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ARTICLE

Evolution of Resveratrol and Piceid Contents during the Industrial Winemaking Process of Sherry Wine

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In the Jerez region, the sherry winemaking process involves a stage of aging carried out in a dynamic system known as "soleras" and "criaderas". In the case of fino sherry, this aging takes place in the presence of a yeast film growing on the surface of the wine, which gives it a very specific character. In this work, the influence of the sherry elaboration process on resveratrol and piceid levels has been studied. With this purpose, the contents of resveratrol isomers and piceid during the main stages of the sherry wine production system, from maturation until bottling, were monitored during two vintages. The results showed that resveratrol contents of both the skin and juice, for Palomino fino grape, are very similar to those described for other white grape varieties. Sherry wine fermentation, clarification, cold stabilization, and filtration processes considerably affect resveratrol and piceid contents. However, biological aging has the most important influence, diminishing their contents by 80%. These results were confirmed in several tests performed in the laboratory, in which various factors that could affect the resveratrol contents during aging were taken into account (oxidative phenomena and a combination with acetaldehyde and "flor" biofilm growth).

KEYWORDS: Resveratrol; sherry wine; Palomino fino grape; flor yeast

INTRODUCTION

Recently, there has been increased interest in the beneficial effects of moderate wine consumption on health. These effects seem to be related to the phenolic compounds naturally present in wine and their antioxidant properties. Resveratrol and its corresponding piceids, which are present in wine, have been related to this effect (*1*, *2*). Many studies have reported the biological effects of resveratrol; its antioxidant and antimicrobial effectiveness provides health benefits, such as the prevention of cardiovascular diseases, arteriosclerosis, and cancer (*1*−*6*).

Resveratrol is a phytoalexin considered to be a precursor of other stilbenes such as viniferins and pterostilbene (*trans*-3,5-dimethoxy-4′-hydroxystilbene) by several authors (*7*−*11*). Phytoalexins are low molecular mass substances with microbial inhibitory activity (*7*). Their accumulation in plants takes place during plant−microorganism interactions (*8*) or after their induction by stress factors such as the presence of heavy metal ions, UV light, or mechanical injuries combined with certain inducing substances (*8*, *9*, *12*−*14*).

Resveratrol and piceid content in wines is determined by numerous factors such as grape variety, climatic conditions, fungal infections, UV light exposure, and enological practices (*15*−*19*). In fact, techniques such as the application of UV light in table and wine grapes (*20*), maceration (*19*), the use of selected and transgenic strains (*21*), or the use of enzyme extracts with β-glucosidase activity (*22*, *23*) to increase resveratrol levels in table grapes and wines have been studied.

Resveratrol isomers have not been detected in fino-type wines from the Jerez-Xérèz-Sherry area (*24*, *25*). However, the Palomino fino grape variety, used in the elaboration of this type of wine, is subjected to stress conditions, which could lead to similar levels of resveratrol as in other white varieties. These stress conditions are principally due to the special characteristics of the climate and soil in this area. In fact, the content of resveratrol in Palomino fino grapes is influenced mainly by the vintage climatic conditions, *Botrytis cinerea* infections (*26*), and the pressing system used to obtain the juice (*25*). The aging system used in fino elaboration differs from that of other winemaking methods because the biological aging is carried out under a film of "flor" yeast. During this aging process, the flor vellum yeast development on the wine surface after fermentation produces important changes in both organoleptic and physical−chemical characteristics of wine (*27*).

The objective of this work was to establish the average content of resveratrol, as free isomers and piceid, in grape juice and skin of the Palomino fino variety cultivated in the Jerez-Xérèz-Sherry area. Additionally, the evolution of these compounds during commercial grape maturation and during the industrial fino winemaking process was studied.

MATERIALS AND METHODS

Grape Sampling. To perform the study, samples of Palomino fino grapes were taken during the course of two vintages (2003 and 2004), in 24 plots situated in the Jerez-Xérèz-Sherry wine production area that is

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included in the maturation monitoring program directed by the Laboratorio Agroalimentario y Estación Enológica of Jerez de la Frontera (Cádiz, Spain). The climatic characteristics of these vintages, as well as the geological features of the soil, were described in a previous work (*26*).

