Effects of Skin Contact Temperature on Chardonnay Must and Wine Composition

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Chardonnay grapes from a single vineyard block were held after crushing for 20 to 25 hours at four different temperatures between 9°C and 30°C, pressed, and vinified separately but identically. Must samples taken at five-hour intervals showed greatly increased phenolic extraction rates at higher temperatures, particularly of the flavonoid fraction. Wine results were similar, showing increased phenolic content in warm-skin-contact wines. Temperature control below 15°C minimized wine protein levels and bentonite requirements. Elevated maceration temperatures produced wines of deeper color, increased oxidative sensitivity, and coarser character which matured more rapidly during barrel aging.

Skin contact (maceration) of crushed white grapes prior to pressing has become standard procedure in many white wine-producing areas, generally out of a belief that flavor components are being extracted from the skins. Often there is little choice, as grapes arriving at the winery are crushed but must wait to be processed in a batch press. Field-crushed grapes receive skin contact during transport to the winery for pressing. Commonly, length of time on skins has been the sole variable considered, both in wine production and in research; the question of the temperature at which skin contact took place has generally been overlooked. However, the chemical changes which occur in grape juice during maceration on the skins might be expected at the very least to change their rates with temperature, and possibly the extraction, hydrolysis, evaporation, oxidation, or other reactions of various grape constituents may be affected in different fashions by temperature changes.

Most investigations of skin contact have focused on differences occurring over time at a constant temperature (1,15,16,17,18,23,26,27).

Du Plessis *et al.* (5,6) reported that seeds, stems, and skins provided equal quantities of extractives during maceration at cooler temperatures, but that at warmer temperatures the skins provided more. Ough and Berg (19) found that increased temperature resulted in more deeply colored white wines, higher pH, and higher levels of potassium, proline, and total phenols. Pallotta and Cantarelli (20) reported increases in catechins of 160%, leucoanthocyanins 58%, tannins 31%, and total polyphenols 42% for a temperature increase from 5°C to 15°C over 72 hours. Bertrand (2) followed aromatic differences in wine produced by settling white grape juice at 0°C versus 22°C. He found increases in higher alcohols at warmer settling temperatures, but decreases in yeast-produced esters. Lateyron (10) examined compositional differences in Chardonnay must and wine produced following skin contact for six hours at 15.5°C, 20°C, and 24°C. She found lower total acidity, higher pH, higher total nitrogen, deeper color, and higher phenolic levels with increased skin contact temperature.

This research was undertaken to examine differences in white musts and wines which would arise due to different holding temperatures during a standard period of skin contact. In particular, rates of phenolic appearance in must were to be compared.

Materials and Methods

Design: During the 1981 vintage, 35 tons of Mendocino County Chardonnay were hand harvested and transported to Simi Winery, Healdsburg, California, for crushing. Half of the grapes were picked and crushed in the morning at a must temperature of 15.5°C; the other half were picked and crushed that afternoon at a must temperature of 28.6°C. All must was pumped through a 9.5 m^2 tube-in-tube heat exchanger, with only half of the morning and half of the afternoon must being chilled. This resulted in four must lots: 9.7°C, 15.5°C, 23.0°C, and 28.6°C at the tank tops, where must samples were taken. No sulfur dioxide was added prior to fermentation. Average harvest data were 23.9°Brix, 8.42 g/L titratable acidity (as tartaric), pH 3.46, and 4.34 g/L malate.

The four lots were separated into glycol-jacketed drain tanks. One-liter juice samples were collected through a sieve at the tank tops at five-hour intervals and were frozen for later analyses. After 20 to 25 hours of skin contact, each lot was drained and pressed separately, the drain and press juice being combined for each temperature variation. The temperatures of the juice lots immediately after pressing were taken to be the average skin contact temperature for the four wines: 9°C, 15°C, 19.5°C, and 27°C. The 27°C treatment showed signs of spontaneous fermentation at this point. The juice was then cooled to 10°C and settled overnight to less than 0.5% solids by volume before racking to separate stainless steel fermenters. The juice was acidulated with tartaric acid to 9.0 g/L, inoculated with 240 mg/L dried Champagne yeast (*Saccharomyces bayanus*, UCD #595), and fermented to dryness at 11°C to 14°C. After fermentation, samples of the wines were pad filtered into 750-mL cork-finish bottles for analysis. The remainder of the lots were racked to 225-L French oak barrels for standard commercial aging.

Analyses: Must volatiles were analyzed as follows. Thawed juice samples were centrifuged at 2500 rpm for

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five minutes to eliminate grape solids. To 400 mL clarified juice, 5 mL of a 2% by volume ethanol-water solution containing 1.6 mg/L 1-decanol were added as an internal standard. The juice was placed in a 500-mL conical flask and extracted five times with dichloro-methane under magnetic agitation. Solvent volume was 20 mL for the first extraction and 10 mL for the succeeding four. Agitation time was five minutes for each extraction. After each extraction, the original phase was separated by centrifuging five minutes at 2500 rpm, and all five extracts were combined. The solvent was then evaporated under nitrogen to a final extract volume of 0.5 mL. For each must sample, two series of extractions were performed.

