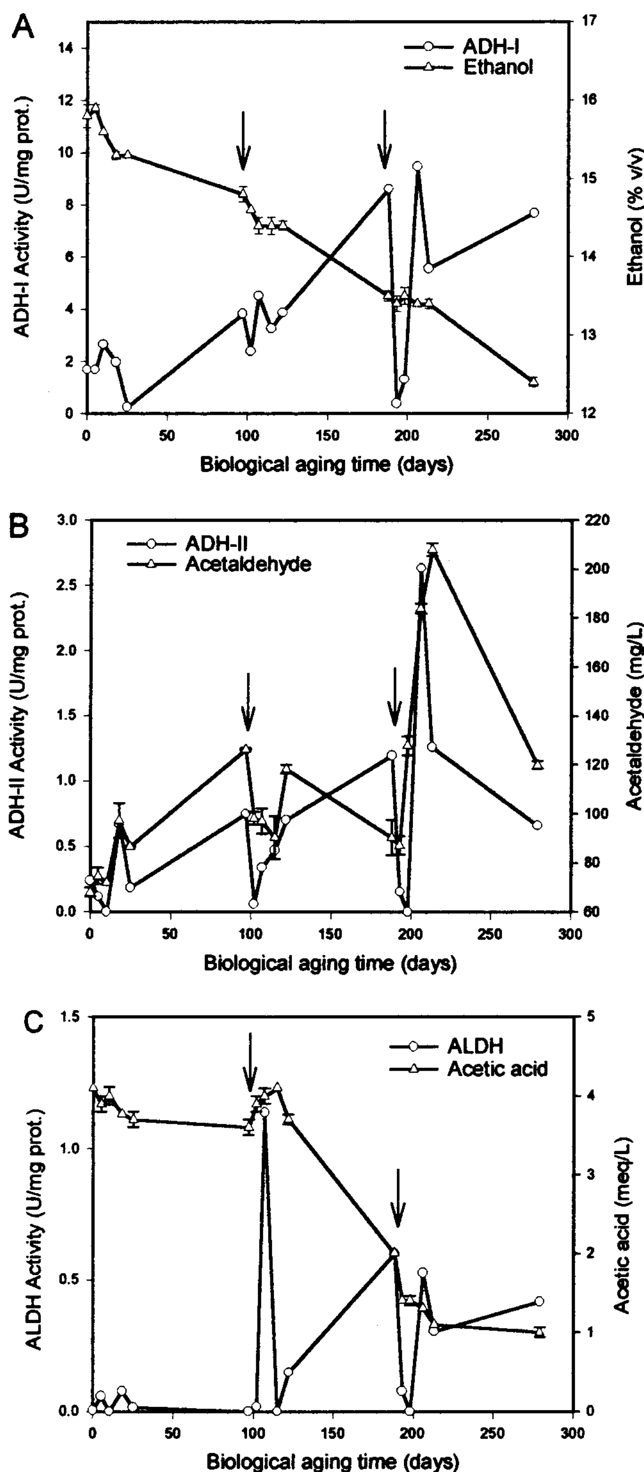

Figure 2. Activity changes in alcohol dehydrogenase I (ADH-I) (A), alcohol dehydrogenase II (ADH-II) (B), isoenzymes and aldehyde dehydrogenase (ALDH) (C) in flor yeast cells and the ethanol (A), acetaldehyde (B), and acetic acid (C) concentrations in Fino wine during biological aging. Arrowheads indicate times of aeration.

deamination, in keto acids, which are decarboxylated to their corresponding aldehydes and reduced by ADH to their higher alcohols (Figure 4) (*25–27*). This pathway plays a prominent role in yeast metabolism as it enables the reoxidation of NADH to maintain the redox potential of the cells (*28*) and the removal of toxic compounds such as aldehydes (*23, 29*).

Acetoin, an acetaldehyde derivative, is the most important ketoinic component of the aroma of Fino wine.

