

Green characters in Cabernet Sauvignon

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Introduction

Anecdotal evidence has suggested characters such as ‘herbaceous’, ‘vegetative’, ‘grassy’, ‘capsicum’ and ‘green’ are distinctive of some Cabernet Sauvignon wines originating from the Margaret River appellation and to an extent may typify wine style for Cabernet Sauvignon from this region. Such characters could be attributed to the presence of methoxypyrazines at elevated concentrations. The methoxypyrazines, 3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP) and 3-*sec*-butyl-2-methoxypyrazine (SBMP) are potent aroma compounds (Figure 1) with detection thresholds in water of 1 to 2 ng/L (Buttery et al. 1969a; Murray et al. 1970). IBMP was first identified in bell peppers (Buttery et al. 1969b) and the methoxypyrazines have since been found to occur widely in nature (Murray et al. 1970; Curioni and Bosset 2002). To date the origin of these compounds remains unclear, although a biochemical pathway has been postulated (Murray and Whitfield 1975). The methoxypyrazines have been identified in wine derived from several grape varieties, including Cabernet Sauvignon, Cabernet Franc, Sauvignon Blanc and Merlot (Harris et al. 1987; Allen et al. 1994; Hashizume and Umeda 1996; Kotseridis et al. 1998). At low concentrations (up to 15 ng/L) methoxypyrazines can contribute to the complexity and varietal character of wine, but at higher concentrations they can dominate wine aroma and be considered detrimental to wine quality (Allen et al. 1998). Of the three methoxypyrazines the most abundant is IBMP, typically present in wine at concentrations between 3.6 and 56.3 ng/L (Allen et al. 1994). IBMP is therefore likely to be of greatest significance from a sensory and wine quality perspective.

The IBMP content of wine is influenced by viticultural and environmental conditions. For example, vine vigour and canopy density, crop yield, irrigation, temperature and light exposure have all been shown to correlate with berry or wine IBMP (Morrison and Noble 1990; Hashizume and Takashi 1999; Roujou de Boubée et al. 2000; Chapman et al. 2004a; Chapman et al. 2004b; Sala et al. 2004; Sala et al. 2005). IBMP has been shown to accumulate during the early stages of berry development and then decline rapidly during ripening (Allen and Lacey 1998). This suggests a link between grape berry maturity (i.e. ripeness) and IBMP levels. Increased IBMP levels and ‘herbaceous-vegetative’ characters are often associated with grapes and wine produced in cooler regions which can struggle to reach maturity targets (Allen and Lacey 1993).

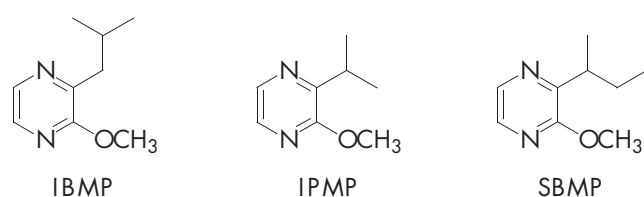


Figure 1. Chemical structures of 3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP) and 3-*sec*-butyl-2-methoxypyrazine (SBMP).

This study comprised a vineyard trial incorporating two experimental treatments: variation in vine vigour class (determined by remote sensing) and variation in crop yield. Vine vigour was selected as a treatment since it is established that increased canopy density and bunch shading results in increased IBMP concentrations (Morrison and Noble 1990). Crop yield was selected as a treatment because it can be easily manipulated and is thought to affect ripeness (Bravdo et al. 1984). The viticultural trial aimed to investigate management practices which could be employed to reduce grape and wine IBMP levels, and associated green characters. A method to quantify IBMP in wine by stable isotope dilution gas chromatography-mass spectrometry (GC-MS) was developed, employing solid-phase microextraction (SPME) as described in Chapman et al. (2004a). In addition to the wines produced from the vigour and bunch-thinning treatments in the vineyard trial, the method was used to determine the IBMP content of several commercial Margaret River Cabernet Sauvignon wines. A sensory study was undertaken on these wines to rank in order of increasing green character and to rate the intensity of several aroma descriptors associated with green character for each wine. The sensory trial aimed to investigate correlations between the nature and intensity of perceived green characters and the IBMP concentrations of these wines.

