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Evolution of fatty acid contents in Garnacha and Viura musts during fermentation and the aging of wine

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Abstract The evolution of fatty acids during the fermentation of *Vitis vinífera* var. Garnacha and var. Viura musts as well as during the aging of the rosé and white wines produced from the said musts was studied. In Garnacha must, practically all the fatty acids were consumed, with the exception of the medium-chain fatty acids, by the time that 50% of the sugar was used up. During the second half of fermentation 80.1% of the fatty acids were consumed, with 28.8% of the remaining fatty acids being used up during aging. In Viura must, the total fatty acid concentration declined 46.9% during the first half of fermentation (first 50% of sugar), most noteworthy was the high consumption of unsaturated large-chain fatty acids (72.3%); during the second half of fermentation, 77.2% of the fatty acids were used, with high consumption of the large-chain saturated and unsaturated acids. During the aging of wine, medium-chain fatty acids were excreted and a small amount of unsaturated acids was consumed.

Key words Fatty acids · Musts · Fermentation · Wine · GC

Introduction

Fatty acids and sterols influence the metabolism and growth of yeast since both are lipid constituents of its membranes [1]. Unsaturated fatty acids are, under anaerobic conditions, growth factors for yeast [2, 3], they regulate the uptake of nutrients through the membrane [4, 5], and increase the resistance of these microorganisms in the face of elevated concentrations of ethanol [6, 7]. As well as using unsaturated fatty acids for the development and proper function of its plasma membranes, yeast also requires the presence of a specific quantity of saturated fatty acids [8]. However, some saturated fatty acids are toxic to yeast; thus, caprylic and caproic acids can prematurely stop fermentation [9] since they function synergistically with ethanol as toxins to the yeast [10–13].

Studies of the lipid composition of the grape and must are relatively recent. Miele et al. [14] studied the fatty acid composition of different lipid fractions from pulp, skin, must and seeds of *Vitis vinifera* Cabernet Sauvignon; their results show that phospholipids predominate in the pulp and skins while neutral lipids are found primarily in the seeds. Other investigators, such as Higgins and Peng [15], isolated glucolipids, phospholipids and neutral lipids in the skin and pulp of Concord grapes, where the predominant glucolipids are rich in linolenic and pelargonic acids. Bauman et al. [16] observed that the ripening of the grape increases the neutral lipid fraction, the predominant fatty acids being myristic, palmitic and stearic acids. The seed composition was found to be 15% oil, predominantly linoleic acid (71%) [17]. There are indications that the fatty acids linoleic and linolenic function as precursors of aromatic compounds, of six-carbon aldehydes and of higher alcohols [18].

It has also been observed that the lipid composition varies according to the variety of grape. Gallander and Peng [19], after analysing six varieties of grape (Cabernet Sauvignon, White Riesling, DeChaunac, Seyval, Catawba and Niagara), found that the total lipid concentration varied between 0.15% and 0.24%.

The aim of this study was to observe the evolution of the fatty acid content during the fermentative process in musts of *V. vinifera* var. Garnacha and *V. vinifera* var. Viura and also during the aging of the stabilized and bottled rosé and white wines.

Materials and methods

Samples and vinification. Wines were produced from *V. vinifera* var. Garnacha and *V. vinifera* var. Viura grapes, by a previously described procedure [20].

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Table 1 Evolution of general parameters of Garnacha and Viura musts and the wines produced (n = 6, SE = error standard)