Must volatiles were separated gas-chromatographically under the following conditions: column - glass capillary (0.25 mm × 25 m) FFAP stationary phase (equivalent to Supelco SP1000, a modified Carbowax 20M material which gives a moderately polar phase); gas - nitrogen, 0.8 mL/min; temperature program - 40°C to 180°C at 2°C/min; detector - flame ionization. Compounds were identified by comparison of retention times with pure substances and confirmed by GC-mass spectrometry.

Wine volatiles were quantified gas-chromatographically as described by Bertrand (3).

Must and wine total phenols, flavonoid phenols, and nonflavonoid phenols were determined by the methods of Singleton, with due attention to correction of interference by sugar (22).

Wine amino acids were determined gas-chromatographically according to the method of Lhuguenot *et al.* (13).

Wine protein was determined spectrophotometrically utilizing Coomassie Brilliant Blue G-250 (4).

Individual wine phenols were determined as follows. A 100-mL sample of wine was concentrated in a rotary evaporator to 25 mL at 40°C. The concentrate was adjusted to pH 7 by addition of NaOH to drive the acid phenols to their ionized (salt) forms, and the sample was extracted three times with ethyl acetate. This extract of non-acid phenols was then evaporated to dryness in a rotary evaporator and redissolved with 5 mL methanol. The previously extracted wine sample containing the phenolic acid anions was then acidified to pH 2 with hydrochloric acid and extracted three more times with ethyl acetate. This extract was dried as before and redissolved in 5 mL methanol. These two extracts were analyzed by high performance liquid chromatography according to the method of Lea (11).

Results and Discussion

Must analyses. *Volatiles:* The dichloromethane extraction resulted in quantification of 24 volatiles from the musts, of which seven were identified. These were primarily six-carbon alcohols, which are generally herbaceous in character. Their changes in concentration with time at the different temperatures are shown in Figures 1 through 5. Benzyl alcohol (Fig. 1), which has a characteristic pleasant, fruity odor (7), initially increased more

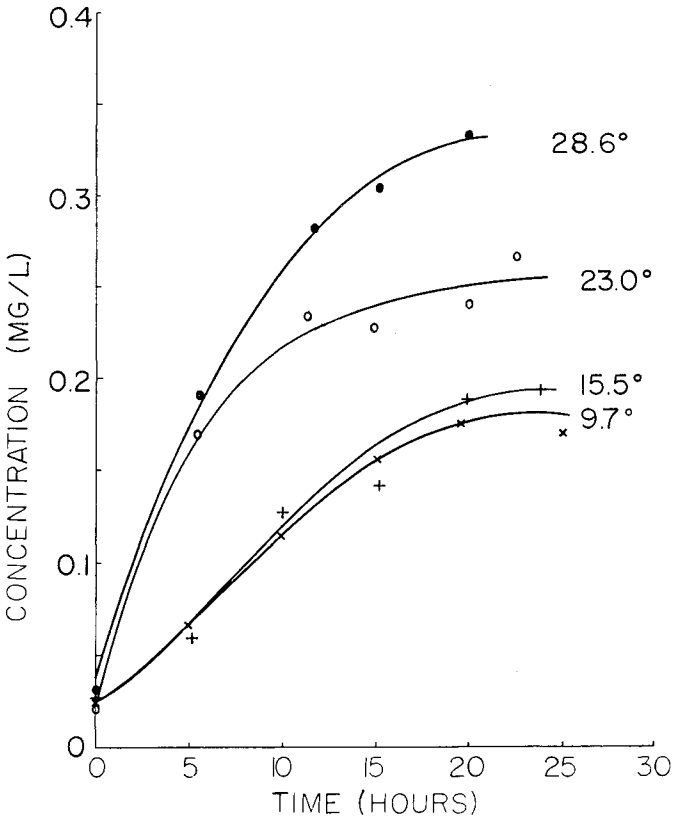

Fig. 1. Benzyl alcohol concentration in Chardonnay must during skin contact.

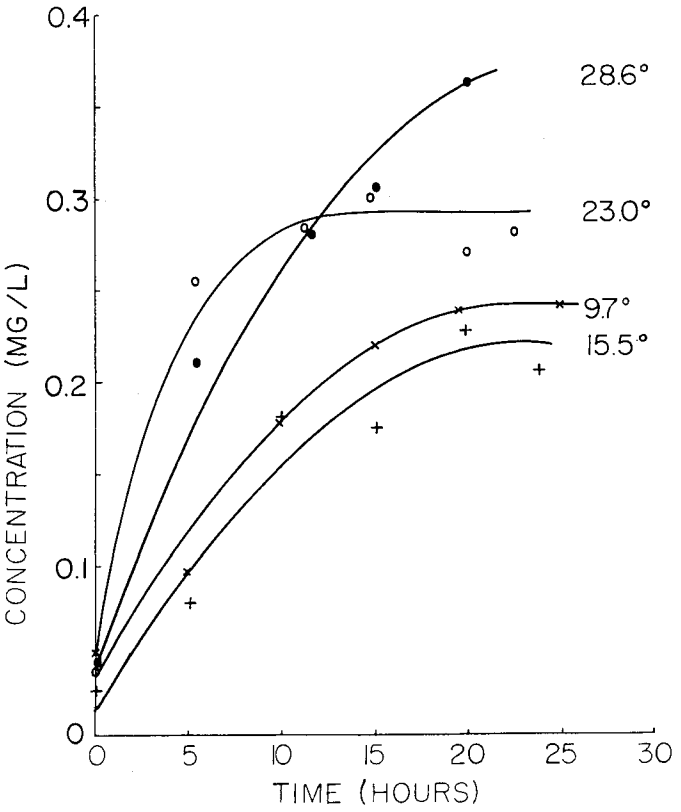

Fig. 2. 2-Phenyl ethanol concentration in Chardonnay must during skin contact.

