Effect of Fermentation, Postfermentation, and Postbottling Heat Treatment on Cabernet Sauvignon Glycoconjugates

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In Cabernet Sauvignon must, total and phenol-free glycosides (expressed as glycosyl-glucose) rose during fermentation while skin concentrations dropped. Wines were heated postfermentation, prior to dejuicing (rising 2 to 3°C per day from 23 to 42°C, and held for one day at 42°C), or after bottling (at 42°C for 21 days) to determine the effect on total glycosides, glycosidic fractions, and anthocyanin complexing. Pre-dejuicing thermal vinification resulted in higher total (12%) and phenol-free (18%) glycosides. Large polymeric pigments rose 208% and small polymeric pigments rose 41%. Skins had lower total glycosides (-16%), and no significant difference in phenol-free glycosides. Postbottling heat treatment resulted in lower total (-15%) and phenol-free (-16%) glycosides, and increased hue (25%). Large polymeric pigments increased 62% compared to control wines.

Key words: Glycoside, thermal vinification, Cabernet Sauvignon, polymeric pigments, GG, glycosyl-glucose

The behavior of grape glycosides during wine processing is not well documented. Glycosides exist mainly as monoglucosides or diglycosides, with sugar moieties occurring as β-D-glucose, 6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranose, 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranose, and 6-*O*-α-L-apiofuranosyl-β-D-glucopyranose [34,35]. Glycoside hydrolysis liberates aglycones, a complex group of chemical compounds that includes aliphatic residues, monoterpenes, sesquiterpenes, norisoprenoids, and shikimic acid metabolites [1,31,36]. Members of this group may affect wine aroma, flavor, color, and structure [19]. Liberation of aglycones may occur enzymatically through β-glucosidases or via heat-induced acid hydrolysis [11,18,37,39,43].

The transfer of glycosides from skins and their subsequent hydrolysis may impact wine aroma, flavor, and color [13,16,30]. Grape glycosides are found primarily in the skin and pulp cells [16]. During wine production, pomace is frequently removed at or before dryness. Wine-pomace contact for up to 50 days postdryness results in increased anthocyanin extraction [30], suggesting the potential for extraction of other glycosides.

Heat treatment of musts and wines has been shown to cause sensory changes [8,23,27,28] by speeding oxidation, esterification, polymerization, precipitation, and hydrolysis reactions [12,42]. Thermal vinification can affect red color through the destruction of grape cell membranes, the hydrolytic release of anthocyanin aglycones [14], and the polymerization of phenolic compounds [27,40]. For example, Gerbaux [14] found that both heating wine to 40°C at the end of vatting and increasing temperatures linearly (up to 42°C) during the vatting period resulted in increased color, tannins, and overall sensory quality. The potential for extraction of glycosidically-bound compounds by postfermentation heat treatment has not been fully explored.

This study determined the effect of fermentation and postfermentation pomace contact, in the presence of heat, on Cabernet Sauvignon glycosides. The effect of postbottling thermal treatment was also evaluated.

Materials and Methods

Fermentation and pre-dejuicing heat treatment. Cabernet Sauvignon grapes (*Vitis vinifera* L.), grown in northern Virginia, were harvested at 21.5 Brix, divided into eight replicates (6.75 kg), destemmed, and crushed. The proportion of broken berries was approximately 70%. Musts were treated with 200 mg/L dimethyl dicarbonate (DMDC, Velcorin™, Bayer Corp., Pittsburgh, PA) and held at 7°C for 48 hr cold soak before inoculation with 120 g/L *Saccharomyces cerevisiae* (Enoferm Bordeaux Red™, Lallemand, Montreal, Canada), hydrated according to the manufacturer's instructions. One hundred sixteen grams of sucrose was added to each treatment replicate to increase the soluble solids level to 22.4 Brix. Diammonium phosphate was added at a rate of 120 mg/L.

Fermentation was conducted at ambient temperature (23 to 24°C) in 7-L polycarbonate containers. Liquid and cap temperatures were recorded every 15 min using a data logger (5100 Logger, Electronic Controls Design, Inc., Milwaukie, OR). Temperatures in the cap and liquid varied from each other by less than 0.5°C. Caps were punched every 8 hr.

