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Changes in Color and Phenolic Compounds during the Raisining of Grape Cv. Pedro Ximenez

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Changes in color parameters and phenolic compounds during the sun-drying grape raisining of cv. Pedro Ximenez to obtain sweet wines are studied. Browning increases during the process as a result of the contribution to a greater extent of the low and medium molecular size polymers than the high molecular size polymers. Raisining decreases hue and lightness and increases chroma, all measured as CIELab parameters, indicating a color change to dark reddish hues that is also preferentially due to low and medium molecular size polymers. Most of the phenols studied increase in concentration during raisining, essentially through the concentration effect resulting from the loss of water in the grapes. The concentration changes, however, are comparatively small for hydroxycinnamic esters and flavan-3-ol derivatives, suggesting that these phenolic fractions undergo predominantly oxidative degradation reactions by enzymatic pathways, contributing strongly to the browning of grapes.

KEYWORDS: Raisining; sweet wines; grape browning; CIELab; phenolic compounds

INTRODUCTION

Pedro Ximenez sweet wine is produced in southern Spanish regions from sun-dried grapes with a cultivar of the same name. Particularly, sun-dried raisining is a common practice in the semiarid climate of the Montilla-Moriles region, with an index above 2597 °C in zone V of the climatic classification of Winkler (*1, 2*). Cv. Pedro Ximenez grapes are typically collected at 13–14 Baumé degrees and extended to sun on mats in slight hills facing south. Depending on the climatic conditions of the particular year, the raisining process can take 5–10 days to complete. During the process, diurnal temperatures can rise above 40 °C (*3*), whereas nocturnal values infrequently fall below 18 °C. The resulting raisins are crushed and pressed at a high pressure in a vertical hydraulic press to ensure a yield of at least 30% (w/v). The must thus obtained is extremely sweet (it can easily contain reducing sugar concentrations above 450 g/L), with a strong flavor of raisins and a very dark brown color. These musts are partially fermented to an ethanol content of 5–9% (v/v) and subsequently fortified up to 12–17%, the resulting wines with higher ethanol contents being subjected to oxidative aging in American oak casks for a variable length of time depending on the commercial criteria.

The brown color of raisins is the combined result of pigments formed by the effects of enzymatic and nonenzymatic reactions (*4, 5*) taking place during grape dehydration (*6*). Several phenolic compounds, particularly hydroxycinnamic acids (*7*), are well-known substrates for oxidative enzymes such as polyphenol oxidase (PPO) (*8*). PPO is a copper-containing enzyme that catalyzes the oxidation of phenolic compounds to quinones in the presence of molecular oxygen, subsequently evolving to brown pigments (melanins). These compounds are responsible for the color alteration, loss in flavor quality, and taste changes (*9*).

The Maillard reaction is induced by thermal treatment in foods containing compounds with free carbonyl and amino groups. The main substrates for this reaction in grapes are monosaccharides and amino acids, which by condensation, dehydration, isomerization, and cyclization lead to the formation of colored compounds, being particularly interesting those polymerized (melanoidins). Some such polymer compounds, the structure of which is poorly known, and other secondary compounds cause not only browning but also changes in the sensory properties of foods. In addition, they possess antioxidant activity, but their efficiency in this respect is influenced by several factors including the types of amino acids and sugars involved and their proportions, temperature, pH, and water activity (*10*). The Maillard reaction is faster with increasing temperatures (especially above 50 °C), and it is favored by pH values over the range from 4 to 7, which are quite usual in foods (*11*). Particularly in sweet wines, Pérez-Magariño et al. (*12*) showed the presence of yellowish brown compounds similar to those found in beer (where they typically appear as a result of the malting of barley). These authors suggested that they might be melanoidins, the most of a high molecular weight, which contribute significantly to the color of wine made from raisins.

It is difficult to evaluate the contribution of each pathway to browning of the grapes used in the production of sweet wines. On the one hand, Radler (*13*) has pointed out that at the end of

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the ripening, and during drying, the grapes contain high concentrations of sugars that may inhibit the browning action of PPO, thereby gradually reducing the contribution of the enzymatic pathway as raisining progresses. On the other hand, the raisining temperature and the gradual decreasing of water activity of grapes can facilitate the progress of the Maillard reactions, leading to the formation of colored polymers of a high molecular weight (*14*).

Depending largely on the climatic features of the particular year, the raisining process can meet with a serious problem because of the growth of fungi producing toxins, such as ochratoxin A (OTA). This compound is a potent nephrotoxin with immunosuppressive, teratogenic, and carcinogenic properties (*15*), in addition to potentially neurotoxic properties (*16*). OTA contamination in grapes and wines is usually introduced by the fungi *Aspergillus nigri* and *Aspergillus carbonarius* (*17*), the latter being the most common by far in Mediterranean regions of Europe (*18*). Because of its potential toxicity, the European Commission has recently established a maximum allowed concentration of ochratoxin A in wines, musts, and grape juices of 2 μg/L (*19*).

The technological replacement of the traditional sun-drying process of Pedro Ximenez grapes with their drying in chambers under controlled conditions would be advantageous from two points of view. On the one hand, the drying process would not be dependent on the climatic conditions of each year, as it would be possible to choose the temperature and degree of humidity in the chambers to minimize the formation of OTA. On the other hand, this lack of dependence on climatic conditions could allow also the degree of the grape ripening to be chosen to obtain an equilibrated berry. However, the change in the grape drying process should consider the wide acceptance of Pedro Ximenez sweet wines obtained from sun-dried grapes, with an annual production completely sold out in advance. Therefore, the traditional sun-drying process should be used as reference in the grape composition to obtain a product as similar as possible. The purpose of this work was to examine changes in color and phenolic compounds during the raisining of cv. Pedro Ximenez grapes in the traditional way (by drying in the sun), as a preliminary study to the technological update of the grape raisining in chamber for the production of sweet wines.