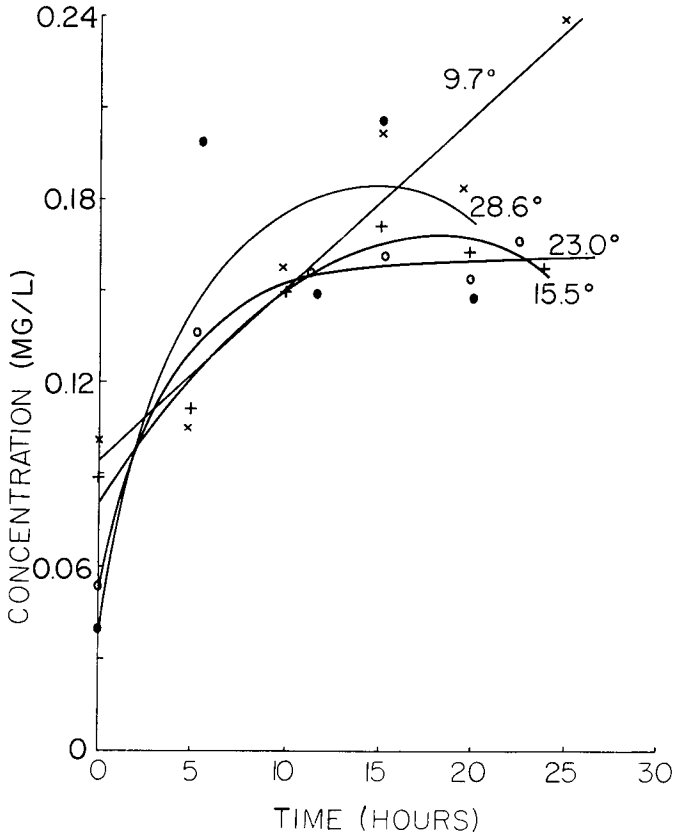

Fig. 3. *Cis*-3-hexen-1-ol concentration in Chardonnay must during skin contact.

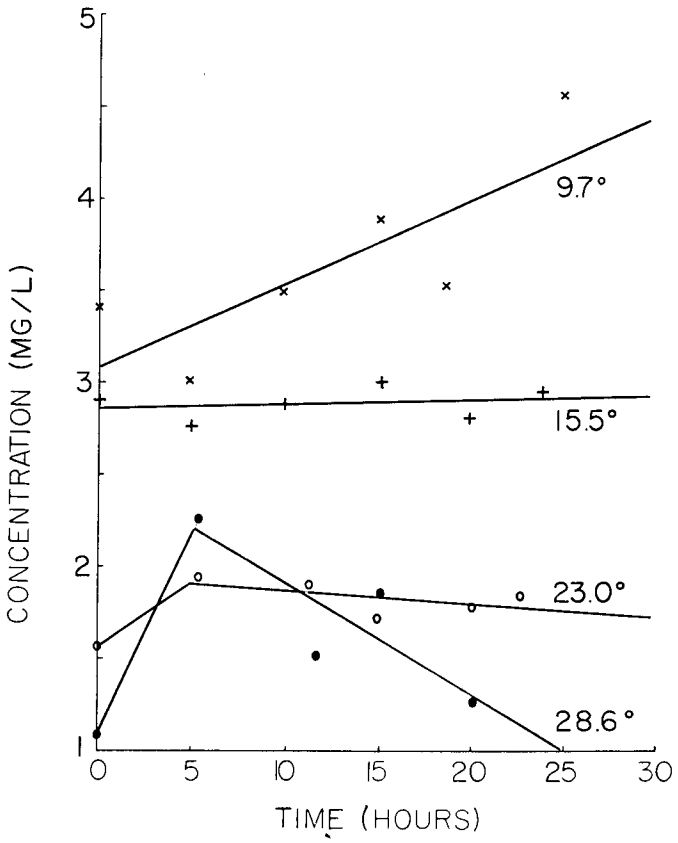

Fig. 4. Hexanol concentration in Chardonnay must during skin contact.

