

Effect of Flash Release Treatment on Phenolic Extraction and Wine Composition

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The flash release (FR) process, consisting of rapidly heating the grapes and then applying strong vacuum, has been proposed to increase the polyphenol content of red wines. Its impact on polyphenol extraction kinetics and on the polyphenol composition of red juice and wines was studied over two seasons on different grape varieties (Grenache, Mourvedre, Carignan). The FR process allows fast extraction of all phenolic compounds (hydroxycinnamic acids, flavonols, anthocyanins, catechins, proanthocyanidins) and can be used to produce polyphenol-enriched grape juices. However, the concentration of all polyphenols dramatically decreased throughout fermentation when pressing was achieved immediately after FR. The FR wines made with pomace maceration were also enriched in polyphenols compared to the corresponding control wines. Increasing the duration of high-temperature exposure in the FR treatment further increased extraction of phenolic compounds but also accelerated their conversion to derived species. The tannin-to-anthocyanin ratio was particularly low in the wine fermented in the liquid phase, higher after FR than in the control, and even higher after longer heating. FR resulted in an increased tannin-to-anthocyanin ratio and an increased conversion of anthocyanins to tannin-anthocyanin adducts showing the same color properties as anthocyanins. The tannin-to-anthocyanin ratio was particularly low in the wine fermented in the liquid phase that also contained larger amounts of orange sulfite bleaching-resistant pigments.

KEYWORDS: Flash release; polyphenols; must; wine; extraction; anthocyanins; tannins

INTRODUCTION

Phenolic compounds are an important group of substances that are responsible for major wine sensorial characteristics such as color and astringency (1, 2) and may also contribute health benefits. Wine phenolic composition depends on the grapes used to make the wine and on the wine-making conditions (3, 4). Factors that determine the extraction of anthocyanins (i.e., the red grape pigments) and of proanthocyanidins (i.e., the grape tannins) are particularly important for wine quality. Besides, both groups of molecules are highly reactive and proceed to various derived tannins and pigments, including tannin derivatives, tannin anthocyanin adducts, anthocyanin polymers, and other anthocyanin derivatives. Wine sensory properties are largely determined by the nature and quantities of these reaction products. Relative amounts of tannins and anthocyanins appear to be particularly important among the various factors controlling these reactions (5) and can be modulated by the extraction conditions (6).

In a recent review, Sacchi et al. have reported that six winemaking variables and techniques increase phenolic con-

centration in red wines (7). Such treatments include thermovincification, must freezing, and use of pectolytic enzymes that may damage the cell and vacuole membranes, and thus increase phenolic extraction into the liquid phase. To complete this review, we studied the flash release (FR) process that is also expected to degrade the cellular structures and to increase phenolic extraction (8). This technique consists of heating the grapes quickly at high temperature (>95 °C) with biological vapor (i.e., steam produced from the water present in the grape, without dilution) at atmospheric pressure and then placing them under a strong vacuum which causes instant vaporization. The vaporization induces a fragilization of the cell wall and a cooling of the treated grapes. A preliminary study showed that the FR technology gives a high increase in the quantity of pigments in the wines. The quantity of total phenolics was reported to be 50% higher than that observed in the control wines (8). However, the wine polyphenol data provided was based only on spectrophotometric indices. Besides, no information is available on the effect of FR process on polyphenol extraction kinetics, so that it is not known whether it enables the production of polyphenol enriched juices.

In the present study, the FR treatment was applied on three grape varieties in different vintages and the evolution of the polyphenolic composition (anthocyanins, flavonols, hydroxy-

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cinnamic acids, and flavanols, including catechin and epicatechin monomers and proanthocyanidins) has been monitored by high performance liquid chromatography coupled with diode array detection (HPLC-DAD) during the fermentation of FR-treated and control musts. Detailed analysis of proanthocyanidin composition was achieved by HPLC after thiolysis, which gives access to total quantity of proanthocyanidins, the percentages of galloylated units (originating mostly from seeds) and of epigallocatechin units (specific of skin tannins), and the mean degree of polymerization. These parameters are of particular importance to the wine taste properties.

MATERIALS AND METHODS

Grapes. Grapes from *Vitis vinifera* var. Grenache (2003 and 2004), Carignan (2004), and Mourvedre (2003) grown in an INRA experimental Unit station located in Gruissan in southern France were harvested at commercial maturity (Grenache 2003, 23.3 °Brix; Mourvedre 2003, 22.1 °Brix; Carignan 2004, 21.8 °Brix; Grenache 2004, 21.7 °Brix).

