DOI:10.1021/jf803720k

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY ARTICLE

Effect of Closure and Packaging Type on 3-Alkyl-2-methoxypyrazines and Other Impact Odorants of Riesling and Cabernet Franc Wines

Amy Blake,[†] Yorgos Kotseridis,[⊥] Ian D. Brindle,[3] Debbie Inglis,[†,§] M. Sears,[⊗] and Gary J. Pickering*[,†,§]

[†]Department of Biological Sciences and [§]Cool Climate Oenology and Viticulture Institute and [3]Department of Chemistry, Brock University, St. Catharines, Ontario L2S 3A1, Canada, [⊥]Department of Food Science and Technology, Agricultural University of Athens, Athens, Greece, and [⊗]Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada

3-Alkyl-2-methoxypyrazines (MPs) represent an important and potent class of grape- and insect-derived odor-active compounds associated with wine quality. Thirty nanograms per liter each of 3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP), and 3-*sec*-butyl-2-methoxypyrazine (SBMP) was added to Riesling and Cabernet Franc wines and monitored with headspace solid-phase microextraction and gas chromatography−mass spectrometry over 18 months to investigate the effects of various closure and packaging options on MPs. Changes in MP concentrations during bottle aging varied with closure/packaging option, with the greatest decrease evident in Tetrapak cartons. After 18 months, IBMP, IPMP, and SBMP in both Tetrapak-stored wines decreased by approximately 45, 32, and 26%, respectively. Similar changes were observed in other impact odorants to previous studies, including a greater decrease in odorant concentrations in wines closed with synthetic corks compared to natural corks and screw caps. These differences are thought to be due to the differential sorptive capacities of the various closure types. Overall, the data suggest that differences in gas permeability/contribution from the different closure and packaging options strongly associate with changes in wine composition during aging.

KEYWORDS: 3-Alkyl-2-methoxypyrazine; methoxypyrazines; wine packaging; wine closures; Cabernet Franc; Riesling; sorption; greenness; ladybug taint; *Harmonia axyridis*

INTRODUCTION

3-Isobutyl-2-methoxypyrazine (IBMP), 3-*sec*-butyl-2-methoxypyrazine (SBMP), and 3-isopropyl-2-methoxypyrazine (IPMP) are three grape-derived volatile compounds that elicit green and vegetative perceptions in wine. Although these MPs can positively influence wine quality in some varieties (*1*), at higher concentrations they are dominant and unpleasant (*2*), can mask "fruity/floral" aromas (*3*), and are associated with wines from cooler climates (*4*−*6*) and under-ripe, low-quality fruit (*5*, *6*). Recently, lady beetles were identified as a second source of elevated MPs in wine that has been named "ladybug taint" (LBT) (*7*). LBT is a wine defect resulting from the undesired incorporation of lady beetles (Coleoptera: Coccinellidae), particularly *Harmonia axyridis* (commonly called the Multicolored Asian Lady Beetle, MALB), into the fermentation process responsible for millions of dollars of lost revenue from downgraded or discarded wine in southern Ontario and parts of the United States (*8*). The prevalence of *H. axyridis* in other wine regions, including Italy, France, Spain, South Africa, and Argentina (*9*), suggests that LBT could be or become a more widespread problem for the wine industry. Regardless of source, MPs can be identified and measured in wines in trace amounts, and due to their extremely low sensory detection thresholds—in the high picograms per liter to low nanograms per liter range (*4*, *10*, *11*)—have the potential to significantly affect wine quality. Efforts to reduce MP levels have included both viticultural (*12*, *13*) and enological (*14*) interventions. However, attempts to decrease MP concentrations in wine using conventional treatments, such as fining, have had limited success (*15*), and novel approaches are required. Closure and packaging options may offer one such approach.

The capacity for packaging to directly remove volatile compounds through sorptive processes is termed flavor scalping and has been well established in the food science/technology literature and exploited commercially. It has been noted particularly with polymer packaging and nonpolar flavor compounds (*16*). In the investigation of the capacity of natural and agglomerate corks to contribute the taint compound 2,4,6-trichloroanisole to wine, it was observed that these closures had an even greater ability to absorb the compounds (*17*). Flavor scalping has since been characterized in wine from a comprehensive bottle-aging trial at the Australian Wine Research Institute, which investigated changes in composition in a Semillon wine after volatile compounds from a range of chemical classes were added and the bottles closed with natural, synthetic, and technical corks and

*Author to whom correspondence should be addressed [telephone: (905) 688-5550, ext. 4715; fax: (905) 688-1855; e-mail: gary.pickering@brocku.ca].

 Published on Web 05/11/2009

Table 1. Closure/Packaging Types, Material, Abbreviation, Brand, and Supplier

closure/ packaging	material/description	abbreviation	brand and supplier
natural cork	natural cork, medium quality	NatC	Sterisun UFB Natural Cork, Scott Laboratories Ltd., ON, Canada
agglomerate cork	small natural cork pieces glued together at high pressure	Agl	Scott Laboratories Ltd., ON, Canada
synthetic cork, extruded	internal thermoplastic elastomer foam and stiff outer polymer layer on sides, covered by food-grade silicone coating	Syn-Ex	Nomacorc Classic+, Funk Winemaking Supplies, ON, Canada
synthetic cork, molded	polyethylene foam, covered by food-grade silicone coating	Syn-M	Supremecorq 45, Malivoire Wine Co., ON, Canada
screw cap	aluminum covered, roll-on−tamper-evident, Teflon liner	Scap	Stelvin, Henry of Pelham Family Estate Winery, ON, Canada
Tetra Pak Prisma aseptic carton	multilayer carton [layers, from inner to outer, polyethylene (PE), PE, aluminum foil, PE, paper, PE]	Tpk	Lanpak Ltd., Andrew Peller Ltd., ON, Canada

screw-cap closures (*18*). Other smaller scale research has examined vanillin permeation through both natural and synthetic corks (*19*) and thiol interactions with screw-cap closures and natural corks (*20*). These studies share common conclusions: that many volatile compounds important in wine aroma show an affinity for synthetic and natural corks that occurs relatively quickly, is more pronounced with synthetic corks than with natural corks, and is not observed in screw caps. Although data on flavor scalping from the popular Tetrapak cartons do not appear in the literature, wine cask bladders ("bag-in-box"), which are made of comparable polymer materials, display marked capacity for flavor scalping of nonpolar compounds (*21*). Additionally, we are not aware of any literature on flavor scalping of MPs in juice or wine.

Winemakers have available to them a range of closure and packaging options that could potentially reduce—or contribute to—MP concentrations in wine. Additionally, Tetrapak cartons (Tpk) have not been investigated with respect to flavor scalping properties, somewhat surprisingly given their widespread use in the wine industry. Therefore, this study examines the effects of a range of commonly used closures and Tetrapak cartons on MP and other key volatile compounds in Riesling and Cabernet Franc wines over 18 months of bottle aging.

MATERIALS AND METHODS

Preparation of Wines and Materials. Riesling and Cabernet Franc wines were chosen for this study due to their importance to the Ontario wine industry and because Cabernet Franc has not previously been investigated for flavor scalping. Filtered, stabilized, bulk wine from the 2006 vintage from grapes grown in the Niagara Peninsula, Ontario, was acquired from a commercial winery (Vincor, St. Catharines, ON). The basic initial chemical composition (±SD) of the Riesling and Cabernet Franc wines, was, respectively, as follows: titratable acidity (g/L), 7.88 ± 0.38, 4.13 ± 0.00; reducing sugars (g/L), 3.86 ± 0.04, 3.01 ± 0.02; ethanol (% v/v), 8.68 ± 0.97, 11.43 ± 0.17; free SO_2 (mg/L), 26.4 ± 0.8, 19.6 ± 2.0; and pH, 2.83 ± 0.04, 3.72 ± 0.00. Measurements were determined after Iland et al. (*22*), except for ethanol, which was measured by gas chromatography−flame ionization detection per ref *23*. The MP concentrations (ng/L) in the base Riesling and Cabernet Franc wines were, respectively, IPMP, 9.0, 17.4; SBMP, 6.7, 2.6; and IBMP, 9.6, 26.4. The method used for MP determination is given below.

IPMP, SBMP, and IBMP were acquired from Sigma-Aldrich, Oakville, ON (97, 99, and 99% purity, respectively). To ensure sensorially relevant concentrations (*5*, *25*) sufficient for quantification over this longitudinal study, 30 ng/L of each MP was added to the base wines. Each MP was first diluted with HPLC grade methanol and subsequently with Milli-RO water and allowed to equilibrate over 24 h, with regular stirring. Equipment and glassware were thoroughly cleaned prior to use to avoid contamination. Deuterated analogues of the MPs ([$^2H_3$]-IPMP, [$^2H_3$]-SBMP, [$^2H_3$]-IBMP) were synthesized for use as internal standards as described in ref *24*.