		Turbidity (NTU[a] ± SE)	Reducing sugars (g/l ± SE)	pH ± SE	Volatile acidity (g/l[b] ± SE)	Ash (g/l ± SE)	SO_2 total (mg/l ± SE)	Alcohol (v/v% ± SE)
Garnacha	Must	1460 ± 14	205 ± 1	3.31 ± 0.01	–	2.80 ± 0.01	53.31 ± 0.01	–
	Midpoint of fermentation		98 ± 3	3.15 ± 0.01	–	2.69 ± 0.01	47 ± 5	–
Rosé wine	Recently produced		0.86 ± 0.04	3.11 ± 0.01	0.19 ± 0.03	2.24 ± 0.06	64 ± 6	12.3 ± 0.1
			0.96 ± 0.01	3.12 ± 0.01	0.27 ± 0.04	1.06 ± 0.03	78.8 ± 0.4	12.4 ± 0.1
Viura	Must	695 ± 7	179.7 ± 1.7	3.51 ± 0.01	–	3.4 ± 0.1	48.5 ± 0.2	–
	Midpoint of fermentation		74 ± 2	3.32 ± 0.01	–	3.1 ± 0.2	48.9 ± 0.2	–
White wine	Recently produced		0.45 ± 0.08	3.28 ± 0.01	0.14 ± 0.01	1.6 ± 0.1	55.6 ± 0.9	10.7 ± 0.1
	Stabilized and aged		0.27 ± 0.02	3.35 ± 0.01	0.23 ± 0.02	1.4 ± 0.1	89.1 ± 0.1	10.7 ± 0.1

[a]Nephelometric turbidity units
[b]As g/l acetic acid

Table 2 Total fatty acid concentrations (mg/l) of Garnacha and Viura musts and in the wines produced

Fatty acids	Initial must	Midpoint of fermentation	Wine recenty produced	Wine stabilized and aged
Garnacha				
Medium chain[a]	1.3	8.2	4.6	4.4
Large-chain, saturated[b]	39.7	17.0	1.6	0.5
Large-chain, unsaturated[c]	29.4	11.4	1.1	0.3
Total	70.4	36.6	7.3	5.2
Viura				
Medium chain[a]	2.1	5.7	1.5	5.1
Large-chain, saturated[b]	12.8	6.6	1.4	1.6
Large-chain, unsaturated[c]	17.3	4.8	1.0	0.8
Total	32.2	17.1	3.9	7.5

[a] Sum of concentrations of $C_{8:0}$, $C_{10:0}$, $C_{12:0}$
[b] Sum of concentrations of $C_{13:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$
[c] Sum of concentrations of $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$

Extraction and GC analysis of fatty acids. The lipid fraction was extracted by the procedure of Darné and Madero-Tamargo [21] based on first adding ethanol (10 ml) and doubly distilled water (10 ml) to 20 ml of sample, homogenization with an Ultra-Turrax T25 (8000 rpm for 2 min), and then extraction with chloroform (20 ml). Fatty acid derivatization was done following the method recommended in the Código Alimentario Español [22], which is based on the formation of methyl esters. The methyl esters formed were extracted using n-hexane. Determination of fatty acids was performed using a Perkin-Elmer 8420 gas chromatograph (Perkin-Elmer Corporation, Norwalk, Conn., USA) equipped with a flame ionization detector and a fused silica capillary column (Supelcowax 10; 30×0.25 mm i.d.). The injector and detector temperatures were both 230 °C. The initial oven temperature was 120 °C, increased at 3 °C per minute up to 200 °C, at which it was maintained for another 35 min. Helium was the carrier gas.

Standard solutions for the analysis of the fatty acids (Matreya, Pleasant Gap, Pa., USA) by GC were prepared for different concentrations between 10 and 250 mg/l. Internal standards were methyl heptadecanoate and methyl undecanoate (Sigma, St. Louis, Mo., USA). For the preparation of methyl esters, sodium methoxide 0.2 M (0.5 g of sodium metal in 100 ml of anhydrous methanol) and hydrochloric acid at 4% (w/w) in methanol (prepared by passing a stream of hydrogen chloride through anhydrous methanol) were used. Reagents employed were from Panreac (Montcada i Reixac, Barcelona, Spain).

Enological parameters. The enological parameters were measured according to the methods described by the Office International de la Vigne et du Vin [23]. The turbidity of the must was determined using a model 18900 Hach turbidimeter (Hach Loveland, Conn., USA) prepared for coloured samples.

Results and discussion

Garnacha must and rosé wine

The turbidity of Garnacha must was 1460 NTU (Table 1). During fermentation to dryness, the pH became slightly more acidic as a result of the precipitation of various salts, especially bitartrates. The pH of the recently produced wine was within the optimum interval (3.0–3.7) for its age [24]. The volatile acidity in the wine were adequate, so they should not change over time. The ash content diminished due to the precipitation of organic acid salts favoured, among other factors, by the presence of ethanol; yeast uses mineral substances for structural purposes and as cofactors [25] which also contributes to this decrease.