MATERIALS AND METHODS

Sample Collection. Pedro Ximenez grapes were collected in the Montilla-Moriles region (southern Spain). Samples were obtained from grapes extended to sun in 10 drying mats about 20 m long each. The grapes were randomly collected in 8 kg batches in triplicate at 8:30 a.m. each day from the start of the raisining process (fresh gapes, day 0) to its end (day 7).

In the laboratory, the grapes were crushed and subsequently pressed in a vertical press similar to those used at the industrial level. The highest pressure reached in each pressing cycle was 300 bar, and each grape batch was pressed in three cycles. The musts thus obtained were centrifuged at 3000 rpm and subjected to the different determinations.

UV–Visible Spectra and Color Measurements. UV−vis spectra were obtained in the range of 250–780 nm using water as reference. The spectrum in the UV zone and absorbances at 280 nm were measured after 1:10 dilution, and A_{420} values were considered to be the browning index. Color analyses were carried out following CIE recommendations (*20*) and using the visible spectrum obtained from 380 to 780 nm (Perkin-Elmer Lambda 25 model). In this work, the following CIELab uniform space colorimetric parameters have been considered: rectangular coordinates L^* (black−white component, lightness), a^* and b^* (chromatic coordinates representing red−green and yellow−blue axes, respectively), and the cylindrical coordinates C^*_{ab} (chroma) and h_{ab} (hue angle). These parameters were measured using as references the CIE 1964 Standard Observer (10° visual field) and the CIE standard illuminant D65.

All spectrophotometric measurements were obtained after filtration of the samples through a filter of HA-0.45 μm pore size (Millipore) and using a Beckman spectrophotometer, DU 600 model, on a 10 mm path length for the absorbances at 280 and 420 nm.

Dialysis. Musts were dialyzed using cellulose dialysis tubing (Sigma-Aldrich) that retained the molecules of a size ≥12000 Da. About 15 mL of must was put into the dialysis tubing, and it was placed in a vessel with 1 L of water. This solution was maintained at 4 °C with stirring for 12 h, followed by a replacement of the water surrounding the dialysis tubing. This procedure was repeated six times. The volume of must that remained in the dialysis tubing was diluted to 25 mL with distilled water. This solution was named the dialyzed fraction.

A_w. The water activity of the entire grapes was determined at 20 °C by using a CX-1 dew-point hygrometer Decagon Devices (Pullman, WA).

OTA Determination. A sample of 5 mL of must was passed through a SPE C18 cartridge, with 1 g of filling (Supelco, Sigma-Aldrich Corp., St. Louis, MO). After preconditioning of the cartridge with 5 mL of acetonitrile/water (10:90) and drying, the OTA was eluted with 1 mL of acetonitrile/water (90:10). Chromatographic analysis was carried out by injection of 10 μL in an HPLC-MS triple quadrupole (Varian 1200L), using a C-18 column (50 mm × 1 mm, 2 μm particle size), and 0.1% aqueous formic acid (solvent A) and acetonitrile/methanol (75:25, solvent B) as mobile phases at a flow rate of 0.2 mL/min. The ionization technique was ESI in negative mode. The column temperature was 40 °C, and the elution phases were as follows: gradient elution, 15−100% CH_3CN in 6 min; isocratic elution, 3 min.

Extraction of Phenolic Compounds. A volume of 25 mL of must was adjusted to pH 7 with 0.1 M NaOH. The sample 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 8 mL of methanol and washed with distilled water, which was adjusted to pH 7 with NaOH (*21*). The cartridge was eluted with 8 mL of water at pH 7. This volume in addition to the volume obtained as a result of the sample run-through prior to the elution was used for the determination of phenolic acids fraction. After preconditioning of the cartridge with 2 mL of water at pH 2, the flavan-3-ol fraction was eluted with 8 mL of 16% acetonitrile in water at pH 2 (*22*). The flavonol fraction was eluted with 10 mL of ethyl acetate. These three collected fractions were concentrated and passed through a filter of 0.45 μm pore size for injection into a Spectra-Physics (San Jose, CA) P4000 HPLC instrument.

Identification and HPLC Analysis. The identification of the phenolic compounds was achieved by comparison with the retention times of the standards, UV spectra obtained by HPLC diode array (Spectra-Physics UV6000LP), and calculation of UV absorbance ratios after co-injection of samples and standards (*23*). The identification of compounds was confirmed by HPLC/ESI-MS analysis (TermoQuest Finnigan AQA quadrupole mass spectrometer). The instrument was operated in both the negative ion and positive ion modes. The ion spray voltage was –4 kV and the orifice voltage, –60 V. Mass data were acquired in two different ways, namely, in the scan mode (by scanning the m/z range from 150 to 1066 at 1.2 intervals) and in the multiple ion mode (by using mass ranges around specific m/z values). Commercial standards were purchased from Sigma-Aldrich Chemical Co. (Madrid, Spain) and Extrasynthese Co. (Genay, France). Caftaric and coutaric acids were isolated according to the method described by Singleton et al. (*24*). The standard purity was 95–99%. Each compound was quantified by comparison with a calibration curve obtained with the corresponding standard, except the caftaric, coutaric, and feftaric acid that were quantified as caffeic, *p*- coumaric, and ferulic acid, respectively, and procyanidins that were quantified as catechin.