At dryness (<0.2% reducing sugar) a gas-flow system was established to continuously blanket the wine with filtered CO_2 (3 µm filter, Pall Gelman Laboratory, Ann Arbor, MI). Treatments were heat (7 days heating; 2 to 3°C increase per day,

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Manuscript submitted October 2002; revised January 2003

beginning at 23°C, ending at 42°C, and held for one day) and ambient control (7 days at 23°C). The four heat-treatment replications were placed in a custom-designed, circulating-water heating tank. After treatment, replicates were placed in a basket press and free-run wine was collected for 20 min. Must and skin samples were taken at crush, postcold soak, dryness, after heat treatment, and at pressing (Table 1). Samples were frozen at -20°C for subsequent analysis.

Analysis. Juice pH was measured with an Accumet® Model 20 pH/conductivity meter (Fisher Scientific, Atlanta, GA), and Brix was determined by refractometer (American Optical 10430 hand refractometer, Scientific Instruments, Warner Lambert, Keene, NH). Fermentable nitrogen was measured as described by Gump et al. [17]. Titratable acidity was determined as described by Zoecklein et al. [41]. Lactic, malic, and tartaric acids were determined via high-performance liquid chromatography (Hewlett Packard Model 1100, Palo Alto, CA) using a Bio-Rad Fast Acid™ column (Bio-Rad, Hercules, CA).

For each replicate, three grams of skins were scraped clean of pulp and homogenized in 30 mL of 50% (v/v) ethanol in a Waring Commercial Laboratory™ blender (New Hartford, CT) for 30 sec on high speed. The homogenate was agitated for 1 hr using a Thermolyne RotoMix™ (Barnstead/Thermolyne, Dubuque, IA), then clarified by centrifugation at 3000 x *g* for 30 min prior to analysis. Juice samples were clarified in the same manner.

Total and phenol-free glycosides were estimated as described by Iland et al. [20] and Zoecklein et al. [42], respectively. The phenol-free glycoside (PFGG) assay measures the concentration of glycosides without phenolic functional groups ionizable at pH 10 [37,38,42]. Total phenols, hydroxycinnamates, and ionized anthocyanins were estimated spectrophotometrically (Genesys 5™, Spectronic Instruments Inc., Rochester, NY) as described by Somers and Evans [32]. Hue (A_{420nm}/A_{520nm}) and intensity ($A_{520nm} + A_{420nm}$) were determined spectrophotometrically as described by Zoecklein et al. [41]. Small and large polymeric pigments were estimated spectrophotometrically as described by Adams and Harbertson [2].

Postbottling heat treatment. Six months postfermentation, one 3.75-L lot of wine, produced as described above for ambient controls, was used for a second study. Total sulfur dioxide concentration was adjusted to 25 mg/L, and the wine was sparged with nitrogen to help displace oxygen. Three treatment wine replicates were decanted anaerobically into standard 750-mL screw-cap glass bottles, flushed with CO_2, capped, and stored for 21 days at 42°C. Three 750-mL control samples were treated in the same manner and held at 10°C (Table 1).

Glycoside concentrations, total phenols, hydroxycinnamates, anthocyanins, hue, intensity, and small and large polymeric pigments were determined as described above.

Data analysis. Data were statistically analyzed using the Student's t-test with JMP™ (SAS Institute, Cary, NC) at a significance of $p \leq 0.05$.

Table 1 Summary of fermentation, postfermentation heat treatment, and postbottling heat treatment of Cabernet Sauvignon wine.

