Balancing Tannin Maturity and Extraction

Studying the relationships between seed maturity, length of maceration and ethanol amount on Merlot wines

By Federico Casassa and James Harbertson

Wine tannins play a pivotal role in defining wine style because they are directly responsible for sensory aspects such as astringency and bitterness; indirectly, wine tannins and their reaction products (such as polymeric pigments) modulate wine color and astringency.

Seed- and skin-derived tannins differ in their chemical makeup, which affects their sensory properties.

Seed-derived tannins endure a generally bad reputation with winemakers, as seeds are particularly rich in monomeric flavan-3-ols that elicit bitterness, while skin tannins have higher molecular weight but would convey a rounder mouthfeel to a final wine due to their association with polysaccharides and mannoproteins.

In studies conducted with model wines (simple aqueous solutions containing about 13% ethanol by volume and adjusted to pH 3.6), extractability of seed tannin into wine has been shown to decrease as grape maturity progresses. As a result, picking decisions based on parameters such as seed color and so-called visual “seed maturity” have been adopted by some winemakers. The rationale is that berries with brown seeds have low extractability of seed tannins and, conversely, higher extractability of skin tannins than berries bearing green seeds.

The need to limit seed tannin extraction into wine by delaying harvest until seeds turn uniformly brown has led to an increase of Brix at harvest: 24.9 ± 0.1) to a target 25 Brix without affecting the total volume of the wine lot significantly (see “Experimental Design for Production of Merlot Wines”).

For the late-harvest treatments (average Brix at harvest: 24.9 ± 0.4), a portion of the must was bled off (saignée), and dechlorinated water adjusted to 4.5 g/L tartaric acid was added to emulate the soluble solids of the first harvest date (target Brix: 20°) without affecting the total volume.

Each adjusted must received 10 days of skin contact (control wines) and 30 days of skin contact (extended maceration wines) to evaluate the chemical and sensory effects of grape maturity and different EtOH concentrations under two contrasting maceration protocols (see “Experimental Design for Production of Merlot Wines”). Fermentations occurred in triplicate in 300-liter stainless-steel jacketed fermentors following standard winemaking practices.

The winemaking protocol was as follows: Fruit was destemmed and pumped to the fermentors with a progressive cavity pump. Sulfur dioxide (50 mg/L SO₂) was added while filling the fermentors. Fermentations occurred in 300-liter stainless-steel jacketed tanks with adjustable lids.

Materials and methods
In this study, the effects of fruit (seed) maturity, maceration length and EtOH concentration on wine phenolics, chromatic composition and sensory attributes of Merlot wines were evaluated. Merlot grapes (Clone 3) were harvested from the Columbia Valley AVA in Washington state at approximately 20.3° and 24.9° Brix in two consecutive years (2011 and 2012). Half of the low-Brix must and half of the high-Brix must were adjusted before alcoholic fermentation to emulate the Brix of the other harvest.

For the early harvest treatments, chaptalization (with a sucrose concentrate (81° Brix) was used to adjust the sugar level in one of the lots (average Brix at harvest: 20.35 ± 0.1) to a target 25° Brix without altering the total volume of the wine lot significantly (see “Experimental Design for Production of Merlot Wines”).

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The must was inoculated seven hours after destemming with 250 mg/L of selected dry yeast (Lalvin EC-1118). Malolactic bacteria (Lalvin VP41 from Lallemand) was added 48 hours after yeast inoculation (10 mg/L). Diammonium phosphate was added to increase the yeast assimilable nitrogen to 225 mg/L before fermentation. Sugar consumption during fermentation was monitored daily, and the tank temperature was maintained at 26° ± 2° C (75.2°-82.4° F).

Cap management consisted of a whole-volume tank pumpeover followed by a five-minute punchdown twice per day during active fermentation. Alcoholic fermentation was completed (reducing sugar less than 2 g/L) after nine to 10 days in all wines. Fermentation kinetics and temperature curves showed good reproducibility for all treatments during both years (data not shown). During post-fermentation, extended maceration (EM) wines received one 1-minute punchdown per day, after which the tanks were sealed and sparged under lid with N₂.

Visual seed maturity was determined following a published protocol (see “Comparing Seed Maturity and Tannins”). Under this protocol, seeds from a representative sample are contrasted against a color chart comprised of 12 colors with corresponding numbers, starting with a bright green (1), through green-yellow, yellow, yellow-brown and dark brown (12) as seeds ripen.18

Fruit and wine analysis included anthocyanins, total phenolics, protein precipitable tannins and wine color (CIELab). Pomace samples were analyzed for tannins recovered in the skins and seeds after the maceration length allotted for each treatment. The proportion of skin- or seed-derived tannins extracted into wine was calculated as the difference between what was found in either the skins or the seeds at harvest, plus the amount remaining in the pomace, and then dividing by the estimated amount of tannin extracted.14

A trained panel provided descriptive sensory analysis (DA) of the wines, which occurred after three months of bottle aging (about 240 days after crushing). Briefly, DA was performed on the 2011 wines for aroma, color, taste and mouthfeel attributes (n = 3), as described in Lawless and Heymann.

