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Formation of Vitisins and Anthocyanin−Flavanol Adducts during Red Grape Drying

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ABSTRACT: This study evaluated the formation of anthocyanin-derived compounds during the production of sweet red wines from Merlot and Syrah grapes previously chamber-dried under controlled-temperature conditions. The musts from both grape varieties were found to contain pelargonidin-3-glucoside throughout the vinification process. Besides, HPLC-DAD-MS revealed the presence of pyranoanthocyanins in unfermented musts from the raisins. These compounds are adducts resulting from the cycloaddition of pyruvic acid (type A vitisins) and acetaldehyde (type B vitisins) to anthocyanin molecules. The analyses additionally revealed the presence of products of the condensation via a methylmethine bridge between anthocyanins and (epi)catechin, which requires the presence of acetaldehyde. The absence of pyruvic acid, acetaldehyde, and ethanol in the musts from fresh grapes and their presence in those from dried grapes support the idea that these compounds result from enzymatic transformations because the vinification of the musts involves no alcoholic fermentation. The drying process alters the permeability of grape membranes by the lipoxygenase activation effect (LOX), a switch to an anaerobic metabolism and the resulting triggering of the alcohol dehydrogenase enzyme (ADH). The activation of these and several other enzymes confirmed the occurrence of enzymatic transformations and the formation of vitisin A, acetylvitisin A, and the B vitisins of malvidin-3-glucoside, peonidin-3-glucoside, peonidin-3-acetylglucoside, and malvidin-3-acetylglucoside, as well as the adducts Pn-3-glc-methylmethine(epi)catechin, Mv-3-glc-methylmethine(epi) catechin, and Mv-3-acetylmethylmethine(epi)catechin.

KEYWORDS: *red raisins, sweet wines, vitisins, anthocyanin adducts*

INTRODUCTION

Off-vine grape sun-drying is a widely used practice for the production of sweet wines.[1,2] A large variety of fruit-drying procedures currently exist, all of which raise the sugar concentration of the fruit by the effect of its dehydration.[3,4] As a rule, the dehydration of the fruit produces a stress situation, which causes substantial alterations in its metabolism.[5] Thus, Bellicontro et al.[6] found that the stress situation took place when the weight loss was 10−15%, causing a change in the metabolism from aerobic to anaerobic. Constantini et al.[7] confirmed the metabolic change and that the formation of ethanol was also accompanied by its oxidation to acetaldehyde. In addition, the dehydration of grapes affects secondary metabolic processes such as the synthesis of volatiles and polyphenols depending on the amount of water loss.[8] This is not so clear for anthocyanins. Thus, Bellicontro et al.[6] found red grapes of the Sangiovese variety dried in a tunnel at 21 °C to exhibit an increase in total anthocyanins relative to slowly dried grapes of the same variety, whereas Moreno et al.[9] found the increase in the anthocyanin content of Pinot noir grapes tunnel-dried at 22 °C to be the exclusive result of water evaporation.

Vinification is known to cause the diffusion of anthocyanins present in grape skin cells to the must.[10,11] Subsequently, these compounds undergo a number of reactions including copigmentation, which involves the bonding of anthocyanins with organic compounds, usually colorless, including some phenolic acids and flavonoids.[12] Also, monomeric anthocyanins initially present in grapes and musts are involved in oxidation, cycloaddition, and polymerization reactions giving new pigments that increase the color stability of the resulting wine.[13]

The processes by which anthocyanins react with flavanols, whether by direct condensation or via a methylmethine bridge, have been issues for many years.[14] Some pigments derived from the acetaldehyde-mediated condensation between flavanols and malvidin-3-glucoside have been studied by some authors in model solution, and the color increase with a shift toward violet was attributed to the formation of new colored compounds.[15−18] The formation of these pigments in experimental red wines is favored at lower pH values.[19] The presence of these derivatives protects the wine color from the effect of pH changes and bleaching by SO_2.[18,20,21] Although these compounds have been detected in red wines from diverse grape varieties vinified and aged in various ways,[22−24] they have never been identified during the drying of red grapes.

Other widely studied types of phenolic compounds are pyranoanthocyanins, which are orangish red colored adducts formed in condensation reactions over the anthocyanins, which become stable oligomers by substitution at the C4 position of the anthocyanins.[25] These oligomers, which are not initially present in the grapes, but formed during alcoholic fermentation

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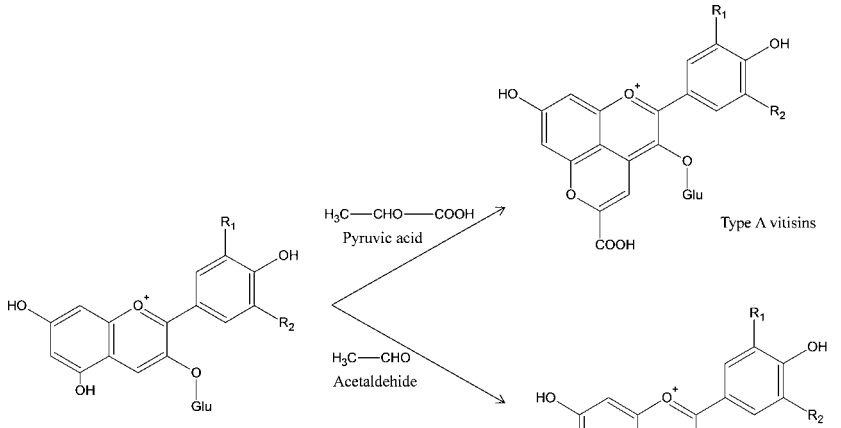

Anthocyanins	R_1	R_2
Delphinidine-3-glucoside	OH	OH
Cyanindine-3-glucoside	OH	H
Petunidine-3-glucoside	OCH_3	OH
Peonidine-3-glucoside	OCH_3	H
Malvidine-3-glucoside	OCH_3	OCH_3

Figure 1. Structures of vitisin A and vitisin B.

and in the following vinification steps, are interesting due to the color stability of the red wines by the effect of their increased resistance to bleaching by sulfur dioxide relative to other anthocyanins.[26,27] Also, they are less prone to hue alterations by the effect of pH changes,[28] so virtually all of these adducts contribute to wine color.