Preparation of Control Trials. Three 100-kg lots of grape clusters of each variety (Grenache, Carignan, Mourvedre) were crushed and destemmed using a destemmer–crusher and put in three 100-L stainless steel tanks to yield triplicate control lots.

Preparation of Flash Release Trials. The treatment by FR consisted of destemming and crushing the grapes, heating them at 95 °C in 6 min with biological vapor, and then submitting them to a strong vacuum (>100 mbar). Three 100-kg lots of the must obtained after FR were used to fill three 100-L tanks. Two additional experiments were performed on the Grenache 2004 sample. The first one consisted of maintaining the grapes longer (15 min) at 95 °C before releasing the pressure, the second one of pressing the must immediately after FR to remove the seeds and skins and fermenting the resulting juice.

Fermentation. All fermentations were carried out in 100-L stainless steel tanks equipped with temperature control (28 °C) unabling to regulate fermentation kinetics. Each lot was sulfited (5 g/100 kg), added with Fermivin yeast (10 g/100 kg), and fermented to completion. The cap was punched down daily to ensure mixture of the marc with the fermenting liquid phase. The fermentation curves were recorded. After 5 days of maceration, when alcoholic fermentation was finished (controlled by sugar analysis), the musts were pressed and the wines were stored in 50-L tanks and added with lactic bacteria to induce malolactic fermentation. Must samples were collected daily during fermentation (after the cap-punching operation) and after pressing (WBM) and were immediately frozen.

The malolactic fermentation was followed by paper chromatography. At the end of the malolactic fermentation, the wine was racked in a 30-L inox tank. Then, it was stored at low temperature (−4 °C) to induce the tartaric stability. After, the wines were bottled and stored in a cellar at 18 °C.

Sample Preparation and Analysis. *Extraction of Plant Material and Pomace.* For the Grenache 2004 trial, grape and pomace samples were also taken, frozen, and freeze-dried.

Fresh grape samples were manually separated into skins, seeds, and pulp. Each part was frozen in liquid nitrogen, ground, and freeze-dried. Pomace samples were taken after pressing, frozen in liquid nitrogen, and ground.

Aliquots of skin, seed, pulp (100 mg each), and pomace (2 g) powders were weighed into Erlenmeyer flasks and extracted with 10 mL (15 mL for pomace) of acetone/water/TFA (60:40:0.05 v/v/v) for 30 min at 4 °C under stirring. The extracts were centrifuged (30 min, 6000 rpm), and the material was re-extracted with 10 mL (15 mL for pomace) of the same solvent (30 min). The combined supernatants were evaporated to dryness in vacuo, and the residue was dissolved in 1 mL (10 mL for pomace) of methanol/0.5% HCl (v/v).

Analysis of Anthocyanins, Hydroxycinnamic Acids, Flavonols, Catechins, and Flavanol Anthocyanin Dimer Adducts in Musts and Wines. Anthocyanins, hydroxycinnamic acids, flavonols, catechins, and flavanol anthocyanin dimer adducts were analyzed by direct injection of the solutions in acidified methanol as described earlier (9).

Must samples were filtered through a 0.45 μm HATF Millipore membrane prior to analysis, and wines were directly injected into the HPLC system. HPLC-DAD analyses were performed using a Waters 2690 system equipped with an autosampler system, a Waters 996 photodiode array detector, and a Millennium 32 chromatography manager software (Waters, Milford, MA). Separation was achieved on a reverse-phase Atlantis dC₁₈ column (250 × 2.1 mm, 5 μm packing) protected with a guard column of the same material (20 × 2.1 mm, 3 μm packing) (Waters, Milford, MA). The elution conditions were as follows, 0.250 mL/min flow rate; oven temperature 30 °C; solvent A, water/formic acid (95:5 v/v); solvent B, acetonitrile/water/formic acid (80:15:5 v/v/v); elution began with linear gradients from 0 to 2% B in 10 min, from 2 to 10% B in 10 min, from 10 to 20% B in 20 min, from 20 to 30% B in 5 min, from 30 to 40% B in 5 min, from 40 to 50% B in 5 min, followed by washing and re-equilibration of the column. The injection volume for all samples was 5 μL. Calibration curves were established using the following external commercial standards of analytical grade: catechin and epicatechin standard (Extrasynthese, France) to quantify, respectively, catechin and epicatechin at 280 nm, t-caftaric acid isolated in our laboratory (10) to quantify hydroxycinnamic acids at 320 nm, quercetin-3-glucoside from Extrasynthese (Genay, France) to quantify flavonols at 360 nm, and malvidin 3-O-glucoside from Extrasynthese (Genay, France) to quantify red pigments at 520 nm.

