

Yield Effects on 2-Methoxy-3-Isobutylpyrazine Concentration in Cabernet Sauvignon Using a Solid Phase Microextraction Gas Chromatography/Mass Spectrometry Method

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A rapid and automated solid phase microextraction (SPME) stable isotope dilution gas chromatography/mass spectrometry (GC-MS) method for 2-methoxy-3-isobutylpyrazine (MIBP) quantification in red wine was developed. Wines with 30% (w/v) NaCl and 2-methoxy-²H₃-3-isobutylpyrazine internal standard were sampled with a 2 cm divinylbenzene/carboxen/poly(dimethylsiloxane) SPME fiber for 30 min at 40 °C and analyzed by GC-MS. The method was used to measure MIBP concentrations in Cabernet Sauvignon wines that were produced from six winter pruning treatments over two vintages. MIBP concentrations were significantly negatively correlated with buds per vine. In addition, the MIBP concentration was directly related to sensory vegetal intensity ratings obtained by descriptive analysis.

KEYWORDS: 2-Methoxy-3-isobutylpyrazine; headspace solid phase microextraction; sensory evaluation; canopy management

INTRODUCTION

MIBP is an extremely potent odorant that is found in vegetables such as bell peppers (1, 2) and french beans (2) and is an important wine grape flavor compound in varieties such as Cabernet Sauvignon and Sauvignon blanc (3). Sets of Cabernet Sauvignon wines have been found to have MIBP concentrations in the range of 3.6–56.3 ng/L by Allen et al. (4) and approximately 3–36 ng/L by Hashizume and Umeda (5). Several studies on Cabernet Sauvignon and Sauvignon blanc have found correlations between MIBP concentrations and viticultural conditions such as growing temperature (4, 6) and light exposure (7, 8). The MIBP concentrations in wine have been shown to correlate with sensory vegetal intensity ratings (9–11). We recently reported that bell pepper and vegetal aromas and flavor intensities decreased as the yield increased when the yield was adjusted by winter pruning in Cabernet Sauvignon (12). Therefore, MIBP is a candidate for completing a vine wine sensory continuum with respect to yield and vegetal character.

Developing such a vine wine sensory continuum in order to understand the connections between vineyard conditions and wine sensory quality is difficult but essential to progress in viticulture. The sensory data must be more specific than simply “quality” measurements. By separating wine qualities into more specific sensory attributes, information more directly related to wine composition may be obtained. The vineyard treatments must create differences in sensory attributes sufficient for

regression analysis with putative flavor compounds, and analytical methods for quantifying flavor compounds present at low concentrations must be available to complete the regressions.

Typically, the analysis of MIBP has been time-consuming due to lengthy sample preparation procedures (4, 5, 13, 14). SPME offers promise as a rapid, solventless, readily automated technique that has been used to quantify various volatiles in wine (15). However, a SPME method for MIBP quantification in wine developed by Sala et al. still required distillation in order to quantify MIBP in wine at sensorially relevant concentrations (16). Hartman et al. developed a SPME method for MIBP quantification in model wine with minimal sample preparation, but its detection limit was approximately 100 ng/L (17), much higher than the 2 ng/L threshold for MIBP in water.

The goal of the current study was to develop a sensitive assay for MIBP that requires minimal sample preparation time so that large numbers of samples could easily be run but still obtain a limit of detection near the sensory threshold. SPME sampling with stable isotope dilution GC-MS was chosen for the method because of its sensitivity, lack of solvents, and ease of use. The method was then used to quantify MIBP concentrations in wines made from six winter pruning treatments over 2 years to determine the effect of vine pruning on Cabernet Sauvignon MIBP concentrations. In addition, sensory ratings of bell pepper aroma collected by descriptive analysis were compared with the wine methoxyypyrazine concentrations.

