

Influence of Blending on the Content of Different Compounds in the Biological Aging of Sherry Dry Wines

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Principal components analysis to examine the effect of blending (viz. the mixing and transfer of wine between cask rows in a “criaderas and solera” system) on metabolic activity in flor yeasts during biological aging of sherry dry wines was carried out. The variables used in the analysis were the wine compounds most deeply involved in the flor yeast metabolism, namely ethanol, acetaldehyde, glycerol, acetic acid, and L-proline. The greatest blending effect was found to be on the third and second “criadera”, which are the stages where the yeasts show a high metabolic activity. The stages holding the oldest wine (viz. the first criadera and the solera) exhibited no differences before and after blending; therefore, the yeasts have a decreased biological activity in them and physical-chemical aging processes seemingly prevail over it.

KEYWORDS: Biological aging; blending; flor yeasts; wine

INTRODUCTION

Biological aging is a biochemical process, carried out by various types of facultative aerobic yeasts (“flor” yeasts), most of which are strains of *Saccharomyces cerevisiae* displaying a great heterogeneity at the level of the nuclear and mitochondrial genome (1), by which sherry dry wine (fine wine) is obtained.

Flor yeasts that grow in wine with a high ethanol concentration (15.5% v/v) and low fermentable sugar content adapt to these conditions by forming a biofilm (flor velum) on its surface (2), and their metabolism causes major changes in its sensory properties and promotes a reducing environment (3, 4). As a result, yeast cells undergo changes in size, morphology, and hydrophobicity (5). Also, their metabolism becomes purely oxidative (respiratory), albeit limited by the availability of dissolved oxygen (6). Aguilera et al. (7) 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 ethanol tolerance and hydrophobicity of flor yeast cells, decreases its density, and facilitates floating on the wine surface, as a result. Flor yeasts use and transform various substances such as ethanol and glycerol, which act as electron donors in redox reactions, and produce acetaldehyde, acetic acid, acetoin, and other intermediate compounds.

Biological aging takes place in oak casks (500–600 liters) through a dynamic process that involves a number of intermediate steps (or scales or rows) and is called the “criaderas and solera” system (8). The system consists of a series of cask rows 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 4 to 6). Commercial sherry wine is collected from the solera and replaced with an identical amount of wine (1/3 of the total volume) from the first criadera, which in turn is replenished with wine from second criadera, and so on, with young wine being added to the last scale to close the cycle (**Figure 1**). The transfer, mixing, and replenishment of wine in the scale system is called blending (“rocio”). The blending contributes the necessary nutrients for the maintenance of the velum and a very beneficial partial aeration that is favorable as much for the wine as for the yeasts (9). Blending is one of the most important steps in making fine wine with a distinct and unique character.

The criaderas and 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 wine to older one, 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 to the wine aging can accelerate the process (10, 11).

The aim of the present work was to study the blending effect on the content of several compounds in fine wine involved with the cellular yeast metabolism during the biological aging process in the criaderas and solera system. For this purpose, a principal components analysis has been used in this study. The principal components analysis has been previously utilized to study as

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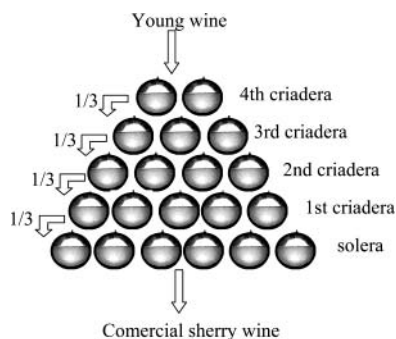


Figure 1. Scheme of the criaderas and solera system.

much the biological aging evolution with different yeast strain (12) as the effect of a small aeration in the accelerated biological aging (11).

MATERIALS AND METHODS

In this work, the biological aging in sherry dry wines produced by the Pérez Barquero winery from grapes grown in the Montilla-Moriles winemaking region (southern Spain) during the 1999 and 2000 years has been studied. In this winery, the casks holding the aging wine are vertically arranged in five rows called solera (at the bottom), first criadera, second criadera, third criadera, and fourth criadera (at the top). An overall 20 casks (4 per row) located at different points in the cellar were randomly chosen for this study. Blending was carried out in May and December both years. Samples were collected for analysis before (BB) and 7 days after blending (7 AB) in May. The data corresponding to the blending time (AB) were calculated theoretically for all the criaderas, taking into account that each blend operation put in contact 1/3 of wine from the preceding criadera (younger wine) with 2/3 of wine from the criadera to replenish (older wine). All results reported herein are the average of all fourth casks.