Proanthocyanidins Analysis. The skin, seed, pulp and pomace extracts dissolved in acidified methanol (100 μL) were added with 100 μL of a thiol solution (5% toluene-α-thiol in 0.2 M HCl in methanol). The mixture was heated at 90 °C for 2 min and then cooled in cold water. Then 10 μL of the end mixture was injected into the same HPLC system as for the previous method.

Must and wine proanthocyanidins were recovered by methanol precipitation as described earlier (11). Proanthocyanidin composition was then analyzed by thiolysis followed by HPLC analysis (9). The concentrations of (+)-catechin, (−)-epicatechin, and benzylthioether derivatives of (−)-epigallocatechin, (+)-catechin, (−)-epicatechin, and (−)-epicatechin 3-gallate and the total quantity of tannins in mg/L were determined from peak areas at 280 nm using calibration curves established using external standards, either commercial ((+)-catechin and (−)-epicatechin) or isolated, and purified in our laboratory (benzylthioether derivatives). The mean degree of polymerization (mDP) was calculated as the ratio between the summed molar concentrations of all released constitutive units and the summed molar concentrations of lower constitutive units.

Colorimetric Measurements. Absorbance measurements were made with a UV mc² spectrophotometer (Safas) as described earlier (12). Absorbance values at 420 (A420), 520 (A520), and 620 nm (A620) were measured directly in a 1-mm light path cell and converted to absorbance values (A420, A520, A620) with a 10-mm light path. Tint (*T*) was calculated as A420/A520 (13) and color intensity (CI) as A420 + A520 + A620 (14).

Color due to derivatives resistant to sulfite bleaching was determined at 520 nm, 10 min after addition of a metabisulfite solution and the percentage of sulfite bleaching-resistant pigments was calculated (15). Total polyphenol index (TPI) was defined as the absorbance at 280 nm of wine diluted in 2% HCl.

RESULTS AND DISCUSSION

The concentrations of hydroxycinnamic acids, anthocyanins, flavonols, and flavanols (monomers and polymers) were monitored during fermentation in control and FR-treated musts.

Effect of Flash Release on Juice Polyphenol Composition. Analysis of the musts before fermentation (Table 1) showed that the control juices contained mostly hydroxycinnamic acids, along with some proanthocyanidins, especially in the Mourvedre variety, which reflects grape flesh composition. Those obtained after FR treatment contained much larger quantities of all polyphenols than the control musts, indicating that FR allowed fast extraction of phenolics from the grape solids. The rather low hydroxycinnamic acid levels measured in the control musts suggest that the samples have undergone some enzymatic

Table 1. Effect of Flash Release on the Must Polyphenol and Proanthocyanidin Compositions (in mg/L)

	Polyphenol			
	anthocyanins	flavonols	hydroxycinnamic acids	catechins
Grenache 2003				
control	1.2 ± 0.4	1.41 ± 0.33	152.8 ± 16.5	1.47 ± 0.42
flash release	165.9 ± 9.0	24.3 ± 0.8	329.9 ± 14.4	55.5 ± 4.3
Mourvedre 2003				
control	2.0 ± 0	0	19.7 ± 3.4	3.33 ± 0.8
flash release	201.7 ± 1.9	57.7 ± 0.8	38.2 ± 3.1	30.4 ± 0
Grenache 2004				
control	0	0	26.8 ± 7.8	0
flash release	100.6 ± 8.7	7.4 ± 1.2	228.8 ± 16.8	43.7 ± 6.4
Carignan 2004				
control	0	0	9.02 ± 2.97	0
flash release	351.1 ± 24.2	35.4 ± 0.4	126.6 ± 8.4	27.0 ± 2.1
	Proanthocyanidin			
	proanthocyanidins	DPm	% gall	% EGC
Grenache 2003				
control	30.8 ± 16.0	6.3 ± 0.7	2.4 ± 0.7	9.6 ± 4.5
flash release	383.3 ± 53.6	4.1 ± 0.4	1.9 ± 0.5	11.9 ± 0.9
Mourvedre 2003				
control	317.2 ± 26.9	19.1 ± 1.9	4.6 ± 0.7	7.2 ± 1.1
flash release	589.9 ± 17.9	8.7 ± 0.2	3.7 ± 0.1	11.4 ± 0.2
Grenache 2004				
control	61.5 ± 23.6	6.9 ± 1.3	4.24 ± 1.33	0
flash release	320.3	2.8 ± 0.5	1.75 ± 0.40	6.35 ± 0.9
Carignan 2004				
control	71.1 ± 12.9	10.5 ± 0.9	2.81 ± 0.31	0
flash release	338.4	4.53 ± 0.1	1.77 ± 0.60	14.6 ± 1.2

oxidation during crushing or sampling. The much higher values measured in the FR musts are due, on one hand, to increased extraction of hydroxycinnamic acids from skins, and on the other hand, to inhibition of grape polyphenoloxidase in the FR process.

