Food Chemistry 119 (2010) 935–944

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of light and temperature on 3-alkyl-2-methoxypyrazine concentration and other impact odourants of Riesling and Cabernet Franc wine during bottle ageing

A. Blake [a], Y. Kotseridis [d], I.D. Brindle [c], D. Inglis [a,b], G.J. Pickering [a,b,*]

[a] Department of Biological Sciences, Brock University, St. Catharines, Canada ON L2S 3A1
[b] Cool Climate Oenology and Viticulture Institute, Brock University, St. Catharines, Canada ON L2S 3A1
[c] Department of Chemistry, Brock University, St. Catharines, Canada ON L2S 3A1
[d] Department of Food Science and Technology, Agricultural University of Athens, Greece

ARTICLE INFO

Article history:
Received 28 January 2009
Received in revised form 2 July 2009
Accepted 29 July 2009

Keywords:
3-Alkyl-2-methoxypyrazines
Bottle hue
Storage temperature
Wine quality
Cabernet Franc
Riesling
Wine flavour
Ladybug taint
Harmonia axyridis

ABSTRACT

3-Alkyl-2-methoxypyrazines (MPs) are grape- and insect-derived odour-active compounds responsible for green and vegetative perceptions in wine, and at elevated concentrations are detrimental to wine quality. We examined the influence of light and temperature during cellaring on MPs and other impact odourants. Riesling and Cabernet Franc wines were supplemented with 30 ng/L of each of 3-isopropyl-2-methoxypyrazine (IPMP), 3-s-butyl-2-methoxypyrazine (SBMP) and 3-isobutyl-2-methoxypyrazine (IBMP), and stored under one of the following conditions: (i) at ambient temperature (22 °C) under fluorescent lighting, (ii) in the dark at ambient temperature, or (iii) in the dark at cellar temperature (12 °C). Additionally, for the light condition, wine was stored in clear, green or amber bottles.

MPs did not vary consistently over time under any of these light or temperature conditions. IBMP decreased over 12 month by approx. 30% under all conditions in both Riesling and Cabernet Franc. Acetate esters also decreased with time, regardless of light or temperature conditions, while phenethyl acetate and isoamyl acetate decreased at a greater rate at 22 °C compared with 12 °C. Free and bound SO_2 retention was higher in light-excluded conditions and influenced by bottle hue. Measures of browning and phenolic content were also affected to varying degrees by bottle hue and storage temperature. These results should assist winemakers in selecting bottle hues, and assist in selection of optimum storage conditions for preserving wine quality in both retail and cellar environments.

1. Introduction

Odour-active compounds are critical determinants of the flavour and overall quality of wine. 3-Alkyl-2-methoxypyrazines (MPs) are powerful, odourous components of many wines, where thresholds have been reported as low as 320 pg/L (Pickering, Karthik, Inglis, Sears, & Ker, 2007). 3-Isopropyl-2-methoxypyrazine (IPMP), 3-s-butyl-2-methoxypyrazine (SBMP) and 3-isobutyl-2-methoxypyrazine (IBMP) are secondary plant metabolites that are most abundant in grape *Vitis vinifera* varieties that originate from the Bordeaux region in France, especially Cabernet Sauvignon (Allen, Lacey, & Boyd, 1994; Kotseridis, Baumes, Bertrand, & Skouromounis, 1999). These MPs can contribute to the desired varietal character of certain wines (Parr, Green, White, & Sherlock, 2007), but, at higher levels, are responsible for vegetative and herbaceous aromas considered detrimental to wine quality (Allen et al., 1994). During the latter stages of grape maturation MPs degrade (Sala, Busto, Guasch, & Zamora, 2004), and their concentrations are higher in under-ripe fruit (Hashizume & Samuta, 1999; Kotseridis et al., 1999) and in grapes grown in cooler climates (Allen et al., 1994; Falcao et al., 2007). Elevated levels of MPs in wine can also be due to the lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae; *HA*). IPMP is a component of *HA* haemolymph, and is released by the beetles when they are inadvertently incorporated in with grapes at harvest (Pickering, Reynolds, Soleas, Riesen, & Brindle, 2005), producing off-flavours known as ladybug taint (LBT; (Pickering et al., 2004)). *HA* can be found in vineyards in large numbers around the time of commercial grape harvest (Koch, 2003), and are found in many winemaking regions of the world, including Italy, France, Spain, South Africa and Argentina (Soares, Borges, Borges, Labrie, & Lucas, 2007).

Pre-fermentation settling of white wines can reduce MP levels (Kotseridis et al., 2008), however, IPMP and SBMP are stable during fermentation and while IBMP levels initially increase with macer-

* Corresponding author. Address: Department of Biological Sciences, Brock University, St. Catharines, Canada ON L2S 3A1. Tel.: +1 905 688 5550.
E-mail address: gary.pickering@brocku.ca (G.J. Pickering).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.07.052

ation, they are also stable during fermentation processes (Sala et al., 2004). Some commercial yeast strains may even produce MPs during the fermentation (Pickering et al., 2008). They are generally resilient to standard wine fining practices (Pickering, Lin, Reynolds, Soleas, & Riesen, 2006), and recent investigations have shown some capacity for post-bottling modification by closures and packaging, likely through migration and sorptive processes (Blake et al., 2009). Thus, other approaches for remediation of high MP wines have been advocated.

Bottle hue, and the light and temperature conditions the wine is exposed to during storage and retail display, may represent additional opportunities for decreasing MP concentrations.

Both light exposure and increased storage temperature have previously been shown to affect some wine constituents (D'Auria, Emanuele, Mauriello, & Racioppi, 2003; Marais & Pool, 1980). In general, wavelengths in the UV spectrum and the blue portion of the visible spectrum (~350–500 nm) adversely affect wine and other food products (e.g., beer, milk), producing "light-struck" or "sunlight" sensory changes (D'Auria et al., 2003). In wine, these changes are believed to result from the photo-activation of riboflavin (vitamin B2), causing the formation of volatile sulphur compounds from sulphur-containing amino acid degradation and/or the selective decomposition of esters (e.g., beer)(D'Auria et al., 2003). The most common hues, clear, green and amber hues, transmit 95%, 50%, and 10% of 350–550 nm light, respectively (Selli, Canbas, & Unal, 2002). Temperature may also affect normal bottle-ageing through mediation of reaction rates (Marais & Pool, 1980). Increased storage temperature produces "quick-ageing" effects associated with advanced oxidation. These include volatile concentration approaching chemical equilibrium (Marais & Pool, 1980), yellow pigments increasing, and, in general, the concentration of phenolic compounds decreasing (Simpson, 1978).