Dissolved Oxygen. The dissolved oxygen content was measured in 250-mL flasks that were filled with 200 mL of wine collected by suction of middle of each cask. Previously, the flasks were first degassed and then refilled with nitrogen gas. The measure was achieved using a sensor of oxygen (Crison, model Oxy-92).

Amino Acids. Free amino acids were determined essentially according to Botella et al. (13). Amino acids were quantified from the absorbance at 254 nm of their dansyl derivatives (14), 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 Analytica (Barcelona, Spain).

Ethanol, Acetaldehyde, Glycerol, and Acetic Acid. Ethanol was determined by the method of Crowell and Ough (15). Acetaldehyde and glycerol were quantified by gas chromatography using the method of the OIV (16) modified by Muñoz et al. (17). Acetic acid was quantified using an enzymatic test from Boehringer Mannheim (Germany).

Statistical Treatment. The statistical software package Statgraphics Plus v. 2, from STSC, Inc. (Rockville, MD.) was used to perform a principal components analysis to study the blending effect and the aging of sherry wine.

RESULTS AND DISCUSSION

The criaderas and solera system allows obtaining year-to-year commercial wine with similar organoleptic profile. The blending not only brings nutrients for the yeasts of the posterior scale but also to homogenizes the wine inside oneself-scale in the criaderas and solera system. On the other hand, the casks contain a normal microbiota of yeasts, whose metabolic state is different even among casks with the same period of aging, in the same scale. Therefore, it should be noted that some compounds exhibited high standard deviations, particularly in the criaderas containing the youngest wine in accordance with Mesa et al. (18), who reported that in the youngest scales is present a high variability of yeast strains, and as consequence, this has a strong influence in the enological parameters in these scales (19).

Variation of the Oxygen Concentration in the Criaderas and Solera System. During biological aging, blending facilitates the mixture of wines from different vintages and their enrichment with nutrients and dissolved oxygen as a result. Because blending involves mixing wine from the preceding row with wine from the current one in a 2:1 proportion and the wine is pumped and aired during the operation, we have checked that the dissolved oxygen concentration increases from 1 to 2 mg L⁻¹ in all cask rows, which is according to Moutounet et al. (20) and Vidal et al. (21). In relation to nutrient, supply diminishes from the fourth criadera (top) to the solera (bottom).

Table 1 shows the variation of the dissolved oxygen concentration in the criaderas and solera system studied. As can be seen, oxygen was consumed 7 days after blending; however, it was not enough to restore the values prior to blending. This suggests that oxygen consumption is very slow, probably because of the need for the oxygen to diffuse from the cask bottom to the wine surface where the yeasts are.

Influence of Blending on Ethanol, Acetaldehyde, Glycerol, and Acetic Acid. During biological aging of sherry dry wine, flor yeasts use ethanol, glycerol, and acetic acid as carbon sources for growing (**Table 2**). Seven days after blending, the ethanol concentration decreased in all cask rows by effect of the yeast metabolism. Also, glycerol was assimilated by the yeasts in the top two rows (third and fourth criadera). The first and second criaderas and the solera, where the glycerol concentration was below 2 g L⁻¹, exhibited low consumption or even release of this compound, with the resulting increase in concentration. Such an increase was probably a result of the

Table 1. Oxygen Concentration in Wine (mg L⁻¹) and Balance in the Aging System before Blending (BB), Just after Blending (AB), and 7 days after Blending (7 AB) during the Years 1999 and 2000^a