Proanthocyanidin composition was also qualitatively different in the control and FR musts (**Table 1**). Their mDP was significantly higher in the control than in the FR-treated musts. This may be due to easier extraction of lower-molecular-weight tannins from the skins and seeds, as described earlier (6), or to cleavage of proanthocyanidins as a result of the heating process (16) in the FR samples. Proanthocyanidins in the musts obtained by FR also contained significantly larger proportions of epigallocatechin units and slightly lower proportions of galloylated units. Since the former are specific of skins (9) and the latter more abundant in seeds (17), these compositional differences indicate that the FR treatment increased extraction of tannins from skins rather than from seeds.

Effect of FR on Phenolic Extraction Kinetics throughout Fermentation. The fermentation curves, recorded for each tank, showed good repeatability of the triplicate fermentation kinetics for all treatments. The lag phase before fermentation started was slightly shorter in the FR musts. This may be due to an increased release of some yeast nutrients by the FR treatment.

Changes in phenolic composition were monitored during fermentation. They are illustrated using the data obtained on Grenache variety in 2004 (**Figure 1**) as similar trends were observed for all three varieties and in both vintages.

Gradual extraction of all phenolic compounds took place during fermentation of the control musts. The levels of catechins, flavonols, and proanthocyanidins also increased during fermentation of FR-treated musts, whereas the concentration of hydroxycinnamic acids remained constant and that of anthocyanins decreased during the first day and then leveled off.

Anthocyanin losses are due to their fast involvement in degradation and/or polymerization reactions, which are known to take place during wine aging but actually occurred very early in the wine-making process. The mDP of proanthocyanidins decreased in the control musts at the beginning of fermentation due to extraction of lower-molecular-weight flavonols, as discussed above. Then, the mDP values remained constant and slightly higher in the control musts throughout fermentation. The concentration of galloylated units increased throughout fermentation in both musts, reflecting the gradual extraction of seed tannins as the ethanol level increases. The proportion of epigallocatechin units first increased and then decreased slowly during fermentation for both control and FR musts. The epigallocatechin units come from skins and are easy to extract due to their polarity and localization (6). During vinification, the quantity of epigallocatechin units increased, indicating extraction of tannins from skins, but their relative contribution to proanthocyanidin composition decreased as other units were proportionally more extracted. The increasing percentage of galloylated units reflects the increasing contribution of tannins originating from seeds as fermentation proceeds.

Effect of FR Treatment and Heating Conditions on Wine Polyphenol Composition. FR wines contained larger amounts of flavonols, catechins, and proanthocyanidins than the control wines and similar (or slightly lower) amounts of hydroxycinnamic acids (**Table 2**). The wine anthocyanin content was also increased after FR, from 4% in the Grenache 2003 wines to 30% in the Grenache 2004 samples, as observed earlier for Cabernet Sauvignon (8). Values of mDP of proanthocyanidins in control and FR wines were almost identical. The FR-treated wines contained higher percentages of galloylated units and lower proportions of epigallocatechin units than the control wines. Again, this presumably reflects different extraction kinetics of tannins from seeds and skins.