Especially prominent pyranoanthocyanins are type A and B vitisins (Figure 1). Vitisins A are adducts resulting from the cycloaddition of a molecule of pyruvic acid to one of anthocyanin.[28] Vitisins B are adducts resulting from the cycloaddition of a molecule of acetaldehyde to one of anthocyanin.[27] Although both types of vitisins are formed during alcoholic fermentation, they exhibit antagonistic kinetics because acetaldehyde can compete with pyruvic acid for anthocyanin molecules.[29]

Bakker[30] found fortified Porto wines to contain greater amounts of vitisins A than did red table wines. Fortification halts fermentation before sugars are depleted. As a result, the formation of type A vitisins is favored because the pyruvic acid concentration remaining in the wine would have been greater if the process had continued to deplete all of the sugar.[29] In addition, fortified Porto wines also contain greater amounts of type B vitisins than do other wines[26] by the effect of the acetaldehyde needed for their synthesis being externally added via distillates.[30]

The purpose of this work was to examine the formation of condensation adducts between anthocyanins and flavanols via methylmethine bridges and pyranoanthocyanins during the drying of Merlot and Syrah red grapes under controlled temperature and moisture conditions. Also, changes in the concentration of these adducts and remaining anthocyanin compounds during the vinification process have been studied.

MATERIALS AND METHODS

Grape Drying. Merlot and Syrah grapes were harvested in the Montilla-Moriles region (southern Spain) in 2009, with initial concentrations of reducing sugars of 211 and 231 g/L, respectively. An amount of about 30 kg of grapes was uniformly distributed in several trays (11 kg/m^2) and allowed to dry in a Frisol Climatronic chamber at an air temperature of 40 °C and a constant relative humidity of ca. 20%. During the drying process, samples were periodically collected, and the weight loss of the grapes was measured. The drying was concluded when the reducing sugar concentration was around 300 g/L.

In the laboratory, the whole bunches of raisins were pressed on a vertical press similar to industrial models. The maximum pressure reached in each pressing cycle was 300 bar, and each raisin batch was pressed in two cycles.

The resulting musts were fortified to 15% (v/v) alcohol wine, holding then maceration with the skins for 48 h at 25 °C. On the basis of previous studies,[31] the maceration time of 48 h was chosen.

Musts from fresh grapes, musts from the grapes after the drying and wines at the end of the process of maceration were taken. All samples were centrifuged at 3000 rpm and filtered prior to analysis. All measurements were performed in triplicate.

Reducing Sugars. The measurement of reducing sugars was performed by refractometry, using a refractometer model Atago Master (Master Baume 2594, Atago, Japan).

Pyruvic Acid. Pyruvic acid was determined enzymatically, using a K-PYRUV 03/07 kit from Megazyme (Wicklow, Ireland). The determination is based on the absorbance change at 340 nm caused by the oxidation of NADH to NAD$^+$ in the presence of the lactate dehydrogenase enzyme as cofactor during the reduction of pyruvic acid to lactic acid.

Ethanol Content. This was determined according to the method of Crowell and Ough.[32] To this end, ethanol in the sample was collected by steam and then reacted with acid potassium dichromate. The reaction was spectrophotometrically monitored via the absorbance at 600 nm against a blank on a Perking Elmer Lambda 25 spectrophotometer.

Extraction of Anthocyanins. A volume of 2 mL of must/wine was passed through a Sep-Pak C18 cartridge, with 900 mg of filling (Long Body Sep-Pak Plus; Waters Associates, Milford, MA) that was previously activated with 5 mL of methanol and washed with aqueous 0.01% (v/v) HCl. The cartridge was washed successively with 10 mL of 0.01% aqueous HCl and 5 mL of ethyl acetate, and anthocyanins were recovered with 5 mL of methanol, which was acidified to pH 2 with HCl. Anthocyanin samples were evaporated to dryness using a vacuum centrifuge thermostated at 35 °C and then dissolved in aqueous 0.01% (v/v) HCl and 10% methanol acidified to pH 2. Samples were passed through a nylon filter of 0.45 μm pore size for HPLC analysis.

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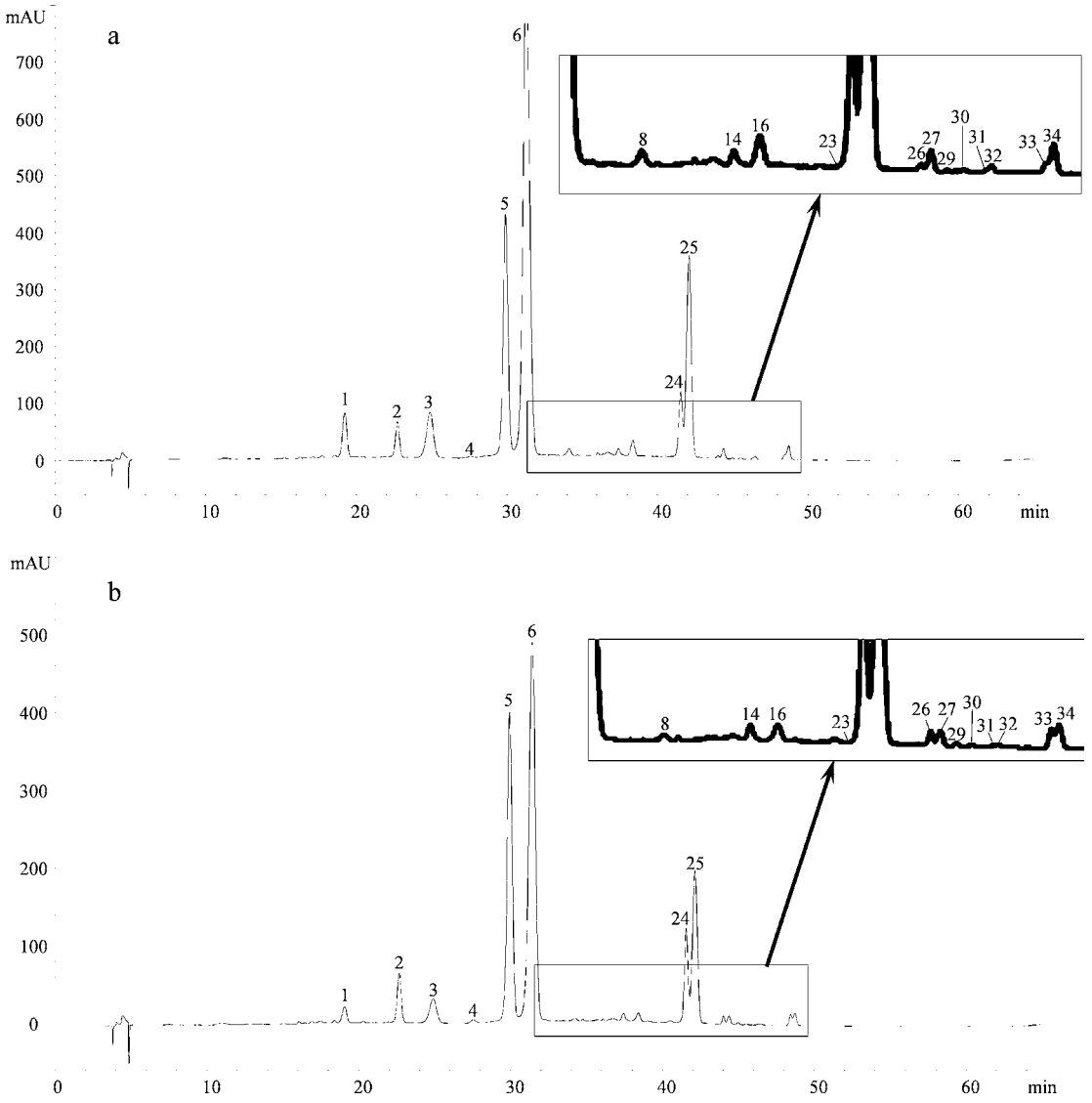