Light and temperature have also been linked to MPs. Levels of MPs in grapes are significantly lower in mature fruit and/or fruit grown in warmer climates as a result of increased light exposure (Hashizume & Samuta, 1999; Ryona, Pan, Intrigliolo, Lakso, & Sacks, 2008) and/or temperature (Falcao et al., 2007). Additionally, IBMP and IPMP have been shown to photo-degrade in aqueous solution (Heymann, Noble, & Boulton, 1986), and IBMP is substantially reduced in red wine subject to thermo-vinification (Roujou de Boubee, 2004). Surprisingly, no research appears in the peer-reviewed literature informing the hypothesis that light, and its mediation by bottle hue, or storage temperature can influence MP composition in finished wines. In addition, information on how cellaring conditions might influence other impact odourants in wine is limited. These considerations form the basis of this study.

2. Materials and methods

2.1. Preparation of wine and materials

Riesling and Cabernet Franc wine from grapes grown in the Niagara Peninsula, Ontario, Canada, were used in this study based on their importance to both the local and global wine industries. Basic chemical composition of the base wines was determined using the methods of Iland, Bruer, Edwards, Weeks, and Wilkes (2004) except for ethanol analysis, which was determined using GC-FID (Nurgel, Pickering, & Inglis, 2004).

IPMP, SBMP and IBMP were acquired from Sigma–Aldrich, Oakville, ON, Canada (97%, 99%, and 99% purity, respectively). In order to achieve ecologically relevant concentrations (Kotseridis et al., 1999; Allen et al., 1994) sufficient for quantification over this longitudinal study, 30 ng/L of each MP were added to the base wines and then equilibrated for 24 h with regular manual stirring. After equilibration, wines were bottled. A further 5 mg/L (Riesling) or 20 mg/L (Cabernet Franc) of SO_2 (as potassium metabisulphite) was added immediately prior to bottling. Wines were bottled in 750 mL glass Bordeaux bottles (Vineco, St. Catharines, ON, Canada) and closed with Sterisun® natural corks (Scott Laboratories, Pickering, ON, Canada) using standard commercial practice. Bottles were placed upright for 7 days before storing horizontally for all conditions to allow time for closures to adjust to the bottle.

Three chambers were prepared for storage of the wines under specific lighting and temperature conditions: Condition 1 ("Light and Ambient Temp"), for examining the influence of clear, green and amber bottle hues at 22 °C; Condition 2 ("Dark and Ambient Temp") and Condition 3 ("Dark and Cellar Temp"), as represented in Fig. 1. Light exposure for Condition 1 was provided by 15 W compact fluorescent light bulbs (*Phillips Marathon*® Energy Saving Mini-Twister) placed ~40 cm above the bottles at 1 bulb per 10 bottles. Bottles in Condition 2 were stored in sealed cardboard wines cases at ambient temperature, and wines under Condition 3 were stored in sealed cases in a Uni-THERM© refrigerator (Grand Haven, MI, USA), at a constant temperature of 12°C.

2.2. Analysis

2.2.1. Sample preparation

Duplicate bottles were retrieved from chambers for analysis at 3, 6, and 12 months after bottling. Samples (100 mL) were poured into Nalgene® HDPE bottles (Sigma–Aldrich, Oakville, ON, Canada) under nitrogen gas, then bottles were closed, covered with laboratory film (Parafilm "M", Pechiney Plastic Packaging, Chicago, IL, USA) and promptly frozen for future analysis.

2.2.2. 3-Alkyl-2-methoxypyrazines

MPs were determined from thawed samples taken at bottling, 3, 6, and 12 months using a stable isotope dilution method that used headspace-solid-phase-microextraction (HS-SPME) coupled to gas chromatography–mass spectrometry (GC–MS) as detailed by Kotseridis et al. (2008) and summarised below.

2.2.2.1. Sample preparation and extraction. Samples were prepared with a mixture of isotopically-labelled internal standards ($[^2H_3]$-IPMP, $[^2H_3]$-SBMP and $[^2H_3]$-IBMP in methanol) to achieve 40 ng/L of each internal standard, and its pH was increased to approximately 6.6 using NaOH, 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, Canada) to improve phase transfer, and a small stirring 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 fibre (StableFlex©Divinylbenzene/Carboxen/PDMS; Supelco, Oakville, ON, Canada) inserted through the septum into the headspace of the vial. After extraction, the fibre 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, Canada) inlet for sample desorption and analysis. The GC–MS program was as follows: in splitless mode, the injector held with no purge at 250 °C for 5 min for sample desorption then purged at 50 mL/min for 5 min to clean the fibre. The oven remained at 40 °C for 5 min, ramped at 3 °C/min up to 110 °C, held for 1 min, and ramped at 25 °C/min up to 230 °C. Helium was used as the carrier gas at a constant pressure (10.36 ψ) with a nominal initial flow (1.2 mL/min). The MSD interface was held at 250 °C while the temperature of the ion source was 200 °C.

2.2.2.2. Identification and quantification. Identification was achieved using select ion monitoring. For IPMP and $[^2H_3]$-IPMP,

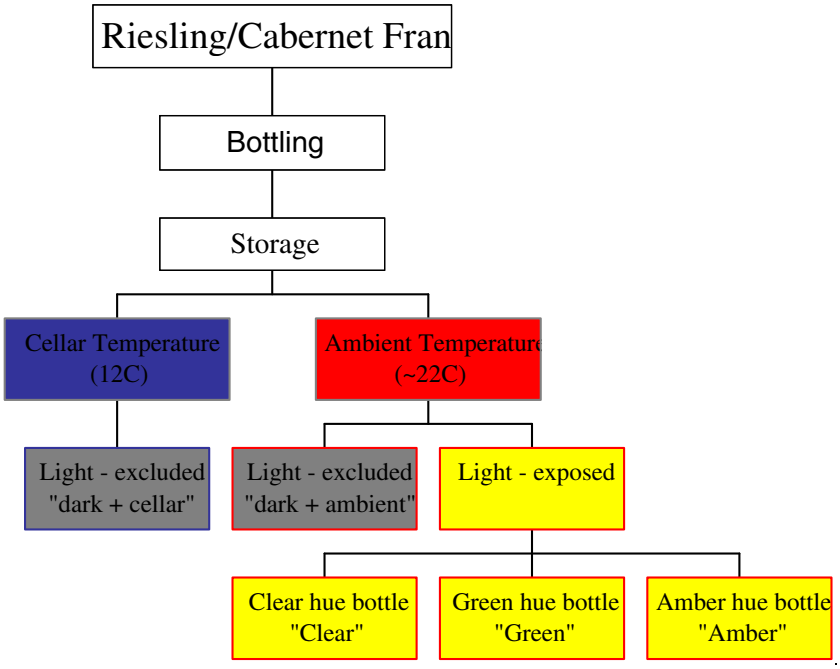

Fig. 1. Experimental design of light/temperature conditions for wine storage trial (modification of (24)).

respectively, selected mass channels were $m/z = 137$, 152 and $m/z = 140$, 155. Ions 137 and 140 were used for quantification, while ions 152 and 155 were used as qualifier ions. For SBMP and [$^2H_3$]-SBMP, respectively, selected mass channels were $m/z = 138$, 124 and $m/z = 141$, 127. Ions 124 and 127 were used for quantification, while ions 138 and 141 were used as qualifier ions. For IBMP and [$^2H_3$]-IBMP, respectively, selected mass channels were $m/z = 109$, 124 and $m/z = 112$, 127. Ions 124 and 127 were used for quantification, while ions 109 and 112 were used as qualifier ions. All samples were analysed 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. A 7-point standard curve with a concentration series ranging from 3 to 80 ng/L was prepared for each MP in a model wine (12.0% (v/v) ethanol, 4.0 g/L tartaric acid, pH 3.5). Standards were extracted and analysed in an identical fashion to wine samples. Average R^2 values for the respective linear regression equations were 0.994 for IPMP and IBMP and 0.993 for SBMP.

