Effect of Steam Treatment of Grape Cluster Stems on the Methoxypyrazine, Phenolic, Acid, and Mineral Content of Red Wines Fermented with Stems

Katsumi Hashizume,* Shigeki Kida, and Takashi Samuta

National Research Institute of Brewing, 3-7-1 Kagamiyama, Higashihiroshima 739-0046, Japan

Steam treatment of Cabernet Sauvignon, Merlot, Pinot noir, and Muscat Bailey A grape cluster stems eliminated >95% of the extractable methoxypyrazines (MPs) from the stems. Steaming of the Cabernet Sauvignon grape stems increased the levels of extractable flavonoid phenolics, the molecular weight distribution and degree of polymerization of which, as estimated by a gel permeation HPLC analysis, were lower than those of nonsteamed stems in a model must solution. Red wines fermented with steamed stems added showed higher absorbance values at 520 nm than those fermented with nonsteamed stems added and also showed lower MP levels than those fermented with nonsteamed stems. The addition of steamed stems increased the level of flavonoid phenolics detectable in the wines. The levels of phosphoric acid (phosphorus), potassium, and calcium were increased as a result of the addition of stems.

Keywords: *Red wine; stem; methoxypyrazine; phenolics; HPLC*

INTRODUCTION

The addition of grape cluster stems to a red wine must provides tannins different from those in the skins and seeds, enriching the astringency and bitterness of the wine, but the addition of stems causes significant color loss and contributes a stemmy flavor to the wine (Boulton et al., 1995b). Consequently, complete stem removal is generally sought before fermentation, but partial or complete addition of stems is occasionally utilized for low-tannin varieties such as Pinot noir in traditional vinifications (Peynaud, 1981; Zoecklein et al., 1995b). Grape stems contribute to higher concentrations of catechins and procyanidins in red wines, and the wine-making procedure employed influences largely the procyanidin content of red wines (Kovac et al., 1992; Ricardo da Silva et al., 1992). Shibasaki et al. (1988) reported that phenolics from Koshu grape stems contribute to the bitterness of the white wine. Thus, stems play a crucial role in the wine-making process; however, few studies have examined the chemical constituents derived from stems in wine.

We recently reported the possibility that 2-methoxy-3-isopropyl- and 2-methoxy-3-isobutylpyrazines (isopropylMP and isobutylMP, respectively) in grape stems might cause the stemmy flavor imparted to wine (Hashizume and Samuta, 1997). The well-balanced methoxypyrazine (MP) aroma provides complexity and varietal character to the wine, but at high levels it becomes overpowering (Allen et al., 1994). It was expected that it might be possible to remove MPs from the stems by some appropriate heat treatment, considering their volatality. Thermal treatment of grape juices and musts is sometimes employed in wine-making, such as high-temperature (80−90 °C) short-time treatments to kill fungi and to denature fungal enzymes or thermovinification (50−60 °C, 10−30 min) to promote extraction of color from those grapes that are poor in pigments (Boulton et al., 1995a), but thermal treatment of grape cluster stems for wine-making has never been studied. Steam treatment is expected to be suitable for this purpose because it is easily applicable to the wine-making process and might be effective for removal of MPs.

It was also expected that steam treatment might facilitate extraction of phenolics from the stems. Therefore, we investigated the effectiveness of steam treatment in the extraction of MPs and phenolics from grape stems in a model must solution. In the course of analysis of the phenolics we applied size exclusion HPLC using an organic solvent for elution and examined the constituents of red wine fermented with stems added to verify the possibility of application of these methods in wine-making.

MATERIALS AND METHODS

Materials. Grapes of *Vitis vinifera* var. Cabernet Sauvignon with a must composition showing Brix = 18.6, titratable acidity as tartaric acid (TA) = 7.7 g/L, and pH 3.48 were obtained from a local vineyard in Kitakoma, Yamanashi prefecture. Grapes of *Vitis vinifera* var. Merlot with a must composition showing Brix = 19.3, TA = 5.0 g/L, and pH 3.72 and var. Pinot noir with a must composition showing Brix = 18.7, TA = 7.1 g/L, and pH 3.68 and grapes of Muscat Bailey A, a hybrid between Bailey (an American species) and Muscat Hamburg (an Oriental−European species), with a must composition showing Brix = 18.6, TA = 5.3 g/L, and pH 3.92 were obtained from our vineyard in Higashihiroshima, Hiroshima prefecture. All grapes were harvested in the autumn of 1996, frozen rapidly, and stored at −85 °C until use.