Figure 2. HPLC-DAD chromatograms at 520 nm of anthocyanins of the musts from fresh grapes of Merlot (a) and Syrah (b) varieties.

HPLC-DAD-MS Analyses. The anthocyanin extracts were analyzed using a Hewlett-Packard 1100 series liquid chromatograph (Agilent Technologies, Waldbronn, Germany). Separation was achieveved on an AQUA (Phenomenex, Torrance, CA) reverse phase C18 column (5 μm, 150 mm × 4.6 mm i.d.) thermostated at 35 °C. The solvents used were (A) 0.1% trifluoroacetic acid in water and (B) 100% HPLC grade acetonitrile. The gradient employed was as follows: isocratic 10% B for 3 min, from 10 to 15% B for 12 min, isocratic 15% B for 5 min, from 15 to 18% B for 5 min, from 18 to 30% B for 20 min, and from 30 to 35% for 5 min, at a flow rate of 0.5 mL min^{-1}.[33] Detection was carried out in a diode array detector (DAD), using 520 nm as the preferred wavelength, and in a mass spectrometer (MS) connected to the HPLC system via the DAD cell outlet. MS detection was performed in an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source and a triple-quadrupole–ion trap mass analyzer that was controlled by the Analyst 5.1 software. Zero grade air served as the nebulizer gas (40 psi) and turbo gas (600 °C) for solvent drying (50 psi). Nitrogen served as the curtain (100 psi) and collision gas (high). Both quadrupoles were set at unit resolution. The ion spray voltage was set at 5000 V in the positive mode. Enhanced MS (EMS) method was used. Setting used were as follows: declustering potential (DP), 41 V; entrance potential (EP), 7.5 V; collision energy (CE), 10 V. Enhanced product ion (EPI) mode was further performed to obtain the fragmentation pattern of the parent ion(s) using the following parameters: DP, 41 V; EP, 7.5 V; CE, 10 V; and collision energy spread (CES), 0 V.

For the quantitative analysis of anthocyanins, a calibration curve was obtained by injection of different concentrations of delphinidin 3-*O*-glucoside (for delphinidin-based anthocyanins), cyanidin 3-*O*-glucoside (for cyanidin-based anthocyanins), petunidin 3-*O*-glucoside (for petunidin-based anthocyanins), peonidin 3-*O*-glucoside (for peonidin-based anthocyanins), malvidin 3-*O*-glucoside (for malvidin-based anthocyanins), and pelargonidin 3-*O*-glucoside standards purchased from Extrasynthèse (Genay, France).

Gas Chromatography. For acetaldehyde quantification, an Agilent 6890 series plus gas chromatograph (Agilent Technologies) with electronic pressure control was used. The column, a CPWAX-57 CB model from Chrompack (Middelburg, The Netherlands), was fused silica 60 m × 0.25 mm and 0.40 μm film thickness. The temperature program was as follows: 50 °C for 15 min and then raised to 190 °C at 4 °C/min for 35 min. The flow rate of helium (carrier gas) was held at 0.7 mL/min for 16 min and then raised at 0.2 mL/min^2 to 1.1 mL/min for 52 min. A 1:30 split ratio and an injector temperature of 275 °C were used. The flame ionization detector temperature was 300 °C, and the hydrogen and air flow rates were 40 and 400 mL/min, respectively. The chemstation software package (Agilent Technologies) was used. One milliliter of a solution containing 1 g/L 4-methyl-2 pentanol as internal standard was added to 10 mL of sample, and an aliquot of 0.5 μL was injected.

Statistical Analysis. All results are given as arithmetic means ± standard deviations for triplicate determinations. Significant differences were established by one-way analysis of variance (ANOVA) at the 99.9% confidence level.

RESULTS AND DISCUSSION

Identification of Anthocyanins by HPLC-DAD-MS. The HPLC-DAD-MS technique allowed a total of 20 monomeric anthocyanins to be identified in musts from fresh Merlot and Syrah grapes (Figure 2), and their chromatographic data are shown in Table 1. The first compound group to be eluted was

Table 1. Anthocyanin Compounds Identified by HPLC-DAD-MS in Merlot and Syrah Musts and Wines