	Crush	Cold soak	Fermentation
Fermentation	10°C	7°C for 48 hr	22-23°C
	Heat treatment		**Control**
Postfermentation	Raised 2-3°C/day for 7 days to 42°C, held 1 day		23°C for 7 days
Postbottling	42°C for 21 days		10°C for 21 days

Results

Cold soak and fermentation. A summary of the processing and postfermentation heat-treatment temperature and duration is provided in Table 1. Mean total glycoside (TGG) concentration increased 194% in juice after two days of 7°C cold soak (Figure 1). Skin TGG and phenol-free glycoside (PFGG) concentrations also rose through the prefermentation maceration period (Figure 2). Total glycoside concentrations in wines continued to rise during fermentation (Figure 1), while skin concentrations dropped (Figure 2). At crush and after cold soak, phenol-free glycosides comprised 50% of total concentration in wines, but dropped to 18% by the completion of fermentation (Figure 1).

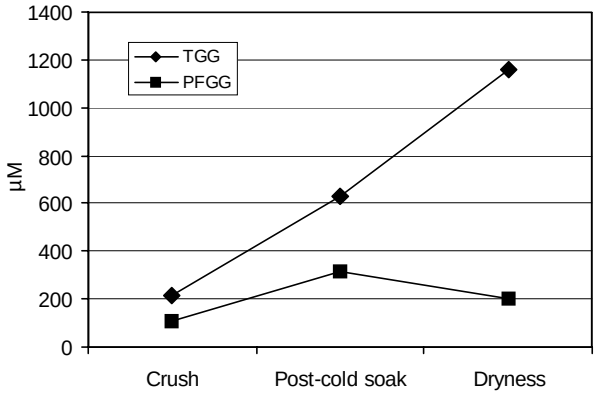

Figure 1 Total (TGG) and phenol-free (PFGG) glycosides (µM) in Cabernet Sauvignon wine during processing. (Values represent the means of replications; n = 8.)

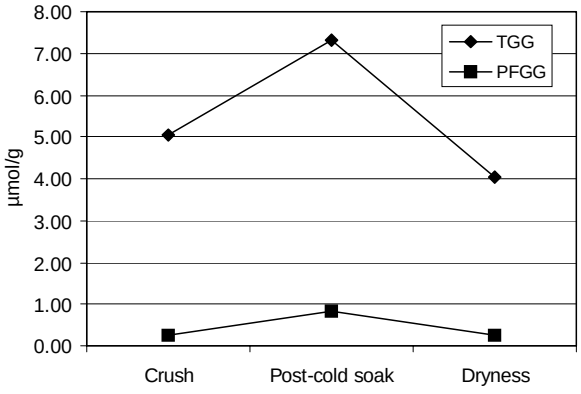

Figure 2 Total (TGG) and phenol-free (PFGG) glycosides (µmol/g) in Cabernet Sauvignon skins during processing. (Values represent the means of replications; n = 8.)

Pre-dejuicing heat treatment. Heat treatment was begun at dryness. Total glycoside concentration in treatment and control wines decreased (15 and 5%, respectively) from dryness until cessation of heat treatment (Figure 3). After pressing, treatment and control wines showed increased total glycoside concentrations (4 and 6%, respectively).

While total glycosides were higher in treatment wines than in ambient controls, treatment skin glycosides were lower than control skins (Figures 3, 4). Total glycoside concentrations in the treatment skins were 4% lower than in control ambient-aged skins when treatment commenced, but dropped to 16% lower than controls by the end of heat treatment (Figure 4).

Treatment and control wines had different PFGG concentrations at dryness (Figure 5). Following heat treatment, PFGG concentration remained 16% higher than control. Phenol-free glycoside concentrations in treatment and control skins were not significantly different at any time (Figure 6). The proportion of phenol-free to total glycosides was similar for control and treatment wines, rising 18 to 20% during ambient postfermentation maceration and 19 to 21% in heat-treated wines. Treatment wines had 208% more large polymeric pigments, and 41% more small polymeric pigments than did control wines (Figure 7). Heat treatment increased color intensity and hydroxy-cinnamates, but had no effect on hue, ionized anthocyanins, or total phenols (Table 2).