In each of these plots, samples of approximately 2 kg per plot were taken in clusters of 10−15 berries. Vines were selected from different parts of the plots to ensure that their size and general characteristics were as representative as possible. The samples were taken weekly, on the same day, and at about the same time, early morning.

Characterization of Juices. Collected samples were mechanically crushed to extract the juice (1.0−1.5 kgf/cm^2). The resulting juice was then filtered (pore size of paper filter ≤0.1 mm), carrying out the analytical determinations required to monitor commercial grape maturity, together with the determination of resveratrol and its derivatives. In parallel, a sample of the residual skin was taken and immediately frozen until analysis could be performed.

In the juice, the Beaumè grade was determined according to the OIV procedure (*28*) with a Dujardin−Salleron density-meter. The titratable acidity was determined by titration according to the method of the American Society of Enologists (*29*). The phenols index (PI) of the juice was determined by measuring the sample's absorbance at 280 nm (*29*) with a UV−visible spectrophotometer (Perkin-Elmer 200, Perkin-Elmer Corp., Norwalk, CT). The industrial maturation index (MI) was obtained from the ratio between sugars (g/L) and total acidity (g of tartaric acid/L).

Determination of Resveratrol and Piceid. Determinations were made on both the juice and the skin. Given the different characteristics of the two types of samples, each was subjected to a different extraction process prior to analysis by high-performance liquid chromatography (HPLC).

For the juice samples, a solid phase extraction (SPE) was performed using C_{18}/600 mg LiChrospher cartridges (Supelco Inc., Bellefonte, PA), following the methodology used by Roldán et al. (*26*). Prior to the extraction stage, the sample was centrifuged and filtered using 0.45 μm Teflon syringe filters (Millex-LCR, Millipore, Bedford, MA). A multiple collector-Visiprep-DL solid phase extraction vacuum manifold (Supelco Inc.), which allowed 12 samples to be extracted simultaneously, was used for the extraction of the samples. The components determination of the concentrated samples was carried out by HPLC.

In the case of the skin (25 g), the samples were macerated with 50 mL of a mixture of MeOH/HCl at 0.1% for 30 min with ultrasound (*26*). Then the samples were centrifuged and filtered prior to analysis by direct injection according to the methodology described by Mattivi (*29*) with the following modifications. A LiChrospher 100 RP reversed-phase column (250 × 4 mm; 5 μm, Merck) at 40 °C was used. The mobile phase involved orthophosphoric acid (one mM) and acetonitrile, with a linear elution gradient that went from 0 to 50% of the acetonitrile over 25 min, employing a flow rate of 1 mL/min and injecting 10 μL of standard (or sample) as required.

The HPLC equipment (Waters, Milford, MA) consisted of a model 2690 separation module, a model 996 aligned photodiode UV−visible light detector, and a PC running Millennium 2010 software for control and processing of the chromatographic data.

trans- and *cis*-resveratrol in the samples were identified by comparison of their retention times and UV spectra with those of the commercial *trans*-resveratrol standard and those of the *cis*-resveratrol obtained by photoisomerization of the trans compound.

Identification and quantitation were accomplished using external calibration with pure standards of piceid and resveratrol (Sigma-Aldrich Química S.A., Madrid, Spain). The calibration equations (eight data points, $n = 3$; range = 0.01−5.0 mg/L) were $y = 451989x + 2577$ ($R^2 = 0.9991$) and $y = 539944x - 15157$ ($R^2 = 0.9990$) for *trans*-resveratrol and piceid, respectively.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the methodology proposed by Bravo et al. (*30*). The concentrations of *trans*-resveratrol and piceid that gave signal-to-noise ratios (S/N) of 4:1 and 5:1, respectively, were considered. LOD and LOQ obtained were 0.02 and 0.06 for *trans*-resveratrol and piceid.

Industrial Process. Studies have been done on resveratrol contents and their changes in an industrial process ("solera" system) used for fino winemaking in the Sherry area (**Figure 1**). The solera system was formed by 2500 oak butts (600 L capacity) and was divided into five stages (500 casks each) of aging called, in ascending order of age, "sobretablas", "third criadera", "second criadera", "first criadera", and "solera", respectively.