Polystyrene molecular weight markers were purchased from Toso Co. (Tokyo, Japan). (±)-Catechin, (−)-catechin gallate, and (−)-epicatechin were obtained from Sigma Chemical Co. 3-Hydroxy-4-methoxycinnamic acid was provided by Aldrich Chemical Co. Standard solutions for inductively coupled plasma analysis, chlorogenic acid, gallic acid, 4-hydroxy-3-

* Author to whom correspondence should be addressed (e-mail hasidume@nrib.go.jp; fax +81 824 20 0803).

10.1021/jf9801771 CCC: $15.00
Published on Web 09/30/1998

Table 1. Effect of Steam Treatment on MP Content[a] of Grape Cluster Stems

	nonsteamed				steamed (100 °C, 60 min)			
	isopropylMP		isobutylMP		isopropylMP		isobutylMP	
grape variety	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Cabernet Sauvignon	10.1	16.4	95.2	97.1			3.1	3.4
Merlot	10.4	13.8	101.1	135.5			1.6	3.6
Pinot noir	74.3	82.3	479.5	619.7			11.3	7.8
Muscat Bailey A	3.8	3.5	22.5	20.6			0.5	

[a] Nanograms per kilogram of stem samples.

methoxycinnamic acid, protocatechuic acid, *p*-hydroxyphenethyl alcohol, and tannic acid were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Preparation of Stem Samples. Frozen grape clusters were separated thoroughly into fruit and stems by hand. Steam treatment of the grape stems was performed in a small cage made from stainless steel by means of a steamer at atmospheric pressure (100 °C) or in an autoclave apparatus, before the stems thawed. Stems were chopped into pieces <20 mm in length before the extraction process.

Extraction of Constituents from the Stems. A model must solution was prepared, consisting of 10% (v/v) ethanol and 0.5% (w/v) tartaric acid, with the pH adjusted to 3.5 by addition of KOH. The stems were added in amounts up to 5.0% (w/v) as raw stems, and extraction was performed at 15 °C with mild shaking at 60 rpm. In a series of experiments, each section was started from the same weight of raw stems. After filtration, the extracted solution was analyzed. These extractions were conducted in triplicate.

Fermentation. After potassium bisulfite was scattered on the stem-free grapes (400 g) at a concentration of up to 200 ppm, they were crushed by hand and then placed in a glass vessel. Twenty grams of stems (12 g in the case of Muscat Bailey A, which has a low stem content), was steamed at 100 °C for 60 min and added to the experimental must sample. Nonsteamed stems were added to other experimental must samples. The must was then inoculated with an activated dry wine yeast, V-1116 (Lallemand Inc.). Fermentation was conducted in the presence of the skin at 25 °C for 7 days. The fermented must was pressed on the seventh day and further fermented at room temperature for 10 days. The wine was centrifuged at 17400*g*, and the supernatant was used for the analysis. The fermentation was carried out in triplicate.

MP Determination. MPs were determined according to a previously reported method (Hashizume and Umeda, 1996) with sample preparation adapted from the method of Harris et al. (1987) and Allen et al. (1994).

Phenolic Analysis. Ethanol extraction of phenolics from the stems was carried out according to the method of Kantz and Singleton (1990). Total phenolics content was determined by using the Folin−Ciocalteu reagent method (Singleton and Rossi,1965), and amounts were expressed in terms of gallic acid equivalents. Flavonoid and nonflavonoid phenolics were separated by HCl·formaldehyde precipitation according to the method of Kramling and Singleton (1969). The extracted phenolics were also measured according to the vanillin·HCl reagent method (Broadhurst and Jones, 1978), and amounts were expressed as catechin equivalents.