It is observed (Table 2) that the total concentration of fatty acids in the musts was 70.4 mg/l and that the unsaturated fatty acids (palmitoleic, oleic, linoleic and linolenic) accounted for 41.8% of the total fatty acids; among the saturated fatty acids, palmitic and stearic acids were most concentrated (Fig. 1), amounting to 51.1% of the total. These results coincide with those of various authors for musts produced from different grape varieties [26–28]. On the other hand, the concentration of unsaturated fatty acids (29.4 mg/l) in Garnacha must was less than that of saturated fatty acids (41 mg/l). The ratio of unsaturated to

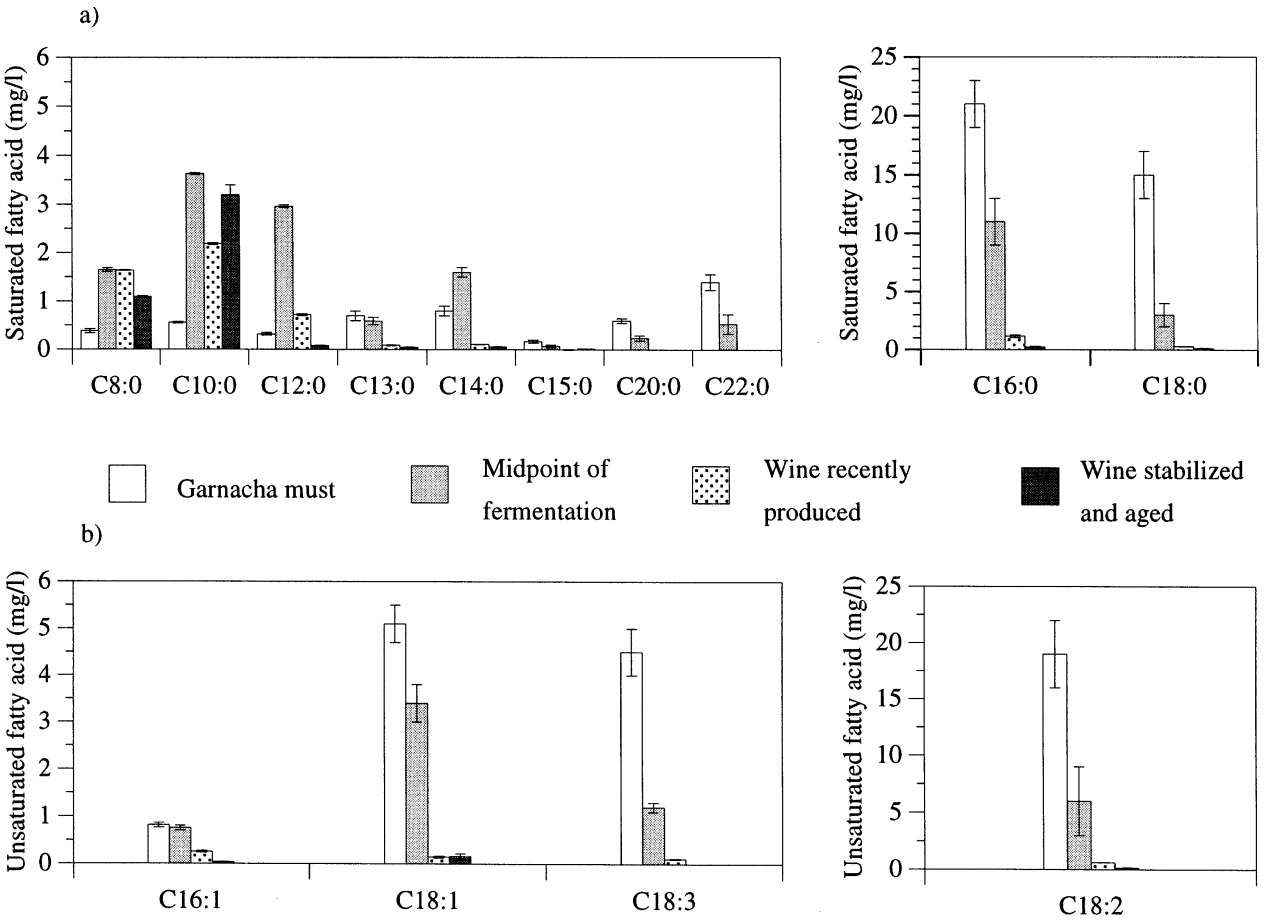

Fig. 1a,b Concentration of fatty acids in different steps of rosé vinification: **a** saturated and **b** unsaturated ($n=6$, values are shown with standard errors)

saturated fatty acids was 0.7, a ratio similar to that determined by Miele et al. [14] for Cabernet Sauvignon must.