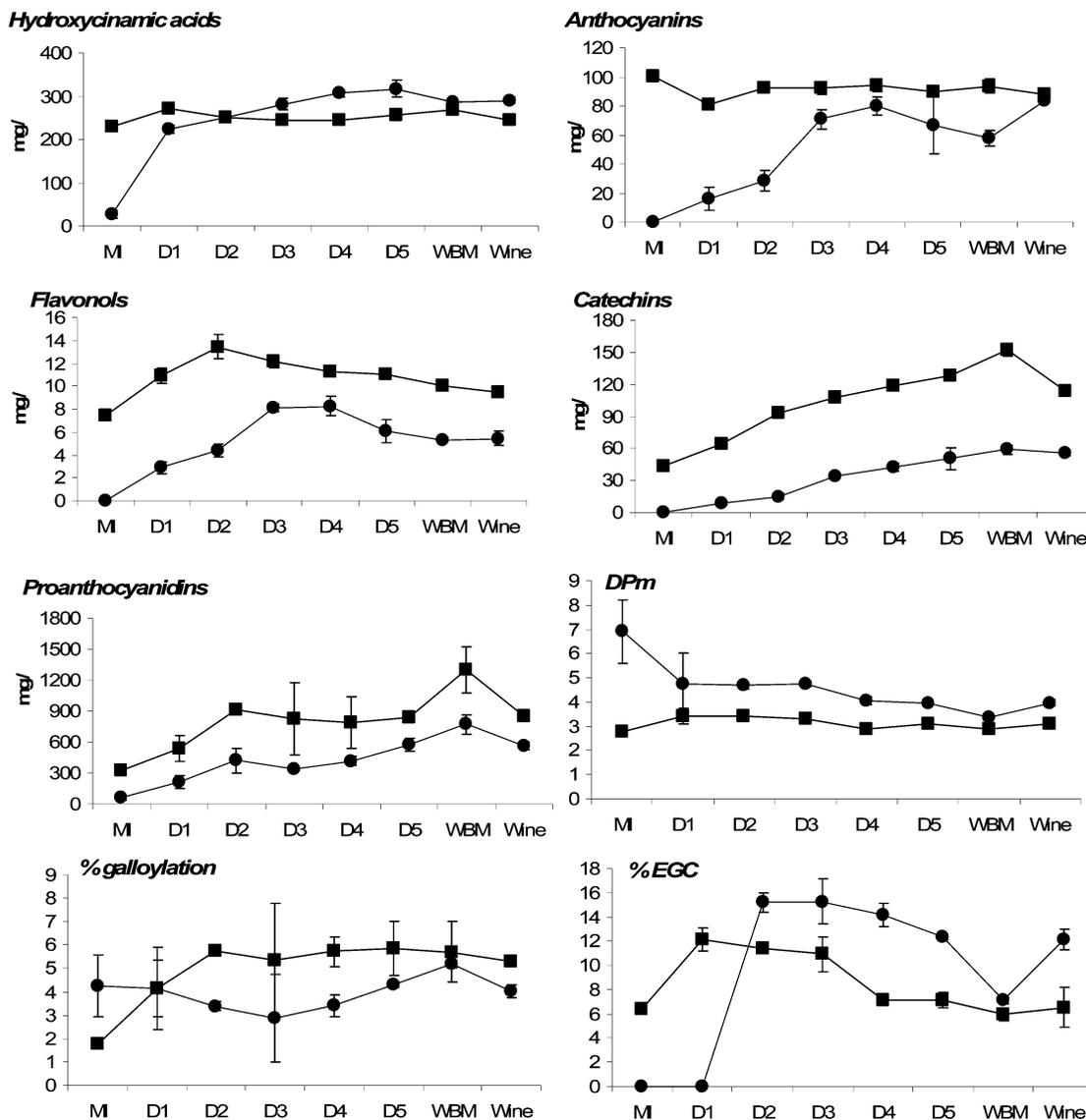


Figure 1. Concentration of phenolic compounds (in mg/L) during the fermentation of control and flash release Grenache musts (2004 vintage). **MI**, Initial Must; **D1**, day 1; **D2**, day 2; **D3**, day 3; **D4**, day 4; **D5**, day 5; **WBM**, wine before malolactic fermentation. Values are the average of three replicates. —●—, control; —□—, flash release

Since application of high temperature (thermovinification) is also used to enhance phenolic extraction in red wine making, an additional experiment aiming at distinguishing between the effect of heat exposure and that of pressure release within the FR treatment was carried out on Grenache in 2004. This was achieved by maintaining the grapes at high temperature for a longer period of time (15 min instead of 6 min) before releasing the pressure. Fermentation was also performed on the juice obtained by pressing immediately after FR to study reactions of phenolic compounds under conditions allowing no further extraction.

Comparison of musts obtained by FR after heating at 95 °C for 6 min or after maintaining them at 95 °C for 9 min more showed that exposure to high temperature induced further increase in the concentration of all polyphenols and especially of catechins and anthocyanins (Figure 2).

Heat also promoted degradation of phenolic compounds so that after 1 day of incubation both FR musts showed identical compositions (results not shown). The concentration of all classes of phenolic compounds dramatically decreased throughout fermentation in the liquid phase, confirming that they rapidly proceed to various degradation and reaction products and

indicating that, in musts fermented on pomace, this was compensated by further extraction.

As a consequence, the wines fermented in the liquid phase contained much lower amounts of all phenolics than all the other wines. Longer heat exposure induced no further increase of phenolics compared to the FR-treated wine and lowered the level of anthocyanins in the final wines. This may be explained by the particular sensitivity of anthocyanins to heat exposure (18).