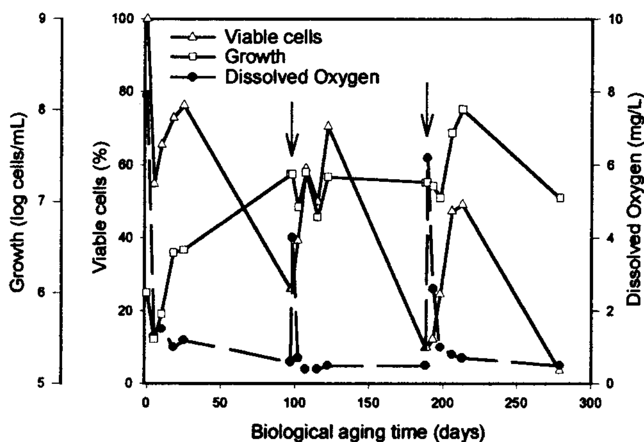

Figure 3. Variation of total and viable flor yeast cells and dissolved oxygen in Fino wine during biological aging.

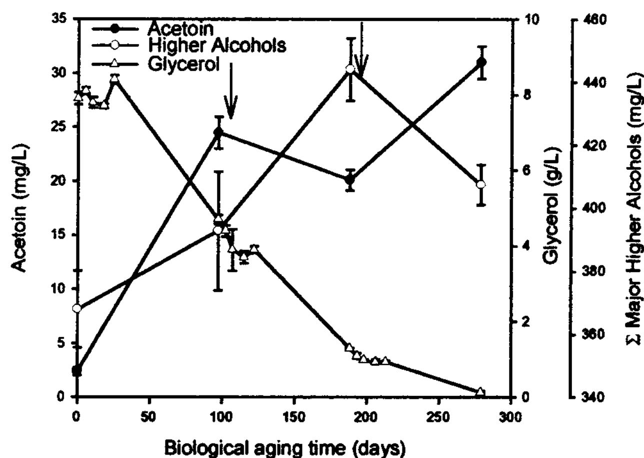

Figure 4. Variation of the acetoin, glycerol, and main higher alcohol contents in Fino wine during biological aging.

Table 1. Intracellular Concentrations of NAD^+, NADH, $NADP^+$, NADPH, and NAD^+/NADH and $NADP^+$/NADPH Ratios in Flor Yeast Cells during Biological Aging of Fino Wine

aging time (days)	μmol/g of cell dry wt				NAD^+/ NADH	$NADP^+$/ NADPH
	NAD^+	NADH	$NADP^+$	NADPH		
0	2.226	7.044	1.866	1.254	0.316	1.488
5	3.627	7.811	2.344	2.113	0.464	1.109
10	1.418	6.155	1.460	0.238	0.230	6.134
18	3.349	5.802	0.886	2.036	0.577	0.435
25	1.651	2.738	0.489	0.195	0.603	2.507
97	2.237	0.783	0.000	0.036	2.855	0.000
102	0.843	1.550	0.071	0.055	0.311	1.290
107	0.965	0.798	0.853	0.328	1.208	2.600
115	0.996	0.497	0.155	0.037	2.000	4.189
122	0.823	1.252	0.853	0.323	0.657	2.640
188	1.102	0.529	0.560	0.191	2.083	2.931
193	0.173	0.229	1.135	0.128	0.756	8.867
198	0.084	0.726	0.354	0.264	0.115	1.340
206	0.567	0.138	0.427	0.365	4.105	1.169
213	0.756	0.175	0.259	0.252	4.300	1.027
279	0.201	0.132	0.172	0.070	1.522	2.457

It is produced during the aging process through reductive acetoinic condensation of two acetaldehyde molecules (*29–31*). Its concentration increased from approximately 2.4 to 25 mg/L (Figure 4). This increase is directly related to the acetaldehyde concentration and the reoxidation of NADH (Table 1; Figure 2).