MATERIALS AND METHODS

Viticulture. Grapevines (*Vitis vinifera* L., cv. Cabernet Sauvignon 110R rootstock) were planted in 1995 at 6' × 8' spacing and trained to

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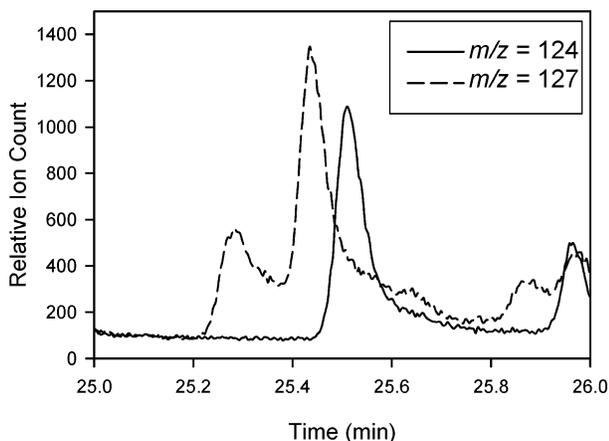


Figure 1. GC-MS selected ion monitoring trace of extracted ions from a model wine. MIBP was quantified at $m/z = 124$, while $[^2\text{H}_3]\text{MIBP}$ was quantified at $m/z = 127$.

bilateral cordons at the Oakville Experimental Vineyard (Oakville, CA). The vines were winter pruned to 12, 18, 24, 30, 36, and 48 buds/vine. The vines were planted in and farmed with standard practices, with the exception of the pruning treatments, which were imposed in a randomized complete block design with six replications. The grapes were harvested at 22 ± 1.1 °Brix in 2000 (early, due to imminent rain) and 23.2 ± 1.3 °Brix in 2001. The pruning treatments resulted in yields that ranged from 6.0 to 22.2 t/ha in 2000 and from 7.1 to 18.0 t/ha in 2001 (12). The fruit was crushed, destemmed, and separated into 55 L plastic fermentation vessels to make three replicate wines from each treatment following a standard experimental winemaking protocol. SO_2 was added (50 ppm), and the musts were inoculated with Premier Cuvée yeast (Red Star, Milwaukee, WI). The musts were punched down twice per day, and the wine was pressed with a single basket press at 2 °Brix. The wines were then inoculated with an active malolactic bacteria culture and were racked after the Brix had stabilized. After malolactic fermentation had completed, the wines were racked again and 25 ppm SO_2 was added before being cold stabilized for 4 weeks and then bottled.

Sample Preparation and Sampling. MIBP for standards was purchased from Pyrazine Specialties (Atlanta, GA) and was 99% pure by GC-MS. The internal standard, $[^2\text{H}_3]\text{MIBP}$, was purchased from CDN Isotopes and was 98.2% pure (Quebec, Canada).

Wine samples (10 mL) were placed in 20 mL round-bottomed headspace sampling vials (Gerstel, Baltimore, MD) with 3 g of NaCl and 10 μL of 50 $\mu\text{g}/\text{L}$ $[^2\text{H}_3]\text{MIBP}$ in EtOH (final internal standard concentration of 50 ng/L). The vials were sealed with magnetic crimp caps (Gerstel) and were carefully shaken to dissolve the NaCl. They were then left to equilibrate overnight in the dark at room temperature.

A 2 cm DVB/CAR/PDMS (Supelco, Bellefonte, PA) 23 gauge SPME fiber was used for sampling. The samples were warmed to 40 °C for 5 min before exposing the fiber for 30 min at 40 °C with agitation.

Instrumental Analysis. The samples were analyzed with an Agilent 6890GC/5973MSD equipped with a Gerstel MPS2 autosampler and a HP-5MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific, Folsom, CA). The injector was held at 260 °C with no purge for 5 min for the analytes to desorb from the fiber, and then, the purge was switched on to 50 mL/min with the fiber in the inlet for an additional 5 min. No carry-over was observed between samples. The oven was kept at 40 °C for 5 min, then increased 2.5 °C/min to 80 °C, 5 °C/min to 110 °C, and 25 °C/min to 230 °C before holding for 5 min. Helium was used as the carrier gas at constant pressure (29.05 psi) with a nominal initial flow of 4.0 mL/min and an average linear velocity of 73 cm/s. The MSD interface was held at 280 °C.