Four cork-type closures, a roll-on-tamper-evident (ROTE) screw cap, and a Tetrapak carton were chosen for investigation because they are closure/packaging types commonly used in the wine industry and represent a range of material types (**Table 1**). Additional SO_2 was added (5 mg/L to Riesling and 20 mg/L to Cabernet Franc) as potassium metabisulfite, to achieve adequate SO_2 protection against oxidative and microbial spoilage (*43*). Wines were filled into 750 mL glass Bordeaux bottles (Vineco, St. Catharines, Ontario) and closed using standard commercial practices. Wines for finishing in Tpk were filled manually. All bottled and Tpk-packaged wines were stored in a wine cellar (14−16 °C) until required for analysis.

Analysis. Duplicate samples (two new bottles/cartons) were retrieved from the cellar 3, 6, 12, and 18 months after bottling. Samples were poured into 125 mL Nalgene HDPE bottles (Sigma-Aldrich, Oakville, ON) under nitrogen gas, and sample bottles were tightly closed and covered with laboratory film (Parafilm "M", Pechiney Plastic Packaging, Chicago, IL) and immediately frozen (−14 °C) for later analysis.

3-Alkyl-2-methoxypyrazines. MPs were determined from thawed samples taken at bottling and 3, 6, 12, and 18 months using a stable isotope dilution method that uses headspace−solid-phase microextraction (HS-SPME) coupled to gas chromatography−mass spectrometry (GC-MS) as detailed in ref *24* and summarized below.

Sample Preparation and Extraction. Samples were prepared with a mixture of isotopically labeled internal standards ([$^2H_3$]-IPMP, [$^2H_3$]-SBMP, and [$^2H_3$]-IBMP in methanol) to achieve 40 ng/L of each internal standard, basified with NaOH, to increase the pH to approximately 6.6 and Milli-RO water for a 2.5-fold dilution. Two 10-mL portions of this solution were poured into glass cylinders that contained approximately 30% w/v sodium chloride (Caledon, Hamilton, ON) to improve phase transfer and a small stir bar and were sealed with a rubber septum for preservation. The sample was then extracted for 30 min with stirring (1100 rpm) at 40 °C on a HS-SPME fiber (StableFlex divinylbenzene/carboxen/PDMS; Supelco, Oakville, ON) inserted through the septum into the headspace of the vial. After extraction, the fiber was carefully retracted and inserted into the GC-MS (Agilent 6890GC/5975B with an HP-5MS 5% phenyl methyl siloxane column (30 m, 0.25 mm i.d., 0.25 μm film thickness), Agilent, Oakville ON) inlet for sample desorption and analysis. The GC-MS program was as follows: in splitless mode, the injector was held with no purge at 250 °C for 5 min for sample desorption and then purged at 50 mL/min for 5 min to clean the fiber. The oven remained at 40 °C for 5 min, ramped at 3 °C/min to 110 °C, held for 1 min, and ramped at 25 °C/min to 230 °C. Helium was used as the carrier gas at constant pressure (10.36 psi) with a nominal initial flow (1.2 mL/min). The MSD interface was held at 250 °C, whereas the temperature of the ion source was 200 °C.

Identification and Quantification. Identification was achieved using selected ion monitoring. For IPMP and [$^2H_3$]-IPMP, respectively, selected mass channels were m/z 137 and 152 and m/z 140 and 155. Ions 137 and 140 were used for quantification, whereas ions 152 and 155 were used as qualifier ions. For SBMP and [$^2H_3$]-SBMP, respectively, selected mass channels were m/z 138 and 124 and m/z 141 and 127. Ions 124 and 127 were used for quantification, whereas ions 138 and 141 were used as qualifier ions. For IBMP and [$^2H_3$]-IBMP, respectively, selected mass channels were m/z 109 and 124 and m/z 112 and 127. Ions 124 and 127 were used for quantification, whereas ions 109 and 112 were used as qualifier ions. All samples were analyzed in duplicate. Area ratios (area of a MP peak/area of corresponding [$^2H_3$]-MP) were calculated from chromatograms and correlated to concentration, based on a standard curve. Three standard curves were developed separately for each MP at various points over the analysis period. Standards were prepared in a model wine (12.0% v/v ethanol, 4.0 g/L tartaric acid, pH 3.5) and extracted in an identical fashion to wine samples. The first curve was based on six MP concentrations (3, 6, 12, 24, 30, and 40 ng/L) and the second two curves on seven (3, 12, 24, 30, 40, 60, and 80 ng/L). The ranges of R^2 values for the linear regression equations were, for IPMP, SBMP, and IBMP, respectively, 0.994−0.998, 0.995−0.997, and 0.990−0.998. The limit of detection for MPs was 1−2 ng/L, and the limit of quantitation was 2−5 ng/L.

Indicator Volatiles. Indicator compounds were chosen to represent the most important classes of wine volatiles on the basis of those previously reported in surveys of a wide range of varietal wines (*26*, *27*). Commercial preparations were obtained (Sigma-Aldrich, Oakville, ON) for five esters with different alkyl groups [phenethyl acetate, isoamyl acetate; ethyl hexanoate, ethyl caprylate (ethyl octanoate), ethyl caprate (ethyl decanoate)], an alcohol (phenylethanol), and a volatile acid (octanoic acid). Indicator volatiles were determined at 3 and 12 months using solid-phase extraction (modification of ref *7*) coupled to GC−flame ionization detection using a single chromatographic run (modification of ref *27*). Three internal standards were selected, which are absent in wines, having chemical similarity to indicator volatiles and distinct elution times: 3-ethyl-2-hydroxyvalerate (for esters), 3-octanol (for phenylethanol), and heptanoic acid (for octanoic acid).

Sample Preparation and Extraction. Samples were prepared with internal standards (1.90 mg/L 3-ethyl-2-hydroxyvalerate, 32.5 mg/L 3-octanol, and 10 mg/L heptanoic acid in HPLC grade methanol) and extracted. The concentrations of internal standards were based on previously reported values for each of the compound classes (*26*). A C-18, reversed phase column (SupelCLEAN, Sigma-Aldrich) was used to extract samples/standards by first conditioning the column (1 mL each of ethyl acetate, 95% v/v methanol, and 10% v/v methanol), then passing 25 mL of wine sample/standard, drying the column for 10 min, and finally passing and collecting two 1-mL aliquots of dichloromethane. All samples were concentrated under a nitrogen gas stream to a consistent volume of 0.5 mL. The extract was then injected into the GC-FID (Agilent GC6890 with DB-Wax, 30 m × 0.255 mm × 0.25 μm; J&W Scientific, Oakville, ON). The GC-FID oven program was as follows: initially 60 °C, ramped at 3.0 °C/min to 200 °C, and then ramped at 15.0 °C/min to 230 °C.

Sample Quantification. Chromatograms were integrated and the peak height ratios (peak height for target compound/peak height for internal standard) were determined and concentrations calculated from calibration curves. A five-point calibration series was used for each compound, ranging from 0.05 to 0.80 mg/L for the esters, from 2.50 to 120 mg/L for phenylethanol, and from 0.50 to 12.0 mg/L for octanoic acid. Standards were prepared in a deodorized wine matrix to mimic actual wine composition. Deodorized wines were prepared by adding 1.5 g/L activated charcoal (Sigma-Aldrich) to a white wine (2006 Pinot Grigio, Andrew Peller Ltd., ON), stirring for approximately 24 h, and filtering the solution through a 0.45 μm filter paper. This process was repeated two or three times as necessary to remove volatiles, as verified by GC-FID, without affecting general wine chemistry parameters, as verified by WineSCAN analysis (*44*, *45*) (data not shown). Average R^2 values for the calibration curves were phenethyl acetate, 0.987; ethyl caprate, 0.984; ethyl caprylate, 0.987; ethyl hexanoate, 0.983; isoamyl acetate, 0.982; phenylethanol, 0.947; and octanoic acid, 0.999.

Other Analytes. General wine chemistry parameters were determined at bottling and after 12 months to elucidate potential changes in basic wine chemistry using the methods of ref *22*: pH [by standardized pH-meter (AB15 Plus Accumet Basic, Fisher Scientific, ON)], titratable acidity (titrated with 0.1 M NaOH to an 8.2 end point), spectrophotometric measures for red and white wines (Genesys 2 spectrophotometer), and free and bound SO_2 by the aspiration method. Determinations were performed in duplicate or triplicate.

Reproducibility and Variability of Analysis. Accuracy and reproducibility of the MP determinations were monitored by quantifying standards of known concentration and by replicate analysis of each wine. After approximately every 15 samples, standards were analyzed to verify methods. The relative standard deviations (RSDs) for standards were IPMP, 5.2%; SBMP, 5.4%; and IBMP, 1.8%. Average RSDs from duplicate measurements across all wine samples for all volatile compounds were IPMP, 7.0%; SBMP, 7.7%; IBMP, 5.7%; phenethyl acetate, 3.7%; ethyl caprate, 1.6%; ethyl caprylate, 2.8%; ethyl hexanoate, 5.5%; isoamyl acetate, 6.0%; phenylethanol, 4.6%; and octanoic acid, 4.1%. Standard and sample RSDs for MPs are consistent with data from ref *24*.