	fourth criadera		third criadera		second criadera		first criadera		solera	
	balance		balance		balance		balance		balance	
1999										
BB	0.75 ± 0.06		1.08 ± 0.37		0.68 ± 0.15		4.58 ± 1.21		4.08 ± 1.48	
AB	2.25 ± 0.06	1.50	2.58 ± 0.37	1.50	2.18 ± 0.15	1.50	6.08 ± 1.21	1.50	5.58 ± 1.48	1.50
7 AB	1.00 ± 0.12	-1.25	0.73 ± 0.15	-1.85	1.08 ± 0.75	-1.10	1.05 ± 0.17	-5.03	1.20 ± 0.16	-4.38
2000										
BB	0.61 ± 0.12		1.50 ± 1.41		0.78 ± 0.12		0.83 ± 0.20		0.88 ± 0.05	
AB	2.11 ± 0.12	1.50	3.00 ± 1.41	1.50	2.28 ± 0.12	1.50	2.33 ± 0.20	1.50	2.38 ± 0.05	1.50
7 AB	1.45 ± 0.33	-0.66	1.75 ± 0.50	-1.25	1.14 ± 0.59	-1.14	1.20 ± 0.97	-1.13	0.70 ± 0.25	-1.68

^a AB was calculated assuming an increase in a uniform manner of 1.5 mg L⁻¹.

Table 2. Ethanol, Glycerol, Acetaldehyde and Acetic Acid Concentration in the Aging System before Blending (BB), Just after Blending (AB), and 7 days after Blending (7 AB) during the Years 1999 and 2000^a