Materials and methods

Sensory study

The intensity rating of green character attributes of five commercial Cabernet Sauvignon wines from the Margaret River appellation were determined according to the Standards Association of Australia method 2542.2.3 (1988), using an expert panel of five judges, comprising winemakers and wine judges. Wine samples (40 mL) were presented in tasting glasses, identified only by three digit random codes, with watch glasses placed over each glass to retain the aromas. The panellists were asked to individually rate each wine for the intensity of attributes on the scorecard using a line-mark scale (labelled as ‘absent’ and ‘extremely strong’ at each end). The attributes assessed included the aroma descriptors: ‘green capsicum’, ‘leafy’, ‘grass cutting’, ‘asparagus’, ‘peas’, ‘mint’, ‘eucalyptus’, ‘vegetative’ and ‘herbaceous’. Panellists were also asked to rank the commercial wines in order of increasing intensity of green character.

Viticultural field trial

Site selection and treatment application

Experiments were conducted on Cabernet Sauvignon in a vineyard located in northern (Jindong) Margaret River, Western Australia. The vineyard comprised vines of seven years of age, grown on their own roots, north-south aligned and trained to unilateral cordons in a vertical shoot positioning trellis system. Aerial digital multi-spectral imaging was used to determine plant cell density (PCD) for the identification of zones comprising high and low vigour vines (data not shown). Six sites were selected from each of the

high and low vigour zones and within each site two target vines in adjacent rows were identified. A 50% bunch-thinning treatment was applied to one target vine at each site (and vines in neighbouring panels within the same row) just prior to véraison, to give four experimental treatments, each with six replicates: control vines of high vigour class; bunch-thinned vines of high vigour class; control vines of low vigour class; and bunch-thinned vines of low vigour class. The assignment of vigour classes was verified by ground-truthing against measurements of: trunk circumference (3 trunks per replicate, being the target vine and two adjacent vines); shoot length (10 shoots per target vine); and light interception (triplicate readings in both the fruit zone and upper canopy for each target vine). Canopy light interception was measured as a percentage of ambient photosynthetically active radiation (PAR) with a handheld ceptometer (LP80, Decagon Devices Inc., Pullman, USA).

Fruit development and composition

Buffer vines adjacent to each target vine were sampled weekly from the time bunch-thinning was imposed, just prior to véraison, until harvest (12 weeks). Berry samples (100 berries) were collected for each replicate of each treatment according to the sampling protocol described in Sala et al. (2004). For each sample, the average berry weight and berry diameter were measured and then the 100 berries were homogenised and centrifuged. The resulting juice was analysed to determine: total soluble solids, using a temperature-compensating digital refractometer (Reichert, Depew, USA); pH and titratable acidity (as tartaric acid equivalents to an end-point of pH 8.2); and anthocyanin and phenolic concentrations according to the method of Iland et al. (1996). At harvest, the fruit from each target vine was removed and the yield (as kg/vine) calculated; bunches were counted and weighed, to determine yield, average bunch number and average bunch weight per vine.

Winemaking

The grapes, approximately 20 kg per replicate, were harvested when total soluble solids (TSS) reached $24 \pm 0.5^\circ\text{Brix}$. The fruit was crushed, destemmed and separated into 30 L plastic fermentation vessels to make three replicate wines from each treatment, following standard small-lot winemaking procedures. Tartaric acid was added to adjust the pH to 3.4 and the musts were inoculated with EC1118 yeast (Lallemand Inc., Montreal, Canada). The fermenting musts were plunged twice per day and the wine was pressed with a single basket press at 3.6°Brix . The ferments were transferred to 15 L glass demijohns and held at 25°C until the residual sugar concentration approached 0 g/L. The wines were then racked and 50 ppm SO_2 was added before being cold stabilised for 4 weeks and then bottled.