Data Treatment. All statistical analyses were performed using XLSTAT-Pro 2008 (Addinsoft, Paris, France). Data were pooled for each analyte for analysis of variance (ANOVA) to test for effects between closures/packages at specific time points and also between times for specific closure types. Bottle replicate was included in all ANOVA tests as a qualitative variable. Fisher's protected least significant difference $(LSD)_{0.05}$ was used as the means separation test. Principal component analysis (PCA) and correlation analysis (R^2) were conducted on all data at 12 months.

RESULTS AND DISCUSSION

3-Alkyl-2-methoxypyrazines. MPs were quantified in wines at bottling and after 3, 6, 12, and 18 months (**Figure 1**).

3-Isopropyl-2-methoxypyrazine. IPMP is associated with perceptions such as "green pea" and "earthy" (*28*), is the second most prevalent MP present naturally in grapes, and is the causal compound in LBT (*7*). During the course of this trial, IPMP concentrations varied among closure/packaging options. IPMP concentrations tended to be lower after 12 and 18 months in Cabernet Franc for all closures, but a similar pattern was not observed for Riesling. The greatest sustained decrease from initial concentrations was with Tpk, for which values were 23 and 41% lower in Riesling and Cabernet Franc wines, respectively. On occasion, particularly with Agl and Scap after 6 months, IPMP concentration was higher than the initial value, possibly suggesting a contribution from the closure.

A closure's ability to contribute volatile compounds to wine has been established for trichloroanisoles (*29*) and also for 2-methoxy-3,5-dimethylpyrazine, an MP constituent of fungally infected corks (*30*). However, to our knowledge, IPMP, SBMP, and IBMP have not been investigated in this regard, although Allen et al. (*2*) suggest postbottling contamination as one source of IPMP in wine. Pickering et al. (*7*) reported an average 39% decrease in IPMP in white and red wines affected by *H. axyridis* over 10 months of aging in wines closed with molded synthetic corks. Here, we observe 10 and 21% decreases after 18 months from concentrations at bottling for white and red wines, respectively, closed with Syn-M. The discrepancy may be related to the different brands of Syn-M used in the two studies and/or the less accurate method (solid-phase extraction without use of deuterated internal standards) used in the former study.

3-*sec*-Butyl-2-methoxypyrazine. SBMP is the least studied of MPs found in wine. Naturally present in grapes at lower concentrations than other MPs, SBMP may still play a role in wine aroma, due to an enhancement effect or other sensory interactions (*31*). Closure/packaging types affected SBMP concentration in a similar way in both wines over time. Concentrations of SBMP were only lower than initial levels in Tpk wines (average decrease of 27%). Values between 3 and 12 months were most stable for NatC (Riesling) and Scap (Cabernet Franc). However, and most notable, SBMP concentrations increased at some time points for most closures, again perhaps suggesting contribution from the closure itself.

3-Isobutyl-2-methoxpyrazine. IBMP, the most prevalent MP present naturally in grapes, is associated with "bell pepper" aroma (*32*) and is the most studied in the wine literature. Concentrations in both Cabernet Franc and Riesling responded similarly to closure/packaging types. Overall, IBMP decreased significantly in all conditions. After 18 months, the greatest decrease was observed in Tpk and Syn-M, and the smallest change was in NatC (Riesling) and Scap (Cabernet Franc). A marked or sustained increase is not observed for any closure/packaging options. Endogenous IBMP concentration has previously been reported as stable during wine storage (*18*, *33*). It is possible that our method has allowed for a more accurate assessment of IBMP changes

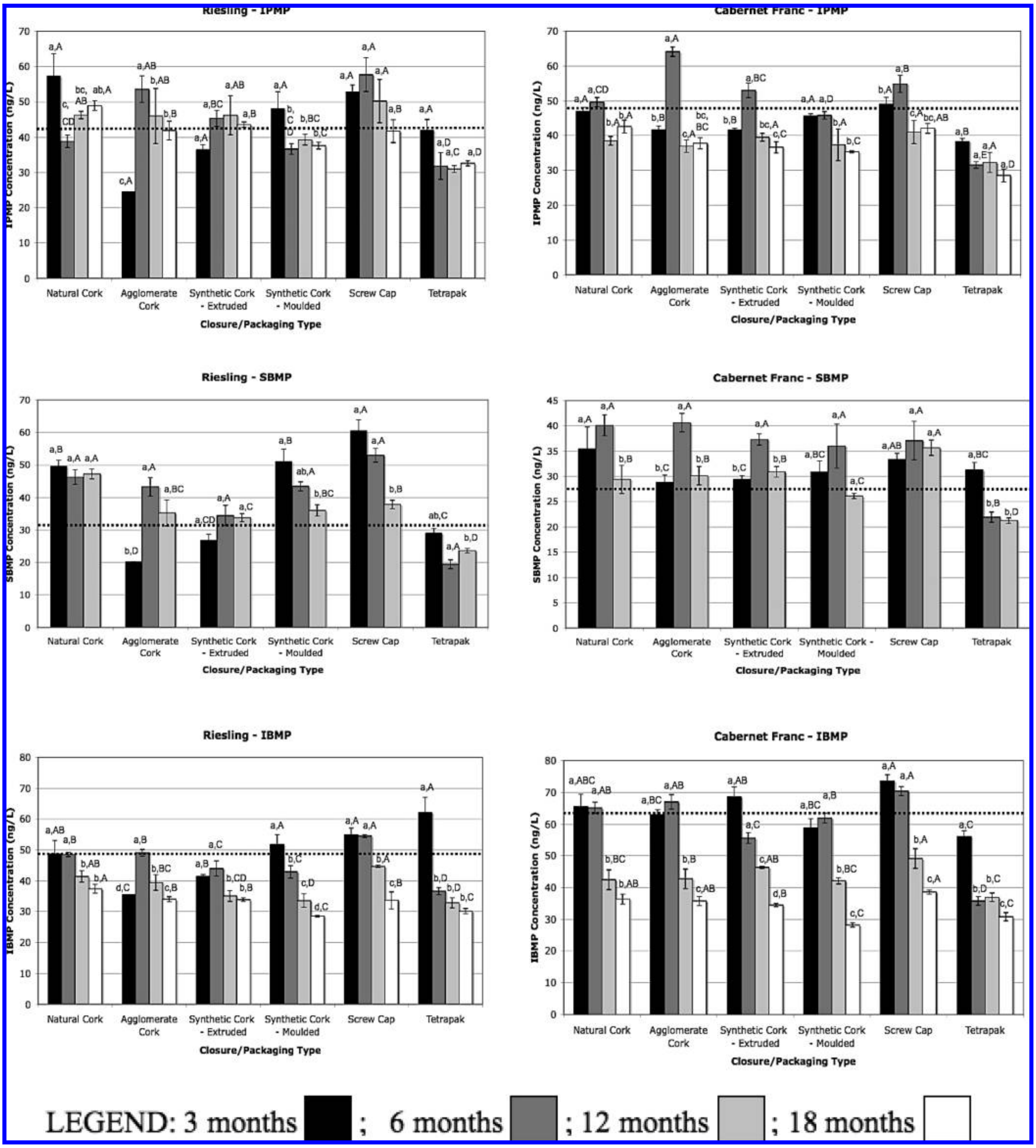

Figure 1. Concentration of 3-alkyl-2- methoxypyrazines (MP) in Riesling and Cabernet Franc wines spiked with 30 ng/L of isopropyl-, *sec*-butyl-, and isobutyl-MP. Data represent mean values of duplicate or triplicate measurements of duplicate bottles ± SEM. Means sharing the same letter do not differ significantly across time (lower case) or at a specific time point (upper case) (Fisher's protected $LSD_{0.05}$). Dashed line indicates initial MP concentration at bottling.

during storage, and/or the elevated concentrations in our wines have negated potential sensitivity issues in prior studies in which levels were closer to the limits of quantitation.

Overall, all three MPs were affected by closure/packaging types to some extent. After 18 months, the greatest decrease is consistently observed in Tpk, followed by Syn-M, and the highest final concentrations were observed in Scap and/or NatC (Riesling only). Odor detection thresholds in red wine for IBMP and IPMP are 3−10 ng/L (*4*, *11*) and 1−2 ng/L, respectively (*10*, *34*), and can be even lower for white wine (*10*). Detection thresholds for SBMP are estimated to be similar to those of other MPs (*13*). This high sensitivity of humans to MPs suggests that the concentration differences observed here between some closure/packaging types may be perceptible. Sensory analysis is required to confirm this speculation, and determination of difference thresholds for MPs in wine would be of value.