carbon source	young wine	fourth criadera			third criadera			second criadera			first criadera			solera		
		BB	AB	7 AB	BB	AB	7 AB	BB	AB	7 AB	BB	AB	7 AB	BB	AB	7 AB
1999																
ethanol (% v/v)	15.40 ± 0.21	15.5 ± 0.10	15.5 ± 0.06	14.8 ± 0.83	15.4 ± 0.91	15.4 ± 0.60	15.2 ± 0.33	15.7 ± 0.86	15.6 ± 0.57	15.4 ± 1.00	15.9 ± 0.42	15.8 ± 0.28	15.1 ± 1.02	16.2 ± 0.45	16.1 ± 0.30	16.1 ± 0.51
	balance			0.00 ± 0.70	-0.70 ± 0.83	-0.20 ± 0.33	-0.20 ± 0.33	-0.10 ± 0.86	-0.10 ± 0.57	-0.20 ± 1.00	-0.10 ± 0.42	-0.10 ± 0.28	-0.70 ± 1.02	-0.10 ± 0.45	-0.10 ± 0.30	-0.00 ± 0.51
glycerol (g L ⁻¹)	9.25 ± 0.59	7.04 ± 2.53	7.80 ± 1.01	7.06 ± 2.53	0.55 ± 1.1	2.81 ± 0.73	1.96 ± 0.24	1.68 ± 1.13	1.30 ± 0.75	1.36 ± 0.9	1.62 ± 0.26	1.64 ± 0.18	1.69 ± 0.08	0.34 ± 0.68	0.77 ± 0.45	0.77 ± 0.80
	balance			0.76 ± 9.53	-0.74 ± 34.0	2.26 ± 31.1	-0.85 ± 21.0	0.06 ± 56.3	-0.38 ± 37.5	0.06 ± 26.6	0.02 ± 35.0	0.05 ± 23.3	0.05 ± 34.1	0.05 ± 25.5	0.43 ± 17.0	0.00 ± 13.0
acetaldehyde (mg L ⁻¹)	121 ± 3.53	156 ± 14	144 ± 9.53	179 ± 34.0	257 ± 46.6	223 ± 31.1	291 ± 21.0	320 ± 56.3	299 ± 37.5	317 ± 26.6	364 ± 35.0	349 ± 23.3	304 ± 34.1	302 ± 25.5	323 ± 17.0	283 ± 13.0
	balance			-12.0 ± 34.0	35.0 ± 46.6	-34.0 ± 31.1	68.0 ± 21.0	-21.0 ± 56.3	18.0 ± 37.5	18.0 ± 26.6	-15.0 ± 35.0	-45.0 ± 23.3	-45.0 ± 34.1	21.0 ± 25.5	0.05 ± 17.0	-0.05 ± 13.0
acetic acid (g L ⁻¹)	0.38 ± 0.00	0.31 ± 0.01	0.34 ± 0.01	0.25 ± 0.07	0.05 ± 0.04	0.14 ± 0.03	0.11 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.03 ± 0.03	0.03 ± 0.02	0.05 ± 0.00
	balance			0.03 ± 0.07	-0.09 ± 0.04	0.09 ± 0.03	-0.03 ± 0.01	-0.01 ± 0.02	-0.01 ± 0.01	-0.03 ± 0.02	0.02 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.03	0.00 ± 0.02	0.02 ± 0.00
2000																
ethanol (% v/v)	15.40 ± 0.21	15.1 ± 0.21	15.2 ± 0.14	14.9 ± 0.36	15.4 ± 0.46	15.3 ± 0.30	15.0 ± 0.48	15.5 ± 0.49	15.4 ± 0.33	14.9 ± 0.38	16.0 ± 0.41	15.9 ± 0.27	14.8 ± 0.6	16.0 ± 0.50	16.0 ± 0.33	14.5 ± 0.85
	balance			0.10 ± 0.36	-0.30 ± 0.46	-0.10 ± 0.30	-0.30 ± 0.48	-0.10 ± 0.49	-0.10 ± 0.33	-0.50 ± 0.38	-0.10 ± 0.41	-1.10 ± 0.27	-1.10 ± 0.6	0.00 ± 0.50	0.00 ± 0.33	-1.50 ± 0.85
glycerol (g L ⁻¹)	9.25 ± 0.59	6.20 ± 2.83	7.22 ± 1.89	6.26 ± 1.75	1.5 ± 1.23	3.07 ± 0.82	3.38 ± 0.45	0.34 ± 0.69	0.41 ± 0.46	0.68 ± 1.37	1.03 ± 0.69	1.07 ± 0.46	1.56 ± 1.06	0.61 ± 0.71	0.91 ± 0.47	2.14 ± 0.16
	balance			1.02 ± 1.75	-0.96 ± 1.23	1.57 ± 0.82	0.31 ± 0.45	0.07 ± 0.69	0.27 ± 0.46	0.27 ± 1.37	0.04 ± 0.69	0.49 ± 0.46	0.49 ± 1.06	0.30 ± 0.71	1.23 ± 0.47	1.23 ± 0.16
acetaldehyde (mg L ⁻¹)	121 ± 3.53	241 ± 87.8	201 ± 58.0	183 ± 53.6	461 ± 35.3	388 ± 23.5	208 ± 32.6	321 ± 42.4	368 ± 28.3	398 ± 28.5	313 ± 32.5	316 ± 21.6	366 ± 10.1	292 ± 30.2	299 ± 20.1	334 ± 20.3
	balance			-40.0 ± 53.6	-18.0 ± 35.3	-73.0 ± 23.5	-180 ± 32.6	47.0 ± 42.4	30.0 ± 28.3	30.0 ± 28.5	3.00 ± 32.5	50.0 ± 21.6	50.0 ± 10.1	7.00 ± 30.2	35.0 ± 20.1	35.0 ± 20.3
acetic acid (g L ⁻¹)	0.38 ± 0.00	0.17 ± 0.07	0.24 ± 0.04	0.23 ± 0.03	0.07 ± 0.01	0.10 ± 0.01	0.13 ± 0.00	0.06 ± 0.04	0.06 ± 0.03	0.05 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
	balance			0.07 ± 0.04	-0.01 ± 0.03	0.03 ± 0.01	0.03 ± 0.00	0.00 ± 0.04	-0.01 ± 0.03	-0.01 ± 0.02	0.01 ± 0.01	0.00 ± 0.01	0.00 ± 0.02	0.00 ± 0.01	0.00 ± 0.01	-0.01 ± 0.01

^a AB was calculated as indicated in materials and methods.**Table 3.** Amino Acids Concentration (mM) in the Aging System before Blending (BB), Just after Blending (AB), and 7 days after Blending (7 AB) during the Years 1999 and 2000