Gel Permeation HPLC. The extract from the stems in the model must solution (2 mL) was lyophilized and then dissolved in 2 mL of *N*,*N*-dimethylformamide. The proportion of soluble phenolics, in the *N*,*N*-dimethylformamide, by comparison with the original extract ranged from 87 to 102%. The HPLC apparatus consisted of a Gilson series 302 pump (Villiers-le-Bel, France) equipped with UV detector (model 1001, M&S Instruments Inc., Osaka, Japan) and connected to a Shimadzu CR-4A integrator (Kyoto, Japan). The detection wavelength was 280 nm. A gel permeation HPLC column (TSKgel G2000H, 7.8 by 300 mm; Toso Co., Tokyo, Japan) was employed, using tetrahydrofuran as the eluate solvent for elution at a flow rate 0.5 mL/min at room temperature (~25 °C). After filtration of each sample using a 0.2 μm membrane filter, 20 μL of sample solution was injected. The column was calibrated using commercially obtained phenolics and standard polystyrenes having known molecular weights as follows: 1.62×10^2, 2.66×10^2, 3.70×10^2, 4.74×10^2, 5.78×10^2, 1.05×10^3, and 2.5×10^3. The recovery of phenolics, as determined by the Folin−Ciocalteu reagent, in this HPLC system, estimated using a commercial tannic acid in an *N*,*N*-dimethylformamide solution (5 mg/mL), was 65 ± 10% by five determinations.

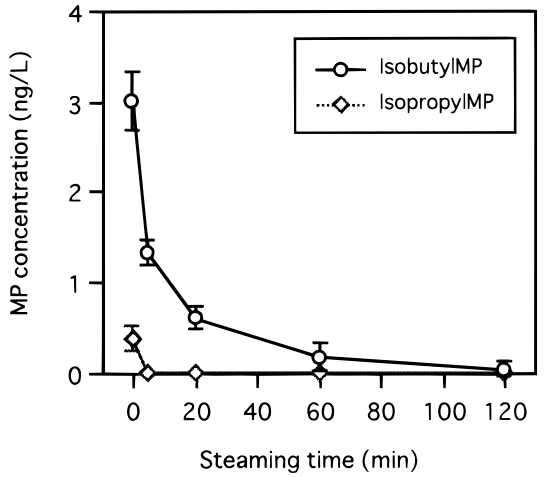

Figure 1. Effect of the duration of steaming of Cabernet Sauvignon grape stems on levels of extracted MPs in a model must solution. Stems were steamed at 100 °C.

Color Analysis. The color of the wines was determined by measuring the absorbance at 520 and 420 nm in a 2 mm cell after filtration through a 0.2 μm membrane.

Other Analyses. The water content of the stems was estimated from the loss in weight after drying at 135 °C for 3 h. Levels of organic and phosphoric acids in the wines were determined by HPLC using a conductivity detector, with isovaleric acid added as an internal standard after 1% PVPP treatment. The mineral content of the wines was determined by means of a plasma spectrometer after microwave digestion with nitric acid and hydrogen peroxide. Glucose and fructose were determined according to an enzymatic method using an F-kit (Boehringer Mannheim) after 1% PVPP treatment.

RESULTS AND DISCUSSION

Removal of MPs from Stems by Steam Treatment. Steam treatment at 100 °C for 60 min eliminated >95% of the extractable isobutylMP from the stems of the four grape varieties tested (Table 1). IsopropylMP was not detected after steam treatment. The results indicate that steam treatment was effective to remove MPs from the stems. The amount of MPs extracted from the Cabernet Sauvignon stems in the model must solution decreased depending on the length of the period of steaming (Figure 1). After the autoclave treatment at 110 or 120 °C for 20 min, isobutylMP was not detected in the model must solution, whereas isobutylMP was detected in the case of the atmospheric treatment (20 min). As the treatment temperature was