Eleven panelists (five males and six females; aged 22 to 74 years, average age 44.1, median age 34; six with previous DA experience) were recruited from students, faculty, staff and retirees from the University of California, Davis, campus. Panelists participated in six one-hour training sessions in two weeks, followed by nine 30-minute evaluation sessions in two weeks.

The wines were evaluated for color, aroma, taste and mouthfeel attributes in individual tasting booths under white light and in pear-shaped black ISO glasses (ISO 1977) labeled with three-digit random numbers, except for color, in which transparent ISO glasses were used.

Results
The main findings of this research indicate the following:

- Wines made from the late-harvest treatment had significantly higher pH and lower titratable acidity compared to wines from the early harvest fruit (data not shown). The final EtOH concentrations confirmed that both the maturity and the EtOH adjustment treatments had the desired effect, yielding an overall difference of 2.7% (v/v) in the EtOH concentration between wines made from fruit with unadjusted Brix and wines produced with chaptalization and/or the saignée/water-back adjustment treatments.
- Seed tannins (on a fresh weight basis) were higher in the early harvest fruit during both years (see “Comparing Seed Maturity and Tannins”), but this higher concentration did not necessarily result in higher extraction into wine. Relative extraction of grape tannins was higher in ripener fruit than in unripe fruit.
- Overall extraction of fruit tannins (seeds and skins) into wine ranged from 12% to 16% and varied as a function of the growing season. Relative and absolute tannin extraction was higher in 2012, which was warmer.
- Extended maceration resulted in a decline in anthocyanins irrespective of fruit maturity and ethanol concentration, which, in turn, resulted in lower wine color saturation.
- Tannin extraction into wine was not affected by the EtOH (11.72% to 14.40% v/v). No effect of the EtOH concentration on the proportion of seed-derived tannins was observed when comparing low EtOH wines with their high EtOH counterparts. Thus, differences in EtOH concentration during maceration as high as 2.7% (v/v) appeared to have no effect on the source of extraction of wine tannins (seeds or skins) or on the overall concentration of extracted tannins.
- Extended maceration resulted in higher tannin extraction into wine. However, tannin extraction was independent of fruit maturity and EtOH concentration during maceration.
- Of the total wine tannin content, extended maceration had about 80% of seed-derived tannins compared to control wines that had about 67% of seed-derived tannins.
• Analysis of tannins recovered in the pomace after maceration showed that the proportion of tannins that could not be accounted for (based upon what was previously measured in the fruit) varied as a function of fruit maturity and maceration length. This “wine matrix” effect modulating the proportion of retained tannins in the wine was of greater relevance in both the late-harvest and extended maceration wines. We speculate that this wine matrix effect is a combination of physical and chemical factors including physical sequestration, non-covalent binding of tannins by cell wall components of grape/yeast origin, precipitation and chemical modification resulting in new tannin structures that are not amenable to protein precipitation.

• Descriptive sensory analysis indicated that wines made with fruit from the later harvest date showed a prevailing and positive effect on the sensory profile of the wines over winemaking factors such as maceration length and EtOH concentration. Wines from late-harvest fruit had a more viscous mouthfeel, sweet taste and fruit-derived aromas than wines from early harvest fruit, which were dominated by fresh vegetal character, higher acidity and lower color saturation.

• Extended maceration shifted the sensory profile toward higher astringency, lighter and yellower color components and cooked vegetal aromas. Chaptalization of the early harvest fruit to 24.9° Brix before fermentation shifted the sensory profile from cooked and fresh vegetable toward sweet taste, alcoholic, floral and chocolate/caramel aromas, astringency and viscous mouthfeel.

Discussion
Analysis indicates that unripe Merlot grapes from the Columbia Valley AVA in Washington state were higher in seed tannins relative to ripe fruit. However, this higher seed concentration did not result in enhanced tannin extraction into wine, whether the alcohol content was increased to what could be achieved by longer ripening or maceration for 20 extra days, or a combination of the two.

It has been postulated that seed tannins oxidize, polymerize and become progressively bound to cell wall components during ripening, which renders them “less” extractable during winemaking.13 Ripening also brings about enzymatic processes that may facilitate extraction,17 thereby counteracting this putative lower extractability due to oxidative polymerization. Our results indicate relatively higher extractability of grape tannins in ripe fruit than in unripe fruit.