nitrogen source	young wine	fourth criadera			third criadera			second criadera			first criadera			solera		
		BB	AB	7 AB	BB	AB	7 AB	BB	AB	7 AB	BB	AB	7 AB	BB	AB	7 AB
1999																
L-proline	11.7 ± 0.71	6.38 ± 0.73	8.15 ± 0.48	6.63 ± 1.68	3.37 ± 0.25	4.37 ± 0.17	3.82 ± 0.30	4.13 ± 0.45	3.87 ± 0.30	3.44 ± 0.46	3.87 ± 0.42	3.96 ± 0.28	3.83 ± 0.54	3.08 ± 0.28	3.34 ± 0.19	4.04 ± 0.62
	balance			1.77 ± 1.68	-1.52 ± 0.25	1.00 ± 0.17	-0.55 ± 0.30	-0.26 ± 0.45	-0.43 ± 0.30	-0.43 ± 0.46	0.09 ± 0.42	-0.13 ± 0.28	-0.13 ± 0.54	0.26 ± 0.28	0.70 ± 0.19	0.70 ± 0.62
L-leucine	2.49 ± 0.30	1.60 ± 0.08	1.89 ± 0.05	2.07 ± 0.18	1.62 ± 0.55	1.61 ± 0.39	2.11 ± 0.14	1.91 ± 0.34	1.84 ± 0.23	1.90 ± 0.19	2.01 ± 0.32	1.98 ± 0.21	1.85 ± 0.43	1.96 ± 0.38	1.97 ± 0.25	2.36 ± 0.43
	balance			0.29 ± 0.18	-0.01 ± 0.55	0.50 ± 0.39	0.50 ± 0.14	-0.07 ± 0.34	0.06 ± 0.19	0.06 ± 0.19	-0.03 ± 0.32	-0.13 ± 0.21	-0.13 ± 0.43	0.01 ± 0.38	0.39 ± 0.25	0.39 ± 0.43
L-glutamic acid	0.22 ± 0.05	ND	0.14 ± 0.08	0.24 ± 0.02	0.24 ± 0.07	0.19 ± 0.5	0.18 ± 0.12	0.21 ± 0.07	0.22 ± 0.04	0.20 ± 0.07	0.24 ± 0.05	0.23 ± 0.04	0.12 ± 0.14	0.28 ± 0.06	0.27 ± 0.04	0.16 ± 0.13
	balance			0.10 ± 0.08	-0.05 ± 0.07	-0.01 ± 0.12	-0.01 ± 0.12	0.01 ± 0.07	-0.02 ± 0.04	-0.02 ± 0.07	-0.01 ± 0.05	-0.11 ± 0.04	-0.11 ± 0.14	-0.01 ± 0.06	-0.11 ± 0.04	-0.11 ± 0.13
α-amino butyric acid	0.10 ± 0.02	ND	0.03 ± 0.00	ND	0.12 ± 0.02	0.08 ± 0.01	0.06 ± 0.06	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.13 ± 0.02
	balance			0.00 ± 0.00	-0.04 ± 0.02	-0.02 ± 0.06	0.00 ± 0.01	0.01 ± 0.02	0.01 ± 0.02	0.01 ± 0.02	0.00 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	-0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.02
γ-amino butyric acid	0.28 ± 0.01	ND	0.17 ± 0.09	0.30 ± 0.04	0.35 ± 0.06	0.26 ± 0.04	0.33 ± 0.06	0.32 ± 0.08	0.32 ± 0.05	0.33 ± 0.07	0.44 ± 0.09	0.40 ± 0.06	0.34 ± 0.10	0.66 ± 0.08	0.58 ± 0.06	0.53 ± 0.08
	balance			0.13 ± 0.04	-0.09 ± 0.06	0.07 ± 0.06	0.07 ± 0.06	0.00 ± 0.08	0.01 ± 0.05	0.01 ± 0.07	-0.04 ± 0.09	-0.06 ± 0.06	-0.06 ± 0.10	-0.08 ± 0.08	-0.08 ± 0.06	-0.05 ± 0.08
2000																
L-proline	11.7 ± 0.71	5.63 ± 1.99	7.66 ± 1.33	5.42 ± 0.84	3.39 ± 1.39	4.12 ± 0.93	5.18 ± 0.92	3.60 ± 0.97	3.53 ± 0.65	3.43 ± 0.95	3.88 ± 0.49	4.00 ± 0.32	2.97 ± 0.29	3.26 ± 0.70	3.53 ± 0.46	2.78 ± 0.54
	balance			2.03 ± 0.84	-2.24 ± 1.39	0.73 ± 0.93	1.06 ± 0.92	-0.07 ± 0.97	-0.10 ± 0.65	-0.10 ± 0.95	0.12 ± 0.49	-1.03 ± 0.32	-1.03 ± 0.29	0.27 ± 0.70	-0.75 ± 0.46	-0.75 ± 0.54
L-leucine	2.49 ± 0.30	1.89 ± 0.29	2.09 ± 0.20	2.12 ± 0.25	1.84 ± 0.73	1.85 ± 0.49	2.10 ± 0.25	1.97 ± 0.47	1.89 ± 0.31	1.87 ± 0.64	2.15 ± 0.28	2.11 ± 0.19	1.68 ± 0.21	1.79 ± 0.31	1.91 ± 0.21	1.64 ± 0.49
	balance			0.20 ± 0.25	-0.03 ± 0.73	0.01 ± 0.49	0.25 ± 0.25	-0.08 ± 0.47	-0.02 ± 0.64	-0.02 ± 0.64	-0.04 ± 0.28	-0.43 ± 0.19	-0.43 ± 0.21	0.12 ± 0.31	-0.27 ± 0.21	-0.27 ± 0.49
L-glutamic acid	0.22 ± 0.05	0.23 ± 0.06	0.23 ± 0.04	0.29 ± 0.06	0.14 ± 0.18	0.17 ± 0.12	0.29 ± 0.07	0.22 ± 0.15	0.19 ± 0.10	0.11 ± 0.12	0.20 ± 0.14	0.21 ± 0.09	0.30 ± 0.11	0.25 ± 0.06	0.25 ± 0.04	0.12 ± 0.15
	balance			0.00 ± 0.06	0.06 ± 0.18	0.03 ± 0.12	0.12 ± 0.07	-0.03 ± 0.15	-0.08 ± 0.10	-0.08 ± 0.12	0.01 ± 0.14	0.09 ± 0.09	0.09 ± 0.11	0.00 ± 0.06	0.00 ± 0.04	-0.13 ± 0.15
α-amino butyric acid	0.10 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	0.08 ± 0.05	0.07 ± 0.08	0.08 ± 0.05	0.12 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0.02
	balance			0.00 ± 0.05	-0.02 ± 0.08	0.01 ± 0.05	0.04 ± 0.01	-0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	-0.02 ± 0.02
γ-amino butyric acid	0.28 ± 0.01	0.25 ± 0.08	0.26 ± 0.06	0.31 ± 0.04	0.35 ± 0.06	0.32 ± 0.04	0.37 ± 0.12	0.38 ± 0.10	0.37 ± 0.07	0.28 ± 0.08	0.39 ± 0.04	0.38 ± 0.03	0.36 ± 0.03	0.49 ± 0.06	0.48 ± 0.04	0.42 ± 0.08
	balance			0.01 ± 0.04	0.05 ± 0.06	-0.03 ± 0.04	0.05 ± 0.12	-0.01 ± 0.10	-0.09 ± 0.07	-0.09 ± 0.08	-0.01 ± 0.04	-0.02 ± 0.03	-0.02 ± 0.03	-0.01 ± 0.06	-0.01 ± 0.04	-0.06 ± 0.08