Gas chromatography-mass spectrometry analysis

Sample preparation

IBMP for standards was purchased from Sigma-Aldrich and isotopically labelled [^3H]-3-isobutyl-2-methoxypyrazine (d_3 -IBMP) was purchased from CDN Isotopes (Quebec, Canada) and used as an internal standard. Wines were analysed using SPME; 10 μL of 50.6 $\mu\text{g/L}$ d_3 -IBMP in ethanol and sodium chloride (ca. 3 g) were added to the wine (10 mL) in a 20 mL screw cap vial with a PTFE seal. A 2 cm divinylbenzene/carboxen/poly(dimethylsiloxane) (Supelco, Bellefonte, USA) 23 gauge SPME fibre was used to sample the headspace above the wine for 30 min at 40°C , immediately prior to instrumental analysis.

Instrumental analysis

Samples were analysed with an Agilent 6890N gas chromatograph

coupled to a 5975 inert source mass spectrometer. The gas chromatograph was equipped with a Gerstel MPS2 autosampler. Samples were analysed using an Agilent HP-5MS capillary column (30 m, 0.25 mm internal diameter, 0.25 μm film thickness) and were injected in a pulsed splitless mode (40 psi for 5 min). The carrier gas was helium (linear velocity 36 cm/s, flow rate 1.0 mL/min). The oven temperature was started at 40°C , held at this temperature for 1 min then increased to 310°C at $5^\circ\text{C}/\text{min}$ and held for 5 min. The injector was held at 260°C and the mass spectrometer interface was held at 310°C . For quantification of IBMP, mass spectra were recorded in the selective ion monitoring (SIM) mode. The ions monitored in SIM runs were: $m/z = 94, 127$ and 154 for d_3 -IBMP (internal standard) and $m/z = 94, 124$ and 151 for IBMP. Ions 124 and 127 were used for quantification (Figure 2) while 94, 151 and 154 were used as qualifiers. Each of the selected fragment ions were monitored for 100 milliseconds each. All samples were analysed in duplicate.

Validation

The method was validated by a series of duplicate standard additions to model wine (10% v/v ethanol in saturated aqueous potassium hydrogen tartrate, pH 3.7). The calibration curve obtained was linear, with a coefficient of determination, $r^2 = 0.999$ (0, and 1.08 to 215.20 ng/L, $n=9 \times 2$) for SPME analysis of IBMP ($y=0.964x+0.044$ for m/z 124 vs. m/z 127) in model wine. The repeatability of the analysis at 53.80 ng/L was determined by spiking eight replicate model wines. The resulting relative standard deviation was 0.61% (Table 1).

Statistical methods

Data were analysed by two-way analysis of variance (ANOVA) using GenStat (8th Edition, VSN International Limited, Herts, UK). Mean comparisons were performed by least significant difference (LSD) multiple comparison tests at $P < 0.05$.

Results and discussion

Commercial wine sensory study and IBMP analysis

The sensory study indicated an apparent correlation between the perceived green character and IBMP concentration of the

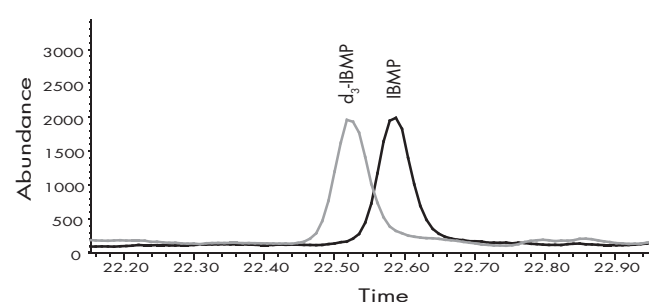


Figure 2. GC-MS select ion monitoring trace of extracted ions for IBMP (53.8 ng/L, $m/z = 124$, $t_r = 22.58$ min) and d_3 -IBMP (50.6 ng/L, $m/z = 127$, $t_r = 22.53$ min) in model wine

Table 1. Repeatability of IBMP analysis in model wine. Model wines were spiked with 53.8 ng/L IBMP and analysed by GC-MS ($n=8$).

spiked IBMP (ng/L)	measured mean IBMP (ng/L)	measured IBMP range (ng/L)	SD ^a (ng/L)	RSD ^b (%)
53.80	53.14	52.58 – 53.56	0.32	0.61

^a where SD equals standard deviation of the mean.