Interestingly, MP concentrations in Tpk decreased between 3 and 6 months after bottling, after which they remained stable

Table 2. Indicator Volatile Concentrations in Riesling (Milligrams per Liter) after Bottle Aging[a]

analyte	time	natural cork	agglomerate cork	synthetic cork, extruded	synthetic cork, molded	screw cap	Tetrapak
phenethyl acetate	bottling	0.213 ± 0.000					
	3 months	0.145bc ± 0.003	0.168a ± 0.008	0.150bc ± 0.001	0.154b ± 0.006	0.154bc ± 0.002	0.145c ± 0.004
	12 months	0.141a ± 0.001	0.141a ± 0.000	0.140a ± 0.002	0.130b ± 0.006	0.130b ± 0.006	0.118c ± 0.001
ethyl caprate	bottling	0.141 ± 0.002					
	3 months	0.137a ± 0.003	0.106b ± 0.003	0.139a ± 0.001	0.142a ± 0.005	0.139a ± 0.002	0.138a ± 0.004
	12 months	0.111c ± 0.001	0.108d ± 0.002	0.112 cd ± 0.003	0.121b ± 0.007	0.123b ± 0.010	0.140a ± 0.001
ethyl caprylate	bottling	0.207 ± 0.011					
	3 months	0.236a ± 0.008	0.127d ± 0.015	0.198b ± 0.015	0.155c ± 0.039	0.089e ± 0.000	0.167c ± 0.046
	12 months	0.108c ± 0.009	0.120c ± 0.006	0.101c ± 0.006	0.120c ± 0.018	0.202b ± 0.050	0.227a ± 0.014
ethyl hexanoate	bottling	0.214 ± 0.005					
	3 months	0.194ab ± 0.014	0.163c ± 0.031	0.172bc ± 0.014	0.175abc ± 0.013	0.194ab ± 0.006	0.197a ± 0.007
	12 months	0.152d ± 0.002	0.170c ± 0.006	0.128e ± 0.009	0.176bc ± 0.014	0.182ab ± 0.017	0.188a ± 0.008
isoamyl acetate	bottling	0.571 ± 0.008					
	3 months	0.338ab ± 0.021	0.252c ± 0.052	0.323b ± 0.019	0.351ab ± 0.033	0.376a ± 0.007	0.325b ± 0.018
	12 months	0.110c ± 0.001	0.130b ± 0.009	0.103d ± 0.002	0.138a ± 0.006	0.133ab ± 0.003	0.110 cd ± 0.005
phenylethanol	bottling	39.121 ± 0.461					
	3 months	36.812a ± 1.464	39.447a ± 4.348	36.823a ± 1.316	38.083a ± 1.005	38.655a ± 0.495	39.669a ± 1.227
	12 months	34.549c ± 1.408	37.506bc ± 1.667	35.232c ± 1.148	40.548ab ± 1.080	43.425a ± 2.195	42.436a ± 1.848
octanoic acid	bottling	6.139 ± 0.204					
	3 months	5.243a ± 0.124	3.480b ± 0.315	5.063a ± 0.168	4.856a ± 0.327	5.031a ± 0.225	5.309a ± 0.111
	12 months	3.966b ± 0.174	4.187b ± 0.264	3.298c ± 0.025	4.414b ± 0.173	5.444a ± 0.492	5.127a ± 0.189

[a] Data represent mean values of duplicate measurements of duplicate bottles ± SEM. Means sharing the same letter do not differ significantly at specific time points (Fisher's protected $LSD_{0.05}$).

for both wines and all MP species. The Tpk material in contact with wine (polyethylene) is well-known to remove flavor compounds through FS (*16*). We speculate that the decrease in MPs may result from their migration to the aluminum surface layer of the container, resulting in adsorption on the surfucuial oxide layer. Surprisingly, given its ubiquitous use, no peer-reviewed literature exists on the influence of Tpk or other multilayer aseptic cartons on wine volatile composition. In wines closed with NatC, all MPs (Cabernet Franc) or IBMP (Riesling) was stable between 3 and 6 months, decreased by approximately 20%, and then were stable again. This trend may be associated with the ingress of oxygen into wine. Lopes et al. (*35*) have reported that oxygen ingress occurs during the first 12 months of storage from within natural corks, after which only trace amounts migrate from the atmosphere to interact with the wine. By contrast, synthetic closures are permeable to atmospheric oxygen after the first month of bottling, whereas screw caps are essentially impermeable to atmospheric oxygen (*35*).

Indicator Volatiles. Indicator volatiles, selected to represent the main chemical classes of common wine odorants, were quantified in wines after 3 and 12 months (**Tables 2** and **3**). As expected, Riesling and Cabernet Franc wines had different initial concentrations of these constituents. Three main mechanisms expected to influence wine volatiles during storage are those that occur within the wine matrix, those that occur due to gas permeation, and those due to direct contact with closure/packaging type. Changes that occurred within the wine matrix, regardless of external factors, are distinguished by their prevalence across all closure/packaging types. For all conditions, acetate esters decreased the most, ethyl esters and octanoic acid either increased or decreased slightly, and phenylethanol remained relatively stable. Over time, changes can occur due to esterification and hydrolysis processes as wines re-establish equilibrium between the esters, alcohols, and acids present immediately after fermentation (*36*). Acetate esters tend to decrease because of the low concentration of acetic acid, whereas ethyl esters are usually at concentrations near their equilibrium following fermentation. Ethyl ester concentration may increase or decrease depending on the extent of ethylation of fatty acids that occurs during fermentation. Higher alcohols are generally stable during wine aging (*37*). These general trends were observed in this trial.

Relating the gas permeability of closures and packages from the literature and the indirect measures included in the present study to changes in volatile concentrations may discriminate effects related to oxygen ingress. Tetrapak cartons appear to have allowed a greater ingress of oxygen into the wines than the bottles, as evidenced by spectrophotometric (A_{420nm}) measurements and changes in free and total SO_2 (*38*, *39*) (**Figure 2**. This suggestion agrees with earlier findings (*38*). Tpk wines had the lowest concentration of acetate esters (in Riesling wine) and the highest concentration of ethyl esters after 12 months, but showed no clear trend for other volatiles. Synthetic closures allow for increased oxygen ingress compared to natural cork (*39*−*41*) and subsequent decrease in fruit aroma intensity, likely due to direct oxidative damage to flavor compounds or indirect masking by the formation of aldehydes (*39*). In the present study, we did not observe a consistent trend of lower concentrations of fruity esters with the synthetic closure types, perhaps due to the relatively low inherent concentration of some volatiles or the relatively short term of the trial.

Closures and packaging can also affect wine volatiles through direct contact through sorption or migration processes. The relative decrease after 3 months in all volatiles, except phenylethanol under Agl, Syn-M, and Tpk (Cabernet Franc), suggests some sorptive capacity compared to other closures. After 12 months, Agl (Cabernet Franc) and Syn-Ex (Riesling) show the lowest concentrations of ethyl hexanoate, ethyl caprylate (Riesling), isoamyl acetate, phenylethanol (Cabernet Franc), and

Table 3. Indicator Volatile Concentrations in Cabernet Franc (Milligrams per Liter) after Bottle Aging[a]

analyte	time	natural cork	agglomerate cork	synthetic cork, extruded	synthetic cork, molded	screw cap	Tetrapak
phenethyl acetate	bottling	1.021 ± 0.044					
	3 months	0.855a ± 0.040	0.855a ± 0.066	0.906a ± 0.016	0.696b ± 0.026	0.890a ± 0.031	0.742b ± 0.064
	12 months	0.371b ± 0.024	0.353b ± 0.009	0.361b ± 0.020	0.371b ± 0.017	0.400b ± 0.009	0.469a ± 0.050
ethyl caprate	bottling	0.105 ± 0.014					
	3 months	0.128a ± 0.001	0.121d ± 0.002	0.124bc ± 0.001	0.121 cd ± 0.001	0.127ab ± 0.001	0.108e ± 0.007
	12 months	0.090c ± 0.003	0.095b ± 0.000	0.090c ± 0.003	0.085d ± 0.000	0.091c ± 0.004	0.104a ± 0.000
ethyl caprylate	bottling	0.122 ± 0.024					
	3 months	0.169c ± 0.046	0.089e ± 0.000	0.145d ± 0.033	0.223b ± 0.006	0.249a ± 0.016	0.162c ± 0.023
	12 months	0.097bc ± 0.006	0.091c ± 0.000	0.104b ± 0.007	0.091c ± 0.000	0.137a ± 0.003	0.148a ± 0.035
ethyl hexanoate	bottling	0.233 ± 0.059					
	3 months	0.190bc ± 0.008	0.162d ± 0.012	0.176 cd ± 0.007	0.166d ± 0.008	0.211a ± 0.007	0.196ab ± 0.008
	12 months	0.187b ± 0.022	0.176b ± 0.005	0.190b ± 0.032	0.183b ± 0.014	0.246a ± 0.013	0.228a ± 0.015
isoamyl acetate	bottling	0.463 ± 0.052					
	3 months	0.355b ± 0.018	0.326bcd ± 0.031	0.345bc ± 0.027	0.289d ± 0.016	0.410a ± 0.006	0.311 cd ± 0.026
	12 months	0.246b ± 0.043	0.198c ± 0.007	0.263b ± 0.050	0.302a ± 0.022	0.308a ± 0.035	0.329a ± 0.024
phenylethanol	bottling	41.339 ± 6.479					
	3 months	35.107a ± 0.720	36.178a ± 1.380	36.345a ± 0.601	36.728a ± 1.524	34.412a ± 0.946	34.479a ± 1.620
	12 months	37.622a ± 3.470	31.139b ± 0.143	37.057a ± 4.166	41.182a ± 2.691	39.658a ± 3.520	38.792a ± 2.501
octanoic acid	bottling	1.967 ± 0.080					
	3 months	1.895bc ± 0.029	1.784d ± 0.069	1.949ab ± 0.047	1.822 cd ± 0.033	2.007a ± 0.026	1.577e ± 0.115
	12 months	1.708b ± 0.126	1.506d ± 0.024	1.599 cd ± 0.079	1.661bc ± 0.066	1.805a ± 0.147	1.698b ± 0.029