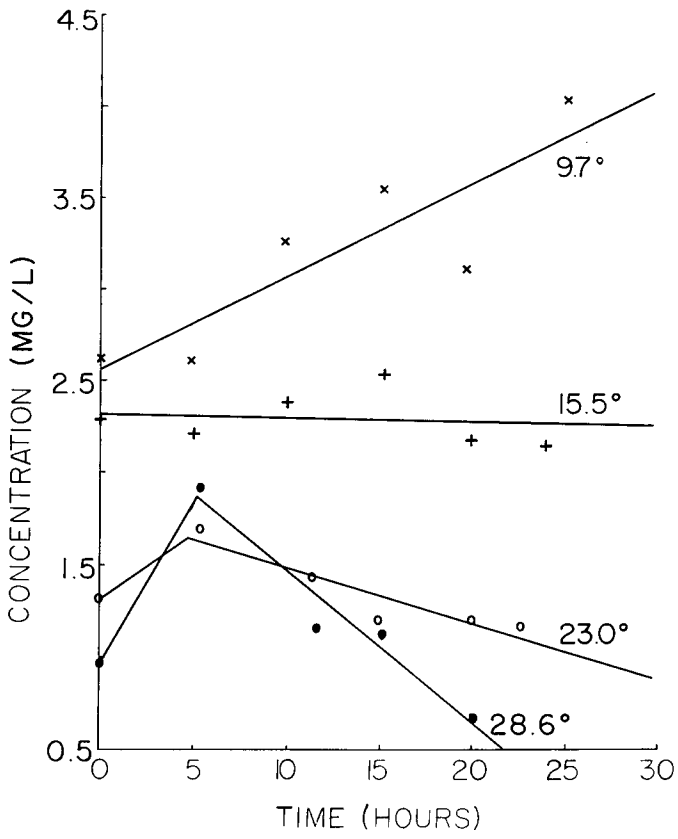

Fig. 5. *Trans*-2-hexen-1-ol concentration in Chardonnay must during skin contact.

rapidly at higher temperatures, as might be expected, and achieved a higher final concentration. 2-Phenyl ethanol (Fig. 2) has a floral, rose-like aroma, and it also showed faster initial extraction rates and higher final concentration at higher temperatures. *Cis*-3-hexen-1-ol (Fig. 3) possesses an herbaceous, leafy odor (its common name is leaf alcohol), and displayed the same increase in initial extraction rate at higher temperatures. It reached a maximum concentration, however, after 10 to 15 hours at the three warmer temperatures, while in the coolest lot, the concentration continued to increase throughout the skin contact period. Hexanol (Fig. 4), which smells only slightly herbaceous, and *trans*-2-hexen-1-ol (Fig. 5), smelling leafy, green, and fruity, both displayed this behavior in even more pronounced fashion. They decreased markedly in concentration after five hours at 23°C or greater, while the coolest lots continued to show increases. These six-carbon compounds related to leafy or herbaceous aromas develop during skin contact, particularly at cooler temperatures, but with commencement of yeast activity would be rapidly converted to the less odiferous *n*-hexanol (8). For the volatiles identified, however, increases in must temperature did not result in uniformly higher levels of aromatic constituents.

Phenols: Nonflavonoid phenolic behavior is graphed in Figure 6. Although this fraction is believed to exist principally in grape juice rather than grape skins (9), it appears that there is some increase in nonflavonoid phenols with skin contact, and more so at higher temperatures.

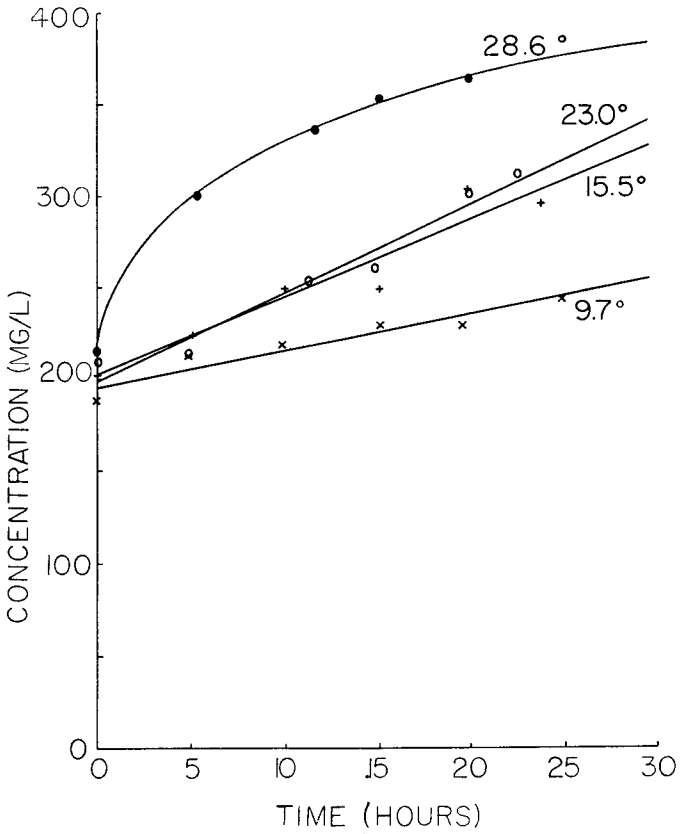

Fig. 6. Nonflavonoid phenol concentration in Chardonnay must during skin contact.