The absence of hexoses (glucose and fructose) from the wine and of transhydrogenase activity in the yeast cells, which allows electrons to be exchanged among different oxidized and reduced coenzymes, force the yeasts to obtain NADPH in other ways (*28*). One is probably the oxidation of acetaldehyde (from ethanol oxidation) to acetic acid by ALDH-NAD(P)$^+$ (Figure 2). Thus, a decrease in the ethanol content in the wine, but not an increase in the acetaldehyde content, was observed at least during the first phase of growth. After 18 days, ethanol consumption was slower, the acetaldehyde content in the wine higher, and the acetic acid content slightly lower. One other way may be the reduction of $NADP^+$ during the oxidation of Δ′-pyrroline-5-carboxylate to L-glutamic acid in L-proline degradation (*32*). Still another possible source of NADPH and biosynthetic intermediates exists: some amino acids that can be assimilated by the yeasts during this period.

Glycerol was released into the wine as a source for the oxidation of NADH during the first 25 days. Subsequently, it was consumed at a constant rate during biological aging (Figure 4).

Adenosine phosphates can affect cellular viability and hence influence the formation and stability of the flor film (*11*, *33*, *34*). The intracellular concentration of AMP remained low throughout the aging process; aeration caused no significant change in its concentration (Table 3). The ATP and ADP concentrations increased during the adaptation phase and decreased during flor film formation.

The adenylate energy charge (EC) remained close to 0.8 during the adaptation period; then, it dropped to ~0.6 and subsequently increased to 0.8 after 3 months, which suggests that yeast cells exhibited a high metabolic activity and that oxygen availability was low at that point (Table 3).

Second Stage. The second stage started immediately after the first aeration. To study the effect of oxygen on flor-forming yeasts, wine with a velum of 3 months was subjected to a short aeration. This boosted the EC of the cells and hence their viability; also, it increased the consumption of dissolved oxygen (Table 3; Figure 3). The activities of the two alcohol dehydrogenase isoenzymes (ADH-I and ADH-II) exhibited a transient decrease (Figure 2) as a result of the activation of the respiratory chain; also, the concentrations of NADH and $NADP^+$ (Table 1) increased by virtue of renewed biosynthesis of cell material. Thus, the NAD^+/NADH and $NADP^+$/NADPH ratios (Table 1) decreased through activation of the synthesis of some compounds (mainly amino acids) (*23*). Once all dissolved oxygen was consumed, the activity of the isoenzymes rose again as a result of its unavailability (Figures 2 and 3).

The intracellular concentrations of ADP and ATP decreased immediately upon aeration and then increased until day 18 (Table 3). EC and glucose 6-phosphate concentration peaked at that time and then decreased as a result of a decreased energy activity in the cells (Table 3).

Ethanol consumption remained constant, although the acetaldehyde concentration in the wine decreased (Figure 2). This was as a consequence of two different reactions, namely, part of the ethanol oxidized to acetaldehyde was again reduced to ethanol by ADH-I, whereas other aldehydes and biosynthetic intermediates were reduced to higher alcohols with reoxidation of NADH. On the other hand, part of the acetaldehyde was oxidized to acetic acid by ALDH immediately after aeration, $NADP^+$ being simultaneously reduced to NAD-

Table 2. Amino Acid and Ammonium Concentrations in Fino Wine during Biological Aging