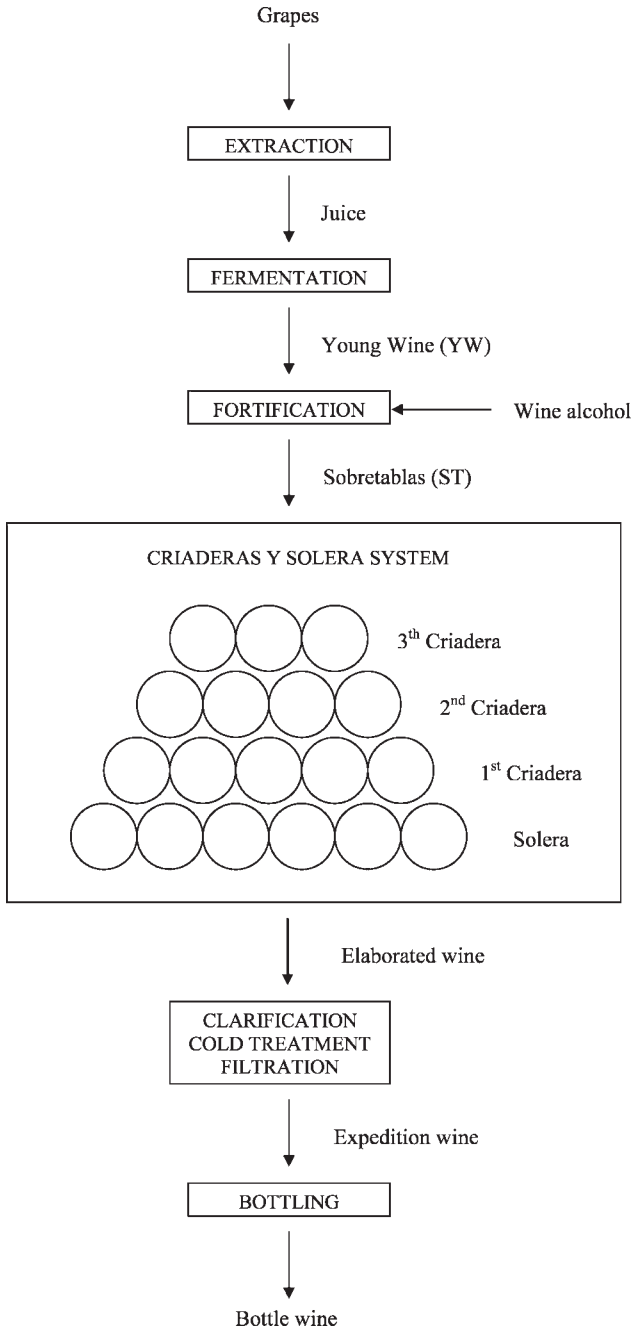

Figure 1. Winemaking process of sherry type wines.

The time of aging for each stage in the solera system of Fino was estimated by Martínez de la Ossa et al. (*27*). The average aging time for the solera system is shown in **Table 1**. The time during which the wine remains in the cask can affect levels of resveratrol.

From the center of each cask, 50 mL samples of wine were taken. For this system and each stage of aging, samples from all 500 butts were blended in a 25 L tank. From these blends, 750 mL samples (one for each stage) were taken and stored at −20 °C for later analysis. Additionally, samples were taken from 750 mL of other stages of winemaking as well as the juice of departure, the fermented wine, the expedition wine, and bottled wine, taking into account the various processes suffered by the wine from one stage to another (i.e., clarification, cold stabilization, filtration, etc.) (**Figure 1**).

Laboratory-Scale Experiments. To determine if the "flor" vellum yeasts were the only ones that were responsible for the modifications in the resveratrol content during the biological aging (or if there were other phenomena that modified this content), an experiment of vellum developed on the laboratory scale was carried out. In this experiment, the

different phenomena that could influence the resveratrol content in wine were evaluated. Thus, we took into account the oxidative phenomena that take place during the storage of the wine, the acetaldehyde presence and, therefore, the possible combination of it with resveratrol, and the growth and development of the yeasts of the vellum flor.

