

Research Article

The Impact of Extended Skin Contact on Phenolic Extraction in Skin-fermented Hybrid White Wines

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Abstract

Background. With growing interest in skin-fermented white wines, more winemakers in North America are producing this style of wine with cold-tolerant, interspecific hybrid grape varieties rather than only *Vitis vinifera* species. **Objective.** In this study we sought to characterize the level of phenolic extraction using extended skin contact post-fermentation using two hybrid white grape cultivars. Alcoholic fermentations were conducted with Cayuga White and Vidal Blanc separately, followed by five months of extended skin contact post-fermentation. Phenolic compounds and color quantification were monitored over the course of post-fermentative aging on grape skins. The parameters were analyzed using standard UV/Visible spectroscopy and HPLC-MS-based methods. **Conclusions.** For both hybrid cultivars, there were no significant changes in phenolic content or in brown or yellow color over five months of post-fermentation skin contact. Under the winemaking conditions used in this study, for the interspecific hybrid grape cultivars Cayuga White and Vidal Blanc, we found that extended skin contact did not increase phenolic extraction beyond the level achieved by completion of alcoholic fermentation, providing useful guidance for winemakers to make production decisions regarding potential benefits (increased compound extraction) and risks (increased spoilage or oxidation potential due to longer periods of atmospheric exposure) of extended skin contact post-fermentation.

Keywords

Orange Wine, Skin-fermented White Wine, Wine Phenolic Compounds, Tannin, Anthocyanin, Interspecific Hybrid Grape

1. Introduction

Skin-fermented white wine is a grape-based still wine with multiple synonymous names, including ‘amber’ and ‘orange’ wine. Coined by David A. Harvey in 2004, the moniker ‘orange’ wine has become popular in the US market, though this often leads to consumers’ incorrect assumption that the wine is produced from the eponymous citrus fruit [1]. In simple terms, skin-fermented white wine is made by processing white grapes in a style commonly used for red winemaking: leaving some portion of the grape pomace (skins, stems and

seeds) in the juice during fermentation, and potentially even for a period of extended maceration following fermentation. One goal of this processing style is to increase phenolic extraction from the grape seeds and skins in an effort to increase color and produce the desired “distinctive, dry and tannic” mouthfeel qualities not commonly associated with traditional white wines [2, 3].

Skin-fermenting white grapes may have been the earliest white wine production method, and is experiencing a recent

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resurgence in popularity [4-6]. Originating from the area that is currently the Republic of Georgia, skin-fermented white wine was traditionally made by fermenting crushed white grapes in buried, wax-lined clay vessels called “kvevri” or “qvevri” [7]. Remnants of these vessels contain some of the oldest biomolecular evidence of winemaking, dating back to 6,000 BCE in the Neolithic period [8]. Today, skin-fermented white wine production has spread worldwide, and the most commonly used grapes globally are *Vitis vinifera* cultivars like Ribolla Gialla, Rkatsiteli, Pinot Grigio and Chenin Blanc [9]. However, more wine producers in North America are exploring the possibility of skin-fermenting the disease-resistant interspecific hybrid grapes cultivars used for traditional white wines in cool and cold climate regions.

Despite the long history of skin-fermented white wines, there is little research on the chemical and sensory effects resulting from this production method. Extended contact with grape pomace has the potential to produce a range of chemical and sensory characteristics not found in typical white wines created from fermented juice. Phenolic compounds, like tannins and anthocyanins, are located primarily in skins and seeds; this fact is exploited in red wine production, where red grapes are skin-fermented to produce wines with desired color, astringency, and aging capabilities. Most extended skin contact research has, understandably, been focused on red wines. However, properties of extended skin contact for red wine production may not be relevant for skin-fermented white wines due to chemical differences, most notably due to the lack of anthocyanins [10]. Some similarities exist, however. With the exception of anthocyanins, white grapes have phenolic content similar in quality and quantity to red grapes, within the expected range of cultivar variation [4]. For example, phenolic content was found to double in skin-fermented vs. traditionally fermented Chenin Blanc [11]. Thus, skin-fermented whites share the “dry,” “bitter,” and “astringent” sensory attributes associated with tannin extraction, and more commonly found in red wines [12].