The detection of trace quantities of MIBP was carried out using selected ion monitoring. Mass channels were $m/z = 124$ and 94 for MIBP and $m/z = 127$ and 154 for $[^2\text{H}_3]\text{MIBP}$ with 100 ms dwell times (Figure 1). Ions 124 and 127 were used for quantification ($\text{M}^+ - \text{C}(\text{CH}_3)_2 = \text{base peak}$), while 94 ($\text{M}^+ - \text{C}_5\text{H}_{12}$) and 154

($\text{M}^+ - \text{C}_3\text{H}_5$) were used as qualifier ions. Different qualifying fragments were used for the analyte and internal standard to avoid interference from other ions with the same m/z ratio. The molecular ion ($m/z = 166$ for MIBP, $m/z = 169$ for $[^2\text{H}_3]\text{MIBP}$) was too small for reliable quantification. All samples were analyzed in triplicate. The three winemaking replications within each viticultural treatment were analyzed over at least two separate days.

Sensory Panels. Descriptive analysis panels were run in the spring of 2001 for the 2000 vintage wines (13 panelists, 9 male, 4 female, ages 21–33) and in the winter of 2003 for the 2001 vintage wines (15 panelists, 7 male, 8 female, ages 21–41). A hybrid consensus training method that combined elements of the QDA (18) and Spectrum (19) methods was used. The panelists were chosen based on interest and availability and were compensated for their participation. The panel was lead through term generation, and in both years, bell pepper aroma, vegetative aroma, and vegetative flavor by mouth were chosen to be included on the final scorecard. Sliced bell pepper in 40 mL of Cabernet Sauvignon base wine was used as a standard to train the panel for the bell pepper aroma descriptor (5 g for the 2000 wines, 1 g for the 2001 wines), while green beans and asparagus were used for the veggie aroma standard (2.5 g of chopped fresh asparagus and 2.5 g of chopped green bean in 2000 and 5 mL of juice from canned green beans and 1 mL of juice from canned asparagus in 2001). During training, the panelists were given three wines per day and were asked to individually rate them for all of the attributes on the scorecard using a 16-point scale (0 = not present, 15 = extremely intense). The panelists then shared their scores, and if there were disagreements, they discussed the wines until a consensus in the rank order of intensities was reached.

Sensory Testing. Wines were stored in the UC Davis wine cellar at 12 °C in the dark and were brought to the sensory laboratory at least 2 h prior to testing. Forty milliliter portions of wine were poured into clear, tulip-shaped glasses coded with three digit random numbers. Plastic polystyrene covers were placed over the glasses to retain the aromas.

The panelists smelled the reference standards to refresh their memories before rating the wines. The standards were available throughout the tests for the panelists to refer to.

All testing was done in individual tasting booths. The 2000 vintage wines were presented monadically under incandescent light with four or five wines per session, while the 2001 vintage wines were rated with a multisample presentation under red light and five or six wines per session. The presentation orders were randomized.

Statistical Methods. Microsoft Excel was used for all linear regressions. The significance of R^2 values was determined using a table for the critical values of the Pearson product moment correlation coefficient (20).

RESULTS

Linearity. A standard curve was created using model wine (12% v/v EtOH and 2 g/L potassium bitartrate in Nanopure water, pH 3.52) to which concentrations between 1 and 50 ng/L of MIBP were added in addition to the $[^2\text{H}_3]\text{MIBP}$ internal standard. Three replications of each of eight standard concentrations (1, 2, 5, 10, 20, 30, 40, and 50 ng/L) were run. The MIBP peak area ($m/z = 124$, $t_R = 25.51$ min) in relation to the $[^2\text{H}_3]\text{MIBP}$ internal standard peak area ($m/z = 127$, $t_R = 25.43$ min) was linearly correlated with the concentration of MIBP in the standards from 1 to 50 ng/L ($R^2 = 0.9943$). The average regression equation was $[\text{MIBP}] (\text{ng}/\text{L}) = 47.39 (A/A_{IS}) - 0.47$ and was used for quantitation of all samples. Three standards (5, 25, and 50 ng/L) were analyzed in duplicate each day, along with the wine samples, to check the calibration. The limit of detection ($3\sigma/S/N$) was 2 ng/L, although the qualifier peak was not present below 5 ng/L, making identification only tentative below this level.