Postbottling heat treatment. TGG and PFGG concentrations of heat-treated wines were 15 and 16% lower, respectively, than

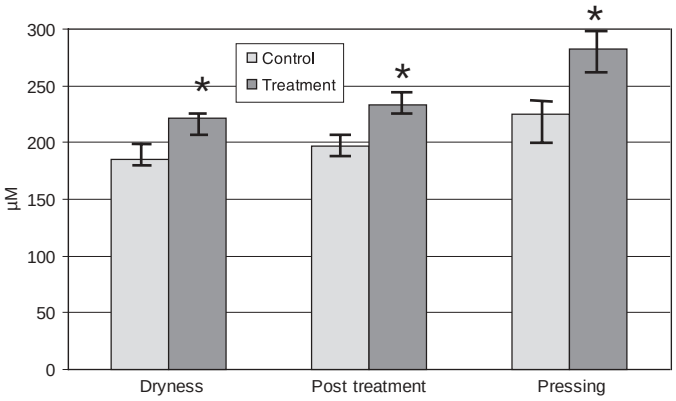

Figure 5 Phenol-free glycosides (µM) in Cabernet Sauvignon wine at dryness, post-heat treatment, and postpressing. (*Indicates significance at $p \leq 0.05$. Values represent the means of replications; n = 4.)

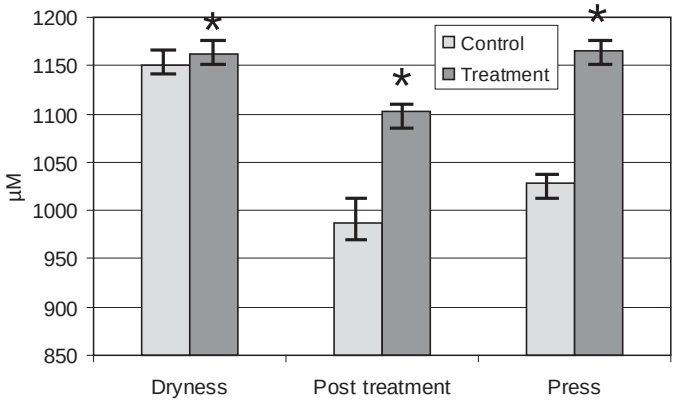

Figure 3 Total glycosides (µM) in Cabernet Sauvignon wine at dryness, post-heat treatment, and postpressing. (*Indicates significance at $p \leq 0.05$. Values represent the means of replications; n = 4.)

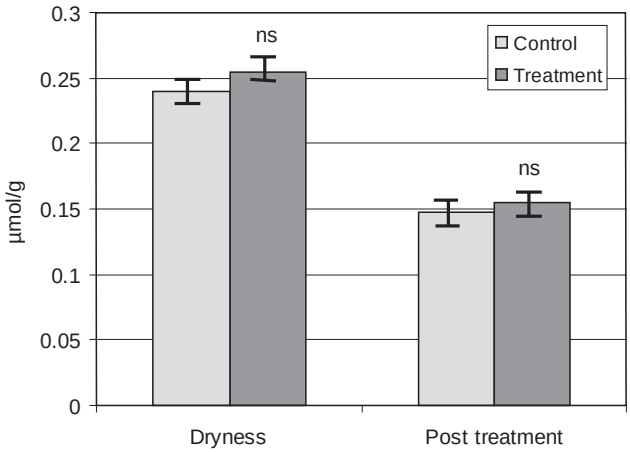

Figure 6 Phenol-free glycosides (µmol/g) in Cabernet Sauvignon skins pre- and post-heat treatment. (ns: indicates no significance at $p \leq 0.05$. Values represent the means of replications; n = 4.)

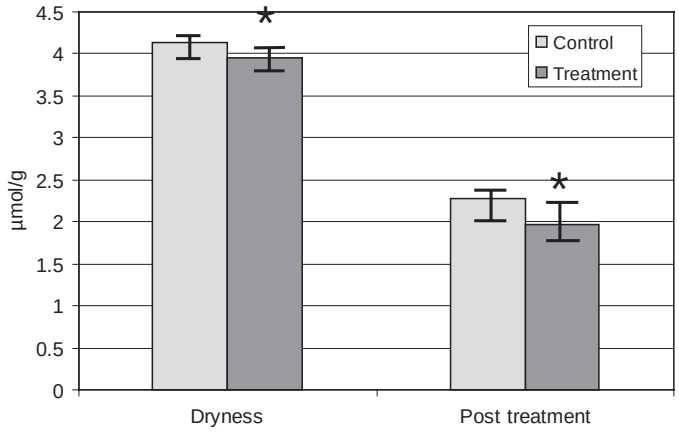

Figure 4 Total glycosides (µmol/g) in Cabernet Sauvignon skins pre- and post-heat treatment. (*Indicates significance at $p \leq 0.05$. Values represent the means of replications; n = 4.)