To provide guidance for winemakers interested in producing skin-fermented white wines from interspecific hybrid wine grapes, we sought to evaluate the role of extended skin contact on phenolic extraction in selected cultivars. We performed this evaluation on wines produced from two commercially important hybrid grape cultivars grown in cool-climate regions like the New York Finger Lakes: Cayuga White and Vidal Blanc.

2. Materials and Methods

2.1. Grape Variety Selection and Harvest

Two interspecific hybrid grape cultivars, Cayuga White and Vidal Blanc, were selected due to their economic importance in New York’s Finger Lakes region and beyond for white wine production. Cayuga White is a cross from the *Vitis labrusca* hybrids Schuyler and Seyval Blanc. Vidal Blanc is a

cross from the *Vitis rupestris* and *Vitis lincedumii* hybrids Rayon d’Or and Ugni Blanc. Both Cayuga White and Vidal Blanc grapes were harvested by hand in Fall 2018 as part of a wine production course from the Cornell University teaching vineyards located in Lansing, NY and stored in a 4°C refrigerated room until further processing.

2.2. Fermentation

Berries for each fermentation replicate were individually picked off the grape rachis and sorted to exclude berries affected by molds, then pressed by hand using cleaned potato mashers to produce 3 L of must inside a screw-cap 3.8 L fermentation vessels with airlocks. Standard parameters for initial juice chemistry and final wine chemistry were determined by ETS Laboratories (St. Helena, CA) (Table 1). Cayuga White and Vidal Blanc had an initial must YAN of 287 ppm and 90 ppm, respectively; Fermid K (Lallemand) was used to make a 30 ppm YAN addition to Cayuga White and 90 ppm to Vidal Blanc. An additional 45 ppm YAN addition of diammonium phosphate (DAP) was made to Cayuga White and 85 ppm to Vidal Blanc on the 4th day of the fermentation in alignment with standard winemaking practices (resulting in a total YAN of 362 ppm and 265 ppm, respectively). Before fermentation, the musts were chaptalized to 22°Brix, and the manufacturer recommended dosage (20g/hL) of commercial *Saccharomyces cerevisiae*, Lalvin ICV-D254 (Lallemand) was rehydrated with 0.3g/L GoFerm (Lallemand) and added according to manufacturer’s specifications. Additionally, 30 mg/L SO₂ (as potassium metabisulfite) was added to the juices immediately before yeast inoculation. Fermentation and post-fermentation extended aging took place in a temperature-controlled room held at 16°C; sugar levels were checked by refractometry, and punch downs were performed twice each day throughout fermentation. After 3 days of fermentation, Cayuga White exhibited steady carbon dioxide bubble generation, and after 6 days had reached an average 3.9°Brix. Vidal Blanc began fermenting vigorously by day 4, and by day 12 it had reached 1.8°Brix. At 21 days, glucose and fructose levels dropped below 0.1g/L, and fermentations were considered complete; 50 mg/L SO₂ was added in accordance with standard winemaking procedures for both antioxidant activity and microbial protection. This 21 day time point corresponds with the initial post-fermentation samples in the figures (listed as 0 months post-fermentation). Notably, 2 of the Vidal Blanc samples had sugar levels between 0-1 g/L; as one sample was below the limit of detection (<0.1 g/L) this was still considered the initial post-fermentation time-point. Each type of fermentation (Cayuga White and Vidal Blanc) was performed in triplicate.

Measurements on initial pressed juice chemistry were performed by ETS Laboratories (St. Helena, CA). A single juice sample was analyzed for both grape varieties. For wine samples, three independent fermentations were analyzed and the average and standard deviation are reported.

Table 1. Initial juice and final wine chemistry parameters.

	Cayuga White Juice	Cayuga White Wine	Vidal Blanc Juice	Vidal Blanc Wine
titratable acidity (g/L)	6.8	8.2 ± 0.44	7.8	8.0 ± 0.61
pH	3.28	3.42 ± 0.05	3.21	3.42 ± 0.06
L-malic acid (g/L)	3.33	2.47 ± 0.07	5.13	3.57 ± 0.12
tartaric acid (g/L)	4.3	not tested	4.0	not tested
°Brix	18.1	not tested	21.1	not tested
glucose + fructose (g/L)	182	< 0.1	218	0.9 ± 0.66
ammonia (mg/L)	22	not tested	<10	not tested
alpha-amino compounds (mg/L as N)	269	not tested	86	not tested
yeast assimilable nitrogen (mg/L as N)	287	not tested	90	not tested
potassium (mg/L)	1480	not tested	1660	not tested
ethanol (% at 20°C)	not tested	10.95 ± 0.45	not tested	12.22 ± 0.34