Accuracy and Precision. The precision tests were run at 5, 20, and 50 ng/L MIBP (Table 1). Relative standard deviations of five replicates over two separate days were 11.6% at 5 ng/L

Table 1. Repeatability Test for MIBP in Model Wine^a

spiked MIBP (ng/L)	measured mean MIBP (ng/L)	measured range (ng/L)	SD (ng/L)	RSD (%)
5.0	4.8	3.9–5.4	0.55	11.6
20.0	20.9	20.0–21.7	0.73	3.5
50.0	50.0	47.4–52.9	2.31	4.6

^a *n* = 5 at each level.**Table 2.** Recovery Trial for MIBP Spiked into Red Wines^a

wine sample	initial MIBP (ng/L)	spike concn (ng/L)	measured MIBP after spike (ng/L)	% recovery of spike
A	16.2	10	27.1	108.7
B	6.9	10	16.9	100.1
C	18.3	10	26.8	85.5
D	9	10	17.7	86.5
A	16.2	50	64.6	96.8
B	6.9	50	52.3	90.7
C	18.3	50	67.9	99.3
D	9	50	57.6	97.2

^a *n* = 5 for initial concentration; *n* = 2 for spikes.

and under 5% at 20 and 50 ng/L. Standard addition tests were also run by adding 10 and 50 ng/L spikes to four different Cabernet Sauvignon wines from the University of California, Davis winery in duplicate (**Table 2**). Recovery of the spikes averaged 95.2% at 10 ng/L and 96.0% at 50 ng/L.

Sensory. MIBP concentrations were linearly correlated with bell pepper aroma intensity ($p < 0.001$ in 2000, $p < 0.05$ in 2001), vegetal aroma intensity ($p < 0.05$ in 2000, $p < 0.001$ in 2001), and vegetal flavor by mouth intensity ($p < 0.1$ in 2000, $p < 0.01$ in 2001) (**Figure 2**). Wines with higher concentrations of MIBP received higher vegetal ratings.

Pruning. MIBP concentrations ranged from 5 to 18 ng/L in 2000 and from <2 to 9 ng/L in 2001. As the number of buds/vine increased, the MIBP concentration decreased (**Figure 3**).

DISCUSSION

Methodology. This SPME GC-MS method for MIBP quantification in wine requires minimal sample preparation and can be automated with a SPME autosampler with a sample heating unit. This makes running large numbers of samples feasible. The sample preparation took under 3 min per sample, and the autosampler injected one sample every 45 min (GC run time plus time for the oven to cool and equilibrate before the next run). The previously developed assays require extensive sample preparation. The methods of Harris et al., Allen et al., and Hashizume and Umeda require distillation, separation with an ion exchange column, and concentration, taking up to 2 days per sample (4, 5, 13). Kotseridis et al. developed a simplified procedure involving solvent extraction and concentration that took 1 h per sample (14). The method of Sala et al. required distillation before SPME sampling for 4 h (16).

A deuterated analogue of MIBP was chosen as an internal standard in order to quantify MIBP at trace levels in the samples. The MIBP and [²H₃]MIBP react nearly identically during isolation and measurement, so that the ratios of the MIBP and [²H₃]MIBP remain constant, despite potential variations in sampling efficiency and GC-MS response (3). Allen and Lacey have shown that deuterated analogues of the analyte of interest are the most reliable internal standards for methoxypyrazine quantification (3).

Sampling conditions were determined by testing several SPME fiber coatings, sampling temperatures, sampling times,