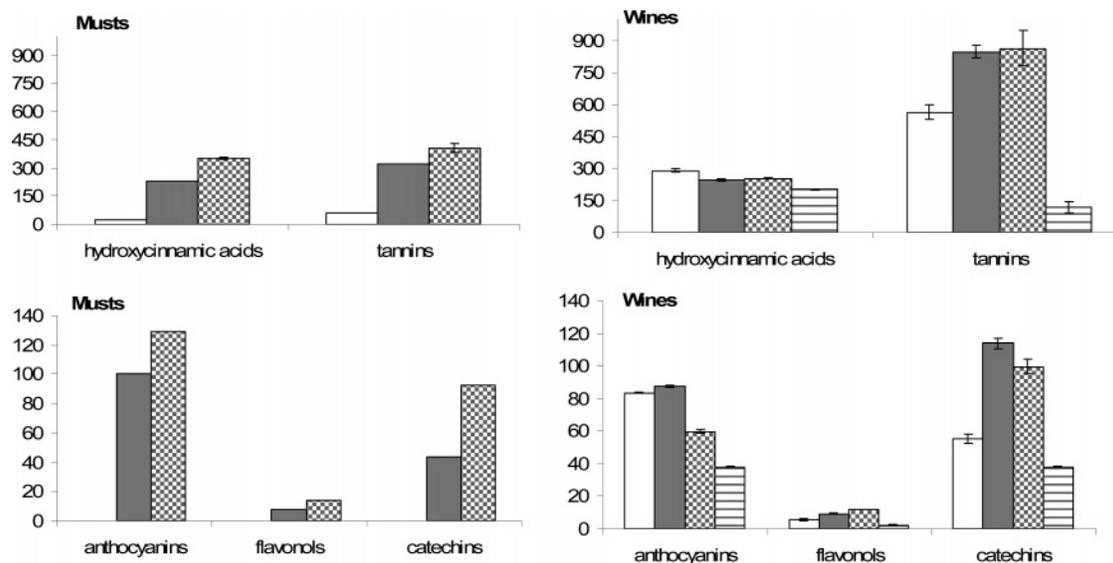
Effect of FR Treatment and Heating Conditions on Polyphenol Extraction and Reactions during Fermentation. Comparison of pomace and wine composition after pressing with the grape polyphenol content is shown in Figure 3.

Wine proanthocyanidins represented about 40% and 60% of the grape proanthocyanidin content, respectively, in the control and FR samples. The remaining percentage was recovered in the pomace, meaning that there was no significant loss of proanthocyanidin units during fermentation.

In contrast, the proportion of anthocyanins recovered in wine and pomace accounted together for only 25% (in control) to 40% (after FR) of anthocyanins, indicating large anthocyanin losses during fermentation. The recovery yield in the FR samples was lower after longer heating, confirming that exposure to high

Table 2. Effect of Flash Release on the Wine Polyphenol and Proanthocyanidin Compositions

	Polyphenol			
	anthocyanins	flavonols	hydroxycinnamic acids	catechins
Grenache 2003				
control	106.1 ± 8.6	20.8 ± 1.4	460.8 ± 12.8	85.2 ± 12.8
flash release	110.9 ± 2.1	30.8 ± 1.1	355.7 ± 7.3	143.3 ± 1.9
Mourvedre 2003				
control	173.2 ± 4.7	36.1 ± 2.0	47.9 ± 5.8	33.9 ± 1.4
flash release	198.7 ± 1.7	67.9 ± 0.3	26.8 ± 1.4	46.8 ± 3.1
Grenache 2004				
control	83.5 ± 0.5	5.4 ± 0.6	316.1 ± 5.4	55.4 ± 2.6
flash release	87.6 ± 0.7	9.4 ± 0.1	270.8 ± 5.7	113.8 ± 3.4
Carignan 2004				
control	161.4 ± 1.9	13.4 ± 1.2	94.1 ± 3.8	26.0 ± 0.7
flash release	210.3 ± 5.6	27.5 ± 0.7	103.2 ± 2.8	32.3 ± 0.8
	Proanthocyanidin			
	proanthocyanidins	DPM	% gall	% EGC
Grenache 2003				
control	751.2 ± 46.9	4.00 ± 0.16	3.42 ± 0.16	10.51 ± 0.4
flash release	997.2 ± 59.1	3.3 ± 0.13	4.8 ± 0.3	7.52 ± 0.3
Mourvedre 2003				
control	819.5 ± 52.1	4.78 ± 0.18	3.1 ± 0.17	13.9 ± 0.6
flash release	1281.7 ± 308.5	4.5 ± 0.2	4.9 ± 0.3	9.5 ± 0.3
Grenache 2004				
control	564.1 ± 35.2	3.95 ± 0.13	4.04 ± 0.28	12.12 ± 0.85
flash release	851.5 ± 36.0	3.11 ± 0.06	5.3 ± 0.16	7.5 ± 0.07
Carignan 2004				
control	308.7 ± 9.2	3.97 ± 0.07	2.08 ± 0.1	19.0 ± 0.66
flash release	356.2 ± 11.2	4.02 ± 0.12	3.0 ± 0.12	17.6 ± 0.2