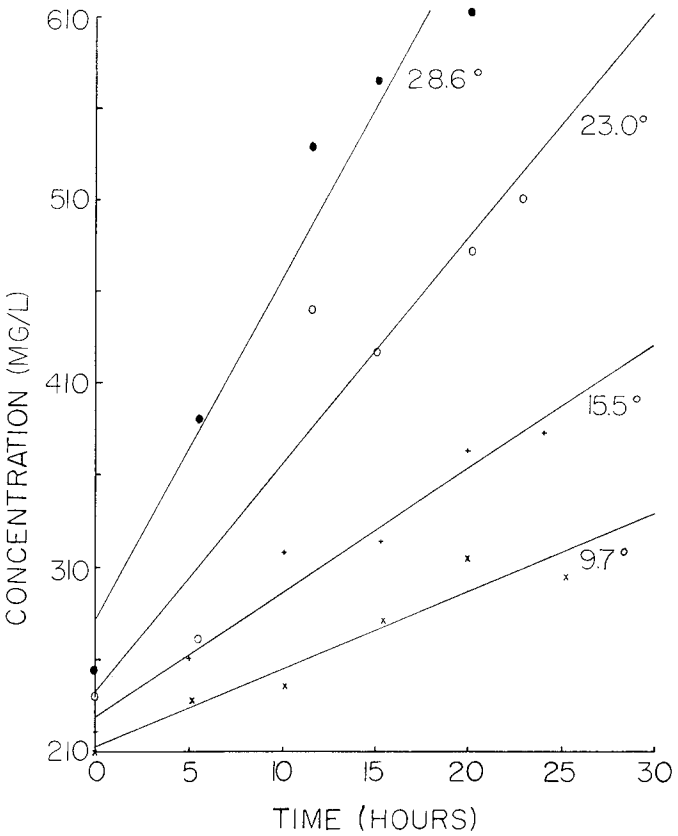

Fig. 8. Total phenol concentration in Chardonnay must during skin contact.

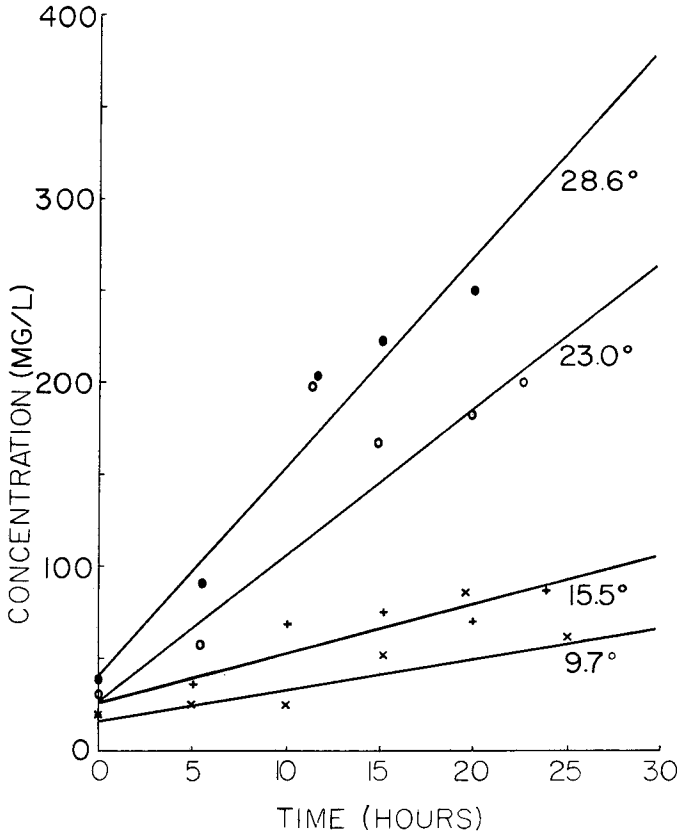

Fig. 7. Flavonoid phenol concentration in Chardonnay must during skin contact.

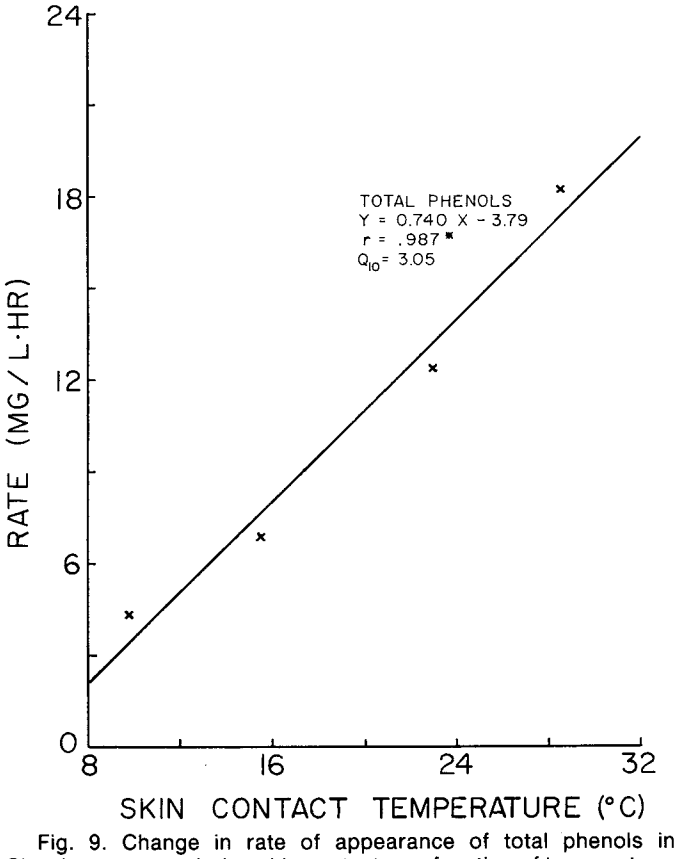

Fig. 9. Change in rate of appearance of total phenols in Chardonnay must during skin contact as a function of temperature.
* = Significant correlation at 95% level.

Flavonoid phenolic behavior is shown in Figure 7; there is a strong temperature dependency. Flavonoid phenols, located principally in grape skins and seeds, contribute bitterness and astringency to white wines, as well as color, turning generally from colorless to yellow to gold to brown as they oxidize. Their content in a white wine can be one of the most significant factors affecting the flavor of that wine (25), with higher levels producing a heavier-bodied wine. In this particular must, as many flavonoids were present after two hours skin contact at 28.6°C as after 25 hours at 9.7°C. It is apparent that flavonoid levels may be dramatically limited by controlling temperature during skin contact to 10°C or below.