2.3. Phenolic Content and Color Determination by Spectrophotometer

Phenolic content and color quantification were analyzed using standard UV/Visible spectroscopy. Absorbance at 440 nm (brown color), 420 nm (yellow), and 280 nm (phenolic content) was recorded to measure the intensity and hue of the samples and reference wines [13]. Reference measurements were taken with the following wines: 2015 Woodbridge Cabernet Sauvignon (a red wine control), 2015 Woodbridge Chardonnay (an oaked white wine control), 2016 Gotsa Chinuri (a *V. vinifera* orange wine control), and 2015 Atwater Dry Riesling (an unoaked white wine control).

2.4. Phenolic Compounds Analysis by HPLC

Samples were submitted to ETS Laboratories (St. Helena, CA) for HPLC-MS-based analysis of the following compounds: gallic acid, catechin, astilbin, tannin, grape reaction product, caftaric acid, caffeic acid, quercetin glycosides and

quercetin aglycone. Samples were analyzed by a proprietary reversed phase HPLC method derived from the method described in Price *et al.* [14]. Samples were evaluated on an Agilent Infinity 1290 system with a Diode Array Detector.

2.5. Statistical Analysis

One-way ANOVA with two tails was used to compare the significance of the means overall followed by a post-hoc Tuckey test to identify significantly different mean values over time for each grape variety [15]. Samples with statistically significant values (with a significance level of 0.05) are indicated throughout Table 2 by different superscript letters.

Samples were collected and analyzed as described in Materials and Methods. One-way ANOVA (two-tailed) was performed to assess statistically significant differences for each compound over the course of fermentation (with a significance level of 0.05). For each column, statistically significant differences within the same grape variety are represented by different superscript letters (e.g., A vs. B). PF: post-fermentation.

Table 2. Specific phenolic compound extraction from extended skin contact post-fermentation.

		Gallic Acid	Catechin	Astilbin	Tannin	Grape Reaction Product	Caftaric Acid	Caffeic Acid	Quercetin Glycosides	Quercetin Aglycone
Cayuga White	Juice	0.20 ± 0.00 ^A	0.20 ± 0.00 ^A	0.40 ± 0.00 ^A	13.2 ± 0.00 ^A	9.80 ± 0.00 ^A	6.8 ± 0.00 ^A	0.20 ± 0.00 ^A	2.50 ± 0.00 ^A	0.20 ± 0.00 ^A
	End of	5.8 ± 0.98 ^B	32.63 ± 11.53 ^B	2.4 ± 0.52 ^B	37.13 ± 4.19 ^B	6.66 ± 1.68 ^B	45.7 ± 7.97 ^B	4.7 ± 0.2 ^B	8.7 ± 1.56 ^A	1.43 ± 0.46 ^B