[a] Data represent mean values of duplicate measurements of duplicate bottles ± SEM. Means sharing the same letter do not differ significantly at specific time points (Fisher's protected $LSD_{0.05}$).

octanoic acid. Interestingly, no increase was observed for any of these volatile compounds, suggesting that migration from closures does not occur. Capone et al. concluded that closure type affects ester concentration such that ethyl esters are all partially absorbed as a function of increasing alkyl chain length, whereas small-chain esters are unaffected. They also showed that absorption of volatiles varied with closure type, with synthetic corks absorbing more than natural cork and no absorption observed with screw-cap closures (*39*). Additionally, Skurray et al. showed that nonpolar volatiles, such as vanillin, permeate synthetic cork to a greater extent than natural cork (*19*). The results of the present study agree with this literature; synthetic corks (Syn-M after 3 months and Syn-Ex after 12 months of storage) have an increased capacity for volatile sorption compared to natural corks and screw caps, although the natural cork-based Agl closure also displayed potential flavor scalping capacity. Overall, the sorption trends were not as clear as some earlier studies, perhaps due to different wine matrices, lower starting concentrations of the selected volatiles, or a shorter aging period.

Odor quality and detection thresholds (μg/L) for these compounds indicate the relevance of changes observed and are as follows: phenethyl acetate, rose/honey/spice, 250; ethyl caprate, fruity/grape, 200; ethyl caprylate, fruity, 5; ethyl hexanoate, apple peel/fruity, 14; isoamyl acetate, banana, 30; phenylethanol, rose/honey/spice, 14000; octanoic acid, cheesy/acid, 500 (*26*, *28*, *42*). All compounds except phenethyl acetate and ethyl caprate were present at suprathreshold intensity in both wines. Further investigations using descriptive analysis techniques may be useful to define the sensory impact of these closure/packaging options.

Other Analytes. Other analytes were quantified after 12 months and included spectrophotometric measures of wine color, phenolics, and free and bound SO_2. Titratable acidity and pH were also measured and did not vary over time or between closure/packaging types (data not shown).

Wines in Tpk had significantly higher A_{420nm} values—an indication of browning—compared with other options (62% higher in Riesling; 44% in Cabernet Franc). Cabernet Franc wine in Tpk also had significantly higher A_{520nm} values (an indication of red pigments), wine color density, and degree of red pigmentation (data not shown). Of the closures we examined, Agl values for these measures were highest. Contrasting with these results, Buiatti et al. found no differences between Tpk and bottle-finished white and red wines for phenolic, A_{420nm}, and A_{520nm} measurements after 24 months of aging.

SO_2 in the free form has both antimicrobial and antioxidant properties that can greatly affect wine quality (*22*), especially during storage. Tpk performed poorly for both free and bound SO_2 retention (**Figure 2**). Scap and NatC (Cabernet Franc) preserved the greatest amount of SO_2, and other closure types were intermediate after 12 months. Previous research on closures found that, in general, free SO_2 loss is greater with synthetic closures, intermediate with cork-type closures, and minimal with screw-capped wines (*39*, *40*). By contrast and in a Riesling wine, screw-cap closures and natural corks are reported to preserve SO_2 to similar extents (*20*). Our results generally agree with these findings, although Agl performed similarly to the synthetic closures. The loss of antioxidants, such as SO_2, is associated with a closure's oxygen permeability, but may also be mediated by its capacity to pick up O_2 during bottling. In previous closure trials, it has been suggested that a minimum of 10 mg/L free SO_2 is critical to protect against development of "oxidized" aroma and other negative quality attributes of white wine (*39*).

Principal Components and Correlation Analyses. Principal component and correlation analyses were conducted on pooled data for both wines after 12 months of aging. PCA of the Riesling

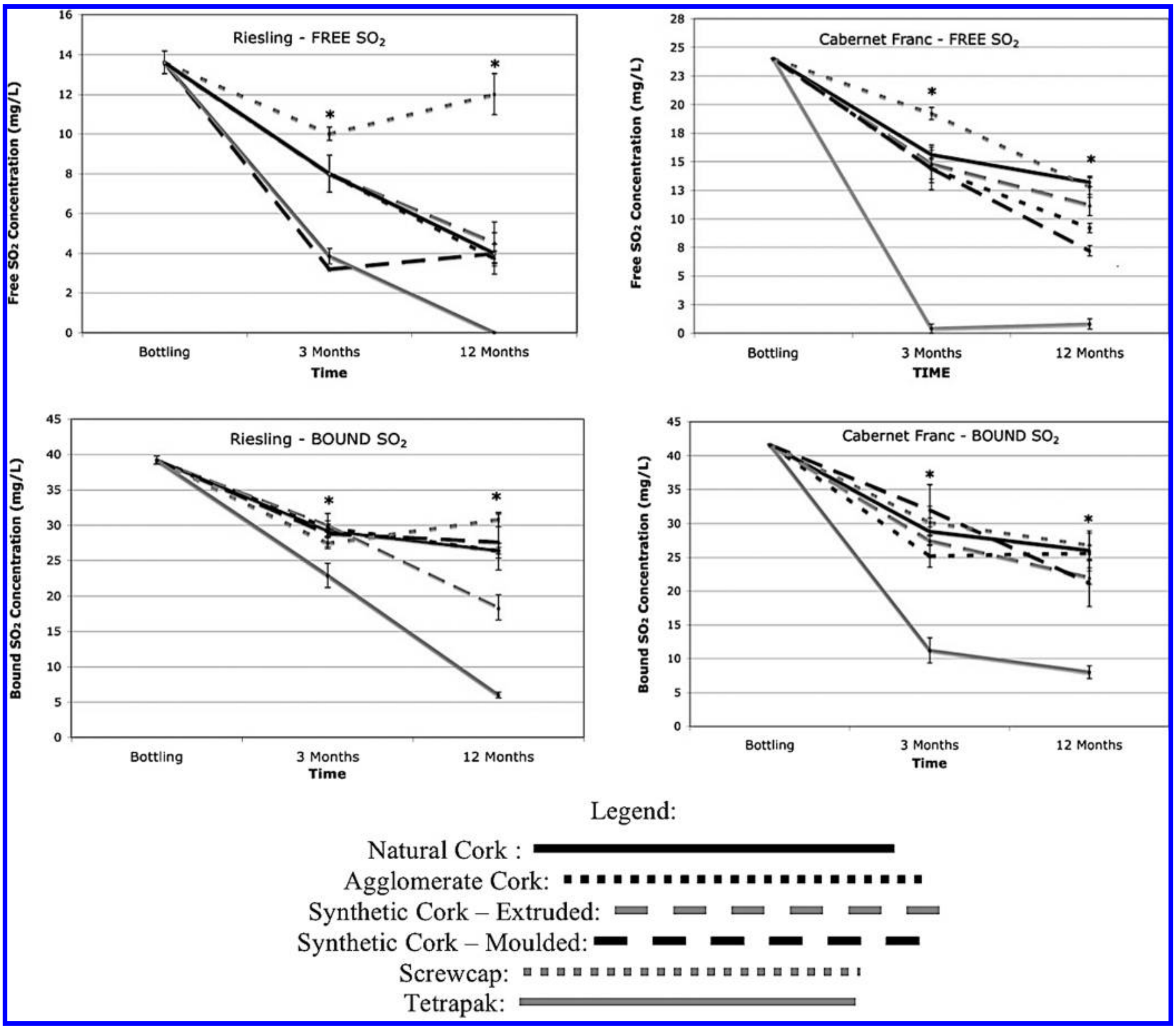

Figure 2. Free and bound sulfur dioxide in Riesling and Cabernet Franc wines. Data represent mean values of duplicate measurements of duplicate bottles ± SEM. An asterisk represents time points with significantly different means (Fisher's protected $LSD_{0.05}$).

wine (**Figure 3**) produced factors 1 and 2, which account for 78.5% of the variation. Factor 1 is defined by positive loadings for titratable acidity and ethyl caprate and highly negative loadings for IPMP and SBMP. Factor 2 is heavily loaded with spectral measures A_{280nm} (total phenolics) and A_{320nm} (total hydroxycinnamates) and isoamyl acetate. The closures/packaging options are well separated within the PCA space, with Tpk well discriminated on the basis of its high A_{420nm} values and low free and bound SO_2. The closures are separated along factor 2, with Scap and Syn-Ex at either end of the axes. Factors 3 and 4 help to further discriminate closure types. Scap is separated from other closure/packaging types on the basis of high concentrations for some volatile constituents, whereas NatC is separated from other closures on the basis of low titratable acidity and high SBMP eigenscores.