Total phenols are graphed in Figure 8. Being the sum of the flavonoid and nonflavonoid fractions, the same behavior is exhibited: strong temperature dependency, and again, equivalent levels after two hours at 28.6°C or 25 hours at 9.7°C.

Changes in rate of phenolic appearance - must: By taking the slope of each curve in Figure 8, a rate constant may be obtained for the appearance of total phenols. This may be taken as an empirical constant for a given compound or class of compounds at a given temperature. If these rate constants are plotted against skin contact temperature, the role that temperature plays in changing rate of appearance may be shown (Fig. 9). Appearance rates of total phenols were positively correlated ($p = 0.05$) with skin contact temperature. Q_{10} is 3.05, based on a temperature shift of 10°C to 20°C, which means that for the 10°C increase, the rate of appearance of total phenols in the must more than tripled.

Wine analyses. *Volatiles:* Wine volatile data are

Table 1. Wine volatiles (mg/L except glycerol, g/L).

Compound	Must temperature 9°C	15°C	19.5°C	27°C
	Must temperature			
Methanol	49	58	67	81
1-Propanol	33	35	29	28
Isobutyl alcohol	47	46	40	26
Active amyl alcohol	43	44	38	25
Isoamyl alcohol	172	163	142	113
Hexanol	4.04	3.35	2.15	2.95
2-Phenyl ethanol	36.7	32.5	31.0	13.1
Glycerol (g/L)	6.4	7.6	6.1	6.3
2,3-Butanediol	579	633	530	579
Ethyl acetate	136	144	150	73
Isoamyl acetate	7.1	8.6	7.6	4.9
Hexyl acetate	0.80	0.83	0.50	0.30
2-Phenyl ethyl acetate	0.99	0.93	1.0	0.18
Ethyl hexanoate	1.24	1.4	1.44	1.41
Ethyl actanoate	1.0	1.45	1.37	1.48
Ethyl decanoate	0.16	0.37	0.32	0.17
Diethyl succinate	0.41	0.33	0.29	0.38
Isobutyric acid	0.77	1.18	1.41	0.69
Butyric acid	1.64	1.39	1.54	2.07
Isovaleric acid	0.58	0.98	1.09	0.60
Hexanoic acid	4.71	4.54	4.62	5.24
Octanoic acid	4.91	5.01	5.80	6.09
Decanoic acid	0.56	1.10	1.20	0.59
Dodecanoic acid	0.29	0.11	0.18	0.24

Results are the mean of triplicate analyses of the same sample.

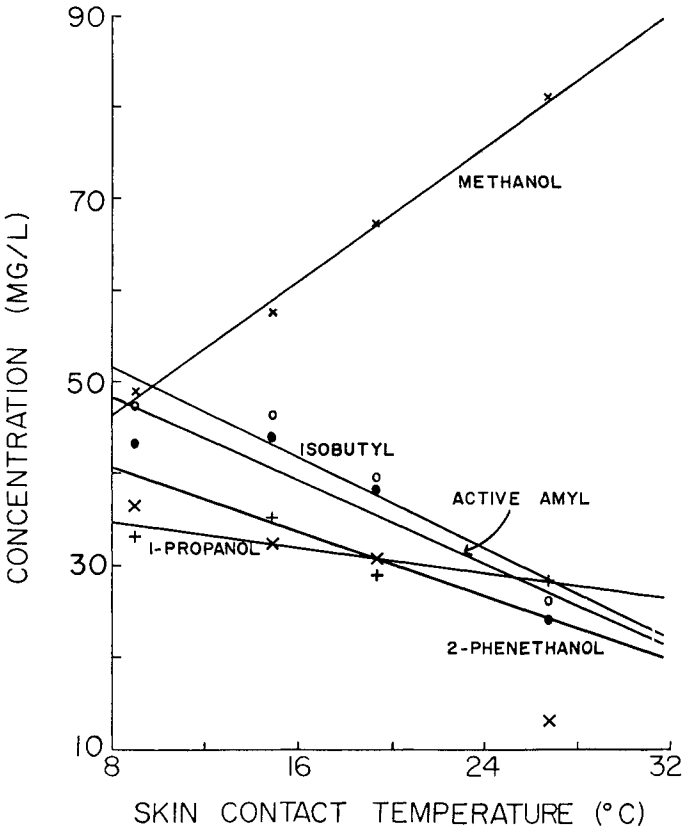

Fig. 10. Concentration of various alcohols in Chardonnay wine as a function of skin contact temperature.