To carry out the trials, a young wine from a winery area was fortified to 15% v/v using wine alcohol. Resveratrol (Sigma-Aldrich) at a concentration of 4.25 mg/L was added to this wine. Three parallel experiments were carried out. In each experiment, 18 glass recipients, with a surface to volume ratio of 190 cm^2/L that also simulated the casks, were used. Weekly sampling by removing three containers of each test was done, and the resveratrol content was analyzed. The weight of the containers was also checked weekly to determine the volume of liquid that was lost by evaporation.

The influence of oxidation phenomena during storage (no flor biofilm growth) was determined by analyzing the resveratrol content in the weekly sampling of a control sample. To study the influence of the presence of acetaldehyde on the resveratrol content, 395 mg/L of acetaldehyde was added to the wine. Finally, to study the influence of vellum yeast on the resveratrol content, several containers were inoculated with certain concentrations of vellum yeasts (4×10^6 cells/mL) with a viability of > 50%.

Statistical Analysis. Means and standard deviations (SD) were calculated, significant differences were calculated by ANOVA, and differences among samplings were tested by post hoc comparison test (LSD) at $p = 0.05$.

RESULTS AND DISCUSSION

Contents in Juice and Skin. In **Table 2**, the resveratrol content in Palomino fino grapes (average of ripening period) is shown. A comparison of the total resveratrol content of the Palomino fino variety with the other 36 white grape varieties reported by Romero-Pérez et al. (*31*) shows that, for all of the varieties studied, the piceids are always found in higher proportions than the corresponding free isomers and that the trans and cis isomer contents depend on the variety. Among these previously studied varieties (*31*), the red grape variety juice appears to be richer in total resveratrol content (average of 4.73 mg/L) than the white grape varieties (average of 0.49 mg/L). The Palomino fino variety appears to contain higher levels of total resveratrol in juice (1.43 mg/L) than other white grape varieties such as Chardonnay or Macabeo (*31*); this can be principally due to the high levels of *cis*-piceid, representing 68% of the total content of resveratrol.

Moreover, a study of the compounds of interest was performed on the skin of the Palomino fino grapes because several authors have stated that the levels of resveratrol are higher in the skin than in the juice (*10*,*32*). As can be observed from the results in **Table 2**, the total resveratrol content in skins (629.3 mg/kg of dry weight) was present principally in piceid form. Compared with studies carried out by other authors (*16*,*33*) the *trans*-resveratrol content of Palomino fino grape skin was lower than that of other white grape varieties such as Chardonnay and Macabeo. However, the skin of this variety presents higher resveratrol content in the glycosylated form (82%), and it is principally like the *cis*-piceid. The same results have been obtained by Romero-Pérez et al. (*16*), which showed that white grape varieties presented higher contents of piceid than free isomers and those piceid were present principally like the *cis*-piceid.

When the distribution of total resveratrol as a molar percentage of each compound was calculated, the greatest percentage of the total resveratrol content of the Palomino fino berry was found in the skin (87.5%), and this is mainly in the glycosylated form, at around 74.5%. Only 12.5% of resveratrol passes to the juice during the extraction process. These data agree with those found by Dixon and Paiva (*34*), who reported that the phenylpropanoids, including resveratrol, accumulate in the vacuoles as glucosides or as other conjugates. Moreover, according to Goldberg et al. (*35*), the high sugar content of the berry and the thin skins of warm-climate grapes, such as the Palomino fino variety, may favor the glycosylation of resveratrol.

Evolution during Maturation. As can be observed in **Table 3**, the total resveratrol contents in juice and skin do not show clear

Table 1. Estimated Average Time for Each Stage (Years) of the Fino "Solera" System[a]

sobretablas (S/T)	1.00
third criadera (3Cra)	1.75
second criadera (2Cra)	2.50
first criadera (1Cra)	3.25
solera (Sra)	4.00

[a] Martínez de la Ossa et al. (*27*).

Table 2. Resveratrol and Piceid Contents in Palomino fino Grape Ripening[a]

	piceid		resveratrol		
	trans-	*cis*-	*trans*-	*cis*-	total
juice (mg/L)	0.08 ± 0.06	0.98 ± 0.15	nq	0.37 ± 0.12	1.43 ± 0.26
skin (mg/kg of dry wt)	126.4 ± 21.8	391.9 ± 61.1	10.1 ± 1.8	100.9 ± 15.4	629.3 ± 100.2

[a] Values are means ± SD ($n = 48$), $p < 0.05$. nq, not quantified.