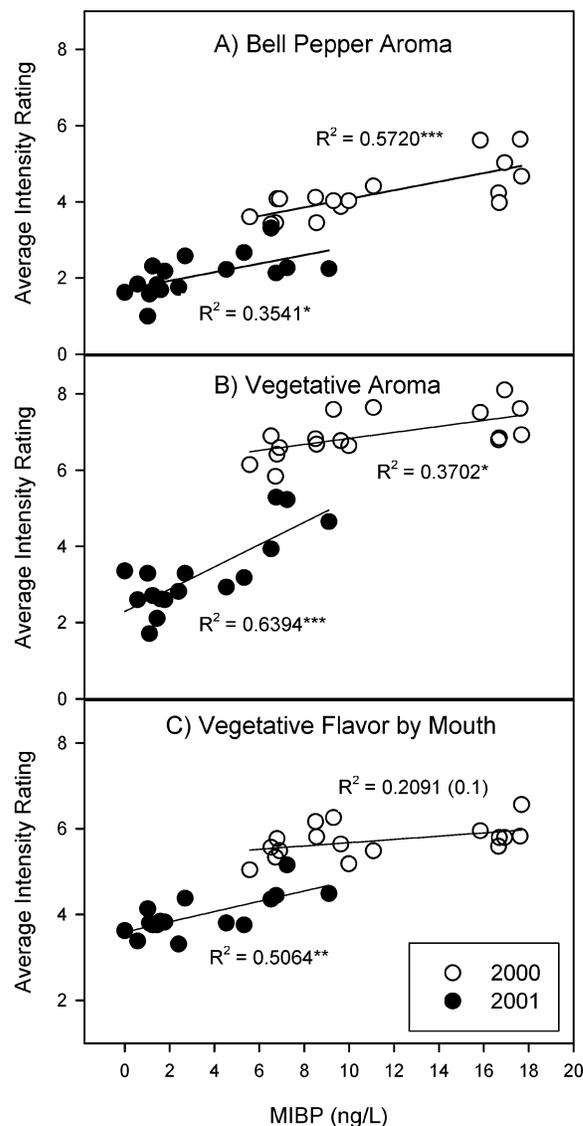


Figure 2. Bell pepper aroma (A), vegetative aroma (B), and vegetative flavor by mouth (C) intensity ratings with response to MIBP concentration for 2000 and 2001 pruning experimental wines. *, **, and *** indicate significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

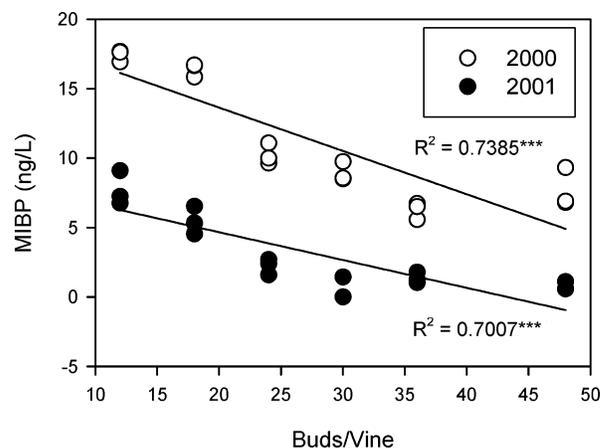


Figure 3. MIBP concentration with response to pruning treatment for 2000 and 2001 experimental wines.

and NaCl concentrations. We found that using 2 cm PDMS/DVB/CARB fibers resulted in 2–3 times better response than using PDMS/DVB fibers. The sampling temperatures between

30 and 60 °C were tested, and 40–45 °C provided the best response. The response leveled off after 30 min sampling time at 40 °C. The addition of 30% NaCl (w/v) more than doubled the sensitivity as compared to 0 or 10% NaCl additions (w/v). These conditions are very similar to the conditions used by Hartman et al. (17).

Although the limit of detection with this method (2 ng/L) is slightly higher than the distillation, ion exchange, and concentration (4, 5, 13) or distillation and SPME sampling (16) methods, this method provides adequate sensitivity with relation to the 2 ng/L threshold of MIBP in water (1) and is equal in sensitivity to the solvent extraction and concentration method of Kotseridis et al. (14). The application of chemical ionization mass spectrometry instead of electron impact mass spectrometry may allow for a lower limit of detection (13) but was not evaluated in this study.

Vineyard Wine Sensory and MIBP. The results build a connection from vineyard practices to wine composition to wine sensory attributes. The pruning treatments affected yield directly (12), and as the yield increased, the MIBP concentrations decreased ($p < 0.001$ in 2000 and 2001). Thus, the results indicate that the MIBP concentration in Cabernet Sauvignon wines is an inverse function of crop yield. This would be similar to the responses of other berry solutes to differences in yield, for example, sugars (21) and anthocyanins (22). The intensity ratings of bell pepper aroma, vegetative aroma, and vegetative flavor by mouth were all positively correlated with the MIBP concentration in the wines. This is also consistent with the literature (9–11) and strongly implies that the differences in vegetal characters among the wines were due to MIBP. Several studies have shown that the MIBP concentration in grapes is directly correlated with the MIBP concentration in the finished wine (5, 14, 23). It is therefore likely that the MIBP differences in the wines arose from the MIBP differences in the grapes.