Our observation that overall tannin extraction from grape (seeds + skins) varies according to the growing season is an empirical fact often observed by grapegrowers and winemakers. For example, in cool climates, heat summation (growing degree-days, or GDD) has been positively associated with an increase in seeds per berry9 and total tannin concentration per berry.16

The figure “Comparing Seed Maturity and Tannins” allows us to draw interesting observations. While visual seed maturity seems to be negatively correlated with seed tannin concentration, our results suggest that there is also a positive correlation between visual seed maturity and seed tannin extraction into wine. In other words, extraction of tannins from ripe seeds (in Merlot) is easier than from unripe seeds.

A previous observation was confirmed: In climates such as eastern Washington state, in which the growing season is typically short but accompanied by fast accumulation of GDD, warmer years (such as 2012) lead to increased accumulation of seed tannins and lower seed maturity than cooler years. In the warmer 2012 vintage, fruit with 25° Brix had essentially the same seed tannin concentration as
In the present work, we assimilate this matrix effect as the proportion of missing tannins that cannot be found in the pomace after maceration and in the wines. We found that the proportion of tannin “bound” was more prominent in ripe fruit than unripe fruit and in extended-maceration wines rather than in control wines. We have evidence that this matrix effect also occurs in Cabernet Sauvignon wines.\(^7\)

Our findings suggest there may be a certain threshold of required EtOH to allow effective tannin extraction from seeds. Above that threshold, further increases in EtOH cause little effect. We observed tannin extraction at EtOH levels as low as 11.7%, and the longer the maceration length, the higher the proportion of seed-derived tannins both at 11.7% and 14.4% EtOH, suggesting that maceration length and not differences in EtOH was the factor responsible for the observed changes.

Either 11.7% represents the lower end of EtOH required to dissolve the lipid outer coat of the seed and/or it represents the minimum EtOH level to disrupt hydrogen bond interactions between previously extracted tannins and cell wall materials toward the end of extended maceration, thereby furthering seed tannin extraction.

While we evaluated an ethanol range between 11.7% and 14.4% EtOH, it is unclear if EtOH levels above 15% will yield similar results. Ethanol levels as high as 15.5% and even 16% are not uncommon in warmer viticultural regions, and thus seed and skin tannin extraction under these conditions should also be studied.

Expected outcomes of extended maceration include lower anthocyanins and wine color saturation and higher extraction of seed tannins into wine (and higher perceived astringency); these outcomes were confirmed in this study.

Extended maceration results in lower color saturation due to progressive loss of anthocyanins, perhaps due to oxidation; polymerization of monomeric anthocyanins into polymeric pigments and pyranoanthocyanins, and progressive binding of anthocyanins to fermentation solids (skins, seeds and stems, if present).

Extended maceration decreased fruitiness and shifted aromas toward cooked and vegetal overtones (see “Sensory Attributes of 2011 Merlot Harvest”). The practice of extended maceration may have resulted in the accumulation of acetaldehyde, which may decrease perceived fruitiness. Increasing skin contact time may result in the loss of fruity nor-isoprenoids such as β-damascenone.\(^2\)

One question this study sought to answer was whether the outcome of extended maceration would be contingent upon fruit maturity at harvest. Our research suggests that tannin extraction into wine during extended maceration is not only independent of EtOH (in the range of 11.7%-14.4%, as stated above) it is, more importantly, also independent of fruit maturity in the range of 20°-24.5° Brix.

In practical terms, even if harvest is delayed, extended maceration should extract a predictable proportion of skin- (about 20%-25%) and seed-derived tannins (about 75%-80%).

An increasing body of research has shown the critical role that grape maturity plays on the sensory profile of a resulting wine. Riper fruit has been found to yield wines of improved chemical and sensory features than wines made from unripe fruit.\(^5,10\)

It is not without reason that winemakers devote substantial effort to chemically and sensorially gauge fruit maturity and establish precise harvest decisions. These results support both this prevailing notion in our industry along with previous research on the topic.

Tannins, aroma and aroma precursors, anthocyanins, native polysaccharides, sugars and acids all evolve during ripening. Expectedly, and consistent with the above research, we found that wines made from ripe fruit had higher color saturation, more fruit-forward aromas, less vegetal and earthy aromas, less sourness and a more viscous mouthfeel (but not less astringency than wines made from unripe fruit (see “Sensory Attributes of the 2011 Merlot Harvest”).

Visual seed maturity and tannin concentration of Merlot seeds at two maturity levels in 2011 and 2012.