The first two factors from the PCA of Cabernet Franc (**Figure 4**) account for 74.5% of the variation. Factor 1 can be partly interpreted as an index of oxidation, with free and bound SO_2 loading negatively, and color density, red pigmentation, A_{420nm}, and A_{520nm} positively loaded. Factor 2 contrasts octanoic acid concentration with wine hue. Tpk is clearly separated from other closure/packaging types along factor 1. Agl is discriminated from other closure/packaging types on the basis of lower values for many of the volatile compounds. Scap-closed wine is well separated from other closures primarily on the basis of its negative association with wine hue. Factors 3 and 4 discriminate between NatC and Syn-Ex primarily on the basis of small differences in titratable acidity values.

Overall, Tpk and Scap were well-separated from other closure/packaging types by PCA, perhaps because they are the most physically different from other closures/packages. Scap was discriminated by its positive association with volatile concentration, consistent with other studies that have reported its efficacy at preserving wine volatile constituents during storage (*20*, *39*). Tpk correlated with A_{420nm} values and was inversely related to free and bound SO_2, phenomena consistent with the increased gas permeation that has previously been observed in Tetrapak cartons (*38*). As shown by the narrow angles of their respective eigenvectors (top plots, **Figures 3** and **4**), MPs were positively correlated to one another. Interestingly, MPs are positively correlated with both free and bound SO_2 in both wines (typical R^2 values range between 0.69 and 0.94), which may

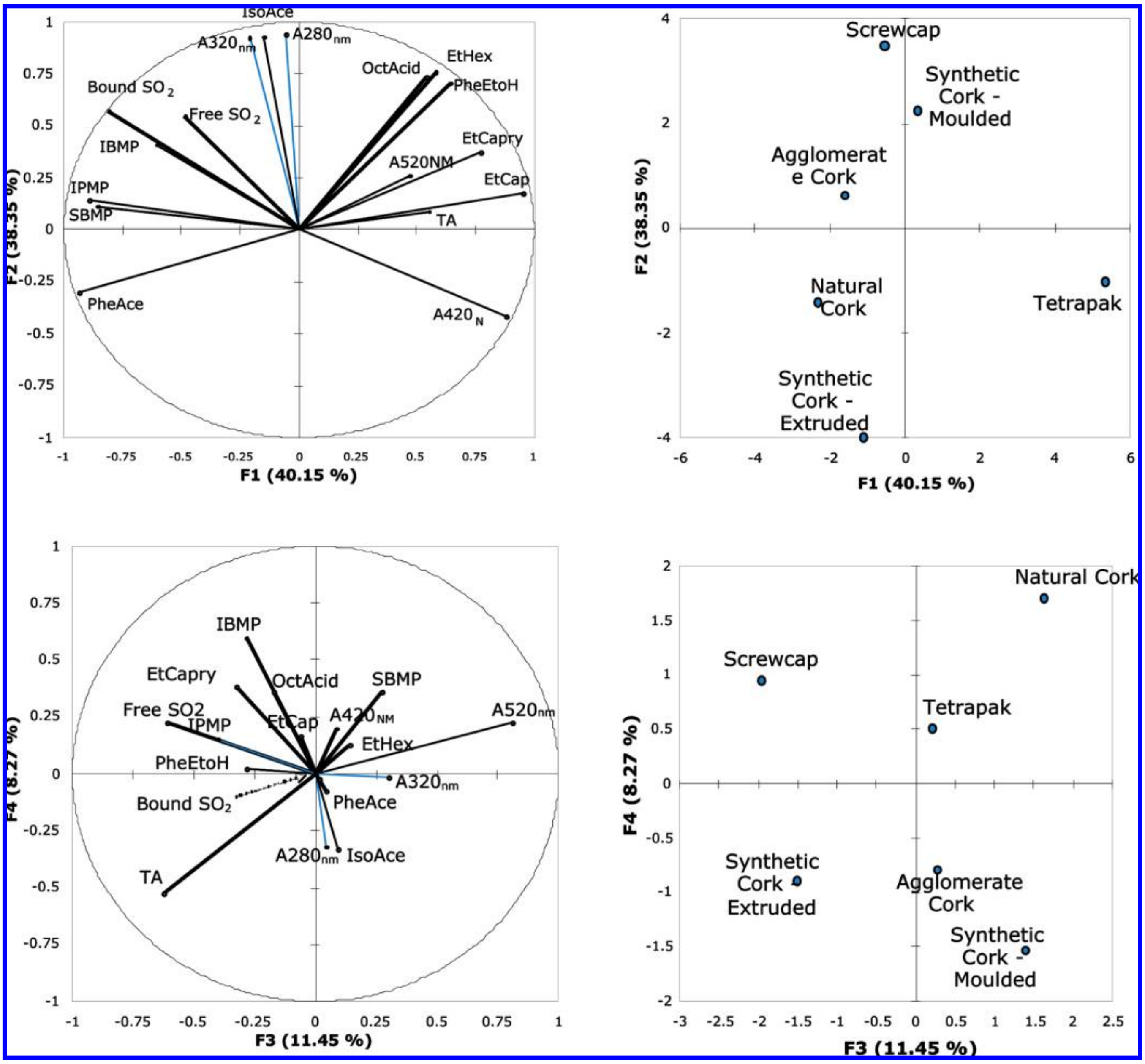

Figure 3. PCA biplot for Riesling wine after 12 months of bottle aging. Factor 1 versus factor 2 (top) and factor 3 versus factor 4 (bottom). Abbreviations: IPMP, 3-isopropyl-2-methoxypyrazine; SBMP, 3-*sec*-butyl-2-methoxypyrazine; IBMP, 3-isobutyl-2-methoxypyrazine; PheAce, phenethyl acetate; EtCap, ethyl caprate; EtCapry, ethyl caprylate; EtHex, ethyl hexanoate; IsoAce, isoamyl acetate; PheEtoH, phenylethanol; OctAcid, octanoic acid.

imply a role for oxygen ingress in MP loss. Ester concentrations were positively correlated with octanoic acid and each other, except for phenethyl acetate, which was negatively correlated with other volatile compounds in Riesling. As expected, spectrophotometric measures of phenolics were positively correlated in Riesling, and wine color measures were positively correlated in Cabernet Franc.

Other Considerations. Overall, the performance of Tpk differentiated it most from the other options, with most differences related to known correlates of elevated oxygen ingress (*38*−*40*). The only other study that we are aware of on Tpk concluded that these multilayer aseptic cartons contribute more oxygen to wines than glass (over a 2 year aging trial), although no significant differences were noted for spectrophotometric measures of phenolics or A_{420nm} and A_{520nm} values (*38*). The present data suggest that Tpk has significantly greater levels of oxygen ingress after 12 months; a timeline consistent with manufacturer information (Jim Dolson, Lanpak, Canada, personal communication) and Italian wine industry legislation (*38*). Tpk wines also had consistently lower concentrations of MPs from 3 months post-packaging, which may be related to flavor scalping processes or a higher level of gas permeability. Whereas this may suggest Tpk as a viable option for remediating wines with elevated MP levels postpackaging, sensory evaluation is required to fully characterize the effects, given that other differences in Tpk wines (e.g., browning and lower SO_2) are generally regarded as negative quality indicators. In future trials of this nature, direct monitoring of dissolved oxygen concentration in the wines would be beneficial. The possible contribution of interfering compounds to MP measurements cannot be ruled out. Further method optimization, including sample cleanup and qualifier ion selection, should be considered in future studies of these analytes during wine storage.