Table 2. Effect of Steaming Conditions on Levels of Phenolics Extractable from the Cabernet Sauvignon Grape Stems

steaming conditions	ethanol extraction		model must extraction for 7 days		
	total phenol[a]	HCl·formaldehyde precipitate[b] (%)	total phenol[a]	HCl·formaldehyde precipitate[b] (%)	vanillin·HCl/Folin−Ciocalteu ratio[b]
nonsteamed	12.06 ± 0.96	98.1	7.49 ± 0.82	95.7	0.47
100 °C, 5 min	16.31 ± 0.23	98.1	14.47 ± 0.18	97.7	0.72
100 °C, 20 min	16.35 ± 1.59	98.0	14.07 ± 0.94	97.3	0.70
100 °C, 60 min	15.73 ± 0.66	98.0	15.12 ± 1.22	97.7	0.66
100 °C, 120 min	13.91 ± 0.83	97.4	14.06 ± 0.62	97.2	0.64
105 °C, 20 min	15.48 ± 0.95	98.1	14.47 ± 0.18	97.5	0.68
110 °C, 20 min	13.17 ± 2.24	97.7	14.06 ± 0.78	97.5	0.67
120 °C, 20 min	12.66 ± 1.30	97.0	13.47 ± 0.48	96.8	0.57 (catechin: 1.00)

[a] Milligrams as gallic acid equivalent per gram of nonsteamed stems. Averages and standard deviations of three determinations. [b] Averages of three determinations.

Table 3. Effect of Steam Treatment[a] on pH, Color, MPs, and Phenolics Content of the Red Wines[b]

grape variety	stem addition	pH	color		MPs[c]		phenolics[d]	
			A_{520}	A_{420}/A_{520}	isopropylMP	isobutylMP	total phenol	HCl·formaldehyde precipitate (%)
Cabernet Sauvignon	none	3.78 ± 0.04	6.51 ± 0.29	0.505 ± 0.005	0.2 ± 0.0	6.5 ± 0.1	1769 ± 32	83.9
	raw	3.85 ± 0.11	4.93 ± 0.14	0.592 ± 0.005	0.7 ± 0.1	8.6 ± 0.2	2160 ± 37	91.0
	steamed	3.87 ± 0.11	5.54 ± 0.29	0.569 ± 0.008	0.2 ± 0.0	6.5 ± 0.2	2307 ± 27	91.8
Merlot	none	3.54 ± 0.01	3.42 ± 0.14	0.630 ± 0.003	0.3 ± 0.1	0.8 ± 0.1	1483 ± 33	78.2
	raw	3.54 ± 0.01	2.58 ± 0.23	0.717 ± 0.017	0.6 ± 0.2	4.4 ± 0.6	1923 ± 55	87.8
	steamed	3.58 ± 0.03	2.72 ± 0.02	0.718 ± 0.010	0.2 ± 0.0	0.8 ± 0.2	2056 ± 75	89.1
Pinot noir	none	3.60 ± 0.01	0.75 ± 0.04	1.020 ± 0.016			1013 ± 67	83.7
	raw	3.69 ± 0.02	0.66 ± 0.04	1.133 ± 0.010	2.9 ± 0.3	16.0 ± 0.9	1100 ± 59	81.7
	steamed	3.65 ± 0.03	0.71 ± 0.04	1.084 ± 0.045		0.6 ± 0.2	1112 ± 57	80.1
Muscat Bailey A	none	3.83 ± 0.04	3.44 ± 0.34	0.758 ± 0.010			1334 ± 168	61.9
	raw	3.95 ± 0.03	3.27 ± 0.08	0.866 ± 0.018		0.4 ± 0.1	1671 ± 39	71.2
	steamed	3.95 ± 0.03	3.35 ± 0.22	0.858 ± 0.012		0.1 ± 0.1	1913 ± 83	75.0

[a] Steam treatment was conducted at 100 °C for 60 min. [b] Results are averages and standard deviations of three determinations. [c] Nanograms per liter of wine. [d] Milligrams as gallic acid equivalent per liter of wine.