2.2.3. Indicator volatiles

Indicator compounds were chosen to represent the most important classes of potent wine volatiles based on those previously reported in surveys of a wide range of varietal wines (Ferreira, Lopez, & Cacho, 2000; Ortega, Lopez, Cacho, & Ferreira, 2001). Commercial preparations were obtained (Sigma–Aldrich, Oakville, ON, Canada) for five esters with different alkyl groups (phenethyl acetate, isoamyl acetate; ethyl hexanoate, ethyl caprylate (ethyl octanoate), ethyl caprate (ethyl decanoate)), an alcohol (phenyl ethanol) and a volatile acid (octanoic acid). Indicator volatiles were determined at 3 and 12 months using solid-phase-extraction (modification of Pickering et al., 2005) coupled to GC-flame ionisation detection using a single chromatographic run (modification of Lopez, Aznar, Cacho, & Ferreira, 2002). Three internal standards were selected which are absent in wines, chemically similar to indicator volatiles, and possess distinct elution times: 3-ethyl-2-hydroxy-valerate (for esters), 3-octanol (for phenyl ethanol) and heptanoic acid (for octanoic acid).

2.2.3.1. Sample preparation and extraction. Samples were prepared with internal standards (1.90 mg/L 3-ethyl-2-hydroxy-valerate, 32.5 mg/L octanol-3 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 (Ferreira et al., 2000). A C-18, reversed phase column (SupelCLEAN©, Sigma–Aldrich, Oakville, ON, Canada) 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 stream of nitrogen gas 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, Canada). The GC-FID oven program was as follows: initially 60 °C, ramped 3.0 °C/min to 200 °C, then ramped 15.0 °C/min to 230 °C.

2.2.3.2. Sample quantification. Chromatograms were integrated and the peak height ratios (peak height for target compound/peak height for internal standard) were determined and concentration calculated from calibration curves. A 5-point calibration series was used for each compound, ranging from 0.05 to 0.80 mg/L for the esters, 2.50–120 mg/L for phenyl ethanol and 0.50–12.0 mg/L for octanoic acid. Standards were prepared in a deodourized wine matrix to mimic actual wine composition. Deodourized wines were prepared by adding 1.5 g/L activated charcoal (Sigma–Aldrich, Oakville, ON, Canada) to a white wine (2006 Pinot Grigio, Andrew Peller Ltd., Grimsby, ON, Canada), stirring for approximately 24 h, and filtering the solution through a 0.45 μm filter paper. This process was repeated 2–3 times as necessary to remove volatiles, as verified by GC-FID, without affecting general wine chemistry parameters, as verified by WineSCAN© analysis (data not shown). Average R^2 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; phenyl ethanol: 0.947 and octanoic acid: 0.999.

2.2.4. 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 (34); pH (by standardised pH metre (AB15 Plus Accumet© Basic, Fisher Scientific, Nepean, ON, Canada)), titratable acidity (titrated with 0.1 M NaOH to an 8.2 endpoint), spectrophotometric measures for red and white wines (Genesys 2 spectrophotometer, California) and free and bound SO_2 by the aspiration method. Determinations were performed in duplicate or triplicate.

2.2.5. 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 sample. After approximately every 15 samples, standards were analysed to verify methods. The relative standard deviation (RSD) for standards was: IPMP: 3.5%; SBMP: 3.7%; IBMP: 3.1%. Average RSDs from duplicate measurements across all wine samples for all volatile compounds were; IPMP, 8.0%; SBMP, 7.1%; IBMP, 8.1%; phenethyl acetate, 3.0%; ethyl caprate, 2.0%; ethyl caprylate, 4.9%; ethyl hexanoate, 4.0%; isoamyl acetate, 3.3%; phenyl ethanol, 5.8% and octanoic acid, 3.6%. Standard and sample RSDs for MPs are consistent with data from Kotseridis et al. (2008).

2.2.6. Data treatment

All statistical analyses were performed using XLSTAT-Pro 2008 (Addinsoft, Paris, France). All data for each analyte underwent 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 Components Analysis (PCA) and Correlation Analysis (R^2) were conducted on all data at 12 months.

3. Results and discussion

Basic composition of the base wines were determined as described above. Values ± SD for Riesling and Cabernet Franc wines, respectively, were; 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 ± 0.91, 11.43 ± 0.16; free SO_2 (mg/L): 26.4 ± 0.8, 19.6 ± 2.0, and pH: 2.83 ± 0.04, 3.72 ± 0.00.

3.1. 3-Alkyl-2-methoxypyrazines

MPs were quantified in wines at bottling and after 3, 6, and 12 months (Fig. 2). Over 12 months, IPMP concentrations were relatively stable or displayed small decreases, and were not consistently affected by light and/or temperature conditions. However, after 12 months IPMP tended to be higher in light-excluded Riesling wines, and, within light treatments, higher in amber bottles compared with other hues for both Riesling and Cabernet Franc. SBMP concentration was unaffected by light or temperature during storage. In Riesling, an average increase of 8 ng/L above concentration at bottling is observed after 12 months, and an increase in IPMP concentration at 3 months in seen in two conditions. IBMP decreased with time in both wine styles regardless of light or temperature condition, which did not consistently affect concentrations.

The pattern of increase in IPMP and SBMP concentration in some wines is consistent with that reported by Blake et al. (2009) in wine closed with natural cork, and we speculate due to migration of these MPs from the closure. Migration of a methoxypyrazine from cork into wine has also been reported by other researchers (Simpson, Capone, & Sefton, 2004). Some evidence suggests that IBMP and IPMP photo-degrade in ripening grapes (Hashizume & Samuta, 1999; Ryona et al., 2008), although the mechanisms are unclear (Ryona et al., 2008). We found no consistent trend indicative of photodegradation of MPs in wine, consistent with Pickering et al. (2006) who reported no significant effect of UV or visible light on IPMP when wine was passed through a light reactor. It is possible that any light-mediated degradation of MPs may be obscured by their apparent migration from some natural cork closures into the wine.

Our data suggest that IBMP shows the greatest reduction during bottle-ageing, consistent with Blake et al. (2009) in their 18 months trial under cellar conditions. While sorption of MPs by cork has previously been noted and may account for some of this loss, it is unlikely sufficient, as alternative closures, including screw-caps, can demonstrate similar decreases during cellaring (Blake et al., 2009). It is possible that MPs, and particularly IBMP, are becoming incorporated into polyphenolic complexes, which may be affecting volatility. This speculation gains some support from the greater drop in IBMP concentration observed in (phenolic-rich) red wine in both Blake et al. (2009) and the current study.