peak	t_R	M^+ (m/z)	MS^2 frag	λ_{max} (nm)	compound
1	19.10	465	303	524	Dp-3-glc
2	22.65	449	287	516	Cy-3-glc
3	24.81	479	317	526	Pt-3-glc
4	27.46	433	271	506	Pg-3-glc
5	29.91	463	301	518	Pn-3-glc
6	31.37	493	331	528	Mv-3-glc
7	32.25	561	399	490	vitisin A
8	33.90	507	303	528	Dp-3-acetylglc
9	34.23	487	325	486	B-type vitisin Pn-3-glc
10	34.43	603	399	490	A-type vitisin Mv-3-acetylglc
11	35.66	517	355	490	vitisin B
12	36.90	779		530	Pn-3-glc-methylmethine(epi) catechin
13	37.25	809	357	530	Mv-3-glc-methylmethine(epi) catechin
14	37.46	491	287	518	Cy-3-acetylglc
15	37.86	809	357	540	Mv-3-glc-methylmethine(epi) catechin
16	38.42	521	317	528	Pt-3-acetylglc
17	38.60	529	325	494	B-type vitisin Pn-3-acetylglc
18	39.07	779		528	Pn-3-glc-methylmethine(epi) catechin
19	39.07	559	355	494	B-type vitisin Mv-3-acetylglc
20	39.28	809	357	542	Mv-3-glc-methylmethine(epi) catechin
21	40.47	809	357	530	Mv-3-glc-methylmethine(epi) catechin
22	40.50	779		528	Pn-3-glc-methylmethine(epi) catechin
23	41.10	641	317	522	Pt-3-caffeoylgluc
24	41.58	505	301	520	Pn-3-acetylglc
25	42.16	535	331	532	Mv-3-acetylglc
26	44.05	625	301	522	Pn-3-caffeoylglc
27	44.41	655	331	534	Mv-3-caffeoylglc
28	44.46	851			Mv-3-acetylglc-methylmethine(epi)catechin
29	44.99	595	287	520	Cy-3-coumaroylglc
30	45.55	625	317	534	Pt-3-coumaroylglc
31	46.20	609	301	526	Pn-3-coumaroylglc *cis*
32	46.50	639	331	538	Mv-3-coumaroylglc *cis*
33	48.44	609	301	524	Pn-3-coumaroylglc *trans*
34	48.71	639	331	538	Mv-3-coumaroylglc *trans*

that of monoglucoside derivatives, five of which are present in most red grape varieties: delphinidin-3-glucoside (peak 1), cyanidin-3-glucoside (peak 2), petunidin-3-glucoside (peak 3), peonidin-3-glucoside (peak 5), and malvidin-3-glucoside (peak 6). Peak 4 was assigned to pelargonidin-3-glucoside, the structure of which is shown in Figure 3, and its MS analysis revealed an $[M^+]$ peak at m/z 433 accompanied by a fragment at m/z 271. This anthocyanin was previously detected in red grapes of the nonviniferous varieties Concord, Rubired, and Salvador[34,35] and, recently, in Garnacha Tintorera grapes.[36] The last authors proposed the use of this compound as a chemical indicator to identify red wines made from this grape variety. This hypothesis, however, could be ruled out on the basis that in this work pelargonidin-3-glucoside has been found in the Merlot and Syrah musts.

The other anthocyanin glucosides detected in musts from the two grape varieties (Figure 2) included the acetic esters of delphinidin (peak 8), cyanidin (peak 14), petunidin (peak 16), peonidin (peak 24), and malvidin (peak 25). In addition, the chromatograms revealed the presence of the caffeoylglucosides of petunidin (peak 23), peonidin (peak 26), and malvidin (peak 27). Finally, peaks 29−34 were assigned to the *p*-coumarylglucosides of cyanidin, petunidin, peonidin, and malvidin. The MS spectra afforded the discrimination of the *cis* and *trans* isomers of the coumarylglucosides of peonidin (peaks 31 and 33), both with a $[M^+]$ ion at m/z 301, and malvidin (peaks 32 and 34), with one at m/z 331.

Figure 4 shows the HPLC-DAD chromatograms obtained at 520 nm for the musts from Merlot and Syrah dried grapes. As can be seen, these musts contained all glucosyl, acetylglucosyl, and caffeoylglucosyl anthocyanins previously detected in the musts from fresh grapes in addition to new compounds formed during the grape-drying process. The formation of pyranoanthocyanins had never previously been observed in the absence of fermentation of *Vitis vinifera* grapes because it requires the presence of pyruvic acid (vitisins A) or acetaldehyde (vitisins B). However, the chromatographic features of peak 7 (viz., an $[M^+]$ ion at m/z 561 and a fragment at m/z 399) and peak 10 (viz., an $[M^+]$ ion at m/z 603 and a fragment at m/z 399) were consistent with those of vitisin A and acetylvitisin A, respectively, which were first identified by Bakker and Timberlake[27] and results from the reaction of pyruvic acid with malvidin-3-glucoside and malvidin-3-acetylglucoside, respectively. These compounds were present in the musts from Merlot and Syrah grapes (Table 1), which suggests that the grape-drying process somehow causes the formation of pyruvic acid.

The formation of vitisin A and acetylvitisin A was confirmed by quantifying pyruvic acid in musts from fresh and dried grapes. The former musts were found to contain very low concentrations of this acid (below the limit of quantitation of the analytical method used, 3 mg/L), whereas the dried grapes contained 16.8 ± 0.791 and 22.2 ± 0.585 mg/L in Merlot and Syrah grapes, respectively. This increase in the pyruvic acid concentration was due, on the one hand, to grape drying (1.54 times in Merlot and 1.47 times in Syrah) as a result of water evaporation. On the other hand, it could be the result of the subsequent synthesis. According to Chkaiban et al.,[8] the loss of water in grapes during the drying process alters their membrane permeability through the activation of the lipoxygenase enzyme (LOX). This suggests a metabolic switch from aerobic to anaerobic in the berries and the consequent activation of the alcohol dehydrogenase enzyme (ADH). Under these anaerobic conditions, other enzymes capable of degrading sugars and/or malic acid in the grapes to pyruvic acid may have been activated, consistent with the contents in this acid found in the musts from dried grapes.

The musts from dried grapes contained also type B vitisins, formed by cycloaddition of acetaldehyde to anthocyanins.