	Gallic Acid	Catechin	Astilbin	Tannin	Grape Reaction Product	Caftaric Acid	Caffeic Acid	Quercetin Glycosides	Quercetin Aglycone	
fermentation										
1 month PF	6.76 ± 1.01 ^B	40.5 ± 8.55 ^B	2.6 ± 0.62 ^B	42.86 ± 2.8 ^B	5.93 ± 0.76 ^B	44.5 ± 1.11 ^B	4.93 ± 0.3 ^B	6.3 ± 1.21 ^A	0.53 ± 0.05 ^A	
2 months PF	7.46 ± 0.77 ^B	42.76 ± 9.7 ^B	2.5 ± 0.3 ^B	43.3 ± 4.21 ^B	6.06 ± 0.51 ^B	41.23 ± 0.45 ^B	5.1 ± 0.34 ^B	5.13 ± 0.66 ^A	0.6 ± 0.1 ^A	
3 months PF	7.76 ± 0.66 ^B	44.63 ± 9.71 ^B	2.33 ± 0.55 ^B	45.63 ± 8.4 ^B	6.2 ± 0.51 ^B	37.76 ± 1.8 ^B	5.06 ± 0.28 ^B	4.13 ± 0.6 ^A	0.53 ± 0.11 ^A	
4 months PF	7.83 ± 0.81 ^B	42.7 ± 8.91 ^B	2.26 ± 0.11 ^B	43.73 ± 5.14 ^B	6.33 ± 1.01 ^B	36.3 ± 2.08 ^B	5.23 ± 0.28 ^B	3.6 ± 0.4 ^A	0.6 ± 0.2 ^A	
5 months PF	6.66 ± 0.65 ^B	40.53 ± 5.74 ^B	2.33 ± 0.05 ^B	46.23 ± 2.26 ^B	5.43 ± 1.04 ^B	32.46 ± 1.85 ^B	4.73 ± 0.15 ^B	1.86 ± 1.44 ^A	0.36 ± 0.05 ^A	
Juice	0.20 ± 0.00 ^A	0.20 ± 0.00 ^A	0.20 ± 0.00 ^A	0.50 ± 0.00 ^A	11.20 ± 0.00 ^A	0.20 ± 0.00 ^A	0.20 ± 0.00 ^A	11.60 ± 0.00 ^A	0.20 ± 0.00 ^A	
End of fermentation	5.37 ± 0.55 ^B	56.43 ± 12.21 ^A	0.53 ± 0.29 ^B	52.17 ± 6.12 ^B	4.70 ± 0.72 ^B	48.07 ± 4.92 ^A	4.83 ± 0.21 ^B	13.47 ± 7.16 ^A	0.70 ± 0.20 ^A	
Vidal Blanc	1 month PF	5.83 ± 0.15 ^B	63.1 ± 4.68 ^A	1.03 ± 0.25 ^B	54.00 ± 5.31 ^B	5.83 ± 0.67 ^B	67.97 ± 5.11 ^A	4.60 ± 0.26 ^B	24.1 ± 9.29 ^A	0.80 ± 0.46 ^A
	2 months PF	6.17 ± 0.61 ^B	59.7 ± 11.66 ^A	0.77 ± 0.06 ^B	53.03 ± 2.24 ^B	5.2 ± 0.44 ^B	58.87 ± 1.66 ^A	4.27 ± 0.06 ^B	17.90 ± 7.73 ^A	0.63 ± 0.21 ^A
	3 months PF	5.93 ± 0.55 ^B	59.57 ± 10.97 ^A	1.40 ± 0.82 ^B	58.73 ± 3.86 ^B	5.37 ± 0.46 ^B	62.80 ± 4.64 ^A	3.9 ± 0.17 ^B	20.83 ± 8.10 ^A	0.63 ± 0.21 ^A
	4 months PF	4.17 ± 0.35 ^B	57.97 ± 14.56 ^A	1.27 ± 0.15 ^B	49.57 ± 1.62 ^B	5.10 ± 0.52 ^B	56.5 ± 1.87 ^A	3.87 ± 0.25 ^B	15.70 ± 7.54 ^A	0.90 ± 0.17 ^A
	5 months PF	3.53 ± 0.23 ^B	61.83 ± 6.96 ^A	0.9 ± 0.99 ^B	49.50 ± 4.86 ^B	5.83 ± 0.91 ^B	80.50 ± 7.45 ^A	3.87 ± 0.35 ^B	29.10 ± 12.32 ^A	1.07 ± 0.29 ^A

3. Results

3.1. Color Measurement by Spectrophotometry

To evaluate whether extended post-fermentation skin contact could increase extraction of wine color compounds, color change was monitored using spectrophotometry. The target color for skin-fermented white wine production has no well-established standard, though consumers expect this style to have a darker color compared to typical white wine. To evaluate yellow and brown color intensities, we measured absorbance at 420 nm and 440 nm, respectively, as described in Iland 2013 [13]. Cayuga White exhibited a slightly lower intensity following 1 month of extended skin contact for both wavelengths, but no further significant changes were observed during the trial period (Figure 1). Vidal Blanc showed

slightly elevated measurements over time, but the wide variation observed resulted in no statistically significant changes in color intensity (Figure 1). Together these data suggest that extended skin contact for Cayuga White or Vidal Blanc beyond alcoholic fermentation would provide little, if any, color change.

Wine samples were collected for measurement immediately after fermentation (glucose and fructose <0.1g/L) and 1, 2, 3, 4 and 5 months post-fermentation. Absorbance at (A) 420 nm as an indicator of yellow color and (B) 440 nm as an indicator of brown color was measured by spectrophotometry for the indicated varietal wines (Cayuga White and Vidal Blanc). Error bars represent the standard deviation of three independent fermentations.