Table 4. Effect of Steam Treatment[a] on the Organic and Phosphoric Acids and Mineral Content of the Red Wines[b]

	Cabernet Sauvignon, stem addition			Muscat Bailey A, stem addition		
	none	raw	steamed	none	raw	steamed
phosphoric acid (mg/L)	795 ± 40	948 ± 4	913 ± 24	669 ± 23	926 ± 27	919 ± 27
organic acids (mg/L)						
citric	571 ± 22	633 ± 10	603 ± 10	729 ± 33	767 ± 13	746 ± 19
tartaric	1446 ± 49	1308 ± 2	1334 ± 40	1105 ± 36	1011 ± 38	1106 ± 39
malic	4220 ± 87	4303 ± 58	4164 ± 75	3787 ± 65	3790 ± 14	3696 ± 16
succinic	1576 ± 70	1486 ± 29	1447 ± 89	1228 ± 18	1032 ± 82	1090 ± 77
lactic	322 ± 73	304 ± 36	351 ± 70	343 ± 44	382 ± 44	411 ± 47
acetic	161 ± 31	165 ± 5	164 ± 12	276 ± 27	247 ± 2	250 ± 21
minerals (mg/L)						
K	1454 ± 29	1927 ± 74	1788 ± 26	2046 ± 90	2476 ± 89	2418 ± 84
P	198 ± 4	277 ± 5	253 ± 8	208 ± 7	389 ± 12	386 ± 16
Ca	62 ± 0.6	69 ± 0.7	68 ± 0.4	74 ± 0.9	90 ± 1.1	90 ± 1.4
Mg	71 ± 1.6	73 ± 1.6	71 ± 1.9	55 ± 0.9	60 ± 0.4	60 ± 2.2
Na	4.4 ± 0.1	4.8 ± 0.4	4.5 ± 0.0	4.7 ± 0.3	4.7 ± 0.1	4.9 ± 0.2
Mn	0.6 ± 0.3	0.8 ± 0.3	0.9 ± 0.3	0.8 ± 0.0	0.9 ± 0.1	0.9 ± 0.1
Fe	5.8 ± 1.4	5.0 ± 0.5	4.7 ± 00	5.1 ± 0.9	7.0 ± 3.2	3.6 ± 0.7
Cu	0.5 ± 0.0	0.9 ± 0.2	0.9 ± 0.3	0.9 ± 0.0	0.8 ± 0.1	0.9 ± 0.2
Zn	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.5 ± 0.0	0.8 ± 0.1	0.9 ± 0.2

[a] Steam treatment was conducted at 100 °C for 60 min. [b] Results are averages and standard deviations of three determinations.

increased from 105 to 120 °C, the amounts of extractable MPs decreased more rapidly, but the high-temperature treatment softened the stem structure. Therefore, high-temperature (>125 °C) autoclave treatment seems to be an unsuitable method.

Extraction of Phenolics from Stems. Steam treatment increased the amounts of phenolics extracted from the stems in the model must solution and the amounts of ethanol extractable phenolics (Table 2). More than 90% of the extracted phenolics in the case of all samples were precipitated, which meant flavonoid phenolics, by the HCl·formaldehyde method (Kramling and Singleton, 1975). The length of the period of steaming had little effect on the amounts of extracted phenolics in the model must solution. Vanillin·HCl/Folin−Ciocalteu values, which serve as an indicator of the degree of flavonoid phenolic polymerization (Goldstein and Swain, 1963; Singleton, 1988), were determined in assays of the extracted phenolics. It was thought that severe heat conditions would enhance the polymerization of the phenolics, but phenolics from nonsteamed stems showed the lowest values. Time

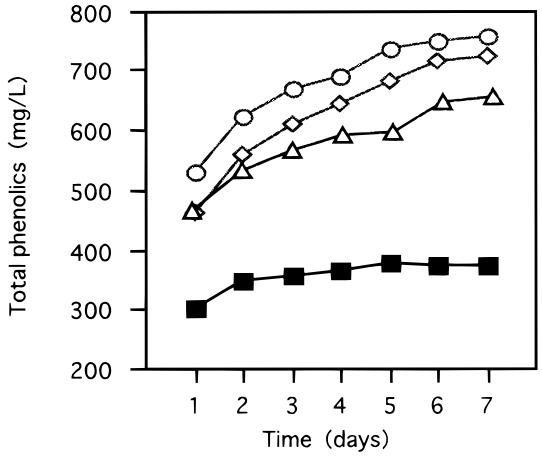

Figure 2. Effect of steaming conditions on the time course of extraction of total phenolics from Cabernet Sauvignon grape stems in the model must solutions: (■) nonsteamed; (◇) 100 °C for 5 min; (○) 100 °C for 60 min; (△) 120 °C for 20 min.

course experiments using the model must solution showed that the steamed stems supplied more phenolics from an early stage than the nonsteamed ones (Figure 2). The increase in amounts of phenolics released from the steamed stems continued until the seventh day. The amounts of the phenolics in the model solutions on the seventh day were comparable with the amounts of ethanol extractable phenolics.

