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Sensory Impact of Extended Maceration and Regulated Deficit Irrigation on Washington State Cabernet Sauvignon Wines

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Abstract: Irrigation practices such as regulated deficit irrigation (RDI) and winemaking practices such as extended maceration have been experimentally evaluated from a chemical perspective but their impacts on sensory composition and interactive effects merit scientific attention. This study evaluated the sensory impact of extended maceration applied to Cabernet Sauvignon grapes sourced from a vineyard subjected to four RDI treatments: replenishment of 100%, 70%, and 25% of full-vine crop evapotranspiration (ET_c) from fruit set until veraison followed by 100% ET_c until harvest (labeled 100% ET_c, 70% ET_c, and 25% ET_c, respectively) and 25% ET_c from fruit set to veraison followed by 100% ET_c from veraison to harvest (labeled 25/100% ET_c). Each RDI treatment was replicated four times (n = 4) and made into wine, with two replicates designated as controls (10 day skin contact) and two as extended maceration (30 day skin contact). Wines were evaluated by descriptive analysis with a trained panel (n = 15) and chemical and sensory data were correlated using canonical correlation analysis. Wine-perceived saturation and purple component ratings were highest in 25% ET_c wines and were highly correlated with the concentration of flavonols, malvidin- and delphinidin-derivatives, and small polymeric pigments. Fruit-based aroma descriptors were highest in the 25/100% ET_c and 70% ET_c wines. Extended maceration increased perceived astringency and bitterness, which were in turn correlated with the concentration of flavan-3-ol and oligomeric proanthocyanidins. These results suggest that moderate RDI protocols such as 70% ET_c and 25/100% ET_c impact positively the fruity aroma component (black and red fruit), whereas extended maceration lowered fruity aromas, possibly due to the masking effect of the oxidized character perceived in these wines.

Key words: extended maceration, regulated deficit irrigation, wine aroma, oxidation, astringency, bitterness

In wines, observed variations in sensory attributes such as color (hue and saturation) and taste and mouthfeel properties (such as bitterness and astringency) are primarily the result of the composition and concentration of two phenolic classes, anthocyanins and proanthocyanidins (Lesschaeve and Noble 2005, Preys et al. 2006). Anthocyanins are pigments that modulate wine color directly due to their spectral properties and indirectly by participating in reactions such as copigmentation resulting in the typical hyperchromic shift (more color) and bathochromic shift (more purple color) observed in young red wines (Boulton 2001). Isolated anthocyanins are tasteless or indistinctly flavored (Vidal et al. 2004). However, when anthocyanins react with proanthocyanidins during winemaking, polymeric pigments are formed and these in turn can modulate astringency (Weber et al. 2013).

Proanthocyanidins (also referred to as tannins) and, to a lesser extent, monomeric flavan-3-ols, display high affinity for proline-rich proteins found in the saliva of humans and other mammals (Mehansho et al. 1987, Poncet-Legrand et al. 2007). The tactile sensation of astringency arises from the formation of proanthocyanidin-protein complexes upon contact of the wine proanthocyanidins with the oral epithelium in a reaction driven by both hydrophobic interactions and hydrogen bonding (Baxter et al. 1997, Simon et al. 2003). Epicatechin-3-*O*-gallate and catechin can precipitate proline-rich proteins when the molar ratio of flavan-3-ols to protein exceeds 27 (Poncet-Legrand et al. 2006), which highlights the potential cooperative role of flavan-3-ols in astringency perception in red wine.

Management of the maceration period during red wine production is arguably the most common practice to achieve the selective diffusion of phenolics, aroma precursors, and free aroma compounds from the skins, seeds, and stems (when present). Extended maceration (EM) is a widely used winemaking technique based on extending the contact of the fermentation solids with the wine after fermentation is

completed (Sacchi et al. 2005, Casassa et al. 2013a). This technique has been used to alter the mouthfeel of the wines, possibly by facilitating proanthocyanidin extraction and the formation of polymeric pigments (Harbertson et al. 2009, Casassa et al. 2013a). However, changes in mouthfeel induced by extended maceration may also arise from modifications in proanthocyanidin structure or size, resulting in a sensory impact beyond that of their concentration in solution.

In addition to its role in the extraction of anthocyanins and proanthocyanidins, contact of the must/wine with the fermentation solids is also needed to extract the precursors of aroma compounds that ultimately define red wine aroma. However, longer maceration time does not necessarily result in an enhancement of fruity aromas in the resulting wines (Harbertson et al. 2009, Casassa et al. 2013a) because the maceration time may have competing effects on the volatile composition depending on the rate of release from the tissues, formation of other compounds (such as acetaldehyde), and/or physical sorption or binding (Callejón et al. 2012).

Reports on the effect of regulated deficit irrigation (RDI) and other irrigation alternatives on the sensory profile of the wines are few and often conflicting. In a study of the sensory properties in Cabernet Sauvignon subjected to three drip irrigation treatments—minimal irrigation, standard irrigation (32 L water/vine/week), and double irrigation (64 L water/vine/week)—descriptive analysis showed that vine water deficits led to wines with fruitier and less vegetal aromas and a reduced astringency, as compared with vines with high vine water status (Chapman et al. 2005). In an evaluation of the sensory attributes of Merlot wines produced from vines under different levels of water stress (35, 70, 100, and 35 to 70% of crop evapotranspiration; ET_c), results indicated that preveraison deficit at 35% ET_c increased fruity aromas and that preveraison deficit followed by ET_c reposition of 70% increased the drying mouthfeel in the resulting wines (Ou et al. 2010). In both of these studies, chromatic properties and perceived color of wines were not examined.

The present study expands on a previous investigation that described the chemical features of wines produced from a combination of two contrasting skin contact treatments (control, 10-day skin contact; extended maceration (EM, 30-day skin contact) applied to fruit produced under four RDI treatments (100% ET_c, 70% ET_c, 25/100% ET_c, and 25% ET_c) (Casassa et al. 2013b). The objectives of the present work were to generate a descriptive analysis of the wines with emphasis on color, aroma, and mouthfeel properties to understand the individual and combined sensory effect of skin contact time and RDI treatments and to elucidate relationships between the chemical and sensory compositions of the wines.