In the first half of fermentation (until 50% sugar consumption) the total fatty acid concentration decreased 48.0% with respect to the initial concentration (Table 2). The start of the fermentation was rapid which probably implies that the yeast had begun its development without the need for synthesizing fatty acids which favoured a good fermentation rate [29]. The concentrations of medium-chain fatty acids studied, $C_{8:0}$, $C_{10:0}$ and $C_{12:0}$, increased during the first half of fermentation, reaching a concentration of 8.2 mg/l (Table 2). At the end of this step, the sum of $C_{8:0}$ and $C_{10:0}$ was 5.3 mg/l, a concentration sufficient, according to Geneix et al. [10] (3 mg/l) to reduce the yeast population in synthetic media. However, this does not influence the evolution of fermentation since it is possible that colloidal substances present in the must absorb these acids, thus impeding their toxic actions [30].

During this step, the total concentration of large-chain fatty acids ($C_{13:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:0}$, $C_{22:0}$) diminished 58.9%. Saturated large-chain fatty acids were consumed (57.2%), but $C_{14:0}$ was excreted (Fig. 1). The consumption of $C_{16:0}$ was 47.6% and the consumption of $C_{18:0}$ was 80%. The presence of $C_{16:0}$ and $C_{18:0}$ in the cellular membranes favours the proper functioning of transport systems for amino acids [31]; in this sense, this must exhibited a good uptake of amino acids [20, 32] possibly favoured, among other factors, by the consumption of these fatty acids. The total consumption of large-chain unsaturated fatty acids ($C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$) was 61.2%. For the mono-unsaturates ($C_{16:1}$ and $C_{18:1}$), considered together with sterols as growth factors for yeast in the absence of oxygen [33–35], 33.3% of $C_{18:1}$ was consumed and the concentration of $C_{16:1}$ was unchanged with respect to the initial values. The polyunsaturates ($C_{18:2}$ and $C_{18:3}$), which cannot be synthesized by *Saccharomyces cerevisiae* [3], were each consumed by about 70% (Fig. 1).

In the second half of fermentation (from 50% sugar consumption until the end of fermentation), it was observed that the total fatty acid concentration decreased in the rosé wine by 80.1% with respect to the fermentation midpoint (Table 2). Total medium-chain fatty acids $C_{10:0}$ and $C_{12:0}$ were consumed by 43.9%, whereas the concentration of $C_{8:0}$ was maintained (Fig. 1).

During this step, the total concentration of large-chain saturated fatty acids ($C_{13:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$) fell by 90.6% in the rosé wine. These fatty acids were each consumed by between 71 and 100%. The consumption of these fatty acids is due to yeast decreasing its growth and biosynthetic activity at the end of fermentation [9] and not being able to synthesize these fatty acids [8]. The large-chain unsaturated fatty acids ($C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$) were