3.2. Indicator volatiles

Indicator volatiles were quantified in wine at bottling and 3 and 12 months post-bottling (Fig. 3). The predominant changes occurring during bottle-ageing involve the transformation of volatile constituents as wines re-establish a chemical equilibrium between acids, alcohols and corresponding esters; reactions that have temperature-dependent rates (Marais & Pool, 1980).

In the present study, concentrations of acetate esters tended to decrease with storage time, regardless of condition, consistent with known equilibrium processes (Rapp & Marais, 1993). However, the acetate esters were most affected by storage conditions, with phenethyl acetate and isoamyl acetate decreasing at a greater rate in ambient temperature conditions compared with 12 °C. This may be due to enhanced ester hydrolysis, which increases linearly with temperature (Ramey & Ough, 1980) and has previously been reported to be most pronounced with acetate esters (Simpson, 1978). Ethyl esters, particularly ethyl hexanoate, tended to be stable with time and did not vary consistently with storage conditions. This result was expected, as ethyl esters are present in young wine at concentrations close to chemical equilibrium (Simpson, 1978) and hydrolyse relatively slowly (Ramey & Ough, 1980). Interestingly, ethyl caprylate in Riesling after 12 months was significantly higher in the Dark + Ambient temperature condition. Bottle hue did not influence acetate or ethyl ester composition.

Both phenyl ethanol and octanoic acid concentration are similar for all treatments and remain relatively stable over time. At 3 months light-exposed treatments tend to have higher concentrations of octanoic acid than wines stored under dark conditions, although this effect is not seen after 12 months. Again, bottle hue did not affect the concentration of these analytes. Overall, these data closely agree with the results from Marais and Pool (1980) who looked at temperature effects during storage of Chenin Blanc wine over 12 months.

3.3. Sulphur dioxide

Free and bound SO_2 measurements were taken at bottling and after 3 and 12 months ageing. Titratable acidity and pH were also measured and did not vary over time or between treatments (data not shown). Free and bound SO_2 retention was higher in light-excluded conditions, while temperature during storage did not affect SO_2 preservation (Fig. 4). Bottle hue also influenced free SO_2 concentration, with retention (averaged across both wine styles)

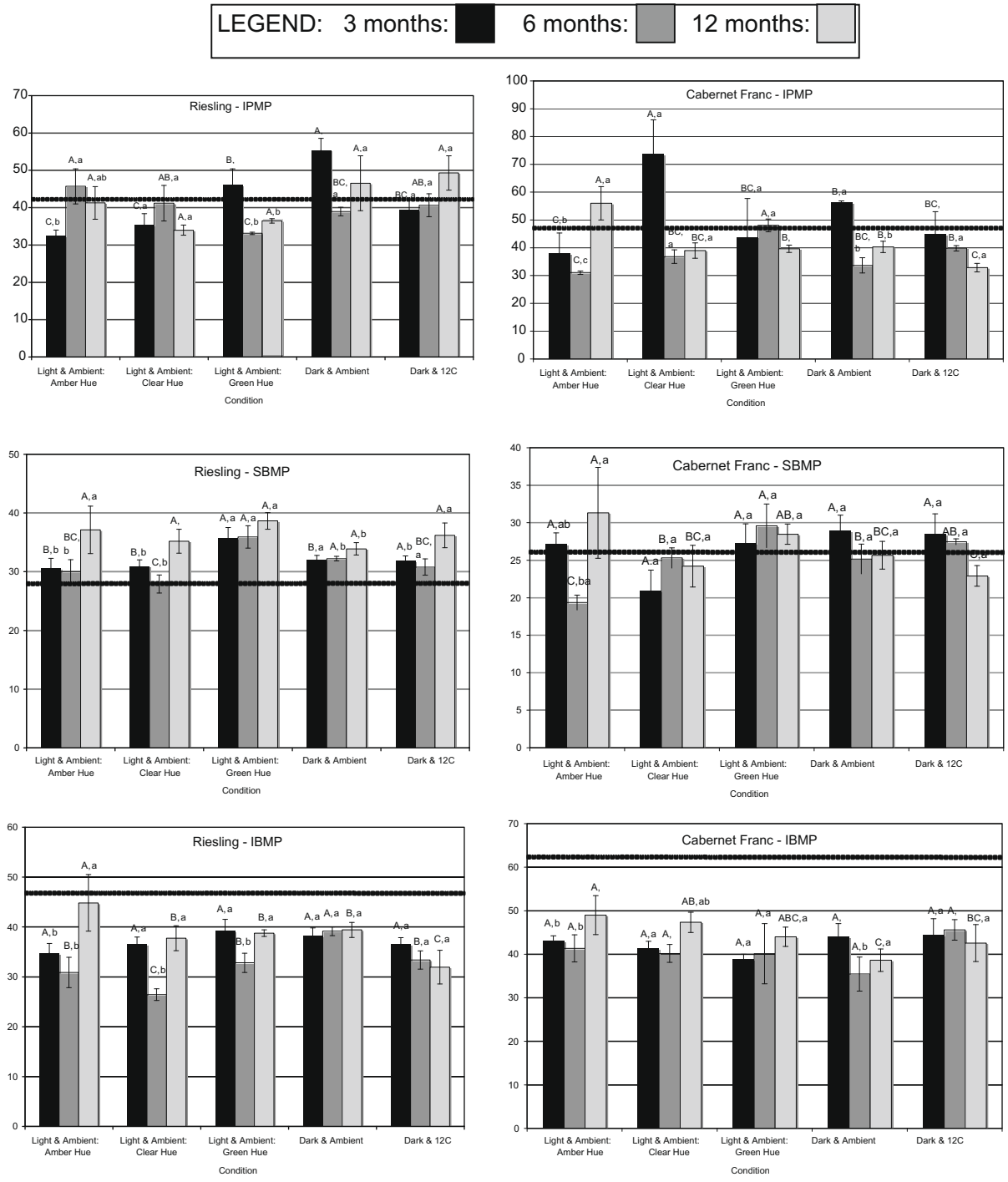

Fig. 2. Concentration of 3-alkyl-2-methoxypyrazines (MPs) in Riesling and Cabernet Franc wine spiked with 30 ng/L of isopropyl-, sec-butyl-, and isobutyl-MP. Data represent mean values of duplicate measurements of duplicate bottles ± SEM. Means sharing the same letter do not differ significantly across time [lowercase] or at a specific time point [uppercase] (Fisher's Protected $LSD_{0.05}$). Dashed line indicates initial MP concentration at bottling.

greatest in amber (55.5%) intermediate with green (42.5%), and lowest in clear bottles (33.8%). A similar pattern is apparent for bound SO_2. The lower retention in clear bottles may be due to the reduction of the photosensitizing compound riboflavin, which when reduced by light, has previously been noted to act as an oxidising agent in wine (D'Auria et al., 2003).