Materials and Methods

Experimental design. Own-rooted *Vitis vinifera* cv. Cabernet Sauvignon (clone 8) grown in the Cold Creek vineyard of Ste. Michelle Wine Estates, southeast of Mattawa, Washington State (lat. 46°57′N; long. 119°89′W) was manually harvested on 12 Oct 2011 and processed at the Washington State University (WSU) research winery facility. The design

consisted of a combination of four RDI treatments and two winemaking skin contact treatments. The current industry standard for RDI was used as a control to replenish 70% of full-vine crop evapotranspiration (ET_c) through harvest (70% ET_c). This standard was derived from a reference crop (grass) evapotranspiration (ET_o) provided by the WSU AgWeatherNet station and a variable crop coefficient (K_c) developed for fully irrigated Cabernet Sauvignon vines in eastern Washington. The remaining treatments were imposed to replenish 100% ET_c and 25% ET_c from fruit set to harvest and 25% ET_c from fruit set to veraison followed by 100% ET_c from veraison to harvest (25/100% ET_c). The field experiment was designed as a randomized complete block with four replicated blocks (n = 4) and the RDI treatments randomly applied (≥6 rows each) within each block. The two skin contact treatments were applied in duplicate using two of the four field replicates of each RDI treatment—control wines, with a 10-day skin contact period, and extended maceration wines (EM), with a 30-day skin contact period—for a total of 16 wines. More details on the vineyard site, winemaking protocol, and chemical analysis of fruit and wines are described in Casassa et al. (2013b). Monomeric anthocyanins and flavonols were determined by HPLC-DAD-MS. Protein precipitable tannins, iron-reactive phenolics, large polymeric pigments, and small polymeric pigments were measured as in Harbertson et al. (2003).

Descriptive analysis. Descriptive analysis of the wines was conducted after three months of bottle aging as described by Lawless and Heymann (2010). A prescreening of the wines by four experienced wine tasters ensured that the wines were different enough to justify a descriptive analysis and also that they were free of sulfur-like or other off-odors. A trained panel was convened (n = 17, 10 females and 7 males, ages ranging from 24 to 63 years). No information about the nature of the study was provided in order to reduce bias, and the WSU Institutional Review Board for human subject participation approved the project. Panelists were screened for both potential color deficiencies and bitterness sensitivity (also known as PROP status) as detailed previously (Casassa et al. 2013a). The results of these tests indicated that none of the panelists had color deficiencies and that the panel was composed of 25% nontasters, 13% medium tasters, and 62% supertasters (Pickering et al. 2004).

Panel training and evaluation. Panelists were trained during seven sessions, each lasting one hour with an additional session for review of standards and self-calibration. After terminology development, four color components (purple, red, brown, and saturation), three aroma attributes (red fruit, black fruit, and oxidized character), and two mouthfeel attributes (astringency and bitterness) were retained upon general consensus. Astringency was defined as the puckering or lack of lubrication sensation around the gums immediately after expectoration of the wine (Gawel et al. 2000). During the training and evaluation sessions, a 15 cm unstructured line scale was used, labeled with terms *low* and *high* at the 1 cm and 14 cm mark from the left side of the scale, respectively. Except for the purple, red, and brown color components, the standards were prepared at low, medium, and high levels

(Table 1), representing anchors located at 1 cm, 7.5 cm, and 14 cm, respectively, from the left end of the unstructured scale. An example of each standard at the three intensity levels was initially presented to the panelists, but the final intensity of each attribute was modified upon panelist feedback (Table 1).

The experimental wines were evaluated during five formal evaluation sessions. Panelists tasted six or seven wines per evaluation session. Wines and their replicates were presented monadically and evaluated twice following a randomized Williams Latin Square block design for control of possible carryover effects, yielding a final count of 480 observations (16 wines × 15 panelists × 2 replicates) for each attribute. Panelists assessed the wines in individual booths (20 ± 2°C), lighted with Lumichrome full spectrum lamps (6,500 K) in the WSU Sensory Laboratory. Aliquots of wine (30 mL) at room temperature were poured into wineglasses coded with three-digit random numbers and covered with aluminum lids to trap volatiles. To reduce buildup and carryover effects, bitterness was evaluated prior to astringency. Prior to astringency evaluation, panelists were instructed to chew one cracker, rinse with deionized water, and wait at least 4 min between samples, during which they assessed the color components of another sample. Results were collected on ballots and responses (in cm) were decoded manually. After the formal evaluation sessions, panel performance was monitored by assessing the correlation of each panelist with the panel mean and by their contribution to the panelist × wine interaction for each attribute. Based on these analyses, it was decided to remove data from two panelists (final n = 15).

Materials and standards. 6-*n*-Propylthiouracil (PROP), caffeine (food grade), and acetaldehyde (>99% purity) were obtained from Sigma-Aldrich (St. Louis, MO). For preparation of reference standards (Table 1), raspberry jam and blackberry jam (Smucker's, Orrville, OH) and blueberry/blackcurrant preserve (Mackays, Arbroath, Scotland) were obtained through local grocery stores. Freeze-dried powdered strawberries were obtained from WSU Prosser. Reference standards for aroma were prepared using a base wine (Paisano Red, Carlo Rossi Vineyards, Modesto, CA) previously stripped of most aroma compounds under reduced pressure (30°C × 45 min/L) using a Büchi Syncore Polyvap (Flawil, Switzerland). Astringency standards were prepared using a 2010 Cabernet Sauvignon wine and a 2010 Merlot wine produced at the WSU winery. Bitterness and color standards were prepared using as a base wine a 2010 Merlot produced at the WSU winery. The color components and saturation standards were obtained by varying the pH and/or by addition of acetaldehyde, H_2O_2, and SO_2, with specifications reported as CIELab units (Table 1). Unsalted crackers (Great Value, Bentonville, AR) and deionized (18.2 MΩ·cm resistivity) water (Milli-Q, EMD Millipore, Billerica, MA) were provided for palate cleansing. To avoid perceptual bias due to color, tulip-shaped cobalt black glasses (Libbey, Toledo, OH) were used for evaluation of aroma and mouthfeel attributes. Clear ISO wineglasses (ISO 1977) were used only for color evaluations.