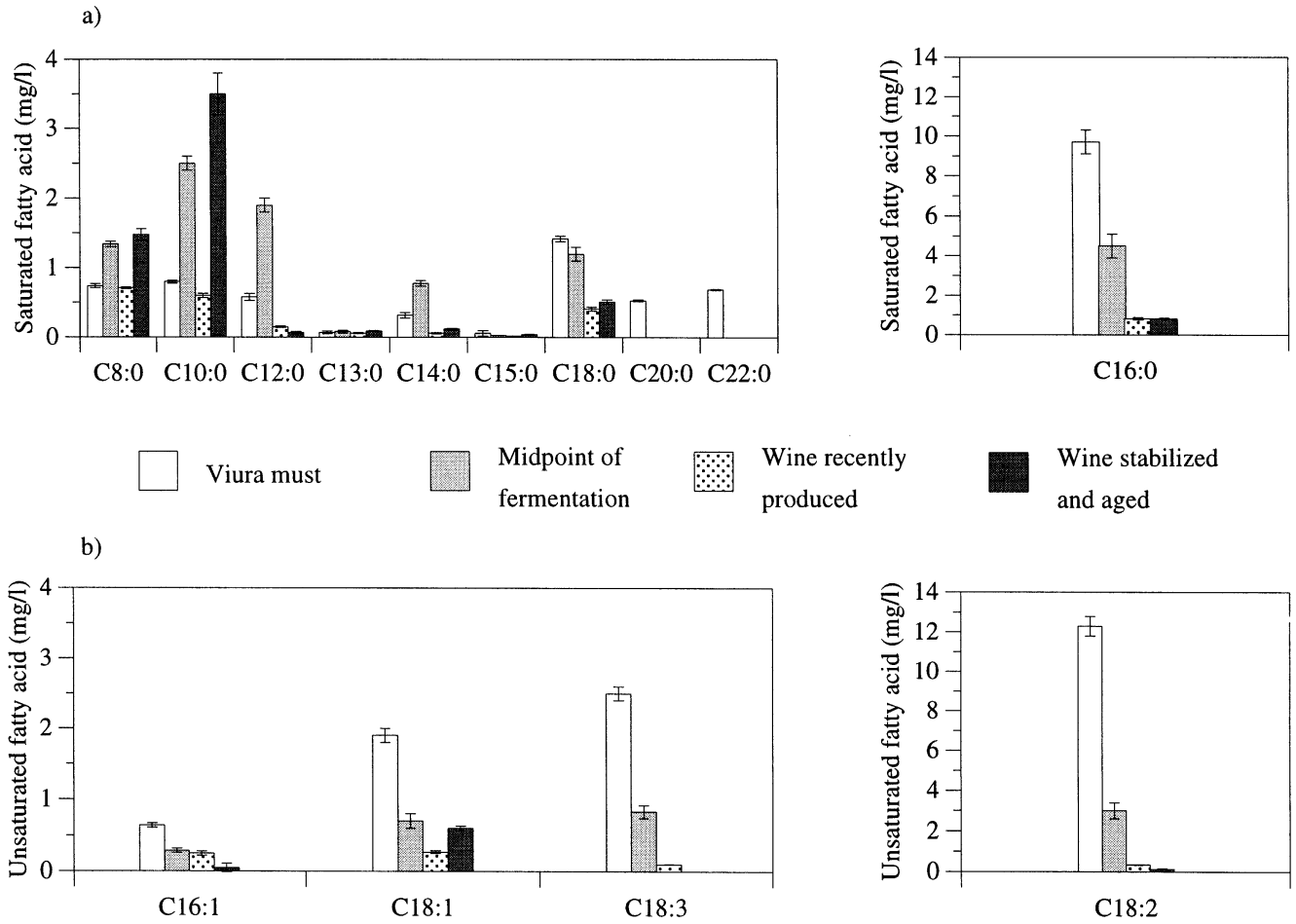

Fig. 2a,b Concentration of fatty acids in different steps of white vinification: **a** saturated and **b** unsaturated ($n=6$, values are shown with standard errors)

consumed by 90.4%. In this respect, Houtman and du Plessis [36] observed that the proliferation of yeast during the fermentative process resulted in a depletion of unsaturated fatty acids in the medium, especially under anaerobic conditions, since these compounds (together with phospholipids, sterols, carotenes and squalene) act like hydrogen acceptors in yeast metabolism [37, 38].

The concentrations of fatty acids in rosé wine that was stabilized and aged in a bottle for 1 year are represented in Fig. 1. The total fatty acid concentration decreased by 28.8% (Table 2). The consumption of fatty acids, despite the fact that autolysis of yeast occurs in this step, can be due, among other factors, to the possible development of residual microorganisms. In this respect, Herraiz et al. [39] found that 38 days after the end of fermentation, a reduction in the concentration of free fatty acids can be produced in the wine and related to possible residual yeast activity.