^a AB was calculated as indicated in materials and methods. ND, not detected.

synthesis and subsequent release of glycerol by the yeasts (22). A similar, albeit less marked, behavior was observed for acetic acid, the consumption of which was very low or null from the second criadera to the solera. Finally, the production of

acetaldehyde was seemingly not affected by blending. Thus, 7 days after blending, all cask rows exhibited synthesis and release of acetaldehyde in some cases but consumption of this compound in others. This differential behavior may be related to

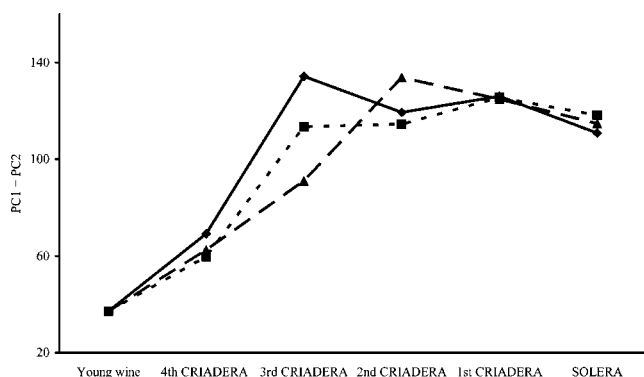


Figure 2. Sum evolution for the first and second components throughout the biological aging process before blending (◆), just after blending (■), and 7 days after blending (▲). The evolution was calculated taking into account that the first component had a weight of 76% relative to the second component.

its concentration in the wine—high concentrations facilitate consumption—and to the metabolic requirements of the yeasts depending on the availability of nitrogen-containing nutrients. Thus, the production of acetaldehyde by flor yeasts is not affected in a direct way by blending, but rather on specific unbalances in redox reactions, which favor the synthesis of this compound rather than to its oxidation in the tricarboxylic acid cycle (22).