Table 1 Ingredients, specifications, and lexicon of sensory analysis standards used during the training and formal evaluation sessions.

Reference standard	Level		
	Low	Medium	High
Aroma[a]			
Red fruit character	1.1 g raspberry jam and 40 mg freeze-dried powdered strawberries	4.5 g raspberry jam and 150 mg freeze-dried powdered strawberries	7.5 g raspberry jam and 250 mg freeze-dried powdered strawberries
Black fruit character	3 g each blackberry jam and blueberry/ blackcurrant preserve	4.5 g each blackberry jam and blueberry/ blackcurrant preserve	9 g each blackberry jam and blueberry/ blackcurrant preserve
Oxidized character	56 µL acetaldehyde	150 µL acetaldehyde	300 µL acetaldehyde
Color[b]			
Purple component	NA	NA	L* = 28.2; C* = 64.6; H* = 25.1; a* = 58.5; b* = 27.4
Red component	NA	NA	L* = 35.4; C* = 58.4; H* = 22.0; a* = 54.1; b* = 21.9
Brown component	NA	NA	L* = 61.3; C* = 40.6; H* = 42.4; a* = 29.9; b* = 27.4
Saturation	C* = 22.9	C* = 50.5	C* = 64.6
Taste/mouthfeel			
Bitterness[c]	No addition	75 mg caffeine	200 mg caffeine
Astringency[d]	188 mg/L PPT	649 mg/L PPT	1583 mg/L PPT

[a]Aroma standards prepared at three levels and dissolved in 750 mL base wine (Paisano Red) stripped of aroma compounds under reduced pressure (30°C × 45 min).
[b]Base wine: 2010 Merlot. NA: not applicable. CIELab values: L*: lightness; C*: saturation or chroma; H*: hue; a*: red component; b*: blue component.
[c]Base wine: 2010 Merlot.
[d]Base wine: 2010 Merlot (188 mg/L protein-precipitable tannins [PPT]); 56/44 blend 2010 Merlot and 2010 Cabernet Sauvignon (649 mg/L PPT); 2010 Cabernet Sauvignon (1583 mg/L PPT).

Data analysis. The significance of effect of RDI, skin contact time, and their interaction on sensory composition was analyzed by a two-way ANOVA with separation of the means accomplished using Fisher's LSD and the significance value established as $p < 0.05$ using XLSTAT ver. 2011 (Addinsoft, Paris, France). Principal component analysis (PCA) using the correlation matrix with no rotation was applied on the wine sensory data, including the replicates, using R software (R Foundation for Statistical Computing, Vienna, Austria). Confidence ellipses indicating 95% confidence intervals were based on the multivariate distribution of the Hotelling's test for $p < 0.05$ and were constructed using SensoMineR panellipse function on R (Husson et al. 2005). Two-way canonical correlation analysis with clustered image maps to relate sensory and chemical data was obtained using the mix0mics package of R software (González et al. 2012).

Results and Discussion

Chemical composition of wines. A detailed discussion of the chemical composition of the wines of this study has been reported previously (Casassa et al. 2013b). Therefore, only a brief summary is given here of the chromatic composition, anthocyanins, polymeric pigments, iron-reactive phenolics and flavonols, and proanthocyanidin composition with the reported proportion of skin- and seed-derived tannins for the individual Cabernet Sauvignon wines (n = 16). In terms of chromatic differences, the control wines had generally lower lightness values (L*) than their EM counterparts (Table 2). With regard to the effect of RDI, the 25% ET$_c$ treatment produced wines with overall higher saturation (C*), hue (H*), and yellow component (b*) values than the other RDI treatments. In terms of phenolic parameters, anthocyanins were generally higher in control wines (with the sole exception of the 25/100% ET$_c$ control and EM treatments), whereas iron-reactive phenolics were consistently higher in the EM wines (Table 3). The RDI treatment applied at 25% ET$_c$ resulted

in an increase in wine anthocyanins and small polymeric pigments. Protein-precipitable tannins, flavan-3-ols, and oligomeric proanthocyanidins were generally higher in EM wines (Table 4). The proportion of skin- and seed-derived tannins affected by the RDI and skin contact treatments showed an unclear trend in the one-way ANOVA, likely due to the rather small sample size and thus insufficient statistical power. However, in an earlier study, a two-way ANOVA captured a significant effect of the skin contact treatment with an overall proportion of 55% of seed-derived tannins in control wines versus 73% of seed-derived tannins in EM wines (Casassa et al. 2013b). Once again, the effect of the RDI treatment on protein-precipitable tannins and proanthocyanidin composition was less evident than the effect of the skin contact treatment.

Descriptive analysis. The main goal of this study was to assess the sensory impact of extended maceration applied to Cabernet Sauvignon grapes produced using four different RDI protocols. To that end, a descriptive analysis with a trained panel was conducted to determine specific sensory effects of both the maceration and the RDI treatments. Potential synergistic or antagonistic sensory effects of extended maceration on the fruit of the different RDI treatments were also explored. A fixed-effect two-way ANOVA with interaction was performed on the sensory data (Table 5). The RDI treatment influenced the sensory profile of the wines, with particular impact on color, taste (bitterness), and mouthfeel (astringency) components. However, the effect of the maceration treatment prevailed in all the sensory attributes, a trend also previously reported for the wine chemical composition.