aging time (days)	L-proline (mM)	L-leucine (mM)	L-tryptophan (mM)	L-threonine (mM)	ammonium (mM)
0	9.977 ± 0.319	0.818 ± 0.078	0.381 ± 0.120	0.318 ± 0.006	0.423 ± 0.000
5	12.524 ± 0.631	0.940 ± 0.018	0.554 ± 0.033	0.397 ± 0.016	0.452 ± 0.000
10	8.781 ± 0.321	0.811 ± 0.055	0.305 ± 0.096	0.315 ± 0.049	0.568 ± 0.000
18	9.309 ± 0.424	0.936 ± 0.074	0.311 ± 0.122	0.324 ± 0.005	0.476 ± 0.019
25	9.357 ± 0.148	0.979 ± 0.100	0.383 ± 0.090	0.328 ± 0.024	0.394 ± 0.044
97	5.127 ± 0.105	0.719 ± 0.039	0.217 ± 0.109	0.304 ± 0.015	0.228 ± 0.000
102	4.719 ± 0.059	0.725 ± 0.038	0.239 ± 0.021	0.282 ± 0.008	0.173 ± 0.000
107	3.821 ± 0.075	0.625 ± 0.027	0.272 ± 0.141	0.227 ± 0.008	0.305 ± 0.000
115	4.196 ± 0.187	0.802 ± 0.163	0.175 ± 0.017	0.250 ± 0.041	0.233 ± 0.000
122	3.415 ± 0.028	0.899 ± 0.020	0.352 ± 0.011	0.295 ± 0.006	0.195 ± 0.055
188	0.583 ± 0.028	0.453 ± 0.027	0.113 ± 0.020	0.264 ± 0.017	0.111 ± 0.012
193	0.506 ± 0.036	0.448 ± 0.070	0.164 ± 0.038	0.249 ± 0.023	0.12 ± 0.033
198	0.470 ± 0.007	0.537 ± 0.019	0.175 ± 0.025	0.273 ± 0.018	0.181 ± 0.038
206	0.309 ± 0.007	0.582 ± 0.015	0.203 ± 0.039	0.248 ± 0.006	0.245 ± 0.000
213	0.255 ± 0.006	0.668 ±0.032	0.122 ± 0.041	0.256 ± 0.023	0.089 ± 0.000
279	0.162 ± 0.002	0.554 ± 0.055	0.261 ± 0.035	0.262 ± 0.012	0.103 ± 0.007

Table 3. Intracellular Concentrations of AMP, ADP, ATP, and Glucose 6-Phosphate (G-6-P) and Energy Charge (EC) in Flor Yeast Cells during Biological Aging of Fino Wine

aging time (days)	nmol/g of cell dry wt				
	ATP	ADP	AMP	G-6-P	EC
0	3.90	3.00	1.20	0.50	0.660
5	17.1	7.80	0.20	0.00	0.836
10	0.50	0.20	0.40	0.90	0.521
18	1.50	1.00	0.70	0.90	0.618
25	1.40	1.80	0.40	0.30	0.635
97	5.50	1.60	0.80	1.10	0.793
102	0.00	0.60	0.20	0.90	0.421
107	0.90	0.00	0.10	0.00	0.867
115	4.40	0.40	0.00	3.20	0.949
122	0.70	0.20	0.10	0.30	0.747
188	0.00	0.00	0.00	0.30	0.500
193	2.00	0.40	0.50	0.00	0.759
198	0.30	0.00	0.00	0.30	1.000
206	0.00	0.70	0.00	0.00	0.437
213	0.00	0.80	0.00	0.00	0.500
279	0.00	0.20	0.10	0.00	0.321

PH. Subsequently, the amount of acetic acid in the wine gradually decreased through consumption in cell biosynthesis processes (Figure 2).

Once the velum is formed, biosynthesis stops and the concentrations of NAD^+ and $NADP^+$ exceed those of NADH and NADPH, respectively (Table 1). Reduction reactions are thus favored after the flor film is formed, when the respiratory electron chain is inefficient because oxygen availability is low (*9*).

L-Proline amino acid is the main nitrogen source for yeast cells during the biological aging process (Table 2) (*6*, *19*). L-Proline accounts for 77.7% of total amino acids and is followed by L-leucine (6.4%), L-tryptophan (3.0%), and L-threonine (2.5%). L-Proline was virtually depleted after 6 months of aging (Table 2). The higher alcohol concentration in the wine was found to gradually increase while it contained some L-proline (Table 2), posibly because of the need to regenerate NAD^+. L-Proline might be converted into various amino acids, starting with L-glutamic acid, which would in turn yield other amino acids to synthesize proteins. Alternatively, they might be used to maintain the redox potential of the cells, which would entail their conversion into keto acids; these would be decarboxylated to the corresponding aldehydes and subsequently reduced by ADH to higher alcohols (*25*, *26*). This is also consistent with the high ADH activity observed during biological aging (*9*).

Third Stage. The third stage started after the second aeration. The cell viability of 6-month-old flor-forming yeasts was again stimulated, and the oxygen was consumed by them. However, viability was lower than after the first aeration (Figure 3), probably because the principal nitrogen source, L-proline, was depleted (Table 2).