In light of the importance of phenolics in relation to the qualities of red wine, vigorous studies have been conducted on the phenolics, applying HPLC or liquid chromatography (Oszmianski and Lee, 1990; Kantz and Singleton, 1990; Cacho and Castells, 1991; Lamuela-Raventos and Waterhouse, 1994), but the molecular weights of polymerized phenolics in grapes or wines have not been satisfactorily characterized. Nakahara et al. (1993) have estimated the molecular weights of oolong tea polyphenols after methylation using the gel permeation HPLC analysis. In this study, we applied the HPLC method to analyze the extracted phenolics from the stems, but omitted the methylation process of the sample constituents because commercial phenolics and polystyrene markers eluted with agreement between their molecular weights and the elution volumes when tetrahydrofuran was used as the eluent (Figure 3). The highest peak corresponded to a molecular weight of ~300, and these major constituents were thought to be flavonoid monomers in the extract from steamed stems, which showed diminished levels as the steaming conditions became severe. A small peak corresponding to a molecular weight of ~300, observed in the analysis of extracts from nonsteamed stems, was indicative of an average molecular weight relatively greater than those of the other samples. These results obtained by gel permeation HPLC analysis agreed well with the results obtained by vanillin·HCl/Folin−Ciocalteu value analysis. Moreover, the brown color of the nonsteamed stem solution was darker than that of the other samples, so it suggests that enzymatic polymerization reactions occurred in the case of nonsteamed stems.

Constituents of Red Wines Fermented with Steamed Stems. Red wines fermented with stems showed slightly lower alcohol and TA contents but higher levels of extract and slightly higher pH values (Table 3), which might be a factor in the instability of the wine color (Sims and Morris, 1985), than did wines

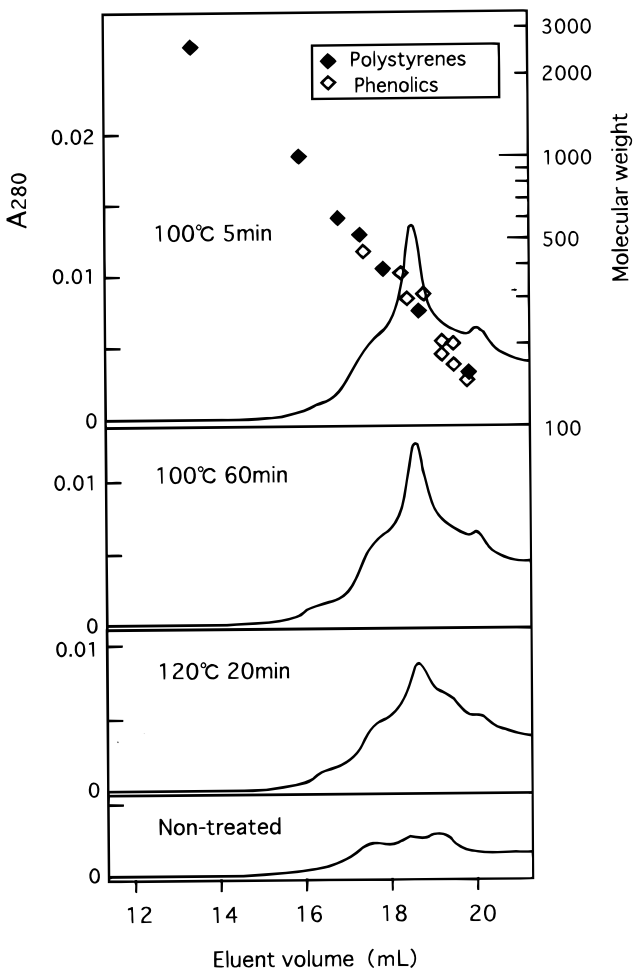

Figure 3. Gel permeation HPLC analysis of phenolics extracted from Cabernet Sauvignon grape stems in the model must solutions. Steaming conditions are shown in each panel. The scale of absorbance at 280 nm in the four chromatograms is the same.