In summary, this research hypothesized that closure and packaging type will affect 3-alkyl-2-methoxypyrazine (MP) concentration and was conducted as a longitudinal trial in

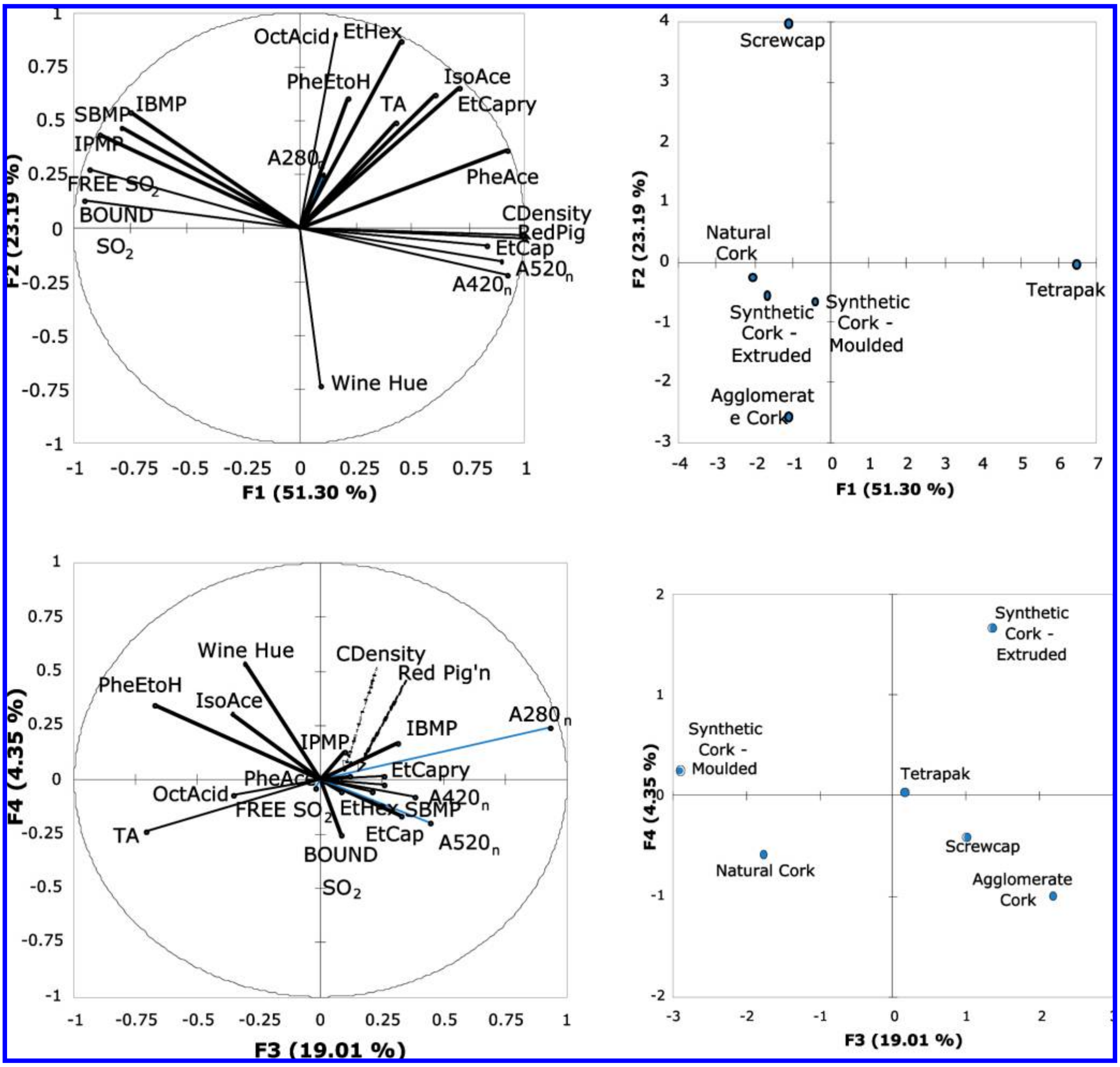

Figure 4. PCA biplot of Cabernet Franc wine after 12 months of bottle aging. Factor 1 versus factor 2 (top) and factor 3 versus factor 4 (bottom). Abbrevations: IPMP, 3-isopropyl-2-methoxypyrazine; SBMP, 3-*sec*-butyl-2-methoxypyrazine; IBMP, 3-isobutyl-2-methoxypyrazine; PheAce, phenethyl acetate; EtCap, ethyl caprate; EtCapry, ethyl caprylate; EtHex, ethyl hexanoate; IsoAce, isoamyl acetate; PheEtoH, phenylethanol; OctAcid, octanoic acid; CDensity, wine color density; RedPig, degree of red pigmentation.

Riesling and Cabernet Franc wines enriched with MPs. All three MPs were affected by closure/packaging type to some extent, with IBMP the most responsive. MP concentration in wines packaged in Tpk decreased the most, followed by the molded synthetic cork closure and screw cap, and natural cork closures retained the highest levels. Wine concentrations of IPMP and SBMP increased under some closure/packaging types after 3 and 6 months, indicating the capacity for some closures to contribute MPs to wine. Some of the indicator volatiles, chosen to represent major chemical classes of odor-active wine compounds, declined with aging, independent of closure/packaging type. Acetate esters showed the greatest decrease, whereas ethyl esters and phenylethanol were generally stable. Agglomerate cork, synthetic corks, and Tetrapak all showed some potential sorptive capacity for esters, higher alcohols, and volatile acids. Tetrapak was distinct from other closure/packaging types after 12 months for many basic wine physicochemical parameters, including SO_2 and A_{420nm}, and we speculate that greater ingress of oxygen accounts for this. Screw caps and natural cork generally preserved higher concentrations of free SO_2. The present study showed the capacity for closure/packaging options to mediate MP concentrations in wine and generally confirmed previous findings for other volatile species important to wine quality. Further study is required to elucidate changes due to direct contact with packaging material and those related to differential gas permeability and should include collection of sensory data.

ABBREVIATIONS USED

MP, 3-alkyl-2-methoxypyrazine; IPMP, 3-isopropyl-2-methoxypyrazine; SBMP, 3-*sec*-butyl-2-methoxypyrazine; IBMP, 3-isobutyl-2-methoxypyrazine; FS, flavor scalping; NatC, natural cork; Agl, agglomerate cork; Syn-Ex, synthetic cork, extruded variety; Syn-M, synthetic cork, molded variety; Scap, screw cap; Tpk, Tetrapak carton.

ACKNOWLEDGMENT

We sincerely thank Vincor, Ontario, for the donation of wine for this project. We thank the Henry of Pelham Family Estate Winery, Ontario, and Melissa Drouin, Lynda Van Zuiden, Kevin Ker, and Dr. Tomas Hudlicky, Brock University, for invaluable technical assistance.