The ATP and ADP concentrations peaked 5 days after aeration and then decreased to zero (Table 3). EC initially increased and then decreased to 0.3, the glucose 6-phosphate concentration falling to zero (Table 3). The second aeration thus decreased all of the biosynthetic activity of the cells by depleting their energy.

Nicotinamide adenine dinucleotides played a different role at this stage, possibly because the principal nitrogen source, L-proline, had been depleted, and as a consequence cells did not use the $NAD(P)^+$ coenzyme for the assimilation of this amino acid until L-glutamic acid (*32*, *35*). This caused the $NADP^+/NADPH$ and $NAD^+/NADH$ ratios to increase much more markedly than after the first aeration (Table 1).

The decreased cell growth facilitated the release of increased amounts of acetaldehyde into the wine as its oxidation to acetic acid was slower (Figure 2). Thus, yeast cells changed their metabolism for NADH oxidation: they did not reduce aldehyde intermediates to higher alcohols (Figure 4). The new pathway involves the production of acetoin from acetaldehyde (Figure 4) (*28*). This is consistent with the good correlation between the increase in the acetaldehyde content and that in ADH-II activity at this stage (Figure 2).

In summary, during biological aging, the flor yeasts grow at the expense of two carbon sources and of nitrogen from ammonium ion and L-proline mainly. The carbon sources were fermentable sugars, glycerol, and ethanol.

Fermentable Sugars and Glycerol. Fermentable sugars are rapidly used during the first few days, whereas glycerol is consumed more slowly. Piruvic acid is formed in the glycolytic pathway. Because this is an anaerobic pathway, glycerol consumption is independent of aeration.

Ethanol. Ethanol assimilation is an aerobic process dependent on cellular respiration (where NAD^+ is reduced to NADH). NAD^+ is required for its oxidation, first to acetaldehyde and then to acetic acid, which is incorporated into cell metabolism as acetyl-CoA. Consequently, ethanol assimilation is accelerated at the beginning of aging and after the first aeration. The second aeration does not increase ethanol consumption; in fact, dissolved oxygen is consumed more slowly. Under these conditions, however, the acetaldehyde concentration in the wine and the ADH-II enzyme

activity increase considerably once oxygen is depleted (Figure 2). This increased activity is a result of the need to reduce some of the NAD^+ produced in respiration, as it cannot be transformed via other pathways.

The redox potential therefore governs the consumption and production of some compounds in the wine (viz., acetoin, acetaldehyde, and higher alcohols), as the yeasts are forced to reduce excess oxidized coenzymes, NAD^+ and $NADP^+$, via other pathways. One such pathway is the oxidation of ethanol to acetaldehyde. The latter is not subsequently oxidized to acetic acid as it is not required for cell metabolism, which is very much inhibited. At this time, there is no nitrogen source available to the yeasts as ammonium ion, L-proline, and other amino acids are at very low concentrations. We thus believe that acetaldehyde is released into the wine when a nutritional imbalance occurs through a lack of nitrogen and that some NAD^+ can be reduced by oxidizing ethanol to acetaldehyde. From then, and until the end of the aging period, the acetaldehyde concentration in the wine decreases while that of acetoin increases, an equilibrium between both being reached that has no influence on the acetic acid concentration (Figures 2 and 4). Thus, the release of acetaldehyde into the wine depends on the physiological conditions of the yeasts (excess oxidized coenzymes) and on a nutritional constraint from the wine (viz., the lack of an oxidizable nitrogen source). This may account for the erratic accumulation of acetaldehyde in wines aging in wineries, as flor-forming yeasts release significant amounts of acetaldehyde only under the above-described conditions.

This paper reports various metabolic changes in a strain of *S. cerevisiae* during a laboratory-simulated process for the production of Sherry wine from a base wine. The goal was to better understand the effect of discrete aeration on the biochemistry of the process and to use this knowledge with a view to optimizing the wine-making procedure. The results obtained in this work may be used as a basis for interesting future projects intended to accelerate the biological aging process.

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