A significant RDI treatment × winemaking interaction was observed for the purple and red color components, black fruit aroma, and bitterness. The significance of the interaction for these attributes called for reevaluation of the treatment effects and first-order interactions by one-way ANOVA (Table 6). The purple component was more (negatively) affected in

Table 2 One-way ANOVA of CIELab color parameters of Cabernet Sauvignon wines subjected to four different regulated deficit irrigation regimes (RDI) and two skin contact time treatments (EM) at day 250 postcrushing.

RDI/skin contact[a]	L*	C*	H*	a*	b*
100% ET$_c$					
Control	44.5 cde[b]	53.5 a	10.7 a	52.8 abc	10.0 a
EM	49.8 f	50.6 a	11.4 a	49.6 a	10.0 a
70% ET$_c$					
Control	40.8 bc	55.4 ab	11.9 a	54.2 bc	11.4 a
EM	49.5 ef	50.6 a	12.2 ab	49.5 a	10.7 a
25/100% ET$_c$					
Control	41.9 cd	55.2 ab	10.9 a	54.2 bc	10.5 a
EM	46.7 ef	52.9 a	11.7 a	51.8 ab	10.8 a
25% ET$_c$					
Control	34.2 a	59.1 b	15.6 bc	56.8 c	15.9 b
EM	36.8 ab	58.9 b	16.3 c	56.5 c	16.5 b
p value	0.001	0.024	0.034	0.029	0.025

[a]RDI treatments: 100% ET$_c$, 70% ET$_c$, and 25% ET$_c$ represent replenishment of 100%, 70%, and 25%, respectively, of full-vine ET$_c$ from fruit set through harvest; 25/100% ET$_c$ represents 25% ET$_c$ from fruit set to veraison followed by 100% ET$_c$ from veraison to harvest. Skin contact treatments: control (10 days skin contact); EM: extended maceration (30 days skin contact).
[b]Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at $p < 0.05$.

the 70% ET_c treatment than in the other RDI treatments upon application of EM. Additionally, a two-way ANOVA applied to the previously reported anthocyanin content of the wines at day 250 (coincident with the sensory analysis) revealed a small yet significant RDI × winemaking effect for anthocyanins ($p = 0.044$) (Casassa et al. 2013b). This effect followed the same direction observed in the visual purple component, which explains the observed sensory interaction.

There was also a significant RDI × winemaking interaction for the black fruit aroma component (Table 5). A one-way ANOVA indicated that the perception of the black fruit aroma decreased proportionally more in the 25% ET_c treatment than

in the other treatments upon application of EM, although the reason for this decrease is unknown. However, extended maceration in general decreased the perception of the black fruit aroma (Table 6). Recently, Callejón et al. (2012) reported that in microfermentations (1 L) of Cabernet Sauvignon wines, some norisoprenoids such as β-damascenone could be selectively bound by the skins and other fermentation solids during prolonged maceration, thereby decreasing their volatility. In turn, β-damascenone has been shown to synergistically increase the perception of the black fruit note (Pineau et al. 2007), which may explain the lower ratings of the black fruit attribute in extended maceration wines. Lastly, bitterness was

Table 3 One-way ANOVA of selected phenolic parameters of Cabernet Sauvignon wines subjected to four different regulated deficit irrigation regimes (RDI) and two skin contact time treatments at day 250 postcrushing.

RDI/ skin contact[a]	Anthocyanins (mg/L malvidin- 3-glucoside)	SPP (AU 520 nm)	LPP (AU 520 nm)	Iron-reactive phenolics (mg/L CE[b])	Flavonols (mg/L quercetin- 3-glucoside)
100% ET_c					
Control	342 bc[c]	1.8 ab	1.0 ab	1857 abc	98 ab
EM	271 a	1.5 a	0.8 a	2384 d	84 a
70% ET_c					
Control	392 c	2.1 bc	1.3 ab	1765 b	109 abc
EM	282 ab	1.8 ab	0.8 a	2423 d	84 a
25/100% ET_c					
Control	352 c	2.1 bc	0.7 a	1665 a	107 abc
EM	325 abc	1.9 b	0.8 a	2239 bcd	101 ab
25% ET_c					
Control	485 d	2.8 d	1.3 ab	1767 ab	139 c
EM	377 c	2.4 cd	1.5 b	2642 d	128 bc
p value	0.0010	0.0017	0.1082	0.0161	0.0298

[a]RDI treatments: 100% ET_c, 70% ET_c, and 25% ET_c represent replenishment of 100%, 70%, and 25%, respectively, of full-vine ET_c from fruit set through harvest; 25/100% ET_c represents 25% ET_c from fruit set to veraison followed by 100% ET_c from veraison to harvest. Skin contact treatments: control (10 days skin contact); EM: extended maceration (30 days skin contact).
[b]Catechin equivalents.
[c]Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at $p < 0.05$.

Table 4 One-way ANOVA of protein-precipitable tannins (PPT), proanthocyanidin (PA) composition, and proportion of skin- and seed-derived tannins of Cabernet Sauvignon wines subjected to four different regulated deficit irrigation regimes (RDI) and two skin contact time treatments at day 250 postcrushing.

RDI/ skin contact[a]	PPT (mg/L CE[b])	Flavan-3-ols (mg/L CE)	Oligomeric PA (mg/L CE)	Polymeric PA (mg/L CE)	Skin-derived tannins (%)	Seed-derived tannins (%)
100% ET_c						
Control	732 abc[c]	133 a	366 a	491 a	29 abc	71 bcd
EM	878 cd	285 bc	989 c	688 abc	23 ab	76 cd
70% ET_c						
Control	703 abc	279 bc	578 b	437 a	59 d	41 a
EM	949 de	372 c	1077 c	1003 c	21 a	79 d
25/100% ET_c						
Control	568 a	232 b	535 ab	592 ab	44 bcd	56 abc
EM	768 bc	341 c	893 c	615 ab	31 abc	69 bcd
25% ET_c						
Control	613 ab	286 bc	424 ab	385 a	46 cd	54 ab
EM	1067 e	385 d	974 c	877 bc	37 abcd	63 abcd
p value	0.0002	0.0012	< 0.0001	0.0165	0.0119	0.0119

[a]RDI treatments: 100% ET_c, 70% ET_c, and 25% ET_c represent replenishment of 100%, 70%, and 25%, respectively, of full-vine ET_c from fruit set through harvest; 25/100% ET_c represents 25% ET_c from fruit set to veraison followed by 100% ET_c from veraison to harvest. Skin contact treatments: control (10 days skin contact); EM: extended maceration (30 days skin contact).
[b]Catechin equivalents.
[c]Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at $p < 0.05$.

also comparatively more accentuated in the 70% ET_c treatment upon EM than in the wines of the other RDI treatments.