Table 3. Resveratrol and Piceid Contents in Skin (Milligrams per Kilogram of Dry Weight) and Juice (Milligrams per Liter) during the Maturation Period[a]

		4 WBH	3 WBH	2 WBH	1 WBH	harvest
skin	*trans*-piceid	161.7 ± 42.1	82.6 ± 3.3	116.1 ± 20.6	134.6 ± 25.4	145.5 ± 16.0
	cis-piceid	430.9 ± 130.6	372.3 ± 83.6	443.2 ± 127.9	331.4 ± 85.2	408.4 ± 120.0
	trans-resveratrol	11.0 ± 0.9	8.0 ± 1.8	10.1 ± 1.6	9.6 ± 1.0	11.7 ± 1.4
	cis-resveratrol	73.1 ± 18.9	76.9 ± 7.5	125.1 ± 6.5	124.4 ± 17.4	105.0 ± 0.5
	P/R	8.29	6.32	4.88	4.10	5.60
juice	*trans*-piceid	0.090 ± 0.056	0.068 ± 0.045	0.085 ± 0.044	0.079 ± 0.041	0.104 ± 0.074
	cis-piceid	0.734 ± 0.137	1.134 ± 0.554	1.189 ± 0.551	0.835 ± 0.523	1.064 ± 0.278
	trans-resveratrol	0.032 ± 0.001	0.044 ± 0.026	0.017 ± 0.004	0.014 ± 0.005	0.074 ± 0.002
	cis-resveratrol	0.462 ± 0.023	0.368 ± 0.153	0.354 ± 0.088	0.319 ± 0.079	0.342 ± 0.097
	P/R	1.81	3.25	3.84	3.01	3.13
	MI	26.1 ± 4.1	37.1 ± 6.6	48.6 ± 8.4	57.0 ± 6.1	60.8 ± 7.5
	TPI	25.0 ± 9.6	26.2 ± 10.6	23.2 ± 10.4	21.6 ± 8.3	28.6 ± 16.7

[a] Values are means ± SD ($n = 48$), $p < 0.05$. WBH, weeks before harvest; P/R, piceid (P) and resveratrol (R) relationship; MI, maturation index; TPI, total phenol index.

Table 4. Resveratrol and Piceid Contents during the Sherry Winemaking Process (Milligrams per Liter)[a]

		piceid		resveratrol		
		trans-	*cis*-	*trans*-	*cis*-	total
	juice	0.09 ± 0.043	0.98 ± 0.19	nq	0.37 ± 0.07	1.44 ± 0.25
biological aging	sobretablas	0.09 ± 0.04	0.13 ± 0.05	0.75 ± 0.13	0.08 ± 0.020	1.06 ± 0.20
	third criadera	0.09 ± 0.04	nq	0.11 ± 0.02	nq	0.19 ± 0.05
	second criadera	0.09 ± 0.05	nq	0.16 ± 0.03	nq	0.32 ± 0.09
	first criadera	0.10 ± 0.05	nq	0.20 ± 0.03	nq	0.30 ± 0.04
	solera	0.12 ± 0.04	nq	0.06 ± 0.02	nq	0.17 ± 0.08
	expedition wine	0.10 ± 0.05	nq	nq	nq	0.10 ± 0.05
	bottle wine	0.10 ± 0.04	nq	nq	nq	0.10 ± 0.04

[a] Values are means ± SD ($n = 5$), $p < 0.005$. nq, not quantified.

evolution during the maturation process. Indeed, a correlation is not observed between the total contents and the maturation index (MI) or the total phenol index (TPI). In light of these results, it can be stated that the levels of resveratrol in the juice and skin during maturation do not vary significantly and do not appear to be related to the state of maturity of the fruit. As far as the different isomers of resveratrol are concerned, their distribution in the juice and skin remains practically constant from the beginning of maturation until harvest.