3.4. Principal components and other analyses

Principal Components Analysis and Correlation Analysis were performed on all data after 12 months. Included in this were spectrophotometric measurements of wine colour and phenolics. Factors 1 and 2 of the PCA analysis of Riesling after 12 months storage account for approx. 70% of the total variation (Fig. 5). Factor 1 is not closely associated with any particular eigenvector, while Factor 2 is heavily loaded with bound SO_2 and, to a lesser extent, free SO_2. Wine from the Dark + Ambient condition is well separated from other wine largely by its higher values for these parameters. Wines of varying bottle hue are separated by Factors 3 and 4, which together account for approx. 30% of the variation. Separation is largely based on their relative values for $A420_{nm}$, phenyl ethanol, ethyl hexanoate and SO_2. Factors 1 and 2 of the PCA analysis of

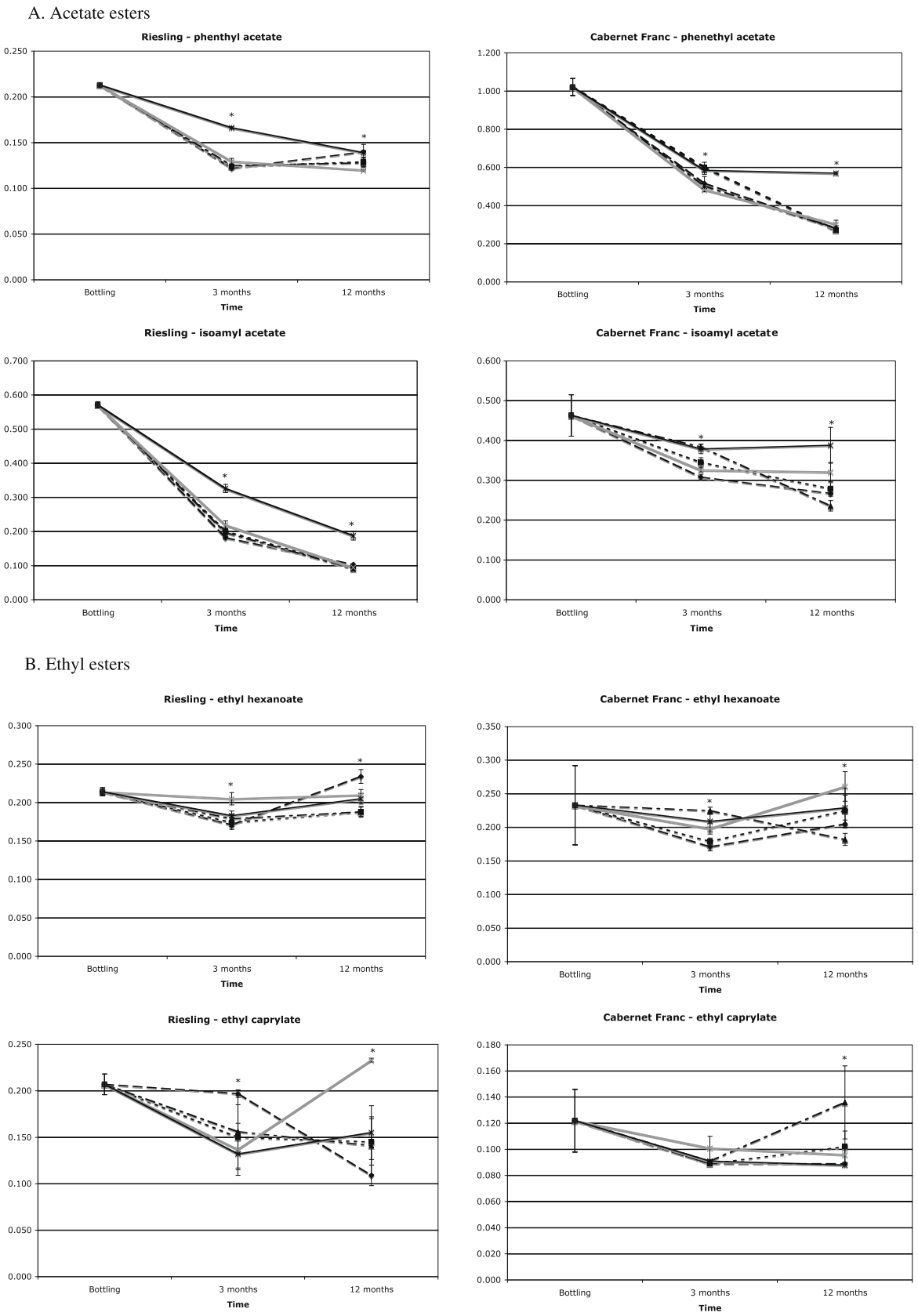

Fig. 3. Indicator volatiles, (A: acetate esters; B: ethyl esters; C: alcohol and acid) in Riesling and Cabernet Franc. Means that are significantly different (Fisher's Protected $LSD_{0.05}$) indicated with have an asterisk above time period as per Fisher's Protected $LSD_{0.05}$. Error bars represent ±SEM. Legend: Light and Ambient: Amber Hue: – – –; Light and Ambient: Clear Hue: .....; Light and Ambient: Green Hue: – - –; Dark and Ambient: ▬; Dark and Cellar ▬.