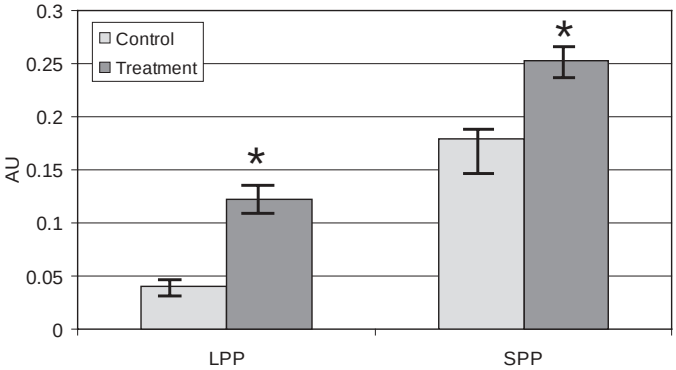

Figure 7 Large (LPP) and small polymeric pigments (SPP) in Cabernet Sauvignon wine post-heat treatment. (*Indicates significance at $p \leq 0.05$. Values represent the means of replications; n = 4.)

Table 2 Spectral analysis of Cabernet Sauvignon wines pre- and post-thermal treatment.

		Treatment wine		Control wine	
		Pre	Post	Pre	Post
Intensity	$A_{420\ nm} + A_{520\ nm}$	2.07 a[a]	2.20 a	2.25 a	1.92 b
Hue	$A_{420\ nm} / A_{520\ nm}$	0.51 b	0.63 a	0.49 c	0.55 a
Total phenols	$A_{280\ nm}$	1.02 b	2.70 a	1.10 b	2.96 a
Ionized anthocyanins	$20 * A_{520\ nm}$	167 a	115 b	168 a	125 b
Total hydroxycinnamates	$A_{320\ nm} - 1.4$	1.95 b	2.31 a	1.98 b	1.92 b

[a]Different letters within rows of each column indicate significance of t-test of treatment means at $p \leq 0.05$; n = 4.

in control wines (Figure 8). Phenol-free glycosides accounted for 52% of total concentration in both control and treatment wines.

Hue increased by 25%, intensity decreased by 4%, and no significant differences were seen in total anthocyanins, total phenols, or total hydroxycinnamates between control and treatment wines (data not shown). Large polymeric pigments were 62% higher in treatment wines, and no significant difference was seen in small polymeric pigments (Figure 9).

Discussion

Cold soak and fermentation. The 194% increase in total glycoside (TGG) concentration in juice after two days of 7°C cold soak (Figure 1) is consistent with McMahon et al. [25], who reported a 103% increase after three days at 10°C. The degree of fruit maturity and berry breakage, along with the hydrolysis of complex macromolecular precursors as suggested by Williams et al. [38], may have influenced glycosidic extraction. Phenol-free glycoside (PFGG) concentration in the juice rose 200% from crush to cold soak (Figure1), consistent with previous research [25].

Skin TGG and PFGG concentrations rose throughout prefermentation maceration (Figure 2). In Cabernet Sauvignon, glycosidically bound aroma, flavor, and phenolic compounds have been found in highest concentrations in the skins [5,16], located primarily in vacuoles of internal skin cell layers [7,10]. The increase in phenol-free skin glycoside concentration may be the result of the activity of arabinosidase, rhamnosidase, and pectinolytic enzymes, which are capable of degrading macromolecules [37]. The adsorption of vacuole contents is also possible; anthocyanins are adsorbed onto grape solids, for example [32].