^b where RSD equals relative standard deviation of the mean.

commercial wines (Table 2). The panel readily identified wine 501 as exhibiting the least intense green character and wine 650 as the wine exhibiting the most intense green character. These rankings were supported by the quantitative GC-MS analysis. Wine 501 contained the least IBMP with a concentration of 12.67 ng/L, whereas wine 650 had the highest IBMP concentration at 48.32 ng/L. The panel was less readily able to distinguish the green character intensity of wines 415, 728 and 937. However this could be attributed to the extremely similar levels of IBMP present in these wines.

The aroma intensity ratings for wines 501 and 650 as determined by the sensory panel are shown in Figure 3. The aroma profiles corresponding to the two wines are clearly distinctive. Wine 501 exhibited relatively high intensity ratings for ‘leafy’, ‘eucalyptus’ and ‘mint’ aromas, but as the IBMP results would predict, low intensity ratings for ‘capsicum’, ‘herbaceous’ and ‘vegetative’ aromas. By comparison, wine 650, which according to the panel exhibited the

Table 2. Concentration of IBMP (ng/L) in commercial Cabernet Sauvignon wines derived from the Margaret River appellation. Wines were ranked by an expert panel on the basis of green character.

commercial wine	green character ranking	IBMP content ^a (ng/L)
501	1	12.67 (0.33)
415	2	21.37 (2.18)
728	3	20.27 (4.19)
937	4	23.93 (1.22)
650	5	48.32 (0.81)

^a average values and standard errors (in brackets) for measurement of IBMP (in duplicate).

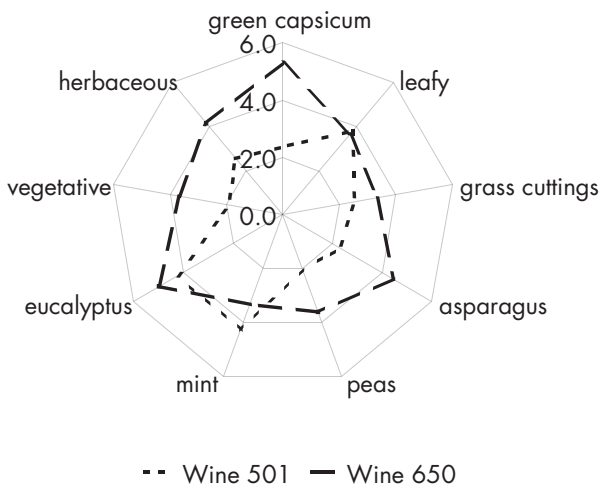


Figure 3. Aroma intensity ratings of green character attributes in two commercial Cabernet Sauvignon wines, 501 (ranked as least intense green character) and 650 (ranked as most intense green character), derived from the Margaret River appellation.

most intense green characters, scored higher intensity ratings for almost all of the green aroma descriptors. As would be expected for a wine with a high IBMP concentration (i.e. 48.23 ng/L), wine 650 afforded high scores for ‘capsicum’, ‘herbaceous’ and ‘vegetative’ characters (aromas associated with IBMP), as well as ‘asparagus’ and ‘eucalyptus’ characters.