3.2. Bulk Phenolic Profile Assessment by Spectrophotometry

Bulk phenolic compounds were measured via absorbance at 280 nm using a spectrophotometer, which detects the phenolic ring present in tannins, anthocyanins, and any other phenolic compounds in the wine matrix [13]. No significant changes were observed in the bulk phenolic content during the period of post-fermentation skin contact for Cayuga White or Vidal Blanc (Figure 2). This suggests that the extending skin-contact post-fermentation does not increase phenolic compounds in general. For comparison, we also examined the phenolic content of commercial wines (unoaked white, oaked white, skin-fermented white, and red). The skin-fermented white wines produced from Cayuga White and Vidal Blanc had a higher phenolic content than unoaked white wine, and were similar to oaked white wine (Figure 2).

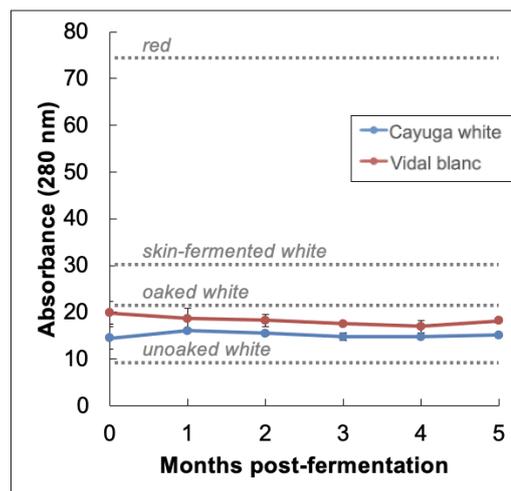


Figure 2. General phenolic extraction from extended skin contact post-fermentation.

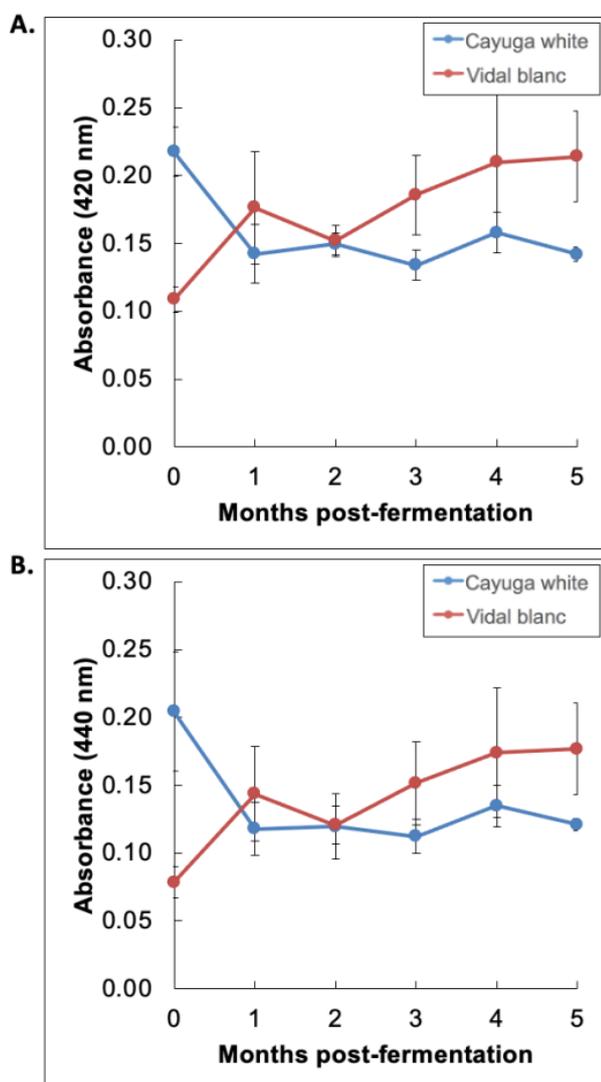


Figure 1. Color extraction from extended skin contact post-fermentation.

Wine samples were collected for measurement immediately after fermentation (glucose and fructose <0.1g/L) and 1, 2, 3, 4 and 5 months post-fermentation. Absorbance at 280 nm as an indicator of phenolic content was measured by spectrophotometry for the indicated varietal wines (Cayuga White and Vidal Blanc). For comparison, measured values for a commercial red, oaked white, unoaked white, and skin-fermented white wine are also shown. Error bars represent the standard deviation of three independent fermentations.