The rise in wine TGG during fermentation (Figure 1), coupled with the converse in skin concentration (Figure 2), may signify extraction. In this study, total glycosides rose throughout fermentation, while prior work found Shiraz glycosides remained steady after reaching maximum concentration [37]. Extraction rates may vary due to varietal differences, degree of maturity, and berry breakage.

Wine PFGG concentrations at dryness (Figure 1) were lower than at the completion of cold soak, consistent with previous studies [25,37]. A drop in the ratio of wine PFGG to TGG, from 50 to 18%, occurred by the end of fermentation (Figure 1), consistent with previous research [25]. The increased extraction of phenolic compounds from skins, described above, may have caused the decrease in phenol-free proportion by the end of fermentation.

Pre-dejuicing heat treatment. The overall decrease in TGG concentrations in all wine from dryness until cessation of heat treatment (Figure 3) may have resulted from a combination of factors, including adsorption, precipitation, and hydrolytic activity. Lebert [21] reported adsorption by lees, and variations in adsorption rate have been recorded among yeast strains [24]. Bourzeix et al. [6] and Somers and Evans [32] demonstrated that anthocyanin glycosides may be adsorbed by yeast lees and/or precipitate following heat treatment. In addition, some limited glycoside hydrolysis may have resulted from endogenous enzyme activity. Treatment temperature of 42°C approached the optimum temperature for *S. cerevisiae* β-glucosidase activity (50°C) [9]. Increased must temperature results in increased ex-

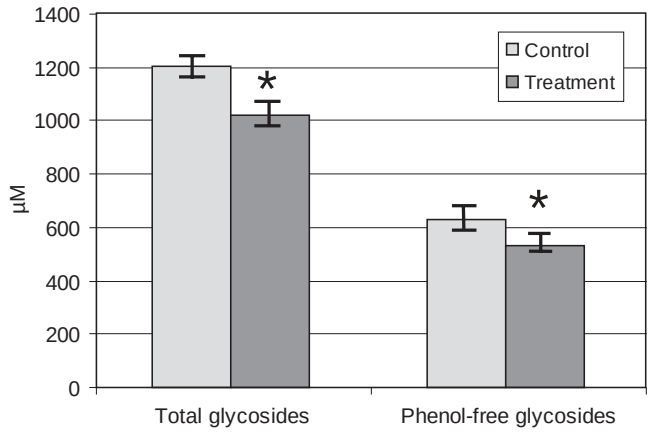

Figure 8 Total and phenol-free glycosides (µM) in postbottling heat-treated Cabernet Sauvignon. (*Indicates significance at $p \leq 0.05$. Values represent the means of replications; n = 4.)

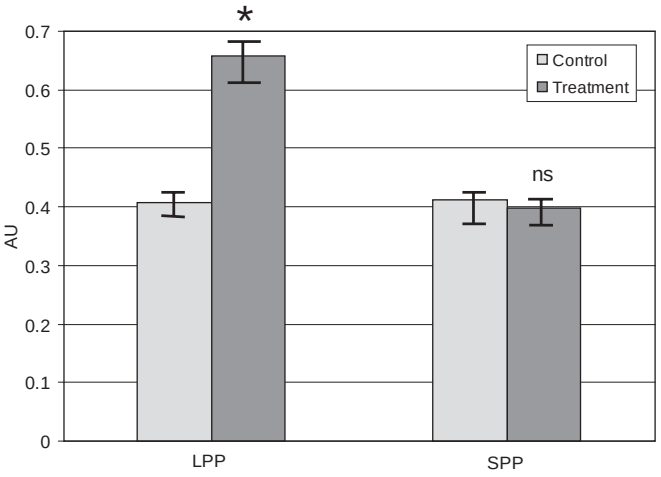

Figure 9 Large (LPP) and small polymeric pigments (SPP) before and after postbottling heat treatment of Cabernet Sauvignon. (*Indicates significance at $p \leq 0.05$. ns: indicates no significance at $p \leq 0.05$. Values represent the means of replications; n = 4.)

traction of grape constituents into wine [8,15,27,32]. Higher glycoside concentrations in treatment wines may have resulted from increased extraction as a result of contributions from the skins, as indicated by the decrease in total skin glycosides. The increased TGG concentration in both treatment and control wines after pressing may have been caused by the physical release of glycosides due to cell rupture in skins and pulp.