These results showed that the resveratrol content and its distribution in the berry were defined almost from the beginning of the ripening process (véraison) (data not shown). Very small variations take place in the total content of resveratrol in the berry during the maturation period. However, the distribution of the different isomers, both resveratrol and piceid, would be strongly influenced by the climatic conditions experienced by each vintage, as can be deduced from the wide range obtained in the data (**Table 3**).

On the relationship between piceid and resveratrol (P/R), an increase in the P/R relationship in the juice and a decrease in the P/R relationship in the skins were observed during maturation (**Table 3**). These results indicate that the hydrolysis of piceids in the skins occurs during this period as a result of glycosidase activity, mainly located in the solid parts of the grapes (*36*). In the juice, however, the formation of piceid appears to be slightly favored by the sugars increase, resulting in a decrease in the P/R ratio.

At harvest, the P/R ratio presents a different evolution, which could be related to the phenomenon of grape concentration (postmaturation) and the stress conditions of the vineyards at the time.

Evolution during the Industrial Process. Importantly, the production of sherry wine is very similar to a white wine elaboration. Therefore, we try to minimize the contact of juice with the solid parts, thereby minimizing component extraction from the skin. **Table 4** shows the results obtained in the resveratrol isomers content at different stages in the industrial process of winemaking of the sherry wine fino type. As shown in this table, the juice of the Palomino fino grape shows low levels of resveratrol despite the high skin content (**Table 2**). Additionally, the levels of piceid in the juices are higher than the levels of the free isomers (ratio of 3:1), the cis isomer being the most important (70%).

During fermentation, the total content of resveratrol decreases by approximately 35%. The increase of *trans*-resveratrol is 20-fold as a result of the enzymatic hydrolysis of piceid and the balancing and isomerization of the cis/trans ratio (*37*). However, decreases of the cis isomers (free and glycosylates) (77 and 89.7%, respectively) have been observed. This could imply that, during alcoholic fermentation, not only hydrolytic phenomena occur but also flor yeasts in the fermentative phase preferably metabolize the cis isomers.

These results agree with those obtained by other authors (*17*, *37*−*39*) who observed that resveratrol isomers were hydrolyzed and metabolized by the yeasts in different proportions depending of the strain used. On the other hand, the decrease of *cis*-piceid content during the alcoholic fermentation could be due to increased β-glucosidase activity during the exponential phase of yeast growth (*17*).

The stage of the process that most influences the resveratrol levels in sherry wines is the biological aging in the criadera and solera systems. Before this study, that the sherry wines had very low or no levels of resveratrol (*24*, *25*) compared to other types of white wines was shown; however, it was justified by the low resveratrol levels in the Palomino fino grapes and by the influence of pressing on resveratrol extraction (*25*). The results of this study show that the resveratrol content of Palomino fino grapes (mostly like piceid) disappears during the winemaking process and, especially, during biological aging. As can be seen in **Table 4**, a reduction of 82% in the resveratrol levels is produced from young fortified wine or sobretablas to third criadera wines. This decrease affects all of the isomeric forms of resveratrol, and it affects *cis*-piceid (< 90%) and *trans*-resveratrol (85.8%) in a very important way. In the following stages, called the criaderas and the solera stages in the system (second and first criaderas), the levels of total resveratrol remain practically invariable. Only a low increase is observed, which is probably due to the "merma" effect (concentration by evaporation losses). From the first criadera to the solera phase, a decrease in the resveratrol levels is again produced, which affects *trans*-resveratrol (71.7%). These results show that, in the fermentative phase, the flor yeast has a greater tendency to hydrolyze and metabolize cis isomers. However, the same yeast in the filmogenic phase hydrolyzes and metabolizes resveratrol like trans isomers. This phenomenon could suggest that the growth stage of flor yeast (fermentative or filmogenic phase) influences their tendency to hydrolyze and metabolize the trans or cis isomers. However, it would be necessary to carry out new works in this sense to confirm these results.

The yeasts of the flor vellum and their biological activity seem to be mainly responsible for the reduction of resveratrol levels in sherry wines. As in the fermentative stage, the hydrolytic activity of these yeasts has been shown. They have a great ability to reduce resveratrol levels during fermentation. Their hydrolytic activity and isomerization equilibrium are important, and these yeasts are also able to metabolize resveratrol, removing it from the wine. Because resveratrol, besides its antioxidant capacity, has an antifungal capacity even in yeasts, the yeasts were able to degrade it at low concentrations.