Viura must and white wine

The turbidity of Viura must was 695 NTU (Table 1). The pH decreased during vinification and its value in the recently produced wine was within the optimum interval (3.0–3.7) for its age [23]. The volatile acids were within the normal range for conservation of the wine. The ash content diminished during the fermentation.

The total concentration of fatty acids in Viura must was 32.2 mg/l (Table 2), a value much smaller than that encountered in the red variety Garnacha must (70.4 mg/l). These results are similar to those of Gallander and Peng [19] who found greater lipid concentrations in red varieties than in white in a study of six different varieties. Additionally, the fatty acid concentration in the must depends on the skin-must maceration time [28] which was greater in the Garnacha than in the Viura must. The concentration of unsaturated fatty acids (17.3 mg/l) was greater than that of the saturated fatty acids (14.9 mg/l). The ratio of unsaturated to saturated fatty acids was 1.2.

In the first half of fermentation, the total fatty acid concentration decreased 46.9% (Table 2) with respect to the initial concentration. The total concentration of large-chain fatty acids ($C_{13:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:0}$, $C_{22:0}$) diminished by 62.1%. In general, saturated fatty acids were consumed but $C_{14:0}$ was excreted (Fig. 2). The consumption of $C_{16:0}$ was 53.6% and that of $C_{18:0}$ was 15.5%. Both saturated acids enable the proper functioning of the glucose and amino acid transport systems of yeast [30] and, therefore, this sample exhibited good utilization of the free amino acids in the medium [32].

During the first half of fermentation, the total consumption of large-chain unsaturated fatty acids was 72.3%. The mono-unsaturate $C_{16:1}$ was consumed 54.7%, and $C_{18:1}$, 63.2% (Fig. 2). The polyunsaturates $C_{18:2}$ and $C_{18:3}$, which cannot be synthesized by *S. cerevisiae* [3], were consumed around 70% each (Fig. 2). The availability of these fatty acids in the medium favours the fermentative process [28] and activates yeast development [40]. The medium-chain fatty acids studied, i.e. $C_{8:0}$, $C_{10:0}$ and $C_{12:0}$, increased during the first half of fermentation, reaching a concentration of 5.7 mg/l (Table 2). At the end of this step, the sum of $C_{8:0}$ and $C_{10:0}$ was 3.8 mg/l.

In the second half of fermentation, it is observed that the total fatty acid concentration decreased 77.2% with respect to the fermentation midpoint (Table 2). The medium-chain fatty acids $C_{8:0}$, $C_{10:0}$, $C_{12:0}$ were consumed by 73.7% (Fig. 2). When studying synthetic media, Rosi and Bertuccioli [7] found that these fatty acids decreased on the 7th and 8th days of fermentation. The total concentration of large-chain saturated fatty acids ($C_{13:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$) fell by 78.8% in the white wine. The high consumptions of $C_{14:0}$ (92.3%), $C_{16:0}$ (81.8%) and $C_{18:0}$ (65.8%) are noteworthy (Fig. 2). The total consumption of large-chain unsaturated fatty acids was 79.2%. The high consumption of $C_{18:3}$ (88%), $C_{18:2}$ (88.7%) and $C_{18:1}$ (61.4%) was also of note; however, $C_{16:1}$ was not consumed.

Figure 2 shows the fatty acid concentrations in the samples after stabilization and aging of the wine in a bottle for 1 year. In general, the quantity of fatty acids increased with respect to the recently produced wine; this increase was due in particular to a significant excretion of medium-chain fatty acids ($C_{8:0}$ and $C_{10:0}$) and of $C_{18:1}$. The liberation of fatty acids into the medium during the aging process is due, possibly, to the predominance of yeast autolytic processes, rather than consumption of these substances by residual microorganisms [41].

The main conclusions of this work are that in spite of different initial concentrations of fatty acids in both musts (i.e. Garnacha and Viura), their evolution during fermentation was similar. In the first half of fermentation, the fatty acids were consumed, predominantly the unsaturated large-chain fatty acids, while those of medium-chain length were excreted. In the second half of fermentation, the fatty acids were consumed, even those of medium chain length. In the rosé wine aged for 1 year in a bottle, fatty acids were consumed, but in the white wine, medium-chain saturated acids were excreted, and large-chain unsaturated fatty acids were used, although not very much.

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