Analyses were carried out on a LiChrospher 100 RP-18 column (250 mm × 4.6 mm, 5 μm particle size) by using 2% aqueous formic acid and acetonitrile as mobile phases at a flow rate of 1 mL/min and detection at 280 nm (phenolic acids and flavan-3-ol fractions), 315 nm (esters of hydroxycinnamic acid), and 360 nm (flavonols).

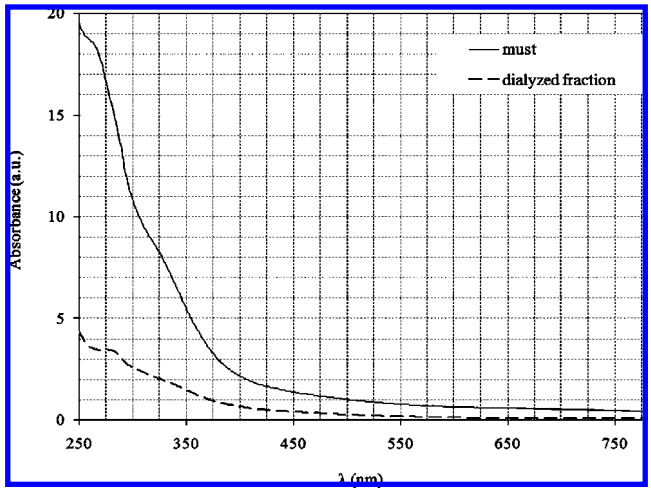

Figure 1. UV−vis spectra for the must and its dialyzed fraction at the end of the raisining process.

The elution phases were as follows: gradient elution from 5 to 10% CH_3CN in 25 min, gradient elution up to 20% CH_3CN in 10 min, gradient elution up to 30% CH_3CN in 10 min, gradient elution up to 100% CH_3CN in 15 min, and isocratic elution for 10 min.

Statistical Procedures. Principal component analyses were performed on the replicated samples by using Statgraphics Statistical Computer Package (Statistical Graphics Corp. v 5.0).

RESULTS AND DISCUSSION

All of the samples obtained during the raisining process showed levels of ochratoxin A below its quantification limit with reliability (<0.1 μg/L). Probably, these low levels were a result of the climate dryness in the Mediterranean area in the past two years (2006 and 2007), particularly in the summer and autumn seasons with virtual absence of nocturnal dew, which does not favor the growth of fungus producer of toxins. **Figure 1** shows the UV−vis spectra for the must obtained at the end of the raisining process (7 days) and its dialyzed fraction. Dialysis isolates brown-colored polymers of a high molecular weight (potentially melanoidins), caramelization products, and phenolic compounds (and/or the products of their browning) of also high molecular weight. As can be seen, the spectrum for the must without dialysis differed from that for the dialyzed fraction, particularly in the UV region. This last spectrum was according to those obtained by other authors working in dialyzed fractions of sweet wines (*14*) and synthetic melanoidins (*12*), which suggests that a substantial portion of the brown polymers may be melanoidins.

Figure 2 shows the change in the absorbance at 280 nm of the musts and their dialyzed fractions during raisining. As can be seen, the A_{280} in musts increased gradually from 5.74 au at the beginning to 14.9 au at the end, therefore increasing by a factor of 2.6 during the raisining process. Because the grapes lost substantial amounts of water during that period, all of their compounds should have gradually grown in concentration. If the increase in reducing sugars, which constitute the major fraction of grapes, is used as a measurement of the concentration effect resulting from the evaporation of water, this effect can be estimated in a concentration increased by a factor of 2.4 (because the sugars increased from 204.8 to 494.2 g/L, that is to say, 2.4 times). As stated above, A_{280} increased by a factor of 2.6, and therefore this increase can largely be ascribed to the concentration effect caused by grape dehydration.

The increase in A_{280} for the dialyzed fraction was less marked (from 1.72 to 3.39 au), which is slightly less than twice. Such a fraction contributed only 30% to the A_{280} at the start of raisining, providing the low and medium molecular weight compounds the main contribution at this absorbance. At the end of the raisining period, this last fraction contributed 77%, showing that the concentrations of the compounds of low and medium molecular weight increased to a greater extent than did those of high molecular weight, assuming that the molar extinction coefficient of both types of compounds is similar in order of magnitude.

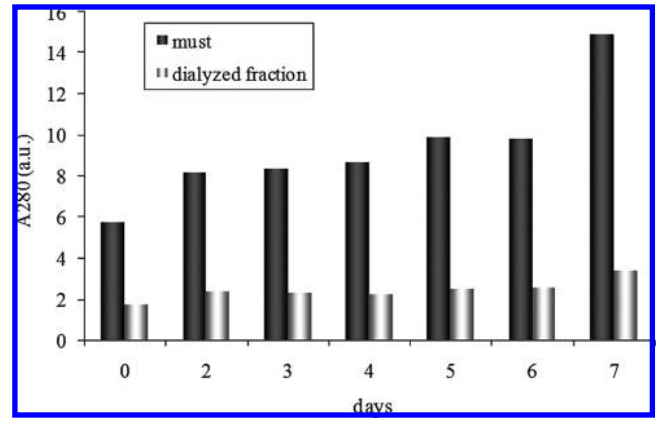

Figure 2. Changes in the absorbance at 280 nm of the musts and their dialyzed fractions during raisining.