3.3. Specific Phenolic Compound Assessment by HPLC

The phenolic compounds in grapes can be categorized into flavonoids and non-flavonoids. Within the flavonoids category, the phenolics can be broken down into tannins, anthocyanins, flavanols and catechins. Flavonoids in wine derive from extracted skins and seeds during maceration and fermentation and can therefore be used as markers to evaluate the effects of extended maceration [16]. Tannin, including condensed tannin from the grapes and hydrolysable tannin from the oak, is one of the key components responsible for the astringent organoleptic sensation in red wine as well as white wine with extended skin contact [16-20]. Anthocyanins and flavanols are found on the skin of grapes, whereas catechins are also found in the seeds. Anthocyanins and flavanols together contribute to the color in red wine, while catechins contribute to the bitterness [21]. The anthocyanin and flavanol compounds measured included catechin, astilbin, quercetin glycosides and quercetin aglycone. Non-flavonoids, on the other hand, are useful to understand the potential oxidative effects of extended skin contact. Caffeic acid and its esterified form bound to tartaric acid (cafteric acid) are responsible for the oxidative browning effect after crushing [16]. They were measured along with grape reaction product in order to eval-

uate the level of oxidation over the course of extended maceration.

The aforementioned specific wine-relevant phenolic compounds were measured using HPLC at ETS Laboratories as described in Materials and Methods. For this phenolic analysis, in addition to post-fermentation samples, juice samples were also evaluated to compare pre- and post-fermentation phenolic compound concentrations. All of the results fit into two patterns: no statistically significant changes over time, or phenolic compound levels changed during alcoholic fermentation, but no further changes occurred post-fermentation even with additional skin contact (Table 2). For both grape varieties, three compounds notably increased during the course of fermentation, including tannin, catechin - a major component of tannin, and caftaric acid - thought to contribute color to white wines [22] (Figure 3, Table 2). These compounds are found primarily in grape seeds and skins. These three compounds, along with the remainder measured, do not exhibit any statistically significant changes post-fermentation despite extended skin contact (Figure 3, Table 2). These results align with the bulk phenolic measurements, both suggesting that extended skin contact beyond alcoholic fermentation does not significantly alter phenolic compound levels.

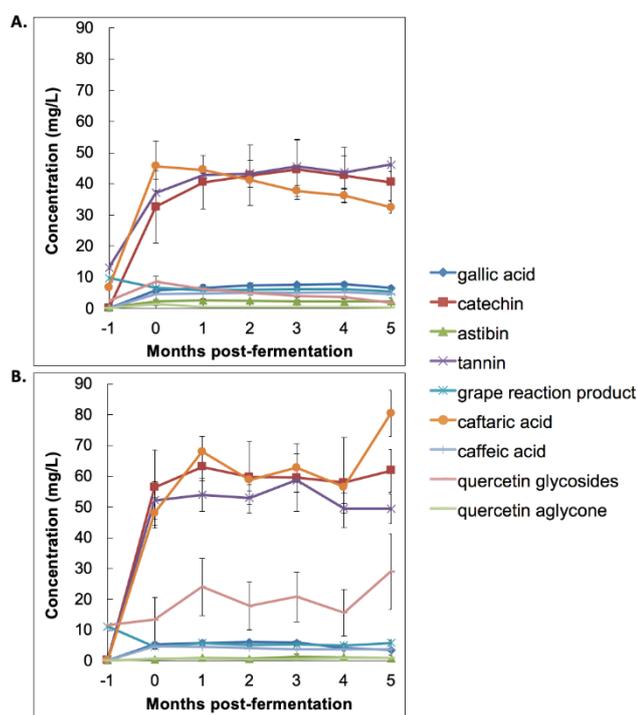


Figure 3. Specific phenolic compound extraction from extended skin contact post-fermentation.

The juice sample, represented as -1, was collected for measurement immediately after pressing grapes. Wine samples were collected for measurement immediately after fermentation (glucose and fructose <0.1 g/L) and 1, 2, 3, 4 and 5 months post-fermentation. Indicated compounds were measured for (A)

Cayuga White and (B) Vidal Blanc wines using HPLC at ETS Laboratories (St. Helena, CA). Error bars represent the standard deviation of three independent fermentations.