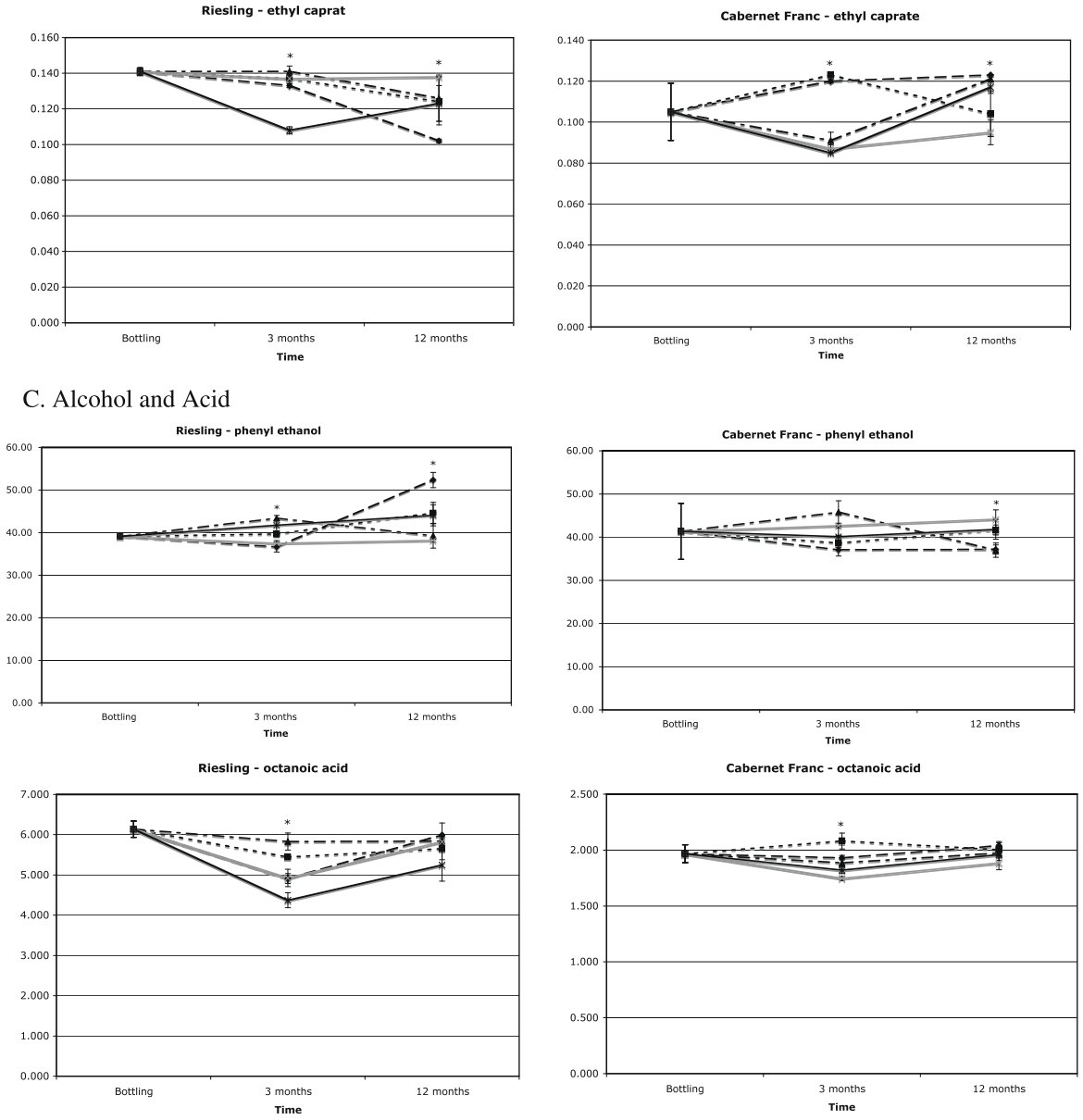

Fig. 3 (*continued*)

Cabernet Franc account for approx. 69% of the total variation (Fig. 6). Factor 1 is positively loaded with isoamyl acetate, total red pigments and wine hue, and negatively loaded with $A420_{nm}$ and degree of red pigmentation. Factor 2 is positively loaded with ethyl caprylate and negatively loaded with bound SO_2. Dark + 12 °C is well discriminated from other wines, largely due to its significantly higher values for A520 nm, total red pigments and colour density (data not shown). Bottle hues are separated by Factors 3 and 4, which account for approx. 31% of the variation. Green is discriminated based on its positive association with ethyl caprylate, and clear bottles are differentiated from those of green and amber hue based on their relatively low scores for A280 nm and total phenolics.

Browning has previously been reported to increase with storage temperature in white wine (Sims & Morris, 1984), in agreement with the trends in $A420_{nm}$ values observed here. Browning in white wine is inhibited by SO_2 (Berg & Akiyoski, 1956), which in this trial was reduced in light-exposed conditions; suggesting that the combination of clear bottles and elevated storage temperature is not optimal for protecting against premature browning and perhaps other negative quality indicators in white wine. While not statistically significant, Cabernet Franc wines stored in clear bottles were 8% and 9% lower for A280 nm and total phenolic measures, respectively, compared with wine stored in amber and green bottles.

Correlation Analysis (data not shown) on Riesling wine produced relatively few associations, while many analytes in Cabernet Franc were positively correlated. Interestingly, octanoic acid is strongly correlated with IBMP in both wines (0.952 in Riesling, 0.986 in Cabernet Franc), an unexpected association. IPMP and SBMP are positively correlated in Cabernet Franc (0.892); however, IBMP in Cabernet Franc and all MPs in Riesling wines, were not. This lack of association is also unexpected, given their similar chemical structure, but supports the earlier result that MPs are not equally affected by storage conditions, including closures, in this trial.

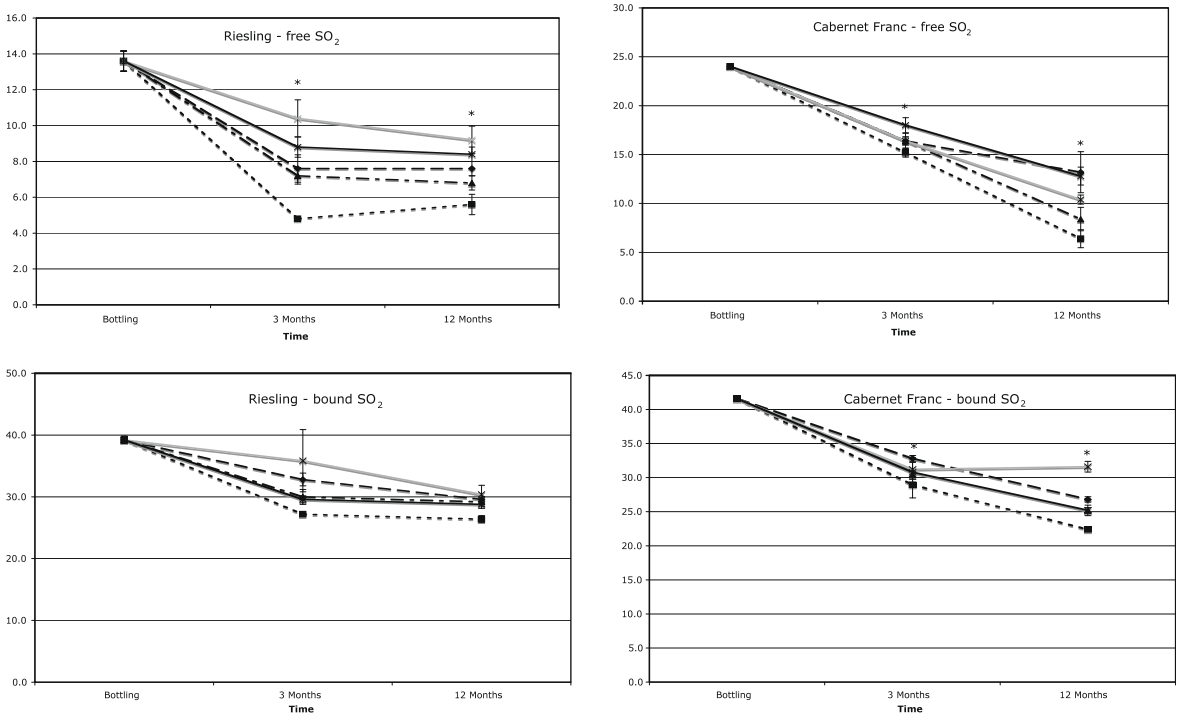

Fig. 4. Concentration of free and bound SO_2 in Riesling and Cabernet Franc wine over 12 months. Error bars represent ± SEM. Asterisks represent significant differences (Fisher's Protected $LSD_{0.05}$). Legend: Light and Ambient: Amber Hue: – – – ; Light and Ambient: Clear Hue: .....; Light and Ambient: Green Hue: – · –; Dark and Ambient: ▬; Dark and Cellar ▬.