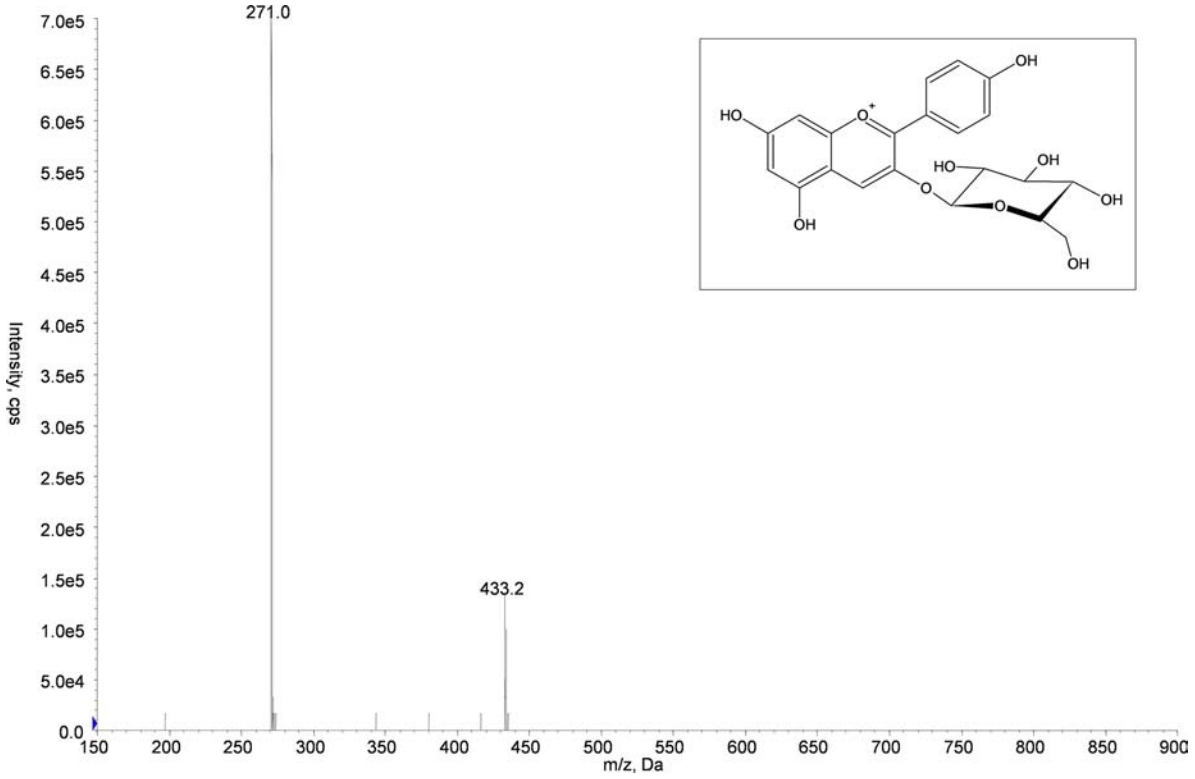

Figure 3. Structure and MS spectrum of pelargonidin-3-glucoside.

Thus, the chromatographic features of peak 11 ($[M^+]$ at m/z 517, a fragment at m/z 355, and λ_{max} = 490 nm) were consistent with those of vitisin B, a pyranoanthocyanin formed by cycloaddition to malvidin-3-glucoside.[22,24] Similarly, peaks 9, 17, and 19 were assigned to the type B vitisins peonidin-3-glucoside, peonidin-3-acetylglucoside, and malvidin-3-acetylglucoside, respectively.

In addition to pyranoanthocyanins, the HPLC-DAD-MS analysis revealed the presence of condensation products between anthocyanins and (epi)catechin via a methylmethine bridge, which inevitably requires the presence of acetaldehyde. These compounds exhibit a UV−vis absorption peak (λ_{max} = 530−540 nm) that is a bathochromic shift in the wavelength with respect to the parent monomer, providing a blue-purple tone in an otherwise red solution.[19] The chromatograms revealed the presence of three compounds of formula Pn-3-glc-methylmethine(epi)catechin (peaks 12, 18, and 22), with $[M^+]$ at m/z 779, and four of formula Mv-3-glc-methylmethine(epi)-catechin (peaks 13, 15, 20, and 21), with $[M^+]$ at m/z 809 and fragmentation ions at m/z 357. The compounds corresponding to peaks 12 and 15 were detected only in the musts from Syrah grapes.

Because the formation of the previous compounds required the presence of acetaldehyde in the musts from dried grapes, the musts were additionally analyzed by GC-FID. The results revealed the absence of this compound in the musts from fresh grapes and its presence at levels of 132 ± 10 and 113 ± 6.0 mg/L in those from dried Merlot and Syrah grapes, respectively. Therefore, the chamber-drying process caused the formation of acetaldehyde in the grapes. Pyruvic acid may have been decarboxylated into acetaldehyde and then may have been converted into ethanol under the action of alcohol dehydrogenase enzyme. This hypothesis was supported by the idea that the ethanol concentration in the musts from fresh grapes was zero, whereas those in the musts from dried grapes were 0.959 ± 0.006 (v/v) for Merlot and 0.559 ± 0.001 (v/v) for Syrah. As before, these contents suggest that acetaldehyde was converted into ethanol in the presence of alcohol dehydrogenase.

Therefore, the presence of pyruvic acid, acetaldehyde, and ethanol in the musts from dried grapes supports the idea that the above-described enzymatic transformations and hence the formation of pyranoanthocyanins and methylmethine-bonded anthocyanin−flavanol adducts occur during the grape-drying process. These anthocyanin derivatives, which possess a high enological interest, had previously been detected only in the presence of fermentation in *V. vinifera* grapes.

The wines were also analyzed by HPLC-DAD-MS after fortification and maceration with grape skin for 48 h. The chromatograms (Figure 5) confirmed the presence of all previously detected monomeric anthocyanins and vitisins (peaks 7, 9−11, and 19), in addition to a type B vitisin (Pn-3-acetylgluc, with $[M^+]$ at m/z 529 and a fragmentation ion at m/z 325). With regard to the anthocyanin−methylmethine−flavanol condensation adducts, the chromatograms revealed the presence of the compounds found in the musts from dried grapes (peaks 12, 13, 15, 18, and 20−22) in addition to a new one (peak 28) corresponding to a compound of formula Mv-3-acetylglc-methylmethine(epi)catechin, with $[M^+]$ at m/z 851, in the wine from Merlot grapes.