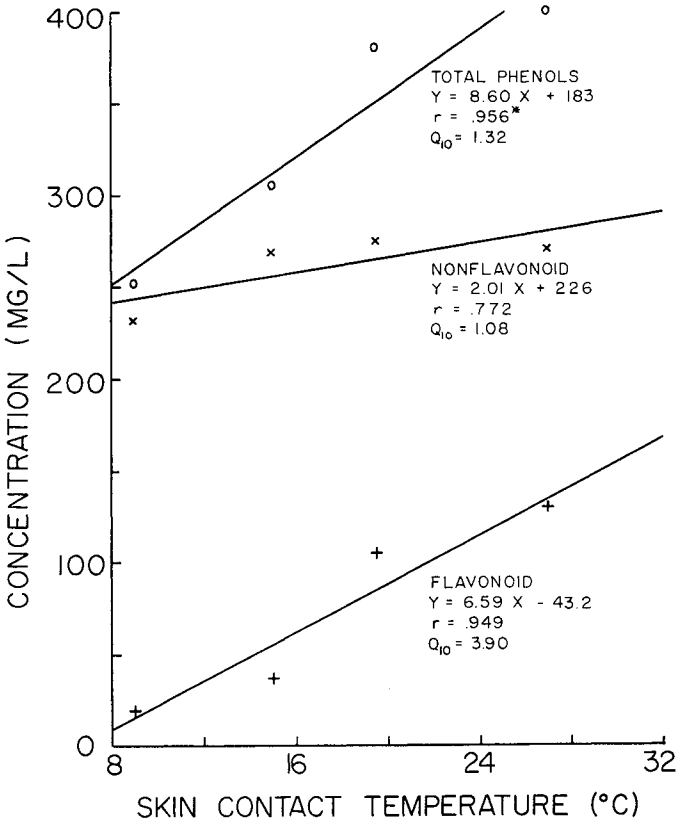

Fig. 11. Flavonoid, nonflavonoid, and total phenol concentration in Chardonnay wine as a function of skin contact temperature. * = Significant correlation at 95% level.

given in Table 1. These are principally yeast-produced compounds rather than grape components, but would be expected to vary according to initial must composition. Volatile esters showed mixed behavior, generally increasing from 9°C to 15°C, then leveling off or decreasing. Lower molecular weight fatty acids (four-, six-, and eight-carbon) increased slightly with temperature and were the only class of wine volatiles to do so. Decanoic, isobutyric, and isovaleric acids increased almost 100% from 9°C to 20°C, then dropped sharply at 28°C. Wine alcohols are graphed in Figure 10. The four higher alcohols decreased at higher maceration temperatures, while methanol displayed a quite linear increase. This is undoubtedly due to increased activity of natural pectin methylesterase at higher temperatures. As with must volatiles, there is no clear trend linking wine volatile concentrations with skin contact temperature.

Phenols: Changes in wine phenols with skin contact temperature are graphed in Figure 11. As with the musts, the nonflavonoid fraction showed only a slight increase, while total phenols increased sharply with temperature. Most of this derives from the flavonoid fraction, which rose from 20 mg/L to 129 mg/L over the 9°C to 28°C range. Q_{10} for 10°C to 20°C is 3.90 for flavonoid phenols, a 290% increase.

Individual nonflavonoid data for the wines are shown in Table 2. The results support the combined nonflavonoid analyses (Folin-Ciocalteu) in showing a slight increase with temperature. The whole of the increase occurred between 9°C and 15°C for all nonflavonoids except monocaffeoyl tartaric acid, for which the entire increase occurred between 15°C and 19.5°C. Analytical recovery of some of the compounds may have been reduced by must oxidation. This would have been most pronounced in the warmer, higher pH musts due to faster rates of enzymatic oxidation; yet those musts still contained higher phenolic levels. The agreement between the HPLC analyses of individual nonflavonoids and the combined nonflavonoid analysis suggests that while most nonflavonoids may exist in grape juice rather than in skins, seeds, or pulp (9), a small amount is contributed by extraction or degradation from either seeds or skins at warmer maceration temperatures. This could contribute harshness to such wines (25).

Table 2. Nonflavonoid phenols - wine (mg/L)[a].

Compound	9°C	15°C	19.5°C	27°C
Hydroxybenzoic acids				
Syringic	0.10	0.13	0.12	0.18
p-Hydroxybenzoic	0.10	0.33	0.17	0.20
Hydroxycinnamic acids				
p-Coumaric	0.55	0.75	0.73	0.85
Caffeic	1.07	1.53	1.32	1.68
Ferulic	0.13	0.23	0.18	0.27
Monocaffeoyl tartaric	27.1	27.3	51.2	48.8
Other nonflavonoid				
Syringaldehyde	0.10	0.22	0.20	0.38
Tyrosol	1.3	1.2	1.1	0.7
Total nonflavonoid	30.5	31.7	55.0	53.1

[a] Results are the mean of triplicate analyses of the same sample, determined by the method of Lea (11).

Table 3. Flavonoid phenols - wine (mg/L)[a].

	Must temperature			
Compound	9°C	15°C	19.5°C	27°C
Flavan-3-ols				
Catechin	1.90	5.53	14.32	20.1
Epicatechin	0.93	4.73	12.77	17.9
Procyanidins				
A_2	0.1	0.6	0.4	1.0
B_1 or $_5$	3.3	9.0	9.5	9.3
B_1 or $_5$	19.7	27.4	38.7	54.8
B_2	0.75	8.9	6.4	8.2
B_4	3.7	5.7	5.1	7.4
Flavonols				
Myricetin	1.1	2.3	3.1	5.3
Quercetin	0.6	1.6	2.3	3.0
Total Flavonoid	32.1	65.8	92.6	127

[a] Results are the mean of triplicate analyses of the same sample, determined by the method of Lea (11).