Our results showed a significant direct correlation between MIBP concentration and sensory vegetal intensity ratings in 2001, a vintage in which all MIBP concentrations in the experimental wines were below 10 ng/L (Figure 2). However, several authors have previously calculated the threshold of MIBP in red wine to be between 10 and 16 ng/L (11, 24, 25). It is possible that the threshold for MIBP in some red wines may therefore be lower than previously reported. In white wine, the detection threshold for MIBP is 2 ng/L (10). Although it is possible that other methoxypyrazines, such as 2-methoxy-3-isopropylpyrazine, were responsible for the vegetal character, this is probably unlikely because they are typically present in Cabernet Sauvignon at less than 13% of the MIBP concentration (unless there is bottle specific microbial contamination), which is well below their sensory detection thresholds (26). It is possible that other vegetative aroma and flavor compounds, such as sulfur compounds (27), may have contributed to the vegetal ratings in these wines.

The perception of MIBP may be a function of its concentration. In 2000, when the concentrations of MIBP ranged from 5 to 18 ng/L, the best sensory correlation with the MIBP concentrations was with the bell pepper descriptor, but in 2001, when the concentrations of MIBP were lower, ranging from <2 to 9 ng/L, there was a better correlation with the vegetal aroma and vegetal by mouth descriptors than with the bell pepper descriptor. The wines from the two vintages were tested by separate sensory panels, which may account for the use of the different descriptors. However, these results may also indicate that at higher concentrations the MIBP is recognized as a bell pepper aroma, while at lower concentrations, it may

only be recognized as a more general vegetal aroma or flavor. Maga found that aroma descriptions of MIBP solutions do change as the MIBP concentration increases (24). Further work to clarify the effects of concentration on sensory properties of MIBP in a wine matrix is needed.

The MIBP concentrations in 2000 ranged from 5.6 to 11.0 ng/L for the 24–48 bud/vine treatments and 15.8 to 17.7 ng/L for the lower yielding 12 and 18 bud/vine treatments (Figure 3). The gap in MIBP concentrations prevents the data from being normally distributed within the overall concentration range. Because the order of introduction of the samples in the GC was randomized across treatments, we are confident that this gap did not occur as a result of the experimental protocol.

It is possible that the treatment differences in sensory ratings and in wine MIBP concentrations were not due to yield directly but were due to another effect of the pruning treatments on berry development, perhaps via the cluster microclimate. The pruning treatments that produced the highest MIBP concentrations in this study had the fewest shoots (and leaves) and therefore the least shading of clusters (28). However, it is clear that MIBP is photodegraded (29), and there is some evidence of higher MIBP in fruit from shaded microclimates (8). In addition, Allen et al. found an excellent negative correlation ($p < 0.005$) between long-term mean January temperatures and MIBP concentration in red wines grown in Australia and New Zealand (4). The results in the present study therefore show the opposite trend that would be predicted by light exposure and temperature alone. However, there is also evidence that light exposure may promote MIBP formation at early developmental stages (7), and it is not clear whether the correlation found by Allen et al. between MIBP concentration and growing temperature (4) reflects an effect of berry temperature per se or other vine responses to temperature.

Our results showed higher MIBP concentrations in 2000 than in 2001. Both Koseridis et al. and Hashizume and Umeda found that MIBP concentrations were lower in years when the grapes were harvested at lower °Brix (5, 14). The wines in this experiment were harvested at a slightly lower °Brix in 2000 (22 ± 1.1 °Brix) than in 2001 (23.2 ± 1.3 °Brix), which is consistent with the higher intensity vegetal ratings in 2000. Several researchers have found higher MIBP concentrations in years with lower temperatures for grapes grown in the same location (6, 25), which is also consistent with our results (1676 degree days in 2000, 1767 degree days in 2001).