Principal component analysis. To further explore the comparative influence of both the maceration and the RDI treatments on the sensory profile of the wines, the full data set was submitted to principal component analysis (PCA), including the wine replicates and the repeated measures performed during the formal sensory sessions. The PCA biplot and confidence ellipses were constructed with 95% certainty according to the Hotteling's test (Husson et al. 2005), which provides significance testing. Only the first two principal components with eigenvalues greater than or equal to one

were retained (Figure 1). The PCA plot showed a bidimensional model that explained 94% of the observed variability. Dimension 1, which explained ~77% of the variability, was a function of the maceration treatment, which suggests a comparatively higher impact of the EM treatment over the RDI treatments on the sensory profile of the wines. Irrespective of the RDI treatment, control wines clustered in the negative region of dimension 1, whereas EM wines grouped on the positive region of dimension 1 (Figure A). There were strong correlations between EM wines and the red and brown color components, oxidized character, astringency and bitterness, all located in the positive side of dimension 1 (Figure 1B).

Table 5 Main effects and interaction of descriptive sensory attributes (n = 15) of Cabernet Sauvignon wines subjected to four different regulated deficit irrigation regimes (RDI) and two skin contact (SC) time treatments.

ANOVA parameter[a]	df	Color components				Aroma components			Taste/mouthfeel	
		Purple	Red	Brown	Saturation	Red fruit	Black fruit	Oxidized character	Bitterness	Astringency
RDI	3									
100% ET_c		10.5 a[b]	7.2 b	1.2 a	10.1 a	4.5 a	6.4 a	1.9 a	6.9 a	10.6 b
70% ET_c		10.9 b	6.9 a	1.2 a	11.1 b	4.7 a	6.9 a	2.0 ab	7.5 b	10.5 b
25/100% ET_c		11.4 c	6.6 a	1.1 a	11.5 c	4.8 a	7.5 b	2.0 a	7.3 ab	10.2 a
25% ET_c		12.5 d	6.7 a	1.1 a	13.1 d	4.9 a	6.6 a	2.3 b	7.9 c	11.4 c
p value		<0.0001	0.004	0.461	<0.0001	0.371	0.001	0.111	<0.0001	<0.0001
Skin contact (SC)	1									
Control		12.1 b	6.1 a	0.9 a	11.9 b	5.4 b	7.6 b	1.0 a	6.6 a	9.5 a
EM		10.6 a	7.6 b	1.4 b	11.0 a	4.0 a	6.0 a	3.1 b	8.3 b	11.8 b
p value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
RDI × SC	3									
p value		<0.0001	<0.0001	0.179	0.161	0.887	<0.0001	0.256	0.006	0.232

[a]RDI treatments: 100% ET_c, 70% ET_c, and 25% ET_c represent replenishment of 100%, 70%, and 25%, respectively, of full-vine ET_c from fruit set through harvest; 25/100% ET_c represents 25% ET_c from fruit set to veraison followed by 100% ET_c from veraison to harvest. SC treatments: control (10 days skin contact); EM: extended maceration (30 days skin contact).
[b]Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at *p* < 0.05. Evaluations made along a 15 cm unstructured line scale.

Table 6 One-way ANOVA of descriptive sensory attributes (n = 15) of Cabernet Sauvignon wines subjected to four different regulated deficit irrigation regimes (RDI) and two skin contact (SC) time treatments.

RDI/ Skin contact[a]	Color component				Aroma components			Taste/mouthfeel	
	Purple	Red	Brown	Saturation	Red fruit	Black fruit	Oxidized character	Bitterness	Astringency
100% ET_c									
Control	11.1 b[b]	7.0 c	1.0 a	10.5 b	5.3 b	6.6 cd	0.9 a	6.2 a	19.6 b
EM	9.9 a	7.4 cd	1.4 b	9.7 a	3.8 a	6.2 bc	2.9 b	7.5 bc	111.6 de
70% ET_c									
Control	12.1 c	6.0 b	0.8 a	11.7 d	5.3 b	7.8 e	1.0 a	6.3 a	9.3 ab
EM	9.8 a	7.7 de	1.6 b	10.5 b	4.1 a	5.8 ab	3.0 b	8.8 d	11.8 e
25/100% ET_c									
Control	12.0 c	6.2 b	0.9 a	11.8 d	5.5 b	8.0 e	1.1 a	6.6 a	8.9 a
EM	10.8 b	7.0 c	1.4 b	11.2 c	4.2 a	7.0 d	2.9 b	8.0 c	11.4 d
25% ET_c									
Control	13.2 b	5.2 a	0.8 a	13.5 f	5.6 b	8.0 e	1.1 a	7.2 b	10.1 c
EM	11.8 c	8.1 e	1.4 b	12.6 e	4.1 a	5.2 a	3.4 c	8.7 c	12.6 f
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a]RDI treatments: 100% ET_c, 70% ET_c, and 25% ET_c represent replenishment of 100%, 70%, and 25%, respectively, of full-vine ET_c from fruit set through harvest; 25/100% ET_c represents 25% ET_c from fruit set to veraison followed by 100% ET_c from veraison to harvest. SC treatments: control (10 days skin contact); EM: extended maceration (30 days skin contact).
[b]Within a column, values followed by the same letter are not significantly different according to Fisher's LSD test at *p* < 0.05. Evaluations made along a 15 cm unstructured line scale.