The figures for the total of individual nonflavonoid phenols fall far short of the Folin-Ciocalteu analyses (30.5 mg/L compared with 232 mg/L for the 9°C wine). This may be explained by: 1) the difference in analytical method; 2) the multiple interferences that the Folin-Ciocalteu analysis is subject to; 3) possible hydrolytic and oxidative losses during preparation for HPLC; and 4) the small number of specific compounds that were quantified by HPLC (eight) compared with the large number that are measured by the traditional analysis. These have proven to be continuing problems (14).

Individual flavonoid data for the wines are shown in Table 3. Much of the increase with temperature occurs in the B-series of the procyanidin fraction, which are dimers of (−)-epicatechin and (+)-catechin (12), and are related to astringency. Procyanidins B_1 and B_5 could not be distinguished because only a mixture of the two pure substances was available to establish retention times. Catechin and epicatechin display 10-fold and 19-fold increases, respectively, across this temperature range. The flavonoid content of the wines determined colorimetrically (Fig. 11) agreed quite well with the sum of the flavonoids determined by HPLC in the same wines (Table 3). There was an appreciable drop in total flavonoids from must (Fig. 7) compared to wines (Fig. 11). This indicates some precipitation, as expected, into the wine lees by combination of these tannins with the proteins present in the fermenter. This also suggests why the monomers (catechin and epicatechin) appear to increase more than the dimers and larger, since catechins do not precipitate in this fashion.

Color/browning: Figure 12 shows the increase in wine color and browning capacity, the latter as measured by the method of Singleton and Kramling (24). Browning capacity and absorbance at 420 nm has previously been correlated with flavonoid content (21,22) and length of skin contact (26); both browning capacity and color

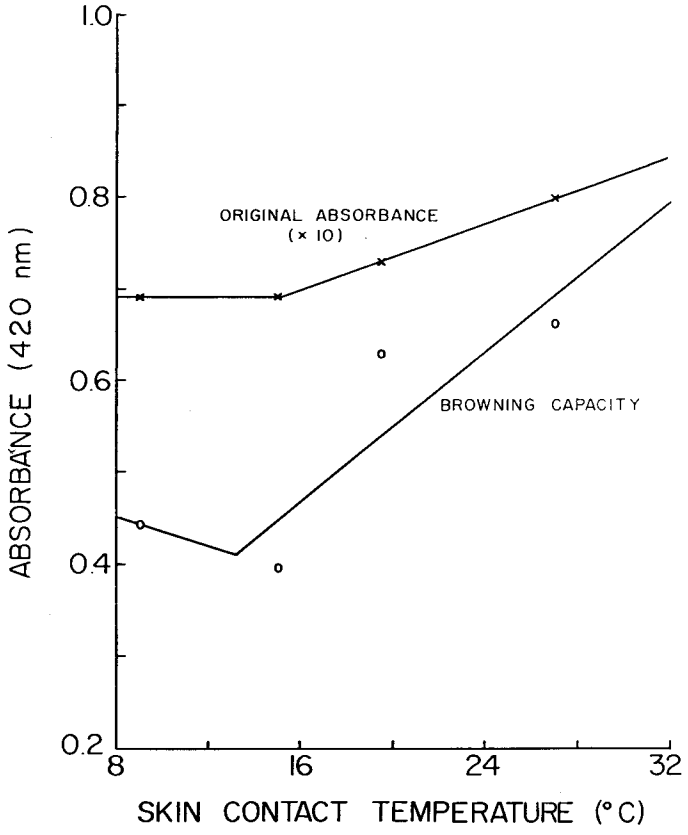

Fig. 12. Absorbance at 420 nm and browning capacity of young (four-month-old) Chardonnay wine as a function of skin contact temperature.

increased with temperature above 15°C. These analyses were performed on young wines. As the wines matured during barrel aging, the color differences became more pronounced, with the 9°C wine being clearly the palest in color and the 19.5°C and 27°C wines a deep gold.

Heat stability: The four wines, 9°C, 15°C, 19.5°C, and 27°C, required 0, 120, 480, and 480 mg/L bentonite, respectively, to achieve commercial heat stability. Heat stability was defined as 1) 24 hours at 38°C, followed by 24 hours at 4°C, without an increase in haze when viewed through a high intensity light, and 2) 24 hours at 60°C, followed by 24 hours at 4°C, with an allowable haze increase but without flocculation. The pH of each of the four wines was 3.33, 3.49, 3.50, and 3.56, respectively, and this would affect the protein stability. The 15°C and 19.5°C wines were very close in pH (3.49 and 3.50), yet the 19.5°C wine required four times more bentonite than did the 15°C wine.

Protein was measured using the Bio-Rad Protein Microassay and found to be 21.9, 31.6, 32.0, and 31.6 mg/L, respectively. The whole of the 50% increase occurred between 9°C and 15°C.

Conclusions

A significant positive correlation exists between skin contact temperature and both flavonoid and total phenols in must and the resulting wine. The higher flavonoid levels produced by warm skin contact resulted in wines which were more deeply colored, had a greater capacity for browning, and displayed a fuller, more coarse character than wines produced with cooler skin contact. Temperature control in the 10°C range was extremely effective in limiting flavonoid extraction.

Volatile components showed mixed behavior. Some compounds increased in concentration with temperature, while others decreased. Skin contact at different temperatures thus did not affect all classes of compounds similarly. Higher phenolic levels provided by warm holding temperatures were not accompanied by uniformly higher concentrations of volatiles in either must or wine.

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