Viticultural field trial

Variation in vine vigour was quantified by direct measurement of trunk circumference, shoot length and canopy light interception, pre-véraison. As would be expected, no bunch-thinning effect on the ground-truthing parameters was observed; the effect of vigour is shown in Table 3. The results indicated that high vigour vines had 25% larger trunks, 50% longer shoots and allowed between 59% and 87% less light to infiltrate the canopy than low vigour vines. The ground-truthing measurements clearly support the Plant Cell Density based assignment of vigour class, and confirm variation in vigour class as an experimental treatment.

The bunch-thinning treatment resulted in yields that varied almost two-fold. High vigour bunch-thinned vines had 45% fewer bunches and a 41% lower yield than high vigour control vines; low vigour bunch-thinned vines had 47% fewer bunches and a 35% lower yield than low vigour control vines. These results verify the effect of bunch-thinning on bunch number and yield per vine, and confirm variation in crop yield as an experimental treatment.

TSS measurements indicated that the target vines were harvested at the same level of grape maturity; i.e. 24 ± 0.5°Brix. Therefore, neither treatment (variation in vigour class or crop yield) had an effect on maturity or sugar ripeness. Excluding pH, a vigour treatment effect was observed for all other berry size and composition parameters, but no crop yield effect was observed (Table 4). Berries harvested from high vigour vines were larger than berries from low vigour vines (37% for berry weight and 13% for berry diameter), consistent with previous reports (Crippen and Morrison 1986). Irrespective of bunch-thinning, the titratable acidity of fruit from low vigour vines was 17% lower compared with fruit from high vines; and the low vigour vine fruit had 30% and 22% higher anthocyanin and phenolic concentrations, respectively, than high vigour vine fruit.

Table 3. Ground-truthing data for vines of high and low vigour class.

experimental treatment	trunk circumference ^a (cm)	shoot length ^a (cm)	light interception ^a (% PAR-ambient)	
			fruit zone	canopy
high vigour	17.6 (0.2) a	139.2 (4.6) a	6.6 (2.3) a	4.1 (4.8) a
low vigour	14.1 (0.2) b	92.7 (3.5) b	16.2 (2.7) b	31.4 (6.0) b

Values followed by a different letter within columns are significantly different.

^a average values and standard errors (in brackets) for measurements of trunk circumference (3 trunks per replicate), shoot length (10 shoots per replicate) and light interception (3 readings per replicate).

Table 4. Size and composition of berries from control and bunch-thinned vines of high and low vigour class, at harvest.

experimental treatment	average berry weight (g)	average berry diameter (mm)	total soluble solids (°Brix)	titratable acidity (g/L)	pH	anthocyanins (mg/g fresh weight)	phenolics (au/g fresh weight)
high vigour control	1.47 a	12.0 a	24.08 n.s.	4.98 a	3.89 n.s.	1.450 a	1.349 a
high vigour bunch-thinned	1.41 a	12.0 a	24.17 n.s.	5.19 a	3.92 n.s.	1.485 a	1.372 a
low vigour control	0.98 b	10.6 b	23.73 n.s.	4.15 b	4.15 n.s.	2.045 b	1.700 b
low vigour bunch-thinned	0.84 b	10.4 b	24.17 n.s.	4.29 b	4.29 n.s.	2.157 b	1.785 b

Values followed by a different letter within columns are significantly different. n.s. is not significant.

Quantitative GC-MS analysis showed 3- to 4-fold higher IBMP concentrations in wines produced from fruit derived from high vigour vines than low vigour vines, in agreement with the literature (Allen and Lacey 1993). This demonstrates a clear vigour effect on IBMP concentration (Table 5).