Heat treatment pre-dejuicing lowered the ionized anthocyanins in wines (-32%) more than ambient aging (-25%) (Table 2). Hue increased 20% in treatment wines, compared to 11% in control wines. Treatment increased total hydroxycinnamates (16%), but did not change total phenols (Table 2). Anthocyanins have been shown to be responsible for 67% of the total grape glycosides in Cabernet Sauvignon wines [25]. Young red wine hue (A_{420nm}/A_{520nm}) is influenced by the equilibrium between colored and colorless anthocyanin forms [5,22]; the shift in absorbance maximum from 520 to 420 nm may be a result of enhanced polymerization with noncolored phenols [26,33]. HPLC analysis of the organic acids confirmed that wines of the study did not undergo malolactic fermentation (data not shown).

The method [2] used to determine the extent of pigment polymerization in this study divides pigments into three classes based upon bisulfite bleaching and protein precipitation. Large polymeric pigments (LPP), which are precipitated by protein and not bleached, increased by 208% as a result of postfermentation heat treatment. Adams et al. [3] reported, and we have observed, that this class of pigments can be impacted by winemaking. Small polymeric pigments (SPP), not precipitated or bleached, increased by 41%. In a static environment, monomeric anthocyanins would be expected to decline as a result of incorporation into LPP and SPPs. In this study, monomeric anthocyanins increased by 30% following heat treatment. This may represent heat-induced extraction, and parallels the increase in wine glycosides and the decline in skin glycoside concentration. The method used underestimates the contribution of the monomeric anthocyanins to the overall color because absorption is measured at pH 4.9 [3]. The increased polymerization, demonstrated by the increased LPP, may indicate changes in phenol structure similar to those found in aged wines [26,30].

The results of this study support previous findings. For example, heating Pinot noir for two days at 40°C post-cold maceration resulted in a 22% increase in wine color intensity, a 15% increase in anthocyanins, and a 16% increase in polyphenols [14]. Zimman et al. [40] found that increasing pomace temperature increased the concentration of proanthocyanidins and copigmented color, consistent with the current study. Such polymerization may result in long-term color stability [29,30] and may alter sensory characteristics [4].

Postbottling heat treatment. TGG and PFGG concentrations of heat-treated wines were lower than those of control wines (Figure 8). Zoecklein et al. [42] found Riesling wines held anaerobically for 20 days at 45°C averaged a 32.9% decrease in glycoside concentrations, suggesting hydrolysis. PFGG accounted for 52% of the total concentration in both control and treatment wines, suggesting release of phenolic and nonphenolic aglycones occurs equally.

The differences found in hue (25%), intensity (-4%), and lack of difference in total anthocyanins, total phenols, and total hydroxycinnamates are contrary to data reported by Somers and Evans [32], who noted a 75% increase in hue, 50% decrease in intensity, and 30% decrease in both anthocyanins and total phenols following heat treatment. Adams et al. [3] found that large polymeric pigments accounted for 37% of color in Syrah wine. In this study, large polymeric pigments were 62% higher in treatment wines, and no significant difference was seen in small polymeric pigments (Figure 9).

Conclusions

This study explored the impact of cold soaking, fermentation, and heat treatment of wine pre-dejuicing and postbottling on glycosides and glycoside fractions. Since glycosides are, in part, important in aroma, flavor, color, and structure, this data may lead to an enhanced understanding of the effect of processing parameters on potential wine quality. Pre-dejuicing heat treatment has the potential to extract, hydrolyze, and impact glycoside polymerization, while the postbottling treatment can affect hydrolysis and polymerization. Pre-dejuicing thermal treatment increased the extraction of total and phenol-free glycosides, but not equally. This processing method, together with postbottling heat treatment, increased the development of large polymeric pigments.

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