Concentration values in mg/g fresh weight (purple) and seed color values (black). Letters (a, b, c, d) within a type color are significantly different.
Sensory results arising from variations in Brix levels at harvest were of greater magnitude than that caused by extended maceration. In other words, between 20.5° and 24.5° Brix, when grapes are harvested seems to be more influential than the technique chosen to conduct red wine maceration.

In spite of EtOH playing a lesser role in tannin extraction, our results suggest that this solvent is critical to other wine sensory aspects not related to wine tannin content. Unripe fruit gave rise to wines that had lower color saturation, a marked vegetal character, higher acidity and lighter body.

However, chaptalization of unripe fruit (and the resulting ethanol from it) improved the sensory profile of the resulting wines by increasing the perception of astringency and wine viscosity and reduction of vegetal aromas, thereby validating this practice. Previous research has shown that ethanol between 10% and 12% and 14.5% to 17.2% (v/v) affects aroma solubility (and, conversely aroma volatility) in different ways, with some aromas declining and others increasing their volatility as EtOH was increased.

For example, it has been previously shown that an increase in EtOH from 14.5% to 17.2% decreased fruitiness. This decrease in fruitiness under increasing EtOH levels has been confirmed in model wine solutions and has been ascribed to the ability of EtOH to form “hydrophobic pockets” that may enhance the solubility (thereby decreasing volatility) of certain hydrophobic aromas.

Our study under actual winemaking conditions suggests that increasing the EtOH level due to chaptalization of unripe fruit has a moderating effect on negative wine aromas and flavors associated with unripe fruit. This may be the result of complex perceptual interactions such as synergistic or masking effects between odorant compounds responsible for the aroma and flavor, which were mediated by the ethanol concentration.

Although chaptalization is not allowed in California and is allowed only during certain years in Washington state, the practice is more common in states such as Florida, New York and Oregon. If our findings in Merlot hold valid for varieties such as Pinot Noir, which is typically harvested earlier than Merlot, chaptalization of unripe fruit in unseasonably cool years may be beneficial.

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**SENSORY ATTRIBUTES OF 2011 MERLOT HARVEST**

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Sensory attributes assessed by a trained panel (n=11) of Merlot wines from the 2011 harvest. Evaluations used a 10-point line scale. (*) Indicates significant differences for Fisher’s LSD and p < 0.05.
Visual seed maturity and tannin extraction

This study was born out of a discussion about the vagaries of “phenolic” and “seed” ripeness and their putative effect on the chemistry and sensory properties of red wines. The results indicated that visual “seed maturity” may play a lesser role in seed tannin extraction than previously thought. In this context, are there other practical reasons to delay harvest until seeds have turned uniformly brown?

The answer is an absolute yes—at least in the maturity range under study (20.3°-24.9° Brix). However, rather than seed tannin extraction, other aspects such as sanitary conditions, aromatic development, sugar accumulation and maintenance of natural acidity should be the points of concern. In the grand scheme of things, “visual seed maturity” appears to be a lesser factor, and winemakers who delay harvest for the sole prospect of getting less seed tannin extraction may be worried for the wrong reasons.

We also wanted to tackle the problem of the potential interaction between length of maceration and fruit maturity. Would extended maceration exacerbate the negative characters associated with unripe fruit? Of practical relevance for winemakers applying this technique, our results indicate that the sensory and chemical outcomes of extended maceration are fairly predictable and independent of fruit ripeness.

These sensory and chemical outcomes include a high concentration of protein-precipitable tannins, a low concentration of anthocyanins and wine color and enhanced astringency. Based on these results, extended maceration seems to have a rather negative impact on the sensory profile of red wines. If the stylistic and/or production goal is to merely enhance tannin extraction, perhaps practices such as saignée can be performed to boost tannin retention while also relieving the logistics of tank turnover in the winery.

This study has brought about new questions whose definitive answer may have important practical implications. Among them: What are the factors that control tannin extraction during extended maceration? Is there a “true” extraction from the seeds occurring during maceration, or rather is there a desorption phenomenon mediated by the action of ethanol on previously bound tannins?

What about seed hydration? Do seeds gain weight during extended maceration resulting from water absorption? (Seeds have comparatively lower water activity than the surrounding must.) If so, may this seed hydration result in cell disruption and release of tannins? Are these findings applicable to other grape varieties?

For early ripening varieties such as Pinot Noir, it will take less time than Merlot to get from 20° to 25° Brix, and thus our results should be applicable to Pinot Noir. Cabernet Sauvignon grapes, on the other hand, ripen under conditions of decreasing rate of accumulation of growing degree-days, and the metabolic changes occurring between 20° and 25° Brix may not be equivalent to those observed in Merlot.

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References and additional graphics for this article are available online at winesandvines.com.

The complete report can be found in the American Journal of Enology & Viticulture ajevonline.org/content/64/4/437.