It has been previously shown that extended maceration for 30 days or more increased astringency ratings by 22% in Cabernet Sauvignon (Scudamore-Smith et al. 1990) and by 34% in Merlot (Casassa et al. 2013a). Similarly, in the present study, astringency ratings were, on average, 25% higher in EM wines relative to the control wines (Table 5).

Bitterness perception was also affected by extended maceration. Although previous reports of the effects of extended maceration on bitterness perception are not conclusive (Yokotsuka et al. 2000, Casassa et al. 2013a), the present work has shown an average increase of 26% in the bitterness ratings of EM wines relative to those of the control wines (Table 5). This increase can also be further corroborated by inspection of the relative position of the ellipses for the EM wines and the loadings for bitterness in the PCA analysis. Monomeric flavan-3-ols content was generally higher in EM wines (Table 4). Because catechin and epicatechin are known to primarily elicit bitterness (Lesschaeve and Noble 2005), higher amounts of these monomers in EM wines may explain the observed higher bitterness ratings. In addition, there is a potential cooperative contribution to overall perceived bitterness perception by the judges from the oligomeric and protein-precipitable tannin content in EM wines (Table 4), but this hypothesis merits further research.

The PCA sensory loadings on the negative side of dimension 1 (Figure 1B) strongly associated the control wines with the purple component and saturation visual descriptors and the black and red fruit aroma descriptors. Overall CIELab chromatic differences between any given control and EM wines were detected at day 250 (Table 2), and these differences favored the control wines. These results suggest that purple component and saturation were the two color components directly related to higher perceived color in the control wines.

Comparatively higher ratings for fruit aroma attributes in the control wines (or lower ratings of both fruit aroma components in EM wines) (Table 5) can be attributed (but may not be limited) either to a genuine increase in the headspace concentration of these two aroma attributes in the control wines or to a masking effect resulting from the oxidized character in EM wines. Many compounds, including heptenal, methional, and phenylacetaldehyde, have been associated with the perception of oxidation in red wines (San Juan et al. 2012). However, in the context of the present study, acetaldehyde formation by metal-catalyzed oxidation of ethanol during extended maceration seems more plausible (Danilewicz 2003). Acetaldehyde at levels above its detection threshold bears a negative sensory connotation, and this compound was used as a standard for the oxidized character during the training of the sensory panel (Table 1). Furthermore, quinones resulting from the coupled oxidation of polyphenols can readily bind nucleophiles such as mercaptans and thiols bearing fruity notes (Nikolantonaki et al. 2010), thus lowering perceived fruitiness. However, the generation of an oxidized character and a decrease in the fruit aroma of the wine may not necessarily be a causative result of the application of extended maceration, as other studies have found no significant increases of the oxidative character upon extended maceration (Casassa et al. 2013a) or no effect of it in the wine's fruit flavor (Harbertson et al. 2009). In the present study, the oxidized character did develop after the extended maceration, although the ratings for the oxidized character in these wines averaged 3 cm on the 15 cm unstructured scale (Table 5), which is in the low perception range of the scale. However low this oxidative character, it was enough to reduce the ratings of red and black fruit in the EM wines by 26% and 21%, respectively, relative to the control wines.

The sensory impact of the RDI treatment was a function of dimension 2, which explained 17% of the residual variability of dimension 1. Wines from the 100% ET_c treatment clustered at the lower end of dimension 2 (Figure 1A), indicating the lowest perception for color, fruity aromas, and mouthfeel attributes. The 100% ET_c treatment was included in the experimental design to explore the relationship between the application of an irrigation protocol based on replenishment of the full evapotranspirative demand and the generation of vegetative aromas in the resulting wines under the premise that a more vigorous canopy would lead to an enhancement of vegetative aromas (Allen and Lacey 1993). However, the vegetal component was confirmed to be absent in the 100% ET_c wines (and in all the wines) and thus was not selected as a discrimination term during the panel evaluations. This result suggests that, under some combinations of cultivar, clone, climate, and soil, supplying the full evaporative demand does not necessarily lead to vegetative flavors in the resulting wines.

The wines of the 70% ET_c and 25/100% ET_c treatments were distributed in the medial region of dimension 2 defined by the aromatic descriptors red and black fruit aroma (control wines) and the chromatic descriptors red and brown component (EM wines) (Figure 1). Although not necessarily causative, the application of different RDI protocols have been linked to enhanced concentration of fruity norisoprenoids in the resulting wines (Qian et al. 2009, Ou et al. 2010). The interconversion of carotenoids to odorant C_{13}-norisoprenoids has yet to be conclusively established, but it is believed that reduced vine vigor may result in increased sun exposure and berry temperature in the fruiting zone, thereby leading to carotenoid degradation with the concomitant increase in C_{13}-norisoprenoids precursors (Kondouras et al. 2006, Bindon et al. 2007). While we did not identify these compounds analytically, the generalized trend for the sensory results presented here is consistent with these previous studies. Finally, the 25% ET_c treatment generated wines with higher purple component and saturation in the control wines, consistent with comparatively higher content of anthocyanins and small polymeric pigments in these wines (Table 3). When the 25% ET_c treatment was processed with EM, the sensory profile shifted toward a pronounced oxidized character, bitterness, and astringency.