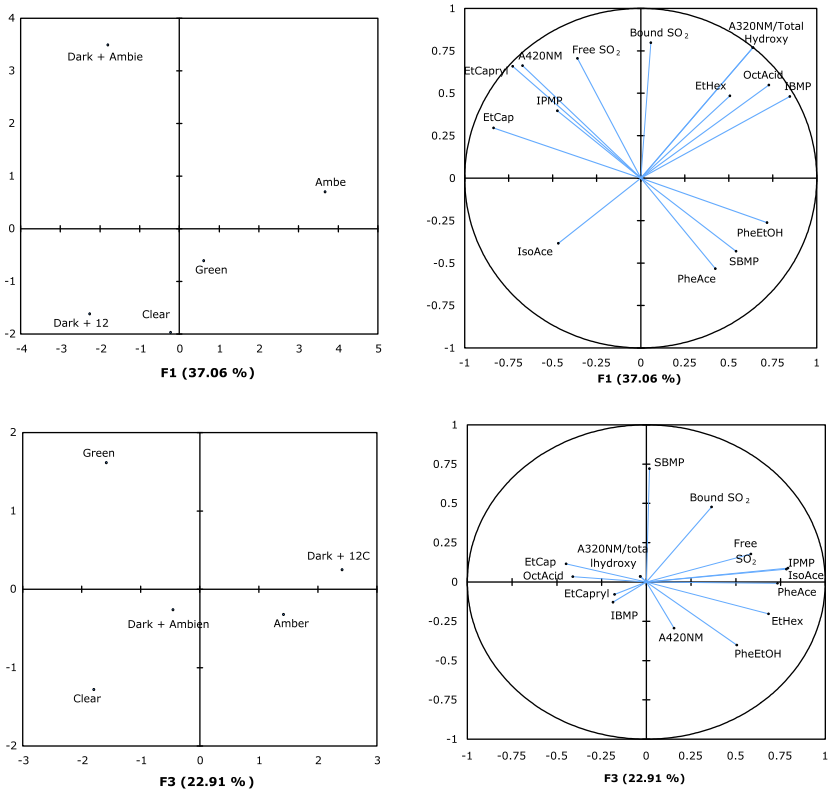

Fig. 5. Principal component 1 vs. 2 and Principal component 3 vs. 4 for Riesling wine after 12 months. Abbreviations: IPMP: 3-isopropyl-2-methoxypyrazine; SBMP: 3-s-butyl-2-methoxypyrazine; IBMP: 3-isobutyl-2-methoxypyrazine; PheAce: phenethyl acetate; EtCap: ethyl caprate; EtCapryl: ethyl caprylate; EtHex: ethyl hexanoate; IsoAce: isoamyl acetate; PheEtOH: phenyl ethanol; OctAcid: octanoic acid.

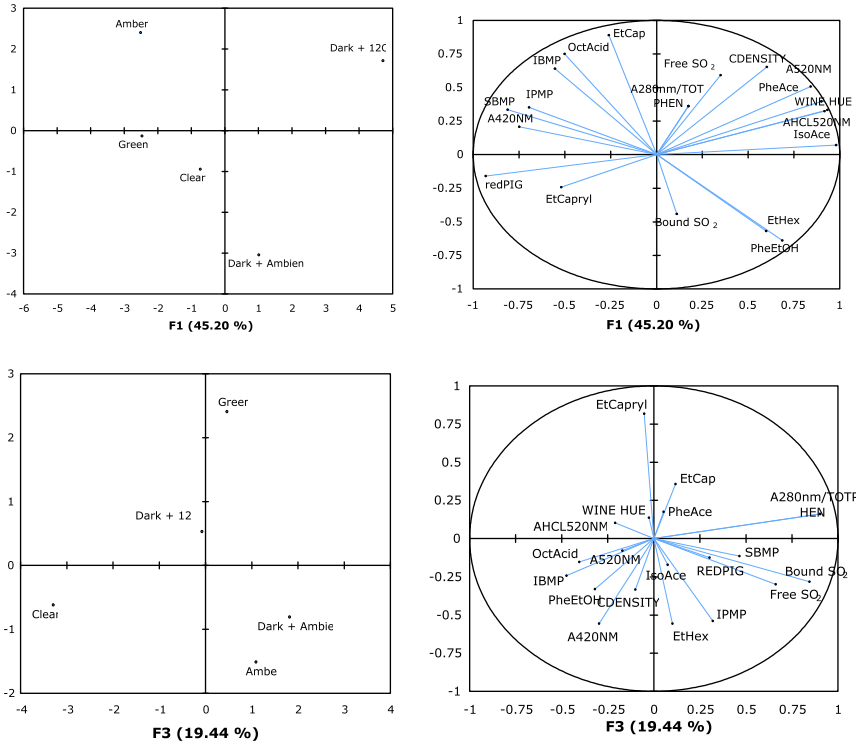

Fig. 6. Principal component 1 vs. 2 and Principal component 3 vs. 4 for Cabernet Franc wine after 12 months. Abbreviations: IPMP: 3-isopropyl-2-methoxypyrazine; SBMP: 3-s-butyl-2-methoxypyrazine; IBMP: 3-isobutyl-2-methoxypyrazine; PheAce: phenethyl acetate; EtCap: ethyl caprate; EtCapryl: ethyl caprylate; EtHex: ethyl hexanoate; IsoAce: isoamyl acetate; PheEtOH: phenyl ethanol; OctAcid: octanoic acid; CDensity: wine colour density; RedPig: degree of red pigmentation.

4. Conclusions

Light exposure and temperature conditions did not consistently influence MP concentrations in Riesling and Cabernet Franc wines during storage. In contrast, both light and temperature affected many of the other volatile and non-volatile constituents examined. The combination of light-exclusion and cooler storage conditions tend to associate with increased retention of acetate esters, free and bound SO_2, phenolic compounds (in red wine), and a lower browning index, all potential indicators of higher wine quality. This finding is consistent with anecdotal information concerning optimal cellaring conditions for wine, but should be further verified with sensory evaluation.

Acknowledgements

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. Thank you to Melissa Drouin, Lynda Van Zuiden, Kevin Ker and Dr. Tomas Hudlicky, Brock University, for invaluable technical assistance.

References

Allen, M. S., Lacey, M. J., & Boyd, S. (1994). Determination of methoxypyrazines in red wines by stable isotope dilution gas chromatography–mass spectrometry. *Journal of Agricultural and Food Chemistry, 42*, 1734–1738.

Berg, H., & Akiyoshi, M. (1956). Some factors involved in browning of white wines. *American Journal of Enology and Viticulture, 7*(1), 1–7.