fermented without stems (blank wines). These differences were slight, however, and no clear differences were observed with respect to the content of glucose or fructose (data not shown). The lower alcohol content might be due to dilution by water derived from the added stems, which contain >70% water but little sugar (Amerine et al., 1972; Rice, 1976). The higher levels of extracted constituents might be due to the release of phenolics and other soluble solids from the stems.

The blank wines showed the highest absorbance values at 520 nm and the lowest hue values (A_{420}/A_{520}), whereas the wines fermented with steamed stems showed higher absorbance values than those of wines with nonsteamed stems added (Table 3). The results indicate that the steaming of stems has a protective effect with respect to color loss in contrast to the evident color decrease that accompanies the addition of raw stems. It was supposed that the steaming treatment might have decreased the anthocyanin adsorption (Boulton et al., 1995b) of raw stems. As expected on the basis of the results of preliminary experiments, wines fermented with steamed stems showed MP levels as low as blank wines. A noteworthy effect was observed in the case of Pinot noir red wines, as the stems of these grapes contain high levels of MPs but there is little in the fruit. The addition of stems increased the levels of flavonoid phenolics in the wines, and steaming of the

stems was effective for enrichment. The effect of steaming on the increase in phenolics in the wines was less than that observed in a test of the model must solution. The enriched phenolics from the stems might contribute to the stability of the red color of the wines in the aging process (Timberlake and Bridle, 1976; Sim and Morris, 1985; Singleton and Trousdale, 1992).

It has been reported that grape stems contain abundant amounts of TA and ash (Rice, 1976), but the addition of stems had little effect on the organic acids content of Cabernet Sauvignon or Muscat Bailey A wines (Table 4). On the contrary, the levels of phosphoric acid (phosphorus), potassium, and calcium in the wines were increased as a result of the addition of stems. The steam treatment of the stems decreased the level of malic acid in the wines, and in the case of Cabernet Sauvignon, the increase of the level of phosphoric acid and potassium caused by the steamed stems was smaller than that caused by raw stems. The increase in levels of potassium might be closely related to the high pH values of the wines (Zoecklein et al., 1995a). The addition of stems clearly did not affect the content of other trace elements.

General Discussion. The steam treatment of grape cluster stems effectively eliminated the amount of extractable MPs and also increased the amounts of extractable phenolics in the red wine must solution. The young wines fermented with the steamed stems showed higher color intensity at 520 nm than did wines with raw stems. These results indicated that the addition of steamed stems, especially instead of the addition of raw stems, might be a worthy process for red wine vinification from low-phenolic grapes. On the other hand, the steamed stems caused disadvantageous color loss: therefore, an overall evaluation including this point must be taken before the application of this vinification style, and some further experiments should be conducted to evaluate the aging process on the chemical and sensory properties of the wines.

LITERATURE CITED

Allen, M. S.; Lacey M. J.; Boyd, S. Determination of methoxypyrazines in red wines by stable isotope dilution gas chromatography−mass spectrometry. *J. Agric. Food Chem.* **1994**, *42*, 1734−1738.

Amerine, M. A.; Berg, H. W.; Cruess, W. V. *The Technology of Wine Making*, 3rd ed.; Avi Publishing: Westport, CT, 1972; p 97.

Boulton, R. B.; Singleton, V. L.; Bisson, L. F.; Kunkee, R. E. *Principles and Practices of Winemaking*; Chapman and Hall: New York, 1995a; p 88.

Boulton, R. B.; Singleton, V. L.; Bisson, L. F.; Kunkee, R. E. *Principles and Practices of Winemaking*; Chapman and Hall: New York, 1995b; p 221.

Broadhurst, R. B.; Jones, W. T. Analysis of condensed tannins using acidified vanillin. *J. Sci. Food Agric.* **1978**, *29*, 788−794.