Chapman et al. (2004b) have investigated the effect of crop yield on the sensory attributes of Cabernet Sauvignon wines, through pruning treatments to reduce the number of buds per vine and cluster-thinning treatments to reduce the number of bunches per vine. Increased vegetative and bell pepper aromas were detected in wines made from low yielding vines compared with high yielding vines, which instead gave increased fresh fruit and red/black berry aromas. In contrast, when crop yield was altered by cluster-thinning, Chapman et al. (2004b) found yield had little impact on wine sensory attributes. In the present study, no significant difference in wine IBMP was observed between control and bunch-thinned vines of low vigour; both treatments resulted in wines containing approximately 10 ng/L IBMP. However, a clear bunch-thinning effect was observed across the high vigour treatment; wines made from low yielding vines had higher concentrations of IBMP than wines made from high yielding vines (41.76 ng/L and 31.71 ng/L respectively). IBMP decline post-véraison is thought to be influenced by light exposure (through photodegradation) and temperature (Hashizume and Samuta 1999; Allen and Lacey 1993). The absence of a bunch-thinning effect on IBMP concentration in wines derived from fruit of low vigour vines may therefore be attributed to the overriding effects of high light interception and berry temperature. By comparison, bunches from high vigour vines experienced reduced light exposure and berry temperature, accounting for increased IBMP levels. However, the reasons behind the elevated IBMP levels in wine derived from high vigour bunch-thinned vines warrants further investigation to establish a plausible explanation.

Conclusion

GC-MS analysis and sensory evaluation of several Margaret River Cabernet Sauvignon wines demonstrated a correlation between perceived intensity of green character and IBMP concentration. Furthermore, wines with higher levels of IBMP had higher aroma intensity ratings for 'herbaceous', 'vegetative' and 'green capsicum' aromas.

Canopy density and vine vigour were found to significantly influence wine quality, with regard to wine colour, phenolics and IBMP concentrations. Wines derived from fruit of low vigour vines were found to have increased colour and phenolic properties, and decreased IBMP concentrations, irrespective of the bunch-thinning treatment. Although wines derived from fruit of high vigour vines had lower colour intensity and phenolic content compared with low vigour vines, these measurements were still high relative to many other wine producing regions. However, it is the increased IBMP levels arising from the high vigour vines, in particular the bunch-thinned vines, that could contribute to increased 'vegetative' and 'herbaceous' green characters. From a wine quality perspective, vine vigour was found to be a more influential parameter than crop yield. Certainly, the bunch-thinning treatment showed no beneficial impact on wine quality and in the case of high vigour vines, bunch-thinning may actually be detrimental to quality.

The results from this study tend not to support the theory that crop reduction pre-véraison produces higher quality wines. With respect to wine IBMP and associated green characters, the use of vineyard management practices to limit vine vigour and canopy density are likely to be more beneficial. Such practices may include:

Table 5. Concentration of IBMP (ng/L) in wines made from fruit harvested from control and bunch-thinned vines of high and low vigour class.

experimental treatment	IBMP content ^a (ng/L)
high vigour control	31.71 (1.80) a
high vigour bunch-thinned	41.76 (1.38) b
low vigour control	10.02 (2.81) c
low vigour bunch-thinned	10.19 (3.13) c

Values followed by a different letter are significantly different.

^a average values and standard errors (in brackets) for measurement of wine IBMP (in duplicate).

reduced fertiliser application rates; reduced or strategic irrigation; and leaf removal or shoot-thinning to reduce vine biomass without affecting crop yield.