4. Discussion

In this study, we sought to evaluate the effect of extended skin contact post-fermentation on phenolic compound extraction for wines produced using two hybrid grape varieties, Cayuga White and Vidal Blanc. Evaluation of color changes over time indicated no changes in color extraction post-fermentation, despite extended skin contact. These results are different than expected based on red grape production, where increased skin contact typically increases red pigment dissolved in the wine [23]. Though abundant in red grapes and wines, anthocyanins are found at concentrations 5,000-60,000 times lower in white grapes [10, 24]. As anthocyanin-tannin complexes are thought to provide stable color during wine aging, and as skin-fermented white wines typically have higher tannin levels, this should provide increased stabilization for any anthocyanins present. White wine color may be conferred by the formation of yellow and orange xanthylum derivatives, though they are generally present at sub-threshold concentrations in traditionally fermented white wines [25, 26]. In model systems, however, xanthylum cations have been shown to form due to a catechin condensation reaction with glyoxylic acid, an oxidized tartaric acid residue [27]. The potential for formation of xanthylum derivatives may exist and confer color properties to skin-fermented white wines, though increased skin-contact post-fermentation did not increase catechin levels or absorbance in the yellow/brown color ranges.

Similarly, other evaluated phenolic compounds associated with wine production did not increase with post-fermentative skin contact, assessed by both evaluating bulk phenolic extraction with absorbance at 280nm and also by measuring specific compounds using an HPLC-MS. Together with the color compound analysis, these results suggest that extended skin contact beyond alcoholic fermentation does not significantly alter phenolic compound levels. Again, these results are different than expected based on red wine production, where increased skin contact typically increases extraction of phenolic compounds [28]. However, these results suggest that using these production methods with Cayuga White or Vidal Blanc, extended skin contact post-fermentation is not beneficial for increasing phenolic extraction. Notably, the phenolic content of both skin-fermented Cayuga White and Vidal Blanc wines was below our commercial *Vitis vinifera*-based skin-fermented white wine standard. It is possible that the phenolic content of these wines is within the range found for skin-fermented white wines as we only included one commercial comparison. Another possibility is differential behavior of hybrid-specific grape varieties compared to *Vitis vinifera* white grape biochemistry, such that the phenolic compound profile and/or functionality is different for

skin-fermented white wines produced from hybrid grapes. For similar reasons, these results may not apply to other grape cultivars, especially *V. vinifera*, as there is growing evidence suggesting that the phenolic profile of native North American and interspecific hybrid grapes may differ significantly from traditional European wine grape cultivars [29]. Recent work has shown that red wines produced from interspecific-hybrid grapes have lower rates of tannin retention, likely due to the presence of pomace proteins that bind with tannins during fermentation [29, 30]. This activity is thought to be responsible for the lower concentration of tannins found in interspecific red-hybrid wines, despite comparable or high levels of tannin found in their source fruit [31]. It is unknown whether such proteins also co-extract and bind tannins in skin-fermented white wines, though recent preliminary work on protein content in white sparkling wines suggests that some interspecific hybrid white grapes may have higher protein content than white *V. vinifera* [32].

5. Conclusions

Taken together, this study demonstrates that post-fermentation skin contact does not increase phenolic content or color extraction in skin-fermented white wines produced using the hybrid grape varieties Cayuga White and Vidal Blanc, though notably some phenolic compounds do increase during the course of fermentation. While not tested in this study, the phenolic compounds extracted during fermentation would still have a measurable sensory impact (astringency, bitterness, mouthfeel) in resulting wines. This information will be directly useful for winemakers, as it indicates that for this production style and these types of grapes, extending skin-contact post-fermentation does not provide any benefits to offset the associated risks (spoilage, oxidation, storing vs. selling, etc.). Further, these results join the growing evidence that phenolic profiles and activities are different in hybrid grape varieties than expected when compared to *V. vinifera*. Future work to characterize these differences has the potential to benefit winemakers throughout the world using cold-tolerant hybrid grape species.

Abbreviations

UV	Ultraviolet
HPLC-MS	High-performance Liquid Chromatography to Mass Spectrometry

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Author Contributions

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Data Availability Statement

The data supporting the outcome of this research work has been reported in the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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