Influence of Blending on Nitrogen Compounds. Young wine was found to contain 16 different amino acids; the most abundant of which were L-proline, L-leucine, L-glutamic acid, α -aminobutyric acid, and γ -aminobutyric acid (Table 3). Only L-proline, and to a lesser extent, L-leucine, were detected at high enough concentrations to enable yeast growth. L-Proline was the major nitrogen source for the yeasts (10). Its consumption peaked between the young wine stage and the fourth criadera. All cask rows exhibited L-proline consumption 7 days after blending; in some rows, the L-proline level fell below that existing prior to blending. Taking into account that oxidizing L-proline requires the presence of molecular oxygen (10), the consumption of this amino acid was restricted by the oxygen availability, so the process stopped when the oxygen concentration in the wine diminished until a certain concentration. At that point, the metabolic reaction may be inverted and small amounts of L-proline released into the wine. The metabolism and growth of flor yeasts is therefore influenced by the L-proline and oxygen concentrations in wine, hence the beneficial effect that the blending has on the development and preservation of flor biofilms.

To examine the effect of blending, we subjected data collected from different criaderas during the 1999 and 2000 years to principal components analysis. The variables included in the analysis were the contents in the compounds most deeply involved in the metabolism of flor yeasts, namely ethanol, acetaldehyde, glycerol, acetic acid, and L-proline. The first two principal components (PCs) were found to account for 86% of the total variance (66% the first and 20% the second). The statistical weights of each variable on the two components are shown in Table 4.

All compounds except ethanol had similar statistical weight on the first PC in absolute value. Note that the weights of ethanol and acetaldehyde were positive, whereas those of glycerol, acetic acid and L-proline were negative. As can be seen from Table 2, the ethanol and acetaldehyde contents increased during biological aging, the former through increased water evaporation and the latter through the action of alcohol dehydrogenase II

Table 4. Statistical Weight of the Compounds Used in the Principal Components Analysis and Explained Variance of the Components 1 and 2

compound	PC1	PC2
variance explained (%)	66	20
ethanol	0.118	-0.980
acetaldehyde	0.458	0.170
glycerol	-0.516	0.027
acetic acid	-0.533	-0.015
L-proline	-0.475	-0.094

(ADH II) isoenzyme on ethanol (6, 9). On the other hand, glycerol, acetic acid, and L-proline were used as nutrients by the yeasts, so their contents decreased throughout the aging process. Ethanol was the compound with the greatest weight on the second principal component.

Figure 2 shows the evolution of the sum of the first and the second principal components throughout the biological aging process. This evolution was calculated taking into account that the first component had a weight of 76% relative to the second component. The variation of the combined first and second component at blending and 7 days after blending was similar. In both, aging was maximal from the fourth to the third criadera.

The variation of the first and second PC 7 days after blending differed from the previous ones. Thus, the greatest differences were those between the third and second criadera, and it was at that point in the process of aging under flor yeasts that the first and second PC peaked.

The differences between the points prior to blending and 7 days after it were most marked in the third and second criadera. It is therefore in these rows where the biological activity of the yeasts is bigger. The criaderas holding older wine (viz. the first criadera and the solera) exhibited no difference by blending effect, so biological activity in the yeasts at these two stages, the last in the process, is weaker than the previous and physicochemical processes, must prevail over it. No differences were observed in the fourth criadera either, possibly, because it received younger wine each year, hence the heterogeneity increase, as has been reflected in the higher variance of its data. However, the analysis of the blending effect on each individual cask in the fourth criadera revealed the significance of the metabolic activity of the yeasts, which was reflected in the changes observed between the young wine and the fourth criadera.

The findings obtained in this study are important in the comprehension of the blending procedure carried out in the criaderas and solera system and for further investigations to accelerate the biological aging process for the making of sherry wine and other wine types with blending procedure involved.

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