Also of significance was the overlap of the 70% ET_c control and 25/100% ET_c control wines on the left side of dimension 1 (Figure 1A). The ellipses represent empirical descriptions of the variability of the sensory evaluations (Husson et al. 2005), and if the ellipses are superimposed, then the wines are not significantly different from a sensory standpoint. Conversely,
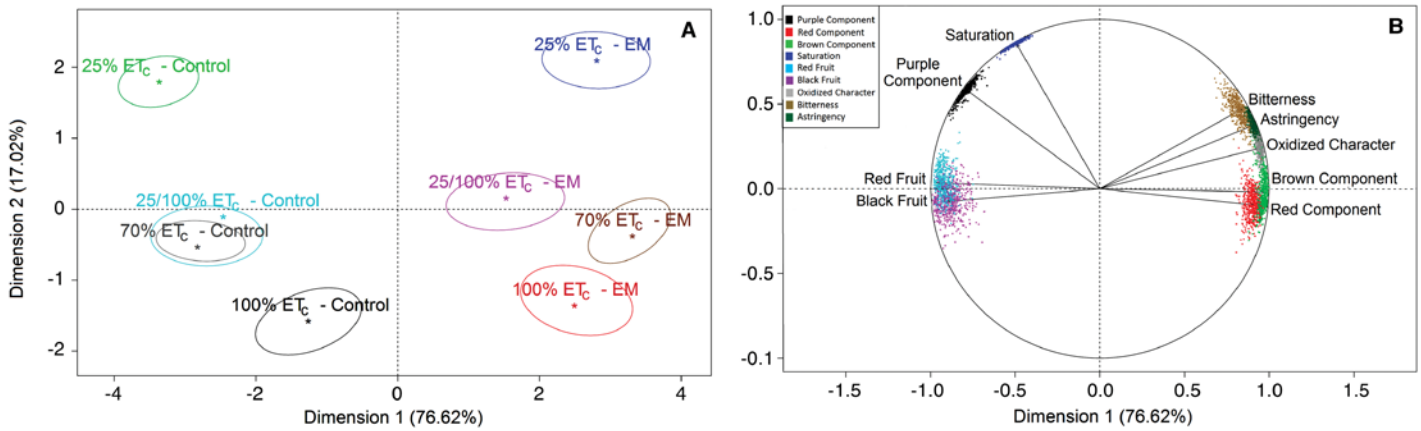
Figure 1 Principal component analysis of descriptive sensory data of Cabernet Sauvignon wines evaluated by a trained panel (n = 15): (**A**) confidence ellipses based on multivariate distribution of Hotelling's test for $p < 0.05$ indicating 95% confidence intervals and (**B**) sensory loadings. RDI treatments: 100% ET$_c$, 70% ET$_c$, and 25% ET$_c$ represent replenishment of 100%, 70%, and 25%, respectively, of full-vine ET$_c$ from fruit set through harvest; 25/100% ET$_c$ represents 25% ET$_c$ from fruit set to veraison followed by 100% ET$_c$ from veraison to harvest. Winemaking treatments: C, control (10 days skin contact); EM, extended maceration (30 days skin contact).
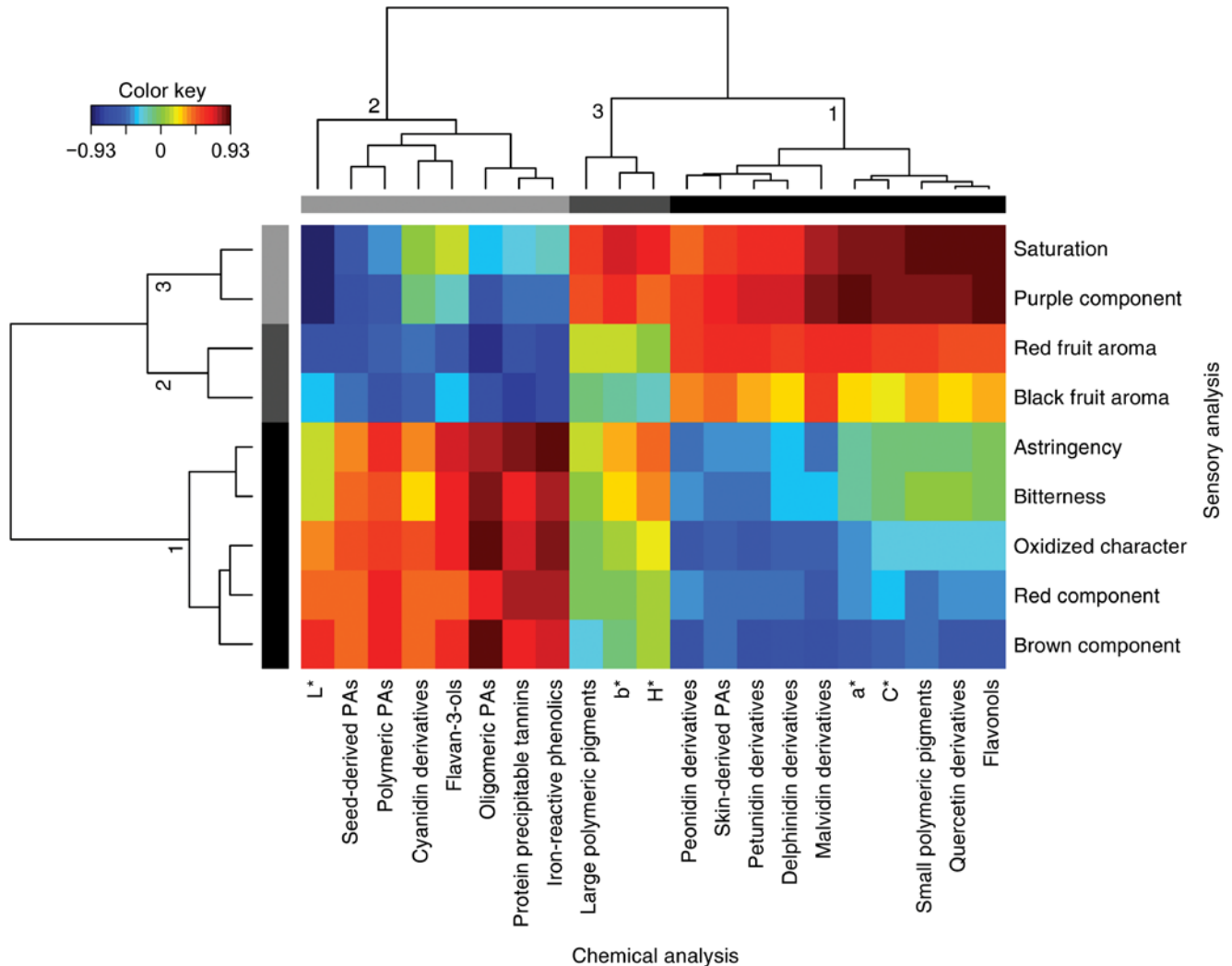