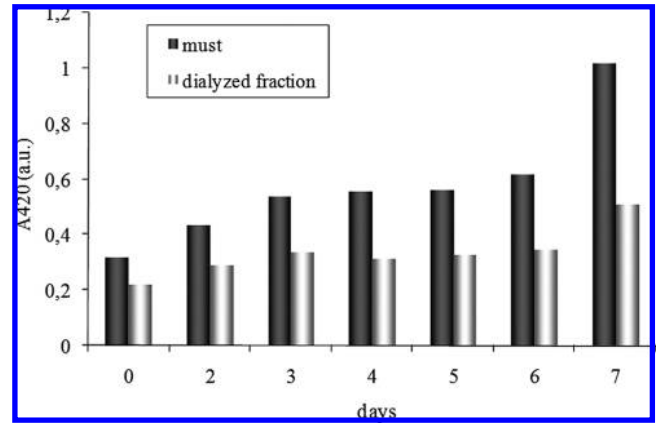

Figure 3. Changes in the absorbance at 420 nm of the musts and their dialyzed fractions during raisining.

Figure 3 shows the changes in browning, measured as the absorbance at 420 nm, in both the musts and their dialyzed fractions over the raisining process. As can be seen, A_{420} increased from 0.316 to 1.02 au in the musts and from 0.218 to 0.507 in the dialyzed fractions (3.2 and 2.3 times, respectively). The dialyzed fraction accounted for nearly 70% of the brown color in the initial must, indicating that most of the colored compounds were polymers of a high molecular weight. However, this proportion fell gradually during the process (to 50% after 7 days), indicating that the colored compounds of low and medium molecular weight grew more markedly in concentration than did those of high molecular weight. Taking as reference the increase in reducing sugars, the growth in the contents of these latter could be reasonably attributed to the evaporation of water during raisining.

The color of musts and their dialyzed fractions during the raisining process can be described in terms of CIELab coordinates (**Figure 4**): the a^*, b^* color plane and the difference of lightness, L^*. As can be seen, musts shifted to the right and up in the color plane as the raisining process developed, with

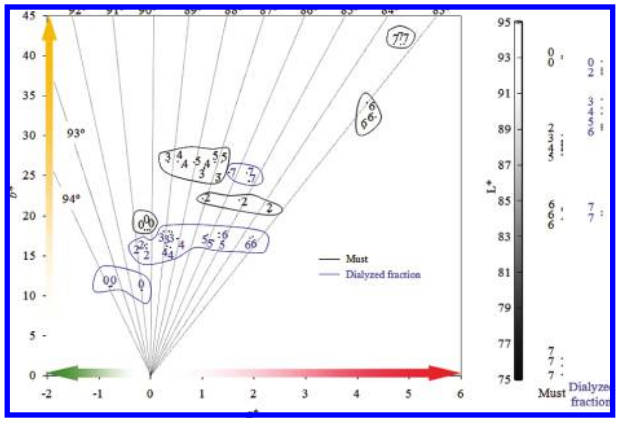

Figure 4. CIELab coordinates for the musts and their dialyzed fractions.

appreciable differences between the initial must and that obtained after 2 days, little changes on days 3–5, and strong variations on days 6 and 7. In addition, color can be expressed in psychochromatic terms by means of the hue angle (h_{ab}) and chroma (C^*_{ab}). The h_{ab} acquires values near 0° for the redness and yellowness in the proximity of 90°. As can be seen, h_{ab} for the studied musts decreased with raisining from about 90° on day 0 to 82–83° on days 6 and 7, indicating that musts reddened with raisining, particularly at the end of the period. C^*_{ab} is a measure of color saturation or intensity, and it is represented by the magnitude of the vector at each point. Such intensity increased during raisining in the musts, particularly for the most raisined grapes (6 and 7 days). Finally, the vertical axis of the CIELab three-dimensional space measures lightness (L^*), which can range from 0 for black to 100 for white. On the basis of its variation, the musts darkened with increasing raisining of the grapes. From a practical point of view, the Euclidian distance ΔE^*_{ab} (which is the distance between two points in a three-dimensional space) is one useful parameter because the human eye can discriminate samples differing by more than 1 in ΔE^*_{ab} (*25*), although some authors establish this value around 3 for red wines (*26, 27*). In any case, the following table shows that each grape must was chromatically different from that obtained from the next sample, except those obtained after 3–4 and 4–5 days of raisining, which cannot be discriminated in terms of ΔE^*_{ab} and consequently be distinguished by the human eye.

raisining time (days)	0–2	2–3	3–4	4–5	5–6	6–7
ΔE^*_{ab}	6.00	4.58	0.39	0.62	7.45	12.50

The dialyzed fraction exhibited the same trends in its color parameters, increasing gradually in yellowish red hues, color intensity, and darkening. Nevertheless, a comparison of the color parameters for the high molecular weight polymers and the must color on each sampling day reveals that the differences were much less marked at the beginning and during the first few days of raisining than they were on days 6 and 7, where the musts exhibited nearly double values in the red and yellow components and color intensity. Finally, the lightness of the dialyzed fractions obtained during the first 6 days of raisining never exceeded that of the must obtained on the second day, and only on day 7 did the dialyzed fraction exhibit a more marked darkening, its L^* being similar to that of the must obtained on day 6. On the basis of the foregoing, high molecular weight compounds contributed to a greater extent to must color CIELab parameters at the beginning of raisining than they did at the end. In other words, low and medium molecular weight colored compounds contribute the most to the color characteristics of the must for obtaining Pedro Ximenez sweet wines.