**Figure 2.** Effect of FR and heating on the polyphenol composition of grenache must and wine (in mg/L): white bars, control; gray bars, flash release (pressure release applied after heating the grapes for 6 min); shaded bars, flash release with longer heating (pressure release applied after heating the grapes for 15 min); lined bars, flash release followed by pressing and fermentation of the liquid phase.

temperature promotes anthocyanin reactions. However, this is not the only factor involved since higher losses were observed in the control.

Anthocyanin losses in wine making and aging are well documented. They result, on one hand, from opening and cleavage of heterocyclic ring, leading to colorless degradation products such as syringic acid, and on the other hand from addition reactions yielding various kinds of derived pigments.

To characterize these changes further, TPI and color indices were measured on the final wines (Table 3).

The TPI was higher in the FR wines and even higher after longer heating. It was lowest after fermentation in liquid phase,

as expected. For a given variety, differences in TPI seem to reflect the levels of proanthocyanidins, which are the major phenolics in wines.

FR increased wine color intensity in most cases (except Grenache 2004) but did not affect tint. Color intensity was positively correlated ($R = 0.90$) and tint negatively correlated ($R = -0.93$) with the anthocyanin content determined by HPLC. However, about half of the color was due to sulfite bleaching-resistant pigments, as expected from the low recovery rate of grape anthocyanins in the wines and marcs. The percentage of sulfite bleaching-resistant pigments was lower in the FR-treated wines than in the corresponding control wines, meaning that

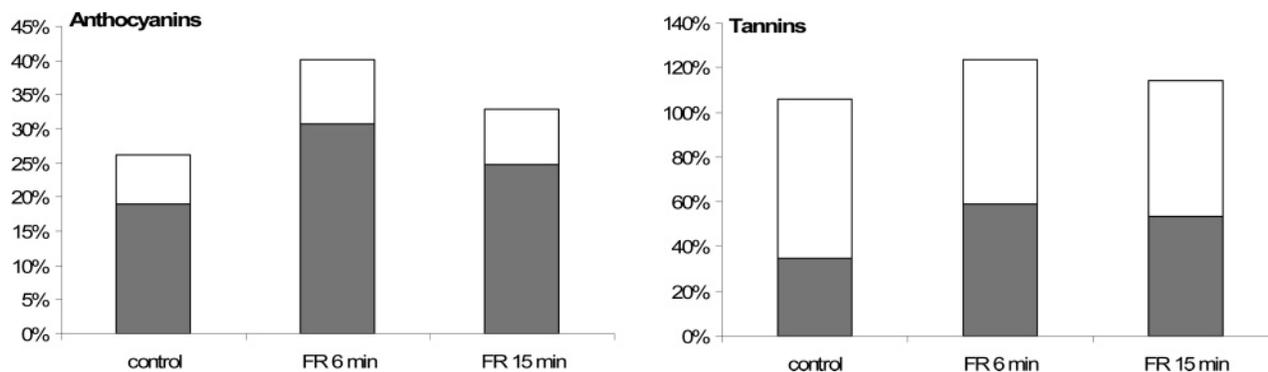


Figure 3. Recovery of grape tannins and anthocyanins in wine and pomace (in % grape content): gray bar, wine after pressing; white bar, pomace.

Table 3. Wine Total Polyphenol Index and Color Characteristics

	TPI	CI	T	% sulfite bleaching resistance
Grenache 2003				
control	30.4 ± 0.7	3.4 ± 0.1	0.96 ± 0.04	51 ± 4
flash release	34.5 ± 1.3	3.7 ± 0.1	0.97 ± 0.01	46 ± 2
Mourvedre 2003				
control	43.2 ± 0.4	10.9 ± 0.1	0.71 ± 0.01	39 ± 1
flash release	47.4 ± 0.3	11.4 ± 0.1	0.71 ± 0.01	36 ± 1
Grenache 2004				
control	27.4 ± 0.8	3.5 ± 0.2	0.93 ± 0.04	59 ± 4
flash release	31.2 ± 0.5	3.4 ± 0.1	0.93 ± 0.02	55 ± 2
flash release 15 min	34.6 ± 0.4	4.0 ± 0.00	0.97 ± 0.01	54 ± 2
liquid phase	14.4 ± 0.5	2.2 ± 0.10	1.11 ± 0.05	68 ± 5
Carigan 2004				
control	26.1 ± 1.0	6.6 ± 0.1	0.67 ± 0.01	40 ± 1
flash release	29.0 ± 0.3	8.6 ± 0.5	0.58 ± 0.02	31 ± 1