Cacho, J.; Castells, J. E. Fractionation of phenolic compounds from grapes by size exclusion liquid chromatography with HPLC instrumentation. *Am. J. Enol. Vitic.* **1991**, *42*, 327−335.

Goldstein, J. L.; Swain, T. Methods for determination of the degree of polymerization of flavans. *Nature* **1963**, *198*, 587−588.

Harris, R. L. N.; Lacy, M. J.; Brown, W. V.; Allen, M. S. Determination of 2-methoxy-3-alkylpyrazines in wine by gas chromatography/mass spectrometry. *Vitis* **1987**, *26*, 201−207.

Hashizume, K.; Samuta, T. Green odorants of grape cluster stem and their ability to cause a wine stemmy flavor. *J. Agric. Food Chem.* **1997**, *45*, 1333−1337.

Hashizume, K.; Umeda, N. Methoxypyrazine content of Japanese red wines. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 802−805.

Kantz, K.; Singleton, V. L. Isolation and determination of polymeric phenols using Sephadex LH-20 and analysis of grape tissue extracts. *Am. J. Enol. Vitic.* **1990**, *41*, 223−228.

Kovac, V.; Alonso, E.; Bourzeix, M.; Revilla, E. Effect of several enological practices on the content of catechins and proanthocyanidins of red wines. *J. Agric. Food Chem.* **1992**, *40*, 1953−1957.

Kramling, T. E.; Singleton, V. L. An estimate of the nonflavonoid phenols in wines. *Am. J. Enol. Vitic.* **1969**, *20*, 86−92.

Lamuela-Raventos, R. M.; Waterhouse, A. L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994**, *45*, 1−5.

Nakahara, K.; Kawabata, S.; Ono, H.; Ogura, K.; Tanaka, T.; Ooshima, T.; Hamada, S. Inhibitory effect of oolong tea polyphenols on glucosyltransferases of mutants streptococci. *Appl. Environ. Microbiol.* **1993**, *59*, 968−973.

Oszmianski, J.; Lee, C. Y. Isolation and HPLC determination of phenolics compounds in red grapes. *Am. J. Enol. Vitic.* **1990**, *41*, 204−206.

Peynaud, E. *Connaissance et Travail du Vin*; Bordas: Paris, 1981; p 168.

Ricardo da Silva, J. M.; Rosec, J. P.; Bourzeix, M.; Mourgues, J.; Moutounet, M. Dimer and trimer procyanidins in Carignan and Mourvedre grapes and red wines. *Vitis* **1992**, *31*, 55−56.

Rice, A. C. Solid waste generation and by-product recovery potential from winery residues. *Am. J. Enol. Vitic.* **1976**, *27*, 21−26.

Shibasaki, S.; Shimizu, M.; Otsuka, K. On extractable phenolic compounds from Koshu grape stem. *J. Brew. Soc. Jpn.* **1988**, *83*, 52−56.

Sim, C. A.; Morris, J. R. A comparison of the color components and color stability of red wine from Noble and Cabernet Sauvignon at various pH levels. *Am. J. Enol. Vitic.* **1985**, *36*, 181−184.

Singleton, V. L. *Wine Analysis*; Springer-Verlag: Berlin, 1988; p 198.

Singleton, V. L.; Rossi, Jr., J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

Singleton, V. L.; Trousdale, E. Anthocyanin-tannin interactions explaining differences in polymeric phenols between white and red wines. *Am. J. Enol. Vitic.* **1992**, *43*, 63−70.

Timberlake C. F.; Bride, P. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* **1976**, *27*, 97−105.

Zoecklein, B. W.; Fugelsang, K. C.; Gump, B. H.; Nury, F. S. *Wine Analysis and Production*; Chapman and Hall: New York, 1995a; pp 77−88.

Zoecklein, B. W.; Fugelsang, K, C.; Gump, B. H.; Nury, F. S. *Wine Analysis and Production*; Chapman and Hall: New York, 1995b; pp 129−130.

Received for review February 24, 1998. Revised manuscript received July 7, 1998. Accepted August 5, 1998.

JF9801771