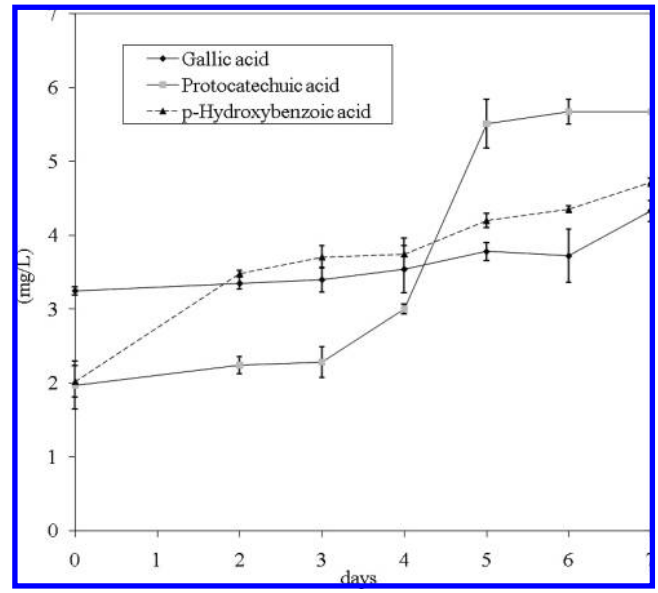

Figure 5. Change in the concentrations of gallic, protocatechuic, and *p*-hydroxybenzoic acids during grape raisining.

As a rule, all phenolic compounds should increase as a result of the grape dehydration during raisining (concentration effect). However, during the raisining process, some phenolic compounds can take part in reactions of the oxidation, mainly by enzymatic pathways led by polyphenol oxidases or peroxidases (*28*). Likewise, it is well-known that some phenols can undergo hydrolysis reactions, and conversely others can increase their contents because of the hydrolysis of higher oligomers, such as procyanidins. Therefore, the final increase (or decrease) in a particular phenol is the result of a balance between losses and gains in concentration terms. **Figure 5** shows the changes in the concentrations of gallic, protocatechuic, and *p*-hydroxybenzoic acids during raisining. As can be seen, gallic acid was the most concentrated of the three at the beginning (3.25 versus 2 mg/L for both protocatechuic and *p*-hydroxybenzoic acids). Raisining increased the concentration of the three compounds, particularly that of protocatechuic acid, which grew by 188%, suggesting an additional increase for this compound by ways other than grape dehydration (increase for reducing sugars by 141%). However, the concentration of gallic acid increased by only 33%, indicating that this compound must be involved in oxidation reactions, possibly followed of polymerization processes, leading to its degradation during raisining (*29*). As stated above, the concentration of *p*-hydroxybenzoic also increased, although it can be reasonably attributed to the evaporation of water from the grapes.

Figure 6 shows the changes in the concentrations of caftaric, coutaric, and feftaric acids during grape raisining. As can be seen, all were present in their cis and trans forms, although the trans configurations of hydroxycinnamic esters are their natural chemical structures in grapes. According to some authors, the cis structures of these compounds could be formed by the effect of UV radiation (*6, 30, 31*). The final balance for the previous three acids was a concentration increased more markedly in the cis structures (38.5, 42.9, and 7.3% for caftaric, coutaric, and feftaric acid, respectively) than for the trans (15.7, 8.7, and 0%, respectively). These increases were lower than expected exclusively because of grape dehydration, indicating a simultaneous degradation of these compounds during the raisining process.

Figure 7 shows the contents in flavan-3-ol monomers and dimers during the studied period. As can be seen, (+)-catechin

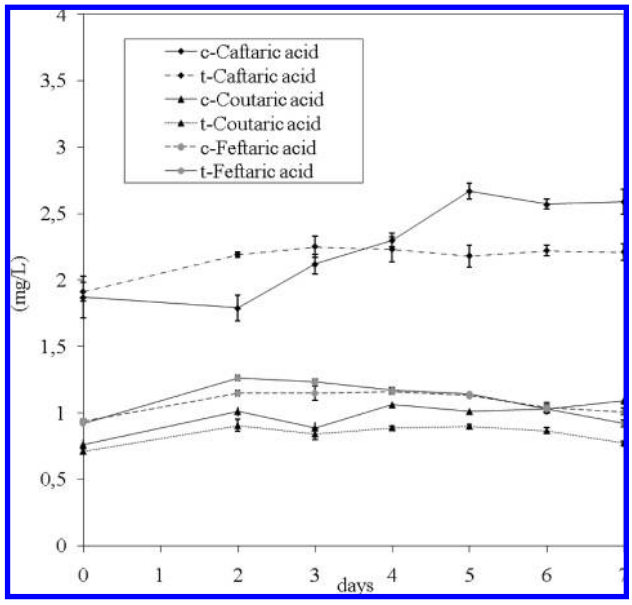

Figure 6. Change in the concentrations of caftaric, coutaric, and feftaric acids during grape raisining.

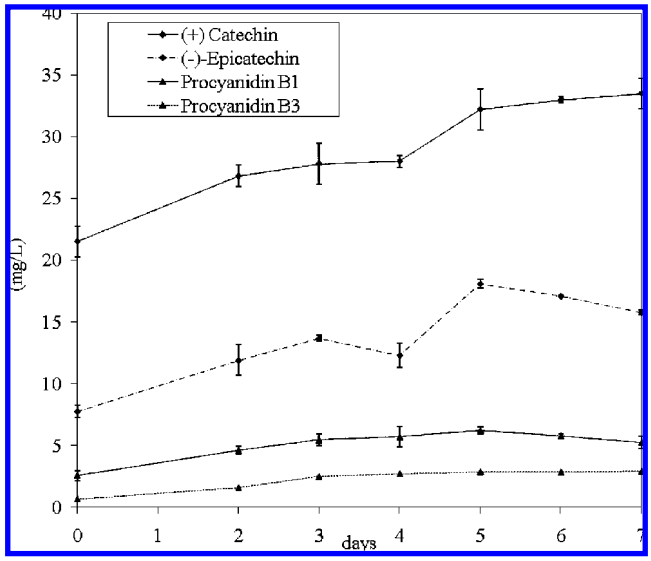

Figure 7. Change in the concentrations of flavan-3-ol monomers and dimers during grape raisining.