the derived pigment compositions were different. Increasing the duration of heating induced an increase of both color intensity and tint, suggesting that it favored conversion of anthocyanins to orange or brown pigments but it did not modify sulfite bleaching resistance. In the FR wine fermented in the liquid phase, color intensity was much lower. Tint and proportion of sulfite bleaching-resistant pigments were much higher than in all other wines, indicating a higher conversion rate of anthocyanins to orange pigments that also showed increased sulfite bleaching resistance. Since FR followed by immediate pressing results in much higher anthocyanin-to-tannin ratio in the must and should thus favor reactions of anthocyanins that do not involve tannins, this suggests that products formed by such reactions are more orange and sulfite resistant than anthocyanin tannin adducts. These color characteristics are common features of pyranoanthocyanins resulting from addition of yeast metabolites onto anthocyanins (19–21).

In contrast, tannin–anthocyanin (T–A) adducts formed by reaction of anthocyanins on the intermediate species released by acid-catalyzed cleavage of proanthocyanidins (22) have the same color properties as anthocyanins themselves (23). Formation of T–A adducts is also expected to depend on the tannin-to-anthocyanin ratio and should thus be influenced by FR treatment and heating (5).

The tannin-to-anthocyanin ratio in the various wines is presented in Table 4. This ratio was particularly low in the wine fermented in the liquid phase, as mentioned above. It was higher after FR than in the control and even higher in the FR wines obtained after heating for 15 min.

For each variety, formation of T–A adducts increased with the tannin-to-anthocyanin ratio and thus with FR and heating. However, the concentration of T–A dimer adducts was higher in the Carignan wines that contained high levels of anthocyanins

Table 4. Tannin-to-Anthocyanin Ratio and Concentrations of Flavanol–Anthocyanin Adducts in Wines

wine	tannin-to-anthocyanin ratio	flavanol anthocyanin dimer adducts (eq. malvidin 3-glucoside, in mg/L)
Grenache 2003		
control	7.2 ± 1.2	0.89 ± 0.06
flash release	8.9 ± 0.4	1.03 ± 0.08
Mourvedre 2003		
control	4.7 ± 0.03	1.99 ± 0.09
flash release	6.4 ± 1.0	2.47 ± 0.07
Grenache 2004		
control	6.7 ± 0.4	0.69 ± 0.07
FR 6 min	9.9 ± 0.3	0.92 ± 0.01
FR 15 min	14.5 ± 1.5	1.25 ± 0.03
liquid phase	3.1 ± 0.7	0.32 ± 0.02
Carignan 2004		
control	1.9 ± 0.04	1.64 ± 0.11
flash release	1.7 ± 0.08	2.03 ± 0.19

and the lowest tannin-to-anthocyanin ratios. Although the recovered dimers are present in rather small amounts, they may be considered as markers of a much wider T–A family. The concentration of T–A dimers in wines appeared negatively correlated with sulfite bleaching sensitivity ($R = -0.90$) and tint ($R = -0.89$), suggesting that formation of these pigments competes with that of sulfite bleaching resistant pigments such as pyranoanthocyanins.

In conclusion, these results allow us to demonstrate that:

The FR treatment resulted in faster extraction of all classes of phenolic compounds, allowing the production of enriched must and juice, but their concentration dramatically decreased throughout fermentation when pressing was achieved immediately after FR.

FR wines fermented with pomace contact contained larger amounts of flavonols, catechins, anthocyanins, and proanthocyanidins than the control wines and slightly lower amounts of hydroxycinnamic acids. Maintaining the grapes at high temperature before the FR treatment induced extraction of higher amounts of phenolic compounds. Heating also accelerated reactions of phenolics and especially conversion of anthocyanins to pigments showing slightly higher tint values. FR increased the tannin-to-anthocyanin ratio and conversion of anthocyanins to T–A adducts that show the same color properties as anthocyanins. Formation of T–A dimers seems limited by availability of both tannins and anthocyanins and competes with that of other types of pigments contributing higher tint values and sulfite bleaching resistance.

Future studies will investigate the effect of composition changes induced by FR on wine flavor properties.

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