Quantitation of Anthocyanins. Table 2 lists the concentrations of anthocyanins in the musts from fresh and dried Merlot and Syrah grapes and shows the homogeneous groups established by one-way analysis of variance (ANOVA) at a confidence level of 99.9% ($p < 0.001$). As can be seen, monoglucosides were the most abundant derivatives in all samples (particularly malvidin-3-glucoside), followed by acetylglucoside derivatives. These two anthocyanin families exhibited variable changes in concentration during the grape-

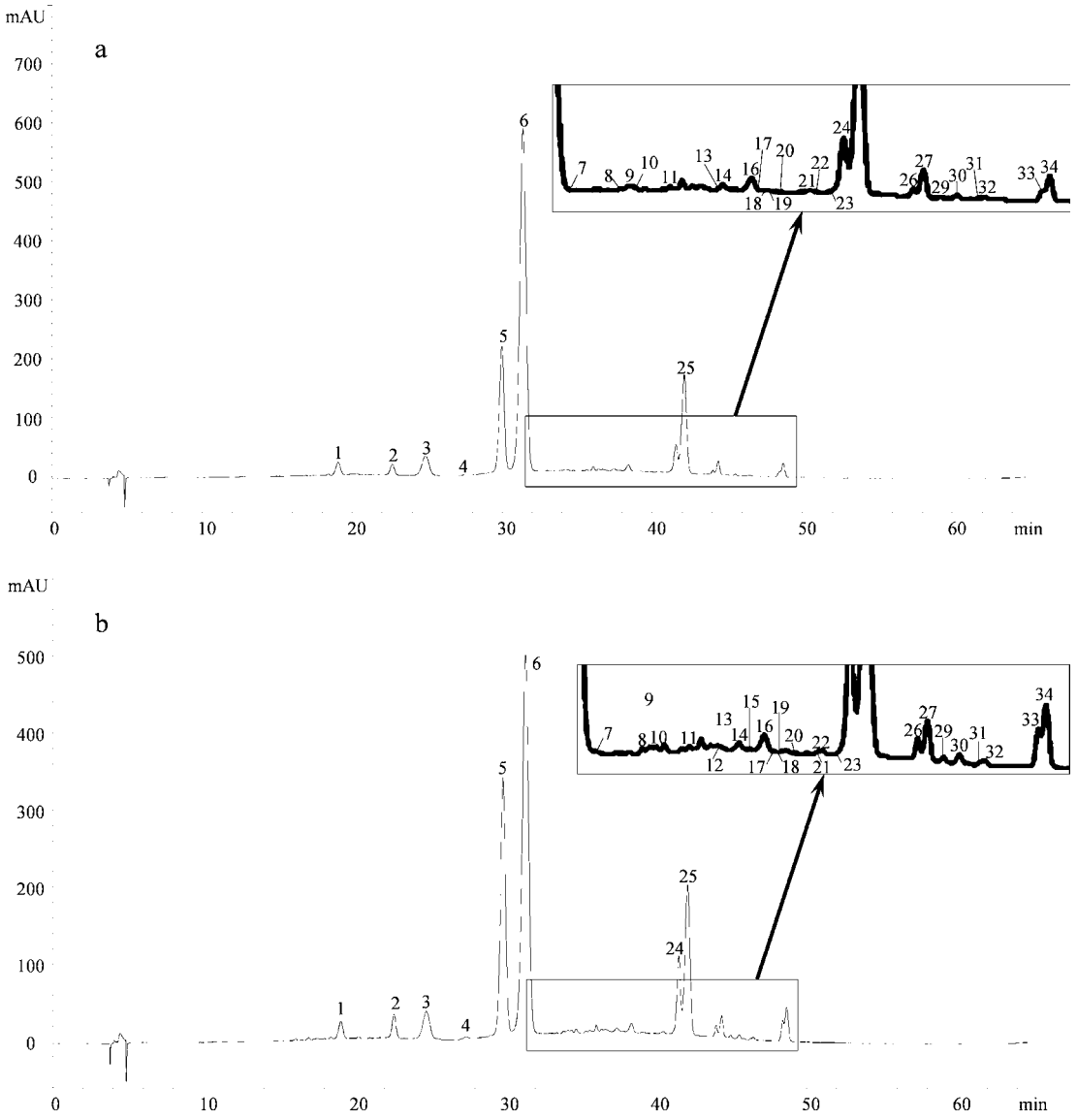

Figure 4. HPLC-DAD chromatograms at 520 nm of anthocyanins of the musts from raisins of Merlot (a) and Syrah (b) varieties.

drying process. However, most of the anthocyanin compounds increased not only by the concentration effect due to water losses by evaporation but also by the effect of the disruption of grape skin layers, which facilitated the release of anthocyanins to the pulp and led to strongly colored musts from the dried grapes.[31] The subsequent vinification process reduced the concentration of some compounds; this can be ascribed to the dilution by the effect of the addition of wine alcohol. Also, according to Boulton,[12] anthocyanins cease to be extracted from grape skins at some point in the maceration process, and an adsorption−desorption equilibrium is then reached between anthocyanins in skin and wine. In addition, during the maceration time, the anthocyanins could take part in different reactions such as polymerization, copigmentation, tannin reactions, and formation of adducts. In fact, most monomeric glucosyl and acetylglucosyl derivatives present in the wines were at low concentrations relative to those musts from dried grapes. Also, pelargonidin-3-glucoside was detected in the two grape varieties, albeit at low levels in both the musts (0.598 and 0.818 mg/L) and the final wines (0.875 and 0.947 mg/L) for Merlot and Syrah, respectively.

The concentrations of all caffeoyl- and coumarylglucosides increased by the effect of vinification in both varieties, because these derivatives are less reactive than the above-mentioned compounds. Also, the *cis* isomers of peonidin-3-glucoside and malvidin-3-glucoside were present at lower concentrations than the *trans* isomers. The concentrations of *cis* isomers were detected at trace levels in some samples. In any case, both types of derivatives were at higher levels in the Syrah wines than in the Merlot wines.

In the musts from dried grapes and their corresponding wines, vitisins were found at very low concentrations, vitisin B being the most abundant, and the type B vitisins were registered in higher concentration than type A vitisins. This fact is not in agreement with other authors;[37] due to the traditional winemaking vitisin A is formed more rapidly than vitisin B, due to the concentration of pyruvic acid produced by the yeasts at the beginning of the alcoholic fermentation. In this winemaking there was no alcoholic fermentation, so the yeasts did not produce pyruvic acid; therefore, the production of this acid was much less according to the above-mentioned enzymatic mechanism and, in addition, this pyruvic acid could be