was the flavan present at the highest concentrations throughout the process (its content rose from 21.5 to 33.5 mg/L), followed by (−)-epicatechin (from 7.76 to 15.8 mg/L) and procyanidins B1 (from 2.56 to 5.26 mg/L) and B3 (from 0.67 to 2.92 mg/L). Procyanidins B2 and B4 were found at very low levels, below their quantification limits. The contents in (+)-catechin, (−)-epicatechin, and procyanidin B1 increased by 56, 104, and 105%, respectively, at the end of the process, these increases being well below that for reducing sugars (141%), particularly corresponding to the former. Taking into account that both the concentration effect resulting from the evaporation of water during raisining and the potential hydrolysis of higher oligomers pointed out by some authors (*32–35*) should lead to higher increases, these compounds must have unavoidably undergone degradation reactions. In some cases, these reactions can lead to a complete degradation of some flavan-3-ols during grape raisining, as some authors have pointed out (*6*). Conversely, the content in procyanidin B3 increased by 334%, suggesting a

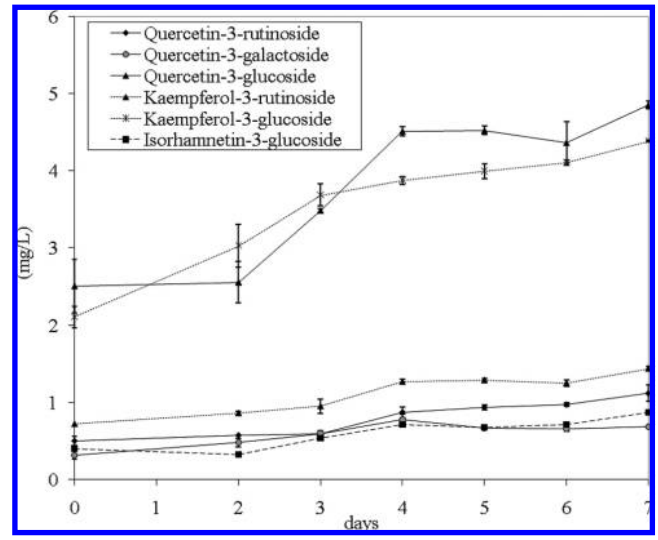

Figure 8. Change in the concentrations of flavonol fractions during grape raisining.

contribution of both evaporation of water and hydrolysis of larger oligomers.

Figure 8 shows the flavonol contents during grape raisining. Flavonols constitute a group of flavonoid compounds ranging from colorless to yellow. Therefore, depending on their particular structure and concentrations, some might slightly contribute to yellowish brown hues measured by A_{420}. Specifically, model solutions of quercetin derivatives (glycoside, rutinoside, and galactoside) at concentrations similar to those in the raisins were found to contribute 0.023 au to A_{420}. In concentration terms, the glycosydes of kaempferol and quercetin prevailed over the others, their contents ranging from 2−2.5 mg/L at the beginning of raisining to 4.4–4.9 mg/L after 7 days (they increased by 108 and 94%, respectively). The other flavanols also increased in concentration by 99 and 123%, which is lower than expected because of the water evaporation, suggesting that all of these compounds could take part in different reactions with results of difficult evaluation in concentration terms. Thus, flavonols are produced in plant tissues by biosynthetic pathways that are strongly influenced by available sunlight. That is why the most strongly lighted grapes are usually those producing the greatest amounts of flavonols (*36, 37*). Nevertheless, UV light is also known to influence the contents in flavonol glycosides of grapes. Specifically, such contents have been found to decrease by the effect of grapes being stored under UV-B light and to increase when stored under UV-C light (*38*). Likewise, although these flavonoid compounds have been found to be much less prone to enzymatic degradation than are hydroxycinnamic acids and flavan-3-ol derivatives, they can be oxidized by coupled reactions (*39*).

To identify the specific parameters most accurately reflecting changes in the grapes during the raisining process, the A_{280}, A_{420}, lightness (L^*), hue angle (h_{ab}), chroma (C^*_{ab}), and concentration data for the different phenolic compounds grouped by chemical fractions were subjected to multivariate principal component analysis. **Figure 9** shows the scores of each sample on the plane defined by the first two principal components (PC) (eigenvalue > 1), which accounted for 90.7% of the total variance and allowed the different samples used in the raisining process to be discriminated. PC1, which accounted for 79.4% of the total variance, increased as raisining progressed by the effect of the increase in the chroma of the musts (greater C^*_{ab}) and of their A_{280}, browning index (A_{420}), phenolic acid and flavonol

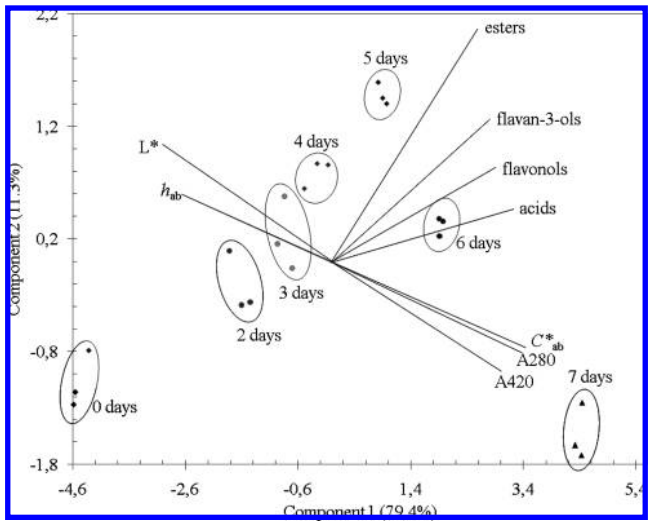

Figure 9. Principal component analysis: biplot representation of must samples and statistical variables.

contents, and darkening (smaller L^*) and reddening values (smaller h_{ab}). On the other hand, hydroxycinnamic esters and flavan-3-ol derivatives were those exhibiting the highest statistical weight for PC2, increasing their contents during the first 5 days and then decreasing through the end, reasonably as a result of their most easy degradation by oxidation.