Blake, A., Kotseridis, Y., Brindle, I. D., Inglis, D., Sears, M., & Pickering, G. J. (2009). Effect of closure and packaging type on 3-alkyl-2-methoxypyrazines and other impact odourants of Riesling and Cabernet Franc wine. *Journal of Agricultural and Food Chemistry, 57*(11), 4680–4690.

D'Auria, M., Emanuele, L., Mauriello, G., & Racioppi, R. (2003). On the origin of "Gout de Lumiere" in champagne. *Journal of Photochemistry and Photobiology A: Chemistry, 158*, 21–26.

Falcao, L. D., De Revel, G., Perello, M. C., Moutsiou, A., Zanus, M. C., & Bordignon-Luiz, M. T. (2007). A survey of seasonal temperature and vineyard altitude influences on 2-methoxy-3-isobutylpyrazine, C-13-norisoprenoids, and their sensory profile of Brazilian Cabernet Sauvignon wines. *Journal of Agricultural and Food Chemistry, 55*, 3605–3612.

Ferreira, V., Lopez, R., & Cacho, J. (2000). Quantitative determination of the odourants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture, 80*, 1659–1667.

Hashizume, K., & Samuta, T. (1999). Grape maturity and light exposure affect berry methoxypyrazine concentration. *American Journal of Enology and Viticulture, 50*(2), 194–198.

Heymann, H., Noble, A. C., & Boulton, R. B. (1986). Analysis of methoxypyrazines in wines. 1. Development of a quantitative procedure. *Journal of Agricultural and Food Chemistry, 34*(2), 268–271.

Iland, P., Bruer, N., Edwards, G., Weeks, S., & Wilkes, E. (2004). In P. Iland, N. Bruer, G. Edwards, S. Weeks, E. Wilkes (Eds.), *Chemical analysis of grapes and wine: Techniques and concepts* (pp. 32–43, 54–58, 64–67, 88–92). Campbelltown, South Australia: Patrick Iland Wine Promotions Pty Ltd.

Koch, R. L. (2003). The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control and non-target impacts. *Journal of Insect Science, 3*(32), 1–16.

Kotseridis, Y., Baumes, R. L., Bertrand, A., & Skouromounis, G. K. (1999). Quantitative determination of 2-methoxy-3-isobutylpyrazine in red wines and grapes of Bordeaux using a stable isotope dilution assay. *Journal of Chromatography A, 841*, 229–237.

Kotseridis, Y., Spink, M., Brindle, I. D., Blake, A., Sears, M., Chen, X., et al. (2008). Quantitative analysis of 3-alkyl-2-methoxypyrazines in juice and wine using stable isotope labelled internal standard assay. *Journal of Chromatography A, 1190*, 294–301.

Lopez, R., Aznar, M., Cacho, J., & Ferreira, V. (2002). Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *Journal of Chromatography A, 966*, 167–177.

Marais, J., & Pool, H. (1980). Effect of storage time and temperature on the volatile composition and quality of dry white table wines. *Vitis, 19*, 151–164.

Nurgel, C., Pickering, G. J., & Inglis, D. (2004). Sensory and chemical characteristics of Canadian ice wines. *Journal of the Science of Food and Agriculture, 84*, 1675–1684.

Ortega, C., Lopez, R., Cacho, J., & Ferreira, V. (2001). 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. *Journal of Chromatography A, 923*, 205–214.

Parr, W. V., Green, J. A., White, K. G., & Sherlock, R. R. (2007). The distinctive flavour of New Zealand Sauvignon Blanc: Sensory characterisation by wine professionals. *Food Quality and Preference, 18*(6), 849–861.

Pickering, G. J., Karthik, A., Inglis, D., Sears, N., & Ker, K. (2007). Determination of ortho- and retro-nasal detection thresholds for 2-isopropyl-3-methoxypyrazine in wine. *Journal of Food Science, 72*(7), S468–S472.

Pickering, G. J., Lin, J., Reynolds, A., Soleas, G., & Riesen, R. (2006). The evaluation of remedial treatments for wine affected by *Harmonia axyridis*. *International Journal of Food Science and Technology, 41*, 77–86.

Pickering, G. J., Lin, J., Riesen, R., Reynolds, A., Brindle, I. D., & Soleas, G. (2004). Influence of *Harmonia axyridis* on the sensory properties of white and red wine. *American Journal of Enology and Viticulture, 55*(2), 153–159.

Pickering, G. J., Reynolds, A., Soleas, G., Riesen, R., & Brindle, I. D. (2005). The influence of *Harmonia axyridis* on wine composition and aging. *Journal of Food Science, 70*(2), S128–S135.

Pickering, G. J., Spink, M., Kotseridis, Y., Inglis, D., Brindle, I. D., Sears, M., et al. (2008). Yeast strain affects 3-isopropyl-2-methoxypyrazine concentration and sensory profile in Cabernet Sauvignon wine. *Australian Journal of Grape and Wine Research, 14*, 230–237.

Ramey, D., & Ough, C. (1980). Volatile ester hydrolysis or formation during storage of model solutions and wines. *Journal of Agricultural and Food Chemistry, 28*, 928–934.

Rapp, A., & Marais, J. (1993). The shelf life of wine: Changes in aroma substances during storage and ageing of white wines. In G. Charalambous (Ed.), *Shelf life studies of foods and beverages. Chemical, biological, physical and nutritional aspects* (pp. 821–891). Amsterdam: Elsevier Science.

Roujou de Boubee, D. (2004). Research on the vegetal green pepper character in grapes and wines. *Vitis Viticulture and Enology Abstracts, 31*, 6–10.

Ryona, I., Pan, B., Intrigliolo, D., Lakso, A., & Sacks, G. (2008). Effect of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. Cv. Cabernet Franc). *Journal of Agricultural and Food Chemistry, 56*(22), 10838–10846.

Sala, C., Busto, O., Guasch, J., & Zamora, F. (2004). 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. *Journal of Agricultural and Food Chemistry, 52*, 3492–3497.

Selli, S., Canbas, A., & Unal, U. (2002). Effect of bottle colour and storage conditions on browning of orange wine. *Food, 46*, 64–67.

Simpson, R. (1978). Aroma and compositional changes in wine with oxidation, storage and ageing. *Vitis, 17*, 274–287.

Simpson, R., Capone, D., & Sefton, M. (2004). Isolation and identification of 2-methoxy-3,5-dimethylpyrazine, a potent musty compound from wine corks. *Journal of Agricultural and Food Chemistry, 52*, 5425–5430.

Sims, C., & Morris, J. (1984). Effects of pH, sulfur dioxide, storage time, and temperature on the color and stability of red muscadine grape wine. *American Journal of Enology and Viticulture, 35*, 35–39.

Soares, A., Borges, I., Borges, P., Labrie, G., & Lucas, E. (2007). *Harmonia axyridis*: What will stop the invader? In H. Roy & E. Wajnberg (Eds.), *Biological control to invasion: Ladybird Harmonia axyridis as a model species* (pp. 127–145). Amsterdam: Springer Netherlands.