Figure 2 Canonical correlation analysis showing clustered image maps of the correlation between significant sensory and chemical attributes considering all wines (n = 16) in the experiment. The dark red and blue colors indicate positive and negative correlation coefficient values, respectively, whereas light green colors indicate near zero correlation coefficient values. Cluster membership within sensory and chemical data is indicated as different shades of grey and black. L*: lightness; PAs: proanthocyanidins; b*: yellow component; H*: hue; a*: red component; C*: saturation.

the general absence of overlap in six out of the eight treatments highlights the magnitude of the sensory differences. Furthermore, the compression of the ellipses is an indication of the reliability of the panel data.

Correlation between chemical and sensory data. The relationship between the significant chemical features of the wines and their sensory properties was examined by canonical correlation analysis (Figure 2). In addition to an agglomerative hierarchical cluster analysis of the chemical and sensory data sets, the correlation coefficients between any given pair of variables in the two retained dimensions are also provided (González et al. 2012). Shared cluster membership indicates the strength of the relationship between the canonical variates for both the chemical and sensory variables. For the chemical and sensory data sets, the cluster containing the highest number of variables was labeled cluster 1, the cluster with the next highest number of variables was labeled cluster 2, and so forth. For the chemical analyses, cluster 1 was composed of all the anthocyanin derivatives (except cyanidin), skin-derived proanthocyanidins, total flavonols, quercetin derivatives, small polymeric pigments, the red CIELab component (a*), and saturation (C*). Cluster 2 was composed of flavan-3-ols, oligomeric and polymeric proanthocyanidins, seed-derived proanthocyanidins, protein precipitable tannins, iron reactive phenolics, lightness (L*), and cyanidin derivatives. Cluster 3 was composed of large polymeric pigments, the yellow CIELab component (b*), and the hue angle (H*). For the sensory data set, three main clusters were identified. Cluster 1 was composed of astringency, bitterness, oxidized character, and red and brown color components. Cluster 2 was composed of red and black fruit aromas, whereas cluster 3 consisted of saturation and purple component. To understand which chemical variables were positively or negatively correlated with the sensory variables, the cluster image map provides the observed correlation coefficient values between any pair of variables. Positive correlation coefficients close to 0.93 were observed between protein precipitable tannins, iron-reactive phenolics, and oligomeric proanthocyanidins as well as astringency, bitterness, and brown component. These sensory attributes defined the sensory profile of extended maceration wines, thus suggesting that for the wines analyzed here, bitterness and astringency are highly correlated with the content of oligomeric proanthocyanidins (mean degree of polymerization up to 5 flavan-3-ols subunits) and also with protein-precipitable tannins, seed-derived proanthocyanidins, and overall phenolic concentration. Likewise, positive correlation coefficients were observed between the color sensory attributes saturation and purple component and the chemical attributes flavonols, quercetin derivatives, small polymeric pigments, a*, C*, and malvidin and delphinidin derivatives. As control wines were chromatically (Table 2) and sensorially (Table 5) more colored than EM wines, the aforementioned chemical variables may most likely be responsible for the perceived color features of control wines. In particular, a positive correlation between small polymeric pigments, C*, and monomeric anthocyanins has been previously reported in Merlot wines obtained after 10 days of skin contact (Casassa et al. 2013a), consistent with the results reported here. Positive correlations were also observed between flavan-3-ol content and the perception of both astringency and bitterness, suggesting that at the concentration of these monomers in EM wines (overall mean: 346 ± 37 mg/L, n = 8), the perception of both bitterness and astringency could be at least partially explained by the simultaneous occurrence of a comparatively higher concentration of flavan-3-ols and oligomeric proanthocyanidins. Nevertheless, since astringency and bitterness were also correlated with multiple phenolic measures (e.g., oligomeric proanthocyanidins, protein-precipitable tannins, iron-reactive phenolics), the occurrence of a single causative effect for both sensory features is unlikely.

Conclusions

The sensory effects of extended maceration and four different RDI treatments and some of their interactive effects were studied under winemaking conditions comparable with those of a commercial scale. While the application of the different RDI treatments primarily produced differences in color, astringency, and bitterness in the resulting wines, the effect of the two contrasting maceration treatments affected all sensory attributes. Thus, under our experimental conditions, the maceration treatments had a comparatively higher impact than the RDI treatments in the sensory and chemical profile of the wines.

Chromatic attributes were significantly enhanced in the 25% ET_c control treatment and were also highly correlated with the flavonol and anthocyanin derivatives content and small polymeric pigments. Red and black fruit aroma attributes were favored in the controls of the 70% ET_c and 25/100% ET_c treatments. These results suggest that moderate RDI protocols such as 70% ET_c and 25/100% ET_c positively impact the fruity component of wine aroma, possibly by favoring accumulation of norisoprenoids, whereas more severe RDI protocols such as 25% ET_c increased perceived color saturation, astringency, and bitterness. Extended maceration resulted in wines with comparatively lower fruit-based aromas and perceived wine color saturation, but enhanced the perception of bitterness and astringency, with the latter possibly arising from the concurrent effect of a high concentration of flavan-3-ols and oligomeric proanthocyanidins in these wines. Thus, extended maceration for 30 days or more appears to have limited merit as a winemaking practice. If wines with high tannin content are sought for stylistic or blending purposes, then practices such as prefermentation runoff (saignée) may be a more rational option in light of optimizing winery logistics.

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