In a previous study using descriptive analysis to qualitatively and quantitatively describe the sensory differences among the wines in the current study, we found that as vegetal aromas and flavors in these wines increased, fruity aromas and flavors decreased (12). Descriptive analysis studies on Cabernet Sauvignon wines often find a separation of vegetative and fruity attributes on the first principal component (12, 30, 31). It is possible that higher MIBP concentrations can mask fruity sensory attributes in wine. However, in this study, no fruity aroma compounds were quantified. Analyzing for chemical markers of fruitiness in wine is not easy, because many compounds, including ethyl esters and acetate esters of fatty acids, may be involved in the perception of fruity aromas and flavors (15). Precedents do exist for the interaction of aroma compounds to create masking effects (32).

Our results provide evidence of a vine wine sensory continuum with respect to winter pruning and vegetal aromas and flavors in Cabernet Sauvignon. The pruning severity was directly related to wine MIBP concentration and to sensory intensity

ratings of bell pepper aroma, vegetative aroma, and vegetative flavor by mouth. These trends were observed over two vintages.

ABBREVIATIONS USED

SPME, solid phase microextraction; GC-MS, gas chromatography/mass spectrometry; MIBP, 2-methoxy-3-isobutylpyrazine; [²H₃]MIBP, 2-methoxy-²H₃-3-isobutylpyrazine; DVB/CAR/PDMS, divinylbenzene/carboxen/poly(dimethylsiloxane).