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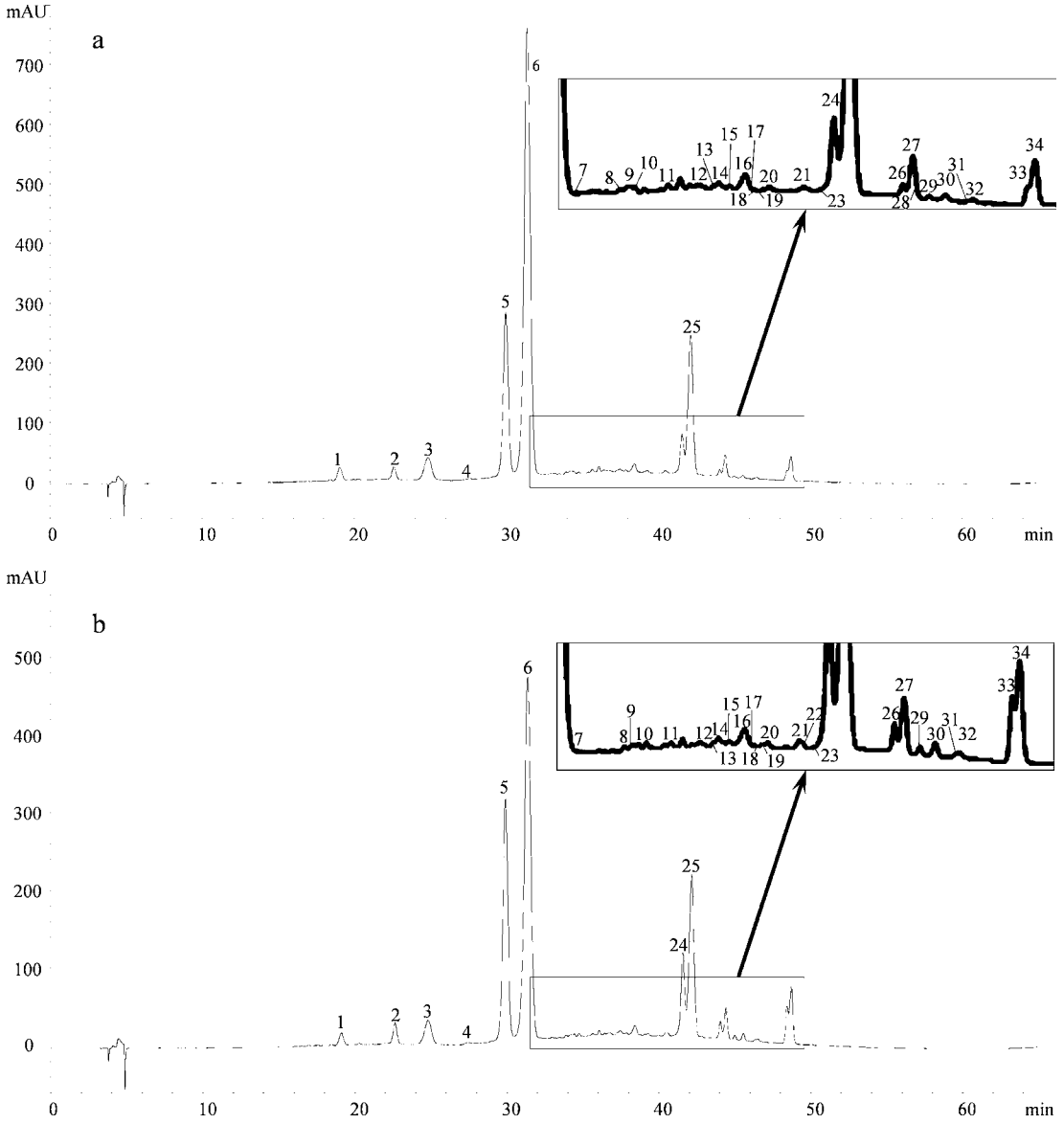

Figure 5. HPLC-DAD chromatograms at 520 nm of anthocyanins of the final wines of Merlot (a) and Syrah (b) varieties.

converted gradually into acetaldehyde, according to our hypothesis. On this basis, and considering the concentration of the both reagents (20 mg/L pyruvic acid and 120 mg/L acetaldehyde), the reaction from pyruvic acid to acetaldehyde is more favored than the formation of the adduct anthocyanin−pyruvic acid, so the formation of type B vitisins could be more probable than the formation of type A vitisins. In addition, the formation of both vitisins seems to follow an antagonistic kinetics, because the acetaldehyde could compete with pyruvic acid by the anthocyanin molecule.[38]

In addition, the four type A and B vitisins from malvidin-3-glucoside increased by the effect of the addition of wine alcohol. The type B vitisin from peonidin-3-glucoside in Merlot grapes was the exception. This suggests that these derivatives are produced at a high rate when all reactants needed for its synthesis are present in the medium.

Most of the addition products of anthocyanins to (epi)-catechin formed by methylmethine bonding present in the musts from dried grapes were at trace levels. Thus, the concentration of Mv-3-glc-methylmethine(epi)catechin (peak 20) in the Merlot must was 0.479 mg/mL. In the Syrah must, the concentrations of the two compounds with the same chemical formula (peaks 15 and 21) were 0.466 and 0.474 mg/L, respectively. The subsequent addition of wine alcohol and maceration of the wines caused the formation of additional compounds, all at higher concentrations than their corresponding musts. Thus, three further compounds (peaks 12, 15, and 21) were quantified at levels >1 mg/L in the Merlot wine.

In conclusion, the HPLC-DAD-MS technique allowed confirmation of the presence of pelargonidin-3-glucoside in musts from both fresh and dried grapes of the Merlot and Syrah varieties, as well as in the resulting wines. In addition, the grapes from off-vine raisining disrupted their inner skin layers and led to anthocyanins diffusing to the pulp and leading to musts strongly enriched with these compounds. Also, chamber-drying altered the membrane permeability of the grapes through the activation of the lipoxygenase enzyme (LOX) and a change in the metabolism from aerobic to anaerobic and, as result, an activation of alcohol dehydrogenase (ADH). The presence of pyruvic acid, acetaldehyde, and ethanol in the

Table 2. Concentrations (Milligrams per Liter) of Anthocyanin Compounds (Mean ± Standard Deviation) in Merlot and Syrah Musts and Wines and Homogeneous Groups ($p < 0.001$)