Overall, the traditional sun-drying grape raisining process used to obtain Pedro Ximenez sweet wines produces strongly sugary and very dark mahogany-colored musts containing increased concentrations of most of the phenolic compounds, which have been deemed healthy because of their antioxidant properties. However, this raisining system is obviously strongly dependent on the climatic conditions of the particular year because it requires high diurnal temperatures and as low environmental humidity as possible. As stated above, the raisins used in this work exhibited very low levels of ochratoxin A (<0.1 μg/L); however, there is a high risk of development of fungi producing harmful toxins in years of high humidity levels during the night or even rain during raisining, the musts and wines reaching levels in OTA that contravene the European regulation. It could be pointed out that water activity levels of entire grapes were measured in this work, which ranged from 0.972 at the beginning to 0.914 at the end of the raisining, they being favorable for OTA production, which according to some authors can be synthesized at a_w values above 0.85 (*40*). In addition, this raisining procedure does not allow one to choose the best grape ripening time as this depends on the weather conditions required for raisining of the grapes (the already mentioned high temperatures and low humidity). However, alternative procedures for raisining in chambers must respect as much as possible the color characteristics obtained by the traditional sun-drying process. Particularly, the ratio between low−medium and high molecular weight polymers is important because of its possible influence on the sensory properties of the musts and, consequently, of the wines. The low increases obtained in this work for the hydroxycinnamic acids and flavan-3-ol derivatives (both pointed out as good substrates for polyphenol oxidase) suggest that the enzymatic pathways contributed strongly to the browning of grapes. However, a system of raisining in chambers with a high temperature and low humidity can favor the nonenzymatic pathways, leading to a different sensory profile of the must. Consequently, further research will be required with a view to developing raisining procedures involving controlled temperature and humidity conditions in chamber, allowing the use of grapes at their optimum ripening stage, minimizing the production of ochratoxins, and maintaining as much as possible the sensory properties of raisins.

LITERATURE CITED

(1) Amerine, M. A.; Winkler, A. J. Composition and quality of musts and wines of California grapes. *Hilgardia* **1944**, *15*, 493–673.
(2) Winkler, A. J. *General Viticulture*; University of California: Berkeley, CA, 1962; 633 pp.
(3) Lopez, P. A.; Moreno, J.; Medina, M. Caracterización del envero y madurez en la uva de *Vitis vinifera* Pedro Ximenez cultivada en la zona Montilla-Moriles. *Rev. Agro. Tecnol. Aliment.* **1988**, *28*, 274–284.
(4) Ramshaw, E. H.; Hardy, P. J. Volatile compounds in dried grapes. *J. Sci. Food Agric.* **1969**, *20*, 619–621.
(5) Grncarevic, M.; Hawker, J. S. Browning of sultana grapes berries during drying. *J. Sci. Food Agric.* **1971**, *22*, 270–272.
(6) Karadeniz, F.; Durst, R. W.; Wrolstad, R. E. Polyphenolic composition of raisin. *J. Agric. Food Chem.* **2000**, *48*, 5343–5350.
(7) Cheynier, V.; Basire, N.; Rigaud, J. Mechanism of trans-caffeoyltartaric acid and catechin oxidation in model solutions containing grape polyphenoloxidase. *J. Agric. Food Chem.* **1989**, *37*, 1069–1071.
(8) Gunata, Y.; Pineau, J.; Cordonnier, R. Détermination de la qualité de la vendage par sa richesse en composés phénoliques. Applications à la vinification. *Rev. Fr. Oenol.* **1987**, *187*, 7–13.
(9) Hidalgo-Togores, J. *Tratado de Enología*; Mundi-Prensa: Madrid, Spain, 2002; Vol. 1, 752 pp.
(10) Lingnert, H.; Hall, G. Formation of antioxidative Maillard reaction products during food processing. In *Amino−Carbonyl Reactions in Food and Biological Systems*; Fujimaki, M., Namiki, M., Kato, E., Eds.; Elsevier: Tokyo, Japan, 1986; pp 273–279.
(11) Morales, F. J.; Jimenez-Perez, S. Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. *Food Chem.* **2001**, *72*, 119–125.
(12) Perez-Magariño, S.; Rivero, M. D.; Gonzalez-Sanjose, M. D. Melanoidins in brown alcoholic beverages. *Czech J. Food Sci.* **2000**, *18*, 108–109.
(13) Radler, F. The prevention of browning during drying by the cold dipping treatment of sultana grapes. *J. Sci. Food Agric.* **1964**, *15*, 684–689.
(14) Rivero-Perez, M. D.; Perez-Magariño, S.; Gonzalez-San Jose, M. L. Role of melanoidins in sweet wines. *Anal. Chim. Acta* **2002**, *458*, 169–175.
(15) Varga, J.; Kozakiewicz, Z. Ochratoxin A in grapes and grape-derived products. *Trends Food Sci. Technol.* **2006**, *17*, 72–81.
(16) Hernandez, M. J.; Garcia-Moreno, M. V.; Duran, E.; Guillen, D.; Garcia-Barroso, C. Validation of two analytical methods for the determination of ochratoxin A by reversed-phased high-performance liquid chromatography coupled to fluorescence detection in musts and sweet wines from Andalusia. *Anal. Chim. Acta* **2006**, *566*, 117–121.
(17) Bornet, A.; Teissedre, P. L. Chitosan, chitin-glucan and chitin effects on minerals (iron, lead, cadmium) and organic (ochratoxin A) contaminants in wines. *Eur. Food Res. Technol.* **2007** (on line).
(18) Leong, S. L.; Hocking, A. D.; Scott, E. S. The effect of juice clarification, static or rotary fermentation and fining on ochratoxin A in wine. *Aust. J. Grape Wine Res.* **2006**, *12*, 245–251.
(19) Commission Regulation (EC) No. 123/2005 of 26 January 2005. *Off. J. Eur. Union* **2005***L25/3*.
(20) C.I.E. *Colourimetry*, 3rd ed.; International Commission on Illumination: Vienna, Austria, 2004.
(21) Jaworski, A.; Lee, C. Y. Fractionation and HPLC determination of grape phenolics. *J. Agric. Food Chem.* **1987**, *35*, 257–259.