Table 5. Resveratrol Evolution during the Laboratory-Scale Process (Milligrams per Liter) and Concentration Effect[a]

time (weeks)	control	AC	FY	cocentration effect (%)
0	3.6 ± 0.3	3.4 ± 0.6	3.6 ± 0.1	
9	3.9 ± 0.3	3.4 ± 0.5	2.9 ± 0.22	7

[a] Values are means ± SD ($n = 24$), $p < 0.001$. Control, oxidative phenomena; AC, combination with acetaldehyde; FY, enzymatic activity of "flor" yeast strain.

Once the stage of biological aging is finished and, in the expedition wine (**Figure 1**), after the clarification process (with bentonite and gelatin) has been completed, and cold treatment (−6 °C for 1 week) and filtration are used (by diatomaceous earth and cellulose filters), there is also a slight decrease of resveratrol (15%) that affects trans isomers. Numerous authors (*17*, *18*, *25*) have shown that clarification and filtration treatments decrease the resveratrol content and that such treatments do not affect all isomers equally. Thus, Castellari et al. (*18*) confirm that bentonite, gelatin, cellulose, and diatomaceous earth treatments reduced the levels of resveratrol by 2−5%, affecting the trans isomers. The amicrobic filtering conducted during the last stage of the process (expedition wine to bottled wine) affects the resveratrol content less, however.

Evolution in the Laboratory-Scale Experiments. During biological aging, several phenomena that affect resveratrol levels can take place. First, the enzymatic and metabolic activity of flor yeast during biological aging can be a cause of resveratrol decrease. With storage in casks, oxidative phenomena that would reduce the resveratrol content by > 50% can take place, subjecting the wines to an oxidative process (*25*). However, one of the main characteristics of the biologically aged wines is that vellum formation avoids the wines' oxidation. On the other hand, during this process, a great quantity of acetaldehyde is formed, which creates a higher combination power for the wine substances (sulfurs, phenolic compounds, etc). The resveratrol combination with the acetaldehyde could be another one of the causes of resveratrol reduction during biological aging. Any one of these three phenomena can take place in any one of the scales of the criadera and solera systems.

As we have seen from **Table 4**, the levels of resveratrol decreased after the third criadera. This decrease could be linked to oxidative phenomena, the combination with acetaldehyde, or the enzymatic activity of flor yeast strains during growth. To verify these hypotheses, a laboratory-scale experiment was conducted (**Table 5**). As can be observed, like an industrial scale, the laboratory scale is also produced by evaporation losses (mermas) up to about 7% after the 9 week period.

In relation to the oxidative phenomenon, the results indicate an increase in resveratrol content by 8%, indicating that resveratrol during that period was not oxidized even without the vellum's presence (control). The resveratrol increase is due to the concentration effect of the merma that affects the wine. Moreover, a combination of acetaldehyde with resveratrol in small proportion (5×10^{-4} mg/L resveratrol/mg/L acetaldehyde) can be observed. Hence, the initial levels in all of the experiments are lower than the applied dose (4.25 mg/L) and even lower in the case of a wine with a higher acetaldehyde content of 400 mg/L. The resveratrol combination with acetaldehyde is favored by the loss of volume by evaporation (concentration effect); then, the resveratrol levels stay about the same as the initial levels.

As can be observed from **Table 4**, the factors that influence the levels of resveratrol in wine are the growth and development of the vellum. Despite the concentration effect, there is a decrease of resveratrol by 19% (27%, considering the merma). This suggests that the dose of resveratrol tested does not inhibit the yeast growth and vellum development, reaching 74% viability in the second week when the vellum is shown with rough aspects (data not shown). However, the vellum yeast is able to degrade and/or absorb *trans*-resveratrol during its growth stage, showing an observed decrease in resveratrol levels of 9.5×10^{-13} mg/cell/h.

These results show that the flor yeast and, therefore, the biological aging stage are primarily responsible for the decreased levels of resveratrol in the winemaking process of sherry wine. Treatments such as clarification, cold treatment, or filtrations influence the amount of resveratrol found in wine. However, the influence of microbiological processes is more important than the physical and chemical processes.

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