LITERATURE CITED

(1) Parr, W.; Green, J.; White, K.; Sherlock, R. The distinctive flavour of New Zealand Sauvignon Blanc: sensory characterisation by wine professionals. *Food Qual. Pref.* **2007**, *18*, 849–861.
(2) Allen, M.; Lacey, M.; Boyd, S. Determination of methoxypyrazines in red wines by stable isotope dilution gas chromatography−mass spectrometry. *J. Agric. Food Chem.* **1994**, *42*, 1734–1738.
(3) Bogart, K.; Bisson, L. Persistence of vegetal characters in winegrapes and wine. *Practical Winery Vineyard* 2006, March/April, 13−22.
(4) Kotseridis, Y.; Beloqui, A.; Bertrand, A.; Doazan, J. An analytical method for studying the volatile compounds of Merlot Noir clone wines. *Am. J. Enol. Vitic.* **1998**, *49*, 44–48.
(5) Lacey, M.; Allen, M.; Harris, R.; Brown, W. Methoxypyrazines in Sauvignon Blanc grapes and wines. *Am. J. Enol. Vitic.* **1991**, *42*, 103–108.
(6) Chapman, D.; Thorngate, J.; Matthews, M.; Guinard, J.; Ebeler, S. Yield effects on 2-methoxy-3-isobutylpyrazine concentration in Cabernet Sauvignon using a solid phase microextraction gas chromatography/mass spectrometry method. *J. Agric. Food Chem.* **2004**, *52*, 5431–5435.
(7) Pickering, G.; Lin, J.; Reynolds, A.; Soleas, G.; Riesen, R.; Brindle, I. The influence of *Harmonia axyridis* on wine composition and aging. *J. Food Sci.* **2005**, *70*, S128–S135.
(8) Pickering, G.; Lin, J.; Riesen, R.; Reynolds, A.; Brindle, I.; Soleas, G. Influence of *Harmonia axyridis* on the sensory properties of white and red wine. *Am. J. Enol. Vitic.* **2004**, *55*, 153–159.
(9) Soares, A.; Borges, I.; Borges, P.; Labrie, G.; Lucas, E. *Harmonia axyridis:* what will stop the invader? In *Biological Control to Invasion: Ladybird Harmonia axyridis as a Model Species*; Roy, H., Wajnberg, E., Eds.; Springer: Amsterddam, The Netherlands, 2007; pp 127−145.
(10) Pickering, G.; Karthik, A.; Inglis, D.; Sears, N.; Ker, K. Determination of orth- and retro-nasal detection thresholds for 2-isopropyl-3-methoxypyrazine in wine. *J. Food Sci.* **2007**, *72*, S468–S472.
(11) Romero, R.; Chacon, J.; Garcia, E.; Martinez, J. Pyrazine content in four red grape varieties cultivated in a warm climate. *J. Int. Sci. Vigne Vin* **2006**, *40*, 203–207.
(12) Sala, C.; Busto, O.; Guasch, J.; Zamora, F. Influence of vine training and sunlight exposure on the 3-alkyl-2-methoxypyrazines content in must and wines from the *Vitis vinifera* variety Cabernet Sauvignon. *J. Agric. Food Chem.* **2004**, *52*, 3492–3497.
(13) Sala, C.; Busto, O.; Guasch, J.; Zamora, F. Contents of 3-alkyl-2-methoxypyrazines in musts and wines from a *Vitis vinifera* variety Cabernet Sauvignon: influence of irrigation and plantation density. *J. Sci. Food Agric.* **2005**, *85*, 1131–1136.
(14) Roujou de Boubee, D.; Cumsille, A.; Pons, M.; Dubourdieu, D. Location of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon grape bunches and its extractibility during vinification. *Am. J. Enol. Vitic.* **2002**, *53*, 1–5.
(15) Pickering, G.; Lin, J.; Reynolds, A.; Soleas, G.; Riesen, R. The evaluation of remedial treatments for wine affected by *Harmonia axyridis*. *Int. J. Food Sci. Technol.* **2006**, *41*, 77–86.
(16) Sajilata, M.; Savitha, K.; Singhal, R.; Kanetkar, V. Scalping of flavor in packaged foods. *Comp. Rev. Food Sci. Food Saf.* **2007**, *6*, 17–35.
(17) Capone, D.; Skouroumounis, G.; Barker, D.; McLean, H.; Pollnitz, A.; Sefton, M. Absorption of chloroanisoles from wine by corks and by other materials. *Aust. J. Grape Wine Res.* **1999**, *5*, 91–98.
(18) Capone, D.; Sefton, M.; Pretorius, I.; Hoj, P. Flavour 'scalping' by wine bottle closure—the 'winemaking' continues post vineyard and winery. *Aust. N. Z. Wine Ind. J.* **2003**, *18*, 16–20.
(19) Skurray, G.; Castets, E.; Holland, B. Permeation of vanillin through natural and synthetic corks. *Aust. Grape Growers Winemakers* 2000, Annual Technical Issue, 121−124.
(20) Brajkovich, M.; Tibbits, N.; Peron, G.; Lund, C.; Dykes, S.; Kilmartin, P.; Nicolau, L. Effect of screw cap and cork closures on SO_2 levels and aromas in Sauvignon Blanc wine. *J. Agric. Food Chem.* **2005**, *53*, 10006–10011.
(21) Pollnitz, A.; Capone, D.; Campbell, J.; Franke, S.; Mclean, H.; Skouroumounis, G.; Sefton, M. Some applications of analyses of volatile flavour compounds to wine. In *Proceedings of the 11th Australian Wine Industry Technical Conference*, Oct 7−11, 2001, Adelaide, South Australia; Blair, R., Williams, P., Hoj, P., Eds.; Australian Wine Industry Technical Conference: Glen Osmond, Australia, 2002; pp 162−164.
(22) Iland, P.; Bruer, N.; Edwards, G.; Weeks, S.; Wilkes, E. *Chemical Analysis of Grapes and Wine: Techniques and Concepts*; Patrick Iland Wine Promotions: Campbelltown, Australia, 2004; pp 40, 41, 56, 57, 65−67.
(23) Nurgel, C.; Pickering, G. J.; Inglis, D. Sensory and chemical characteristics of Canadian ice wines. *J. Sci. Food Agric.* **2004**, *84*, 1675–1684.
(24) Kotseridis, Y.; Spink, M.; Brindle, I.; Blake, A.; Sears, M.; Chen, X.; Soleas, G.; Inglis, D.; Pickering, G. Quantitative analysis of 3-alkyl-2-methoxypyrazines in juice and wine using stable isotope labelled internal standard assay. *J. Chromatogr., A* **2008**, *1190*, 294–301.
(25) Kotseridis, Y.; Baumes, R.; Bertrand, A.; Skouromounis, G. 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.
(26) Ferreira, V.; Lopez, R.; Cacho, J. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80*, 1659–1667.
(27) Ortega, C.; Lopez, R.; Cacho, J.; Ferreira, V. Fast analysis of important wine volatile compounds. Development and validation of a new method based on gas chromatography flame ionisation detection analysis of dichloromethane microextracts. *J. Chromatogr., A* **2001**, *923*, 205–214.
(28) Acree, T.; Arn, H. Isopropylmethoxypyrazine. *Flavornet© and Human Odor Space*, 2004; retrieved from http://www.flavornet.org/info/25773-40-4.html (Nov 25, 2008).
(29) Prak, S.; Gunata, Z.; Guiraud, J.; Schorr-Galindo, S. Fungal strains isolated from cork stoppers and the formation of 2,4,6-trichloroanisole involved in the cork taint of wine. *Food Microbiol.* **2007**, *24*, 271–280.
(30) Simpson, R.; Capone, D.; Sefton, M. Isolation and identification of 2-methoxy-3,5-dimethylpyrazine, a potent musty compound from wine corks. *J. Agric. Food Chem.* **2004**, *52*, 5425–5430.
(31) Allen, M.; Lacey, M.; Harris, R.; Vance Brown, W. Contribution of methoxypyrazines to Sauvignon Blanc wine aroma. *Am. J. Enol. Vitic.* **1991**, *42*, 109–112.
(32) Buttery, R.; Siefert, R.; Guadagni, D.; Ling, L. Characterization of some volatile constituents of bell peppers. *J. Agric. Food Chem.* **1969**, *17*, 1322–1327.
(33) Allen, M.; Lacey, M. Methoxypyrazines of grapes and wines. In *Chemistry of Wine Flavour*; Waterhouse, A., Ebeler, S., Eds.; American Chemical Society: Washington, DC, 1998; pp 31−38.
(34) Maga, J. Sensory and stability properties of added methoxypyrazines to model and authentic wines. In *Flavors and Off-Flavors, Proceedings of the 6th International Flavor Conference*, Rethymon, Crete, Greece July 5−7, 1989; Charalambous, G., Ed.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1989; pp 61−70.
(35) Lopes, P.; Saucier, C.; Teissedre, P.; Gloires, Y. Main routes of oxygen ingress through different closures into wine bottles. *J. Agric. Food Chem.* **2007**, *55*, 5167–5170.
(36) Clarke, R.; Bakker, J. In *Wine Flavour Chemistry*; Blackwell Publishing: Oxford, U.K., 2004; pp 179−180.
(37) Rapp, A.; Marais, J. The shelf life of wine: changes in aroma substances during storage and ageing of white wines. In *Shelf Life*

Studies of Foods and Beverages. Chemical, Biological, Physical and Nutritional Aspects; Charalambous, G., Ed.; Elsevier Science: Amsterdam, The Netherlands, 1993; pp 891−821.
(38) Buiatti, S.; Celotti, E.; Ferrarini, R.; Zironi, R. Wine packaging for market in containers other than glass. *J. Agric. Food Chem*. **1997**, *45*, 2081–2084.
(39) Godden, P.; Francis, L.; Field, J.; Gishen, M.; Coulter, A.; Valente, P.; Hoj, P.; Robinson, E. Wine bottle closures: physical characteristics and effect on composition and sensory properties of a Semillon wine 1. Performance up to 20 months post-bottling. *Aust. J. Grape Wine Res*. **2001**, 62–104.
(40) Francis, L.; Field, J.; Gishen, M.; Coulter, A.; Valente, P.; Lattey, K.; Hoj, P.; Robinson, E.; Godden, P. The AWRI closure trial: sensory evaluation data 36 months after bottling. *Aust. N. Z. Grape Growers Winemakers* **2003**, 59–63.
(41) Lopes, P.; Saucier, C.; Glories, Y. Nondestructive colorimetric method to determine the oxygen diffusion rate through closures used in winemaking. *J. Agric. Food Chem*. **2005**, *53*, 6967–6973.
(42) Guth, H. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem*. **1997**, *45*, 3022–3026.
(43) Rankine, B. *Making Good Wine: A Manual of Winemaking Practice for Australia and New Zealand*; Pan Macmillan Australia: Sydney, Australia, 1998.
(44) Kupina, S. A.; Shrikhande, A. J. Evaluation of a Fourier transform infrared instrument for rapid quality-control wine analyses. *Am. J. Enol. Vitic*. **2003**, *54:2*, 131–134.
(45) Bevina, C. J.; Dambergs, R. G.; Fergusson, A. J.; Cozzolinob, D. Varietal discrimination of Australian wines by means of mid-infrared spectroscopy and multivariate analysis. *Anal. Chim. Acta* **2008**, *62* (1), 19–23.

Received for review November 30, 2008. Revised manuscript received April 8, 2009. Accepted April 9, 2009. Financial assistance was provided by the Natural Sciences and Engineering Research Council of Canada, the Wine Council of Ontario, and the Grape Growers of Ontario.