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References

- Allen, M.S. and Lacey, M.J. (1993) Methoxypyrazine grape flavour: influence of climate, cultivar, and viticulture. *Viticultural and Enology Sciences* 48, 211-213.
- Allen, M.S., Lacey, M.J. and Boyd, S.J. (1994) Determination of methoxypyrazines in red wines by stable isotope dilution gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry* 42, 1734-1738.
- Allen, M.S. and Lacey, M.J. (1998) Methoxypyrazines of grapes and wines. Chapter 3, pp 31-39 in "Chemistry of wine flavour" Ed. Waterhouse, A.L. and Ebeler, S.E. American Chemical Society, Washington DC, USA.
- Bravdo, B., Hepner, Y., Loinger, C., Cohen, S. and Tabacman, H. (1984) Effect of crop level on growth, yield and wine quality of a high yielding Carignane vineyard. *American Journal of Viticulture and Enology* 35, 247-252.
- Buttery, R.G., Seifert, R.M., Guadagni, D.G. and Ling L.C. (1969a) Characterisation of some volatile constituents of bell peppers. *Journal of Agricultural and Food Chemistry* 17, 1322-1327.
- Buttery, R.G., Seifert, R.M., Lundin, R.E., Guadagni, D.G. and Ling L.C. (1969b) Characterisation of an important aroma component of bell peppers. *Chemistry and Industry* 4, 490-491.
- Chapman D.M., Thorngate, J.H., Matthews, M.A., Guinard, J.-X. and Ebeler, S.E. (2004a) Yield effects on 2-methoxy-3-isobutylpyrazine concentration in Cabernet Sauvignon using a solid-phase microextraction gas chromatography/mass spectrometry method. *Journal of Agricultural and Food Chemistry* 52, 5431-5435.
- Chapman, D.M., Matthews, M.A. and Guinard, J.-X. (2004b) Sensory attributes of Cabernet Sauvignon wines made from vines with different crop yields. *American Journal of Enology and Viticulture* 55, 325-334.
- Crippen, D.D. and Morrison, J.C. (1986) The effects of sun exposure on the compositional development of Cabernet Sauvignon berries. *American Journal of Enology and Viticulture* 37, 235-242.
- Curioni, P.M.G. and Bosset, J.O. (2002) Key odorants in various cheese types as determined by gas chromatography-olfactometry. *International Dairy Journal* 12, 959-984.
- Harris, R.L.N., Lacey M.J., Brown, W.V. and Allen M.S. (1987). Determination of 2-methoxy-3-alkylpyrazines in wine by gas chromatography/mass spectrometry. *Vitis* 26, 201-207.
- Hashizume, K. and Umeda, N. (1996) Methoxypyrazine content of Japanese red wines. *Bioscience, Biotechnology, Biochemistry* 60, 802-805.

- Hashizume, K. and Samuta, T. (1999) Grape maturity and light exposure affect berry methoxypyrazine concentration. *American Journal of Enology and Viticulture* 50, 194-198.
- Iland, P.G., Bruer, N., Edwards, G., Weeks, S. and Wilkes, E. (2004) Chemical analysis of grapes and wine: techniques and concepts. Patrick Iland Wine Promotions, Adelaide, Australia.
- Kotseridis, Y., Anocibar-Beloiqui, A., Bertrand, A. and Doazan, J.P. (1998) An analytical method for studying the volatile compounds of Merlot noir clone wines. *American Journal of Enology and Viticulture* 49, 44-47.
- Morrison, J.C. and Noble, A.C. (1990) The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties. *American Journal of Enology and Viticulture* 41, 193-200.
- Murray, K.E., Shipton, J. and Whitfield, F.B. (1970) 2-Methoxypyrazines and the flavour of green peas (*Pisum sativum*). *Chemistry and Industry* 7, 897-898.
- Murray, K.E. and Whitfield, F.B. (1975) The occurrence of 3-alkyl-2-methoxypyrazines in raw vegetables. *Journal of Science and Food Agriculture* 26, 973-986.
- Roujou de Boubee, D., van Leeuwen, C. and Dubourdieu, D. (2000) Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect on environmental conditions on concentrations in grapes during ripening. *Journal of Agricultural and Food Chemistry* 48, 4830-4834.
- Sala, C., Busto, O., Guasch, J. and Zamora, F. (2004) Influence of vine training and sunlight exposure on the 3-alkyl-2-methoxypyrazine content in musts and wines from *Vitis vinifera* variety Cabernet Sauvignon. *Journal of Agricultural and Food Chemistry* 52, 3492-3497.
- Sala, C., Busto, O., Guasch, J. and Zamora, F. (2005) Contents of 3-alkyl-2-methoxypyrazines in musts and wines from *Vitis vinifera* variety Cabernet Sauvignon: influence of irrigation and plantation density. *Journal of the Science of Food and Agriculture* 85, 1131-1136.
- Standards Association of Australia (1988) Australia Standard 2542.2.3, Sydney, Australia.