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LITERATURE CITED

- Buttery, R. G.; Seifert, R. M.; Guadagni, D. G.; Ling, L. C. Characterization of some volatile constituents of bell peppers. *J. Agric. Food Chem.* **1969**, *17*, 1322–1327.
- Murray, K. E.; Whitfield, F. B. The occurrence of 3-alkyl-2-methoxypyrazines in raw vegetables. *J. Sci. Food Agric.* **1975**, *26*, 973–986.
- Allen, M. S.; Lacey, M. J. Methoxypyrazines of grapes and wines. In *Chemistry of Wine Flavor*; Waterhouse, A. L., Ebeler, S. E., Eds.; ACS Symposium Series No. 714; American Chemical Society: Washington, DC, 1999; pp 31–38.
- 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.
- Hashizume, K.; Umeda, N. Methoxypyrazine content of Japanese red wines. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 802–805.
- Lacey, M. J.; Allen, M. S.; Harris, R. L. N.; Brown, W. V. Methoxypyrazine in Sauvignon blanc grapes and wines. *Am. J. Enol. Vitic.* **1991**, *42*, 103–108.
- Hashizume, K.; Samuta, T. Grape maturity and light exposure affect berry methoxypyrazine concentration. *Am. J. Enol. Vitic.* **1999**, *50*, 194–198.
- Allen, M. S.; Lacey, M. J. Methoxypyrazine grape flavour: influence of climate, cultivar and viticulture. *Die Wein-Wissenschaft* **1993**, *48*, 211–213.
- Noble, A. C.; Elliot-Fisk, D. L.; Allen, M. S. Vegetative flavor and methoxypyrazines in Cabernet Sauvignon. In *Fruit Flavors: Biogenesis, Characterization, and Authentication*; Rouseff, R. L., Leahy, M. M., Eds.; ACS Symposium Series No. 596; American Chemical Society: Washington, DC, 1995; pp 226–234.
- Allen, M. S.; Lacey, M. J.; Harris, R. L. N.; Brown, W. V. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Am. J. Enol. Vitic.* **1991**, *42*, 109–112.
- Roujou de Boubée, D.; Van Leeuwen, C.; Dubourdieu, D. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *J. Agric. Food Chem.* **2000**, *48*, 4830–4834.
- Chapman, D. M.; Matthews, M. A.; Guinard, J.-X. Sensory attributes of Cabernet Sauvignon wines made from vines with different crop yields. *Am. J. Enol. Vitic.* **2004**, Submitted for publication.
- Harris, R. L. N.; Lacey, 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.
- Kotseridis, Y.; Baumes, R. L.; Bertrand, A.; Skouroumounis, G. K. Quantitative determination of 2-methoxy-3-isobutylpyrazine in red wines and grapes of Bordeaux using a stable isotope dilution assay. *J. Chromatogr. A* **1999**, *841*, 229–237.
- Ebeler, S. E. Analytical chemistry: Unlocking the secrets of wine flavor. *Food Rev. Int.* **2001**, *17*, 45–64.
- Sala, C.; Mestres, M.; Marti, M. P.; Busto, O.; Guasch, J. Headspace solid-phase microextraction analysis of 3-alkyl-2-methoxypyrazines in wines. *J. Chromatogr. A* **2002**, *953*, 1–6.
- Hartmann, P. J.; McNair, H. M.; Zoecklein, B. W. Measurement of 3-alkyl-2-methoxypyrazines by headspace solid-phase microextraction in spiked model wines. *Am. J. Enol. Vitic.* **2002**, *53*, 285–288.
- Stone, H.; Sidel, J. L. *Sensory Evaluation Practices*, 2nd ed.; Academic: Orlando, FL, 1993.
- Meilgaard, M.; Civille, C. V.; Carr, B. T. *Sensory Evaluation Techniques*; CRC Press: Boca Raton, FL, 1991.
- O'Mahony, M. *Sensory Evaluation of Food: Statistical Methods and Procedures*; Marcel Dekker: New York, 1986.
- Sinton, T. H.; Ough, C. S.; Kissler, J. J.; Kasimatis, A. N. Grape juice indicators for prediction of potential wine quality. I. Relationship between crop level, juice and wine composition, and wine sensory ratings and scores. *Am. J. Enol. Vitic.* **1978**, *29*, 267–271.
- Freeman, B. M.; Kliever, W. M. Effect of irrigation, crop level and potassium fertilization on Carignane vines. II. Grape and wine quality. *Am. J. Enol. Vitic.* **1983**, *34*, 197–207.
- Roujou de Boubée, D.; Cumsille, A. M.; Pons, M.; Dubourdieu, D. Location of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon grape bunches and its extractability during vinification. *Am. J. Enol. Vitic.* **2002**, *53*, 1–5.
- Maga, J. A. Sensory and stability properties of added methoxypyrazines to model and authentic wines. In *Flavors and Off Flavors: Proceedings of the 6th International Flavor Conference*; Charalambous, G., Ed.; Elsevier: Amsterdam, 1990; pp 61–70.
- Kotseridis, Y.; Anocibar Belouqui, A.; Bertrand, A.; Doazan, J. P. An analytical method for studying the volatile compounds of Merlot noir clone wines. *Am. J. Enol. Vitic.* **1998**, *49*, 44–48.
- Allen, M. S.; Lacey, M. J.; Boyd, S. J. Methoxypyrazines in red wines: Occurrence of 2-methoxy-3-(1-methylethyl)pyrazine. *J. Agric. Food Chem.* **1995**, *43*, 769–772.
- Margalit, Y. Aroma and flavor: Sulfur compounds. In *Concepts in Wine Chemistry*; Crum, J., Ed.; Wine Appreciation Guild: San Francisco, CA, 1997; pp 171–174.
- Reynolds, A. G.; Pool, R. M.; Mattick, L. R. Effect of shoot density and crop control on growth, yield, fruit composition, and wine quality of 'Seyval blanc' grapes. *J. Am. Soc. Hortic. Sci.* **1986**, *111*, 55–63.
- Heymann, H.; Noble, A. C.; Boulton, R. B. Analysis of methoxypyrazines in wines. 1. Development of a quantitative procedure. *J. Agric. Food Chem.* **1986**, *34*, 268–271.
- Turbow, S. B.; Block, D. E. Effects of viticultural practices on the aroma of 2000 Napa Valley Cabernet Sauvignon wines. *Am. J. Enol. Vitic.* **2002**, *53*, 249A.
- Heymann, H.; Noble, A. C. Descriptive analysis of commercial Cabernet Sauvignon wines from California. *Am. J. Enol. Vitic.* **1987**, *38*, 41–44.
- Cain, W. S.; Drexler, M. Scope and evaluation of odor counteraction and masking. *Ann. N. Y. Acad. Sci.* **1974**, *237*, 427–439.

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