		Merlot			Syrah		
peak	compound	fresh grapes	dried grapes	wine	fresh grapes	dried grapes	wine
1	Dp-3-glc	2.79 ± 0.125 b	1.75 ± 0.147 a	1.54 ± 0.202 a	0.697 ± 0.003 a	1.45 ± 0.031 c	0.872 ± 0.016 b
2	Cy-3-glc	1.72 ± 0.122 b	1.16 ± 0.052 a	1.33 ± 0.161 a	1.80 ± 0.031 c	1.56 ± 0.003 b	1.30 ± 0.039 a
3	Pt-3-glc	11.6 ± 0.581 a	11.4 ± 0.818 a	10.8 ± 1.12 a	5.90 ± 0.033 a	9.34 ± 0.029 c	7.99 ± 0.031 b
4	Pg-3-glc	0.598 ± 0.003 a	1.04 ± 0.069 b	0.875 ± 0.096 b	0.818 ± 0.035 a	0.870 ± 0.035 a	0.947 ± 0.092 a
5	Pn-3-glc	17.9 ± 1.02 a	20.0 ± 0.207 b	19.3 ± 0.211 ab	16.8 ± 0.886 a	22.7 ± 0.632 b	20.2 ± 1.61 b
6	Mv-3-glc	58.8 ± 3.65 a	74.1 ± 1.97 b	70.8 ± 2.44 b	30.0 ± 1.44 a	47.2 ± 1.71 b	43.1 ± 3.61 b
7	vitisin A	nd	0.121 ± 0.006a	0.185 ± 0.005a	nd	0.105 ± 0.008a	0.119 ± 0.009a
8	Dp-3-acetylglc	0.645 ± 0.008 c	0.393 ± 0.001 a	0.486 ± 0.006 b	tr [a]	0.400 ± 0.002 b	0.372 ± 0.001 b
9	B-type vitisin Pn-3-glc	nd	0.750 ± 0.002 b	0.541 ± 0.010 a	nd	0.383 ± 0.002a	0.397 ± 0.004 b
10	A-type vitisin Mv-3-acetylglc	nd	0.444 ± 0.040 a	0.715 ± 0.143 a	nd	0.527 ± 0.009 a	0.582 ± 0.026 b
11	vitisin B	nd	0.837 ± 0.036 a	1.32 ± 0.157 b	nd	0.760 ± 0.051 a	0.852 ± 0.026 a
12	Pn-3-glc-ethyl(epi)catechin	nd	nd	1.10 ± 0.032	nd	tr	tr
13	Mv-3-glc-ethyl(epi)catechin	nd	tr a	0.650 ± 0.041 b	nd	tr [a]	0.600 ± 0.003 b
14	Cy-3-acetylglc	0.284 ± 0.070 a	0.983 ± 0.017 c	0.534 ± 0.014 b	0.285 ± 0.015 a	0.666 ± 0.056 b	0.353 ± 0.102 a
15	Mv-3-glc-ethyl(epi)catechin	nd	nd	1.01 ± 0.058	nd	0.466 ± 0.001 a	0.917 ± 0.177 b
16	Pt-3-acetylglc	3.74 ± 0.137 a	6.33 ± 0.510 b	7.12 ± 0.403 b	3.12 ± 0.069 a	5.17 ± 0.373 b	4.88 ± 0.019 b
17	B-type vitisin Pn-3-acetylglc	nd	tr a	2.31 ± 0.088 b	nd	0.200 ± 0.019 a	0.369 ± 0.005 b
18	Pn-3-glc-ethyl(epi)catechin	nd	tr	tr	nd	tr	0.402 ± 0.029
19	B-type vitisin Mv-3-acetylglc	nd	tr a	0.626 ± 0.044 b	nd	tr	tr
20	Mv-3-glc-ethyl(epi)catechin	nd	0.479 ± 0.020 b	tr a	nd	tr a	0.828 ± 0.053 b
21	Mv-3-glc-ethyl(epi)catechin	nd	tr a	1.02 ± 0.102 b	nd	0.474 ± 0.058 b	tr a
22	Pn-3-glc-ethyl(epi)catechin	nd	0.500 ± 0.093	nd	nd	tr	tr
23	Pt-3-caffeoylgluc	1.92 ± 0.185 a	2.86 ± 0.022 b	2.99 ± 0.027 b	2.11 ± 0.181 a	2.95 ± 0.098 b	3.10 ± 0.019 b
24	Pn-3-acetylglc	3.44 ± 0.164 a	3.81 ± 0.080 a	5.17 ± 0.152 b	3.65 ± 0.123 a	5.57 ± 0.061 b	6.06 ± 0.493 b
25	Mv-3-acetylglc	15.9 ± 1.34 a	16.7 ± 0.348 a	18.2 ± 0.641 a	8.96 ± 0.599 b	14.3 ± 0.653 c	6.91 ± 0.482 a
26	Pn-3-caffeoylglc	tr a	0.449 ± 0.002 b	0.845 ± 0.001 c	tr a	0.851 ± 0.031 b	1.25 ± 0.015 c
27	Mv-3-caffeoylglc	0.175 ± 0.031 a	1.73 ± 0.020 b	2.45 ± 0.199 c	0.290 ± 0.043 a	1.56 ± 0.052 b	2.45 ± 0.128 c
28	Mv-3-acetylglc-ethyl(epi) catechin	nd	nd	tr	nd	nd	nd
29	Cy-3-coumaroylglc	tr a	tr a	0.297 ± 0.045 b	tr a	tr a	0.476 ± 0.011 b
30	Pt-3-coumaroylglc	1.88 ± 0.055 a	2.36 ± 0.029 b	3.40 ± 0.176 c	1.84 ± 0.060 a	2.54 ± 0.170 b	3.58 ± 0.222 c
31	Pn-3-coumaroylglc *cis*	tr a	tr a	0.184 ± 0.035 b	tr	tr	tr
32	Mv-3-coumaroylglc *cis*	0.054 ± 0.016 a	0.108 ± 0.029 a	0.467 ± 0.007 b	tr a	tr a	0.468 ± 0.032 b
33	*Pn*3-coumaroylglc *trans*	0.038 ± 0.002 a	0.335 ± 0.063 b	0.619 ± 0.007 c	0.197 ± 0.012 a	0.786 ± 0.037 b	1.64 ± 0.137 c
34	Mv-3-coumaroylglc *trans*	0.709 ± 0.058 a	1.45 ± 0.034 b	2.22 ± 0.003 c	0.431 ± 0.005 a	2.00 ± 0.103 b	3.31 ± 0.428c

musts from dried grapes explains their formation by enzymatic reactions. These compounds were the precursors in the formation of pyranoanthocyanins and methylmethine-bonded anthocyanin–flavanol condensation adducts. These anthocyanin derivatives confer to sweet red wines, which are produced in the absence of alcoholic fermentation, color stability against pH changes and SO_2. This warrants the conduct of future research with a view to identifying the specific grape-drying conditions maximizing the synthesis of these anthocyanin derivatives.

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