(22) Oszmianski, J.; Ramos, T.; Bourzeix, M. Fractionation of phenolics compounds in red wines. *Am. J. Enol. Vitic.* **1988**, *39*, 259–262.
(23) Lopez-Toledano, A.; Merida, J.; Medina, M. Colour correction in white wines by use of immobilized yeasts. *Eur. Food Res. Technol.* **2007**, *225*, 879–885.
(24) Singleton, V. L. Wine phenols. In *Wine Analysis*; Linskens, H. F., Jackson, J. F., Eds.; Springer-Verlag: Berlin, Germany, 1988; pp 173–218.
(25) Gonnet, J. F. Colour effects of co-pigmentation of anthocyanins revisited. 1. A colorimetric definition using the CIELAB scale. *Food Chem.* **1998**, *63*, 409–415.
(26) Martinez, J. A.; Melgosa, M.; Perez, M. M.; Hita, E.; Negueruela, A. Visual and instrumental color evaluation in red wines. *Food Sci. Technol. Int.* **2001**, *7*, 439–444.
(27) Perez-Caballero, V.; Ayala, F.; Echavarri, J. F.; Negueruela, A. Proposal for a new estándar OIV method for determination of chromatic characteristics of wine. *Am. J. Enol. Vitic.* **2003**, *54*, 59–62.
(28) Macheix, J. J.; Sapis, J. C.; Fleuriet, A. Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines. *Crit. Rev. Food Sci. Nutr.* **1991**, *30*, 441–486.
(29) Tulyathan, V.; Boulton, R. B.; Singleton, V. L. Oxygen uptake by gallic acid as a model for similar reactions in wines. *J. Agric. Food Chem.* **1989**, *37*, 844–849.
(30) Singleton, V. L.; Timberlake, C. F.; Lea, A. G. H. The phenolic cinnamates of white grapes and wine. *J. Sci. Food Agric.* **1978**, *29*, 403–410.
(31) Rodriguez Montealegre, R.; Romero Peces, R.; Chacon Vozmediano, J. L.; Martinez Gascueña, J.; Garcia Romero, E. Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. *J. Food Compos. Anal.* **2006**, *19*, 687–693.
(32) Timberlake, C. F.; Bridle, P. Interactions between anthocyanins, phenolic compounds and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* **1976**, *27*, 97–105.
(33) Haslam, E. In vino veritas: oligomeric procyanidins and the ageing of red wines. *Phytochemistry* **1980**, *19*, 2577–2582.
(34) Dallas, C.; Ricardo-Da-Silva, J. M.; Laureano, O. Degradation of oligomeric procyanidins and anthocyanins in a Tinta Roriz red wine during maturation. *Vitis.* **1995**, *34*, 51–56.
(35) Dallas, C.; Hipolito-Reis, P.; Ricardo-Da-Silva, J. M.; Laureano, O. Influence of acetaldehyde, pH, and temperature on transformation of procyanidins in model wine solutions. *Am. J. Enol. Vitic.* **2003**, *54*, 119–124.
(36) Spayd, S. E.; Tarara, J. M.; Mee, D. L.; Fergurson, J. C. Separation of sunlight and temperature effects on the compositio of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* **2002**, *53*, 171–182.
(37) Makris, D. P.; Kallithraka, S.; Kefalas, P. Flavonols in grapes, grape products and wines: burden, profile and influential parameters. *J. Food Compos. Anal.* **2006**, *19*, 396–404.
(38) Cantos, E.; García-Viguera, C.; Pascual-Teresa, S.; Tomas-Barberán, F. A. Effect of postharvest ultraviolet radiation on resveratrol and other phenolics of cv. Napoleon table grapes. *J. Agric. Food Chem.* **2000**, *48*, 4606–4612.
(39) Amiot, M. J.; Tacchini, M.; Aubert, S. Y.; Oleszek, W. Influence of cultivar, maturity stage, and storage conditions on phenolic composition and enzymatic browning of pear fruits. *J. Agric. Food Chem.* **1995**, *43*, 1132–1137.
(40) Çağlarirmak, N. Ochratoxin A, hydroxymethylfurfural and vitamin C levels of sun-dried grapes and sultanas. *J. Food Process. Preserv.* **2006**, *30*, 549–562.

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