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Method development and cultivar-related differences of nine biogenic amines in Ontario wines

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Abstract

We have developed a new high-performance liquid chromatographic method using pre-column derivatization with orthophthaladehyde, gradient elution and fluorescence detection to assay the concentrations of nine biogenic amines in wines: histamine, 1-methylhistamine, methylamine, ethylamine, tyramine, tryptamine, 2-phenylethylamine, putrescine and cadaverine. The method shows excellent analytical characteristics. It has been used to measure the concentration of these biogenic amines in 73 monovarietal wines from five red and six white cultivars. All wines were from the Niagara viticultural region of southern Ontario and were certified as to origin. Pinot noir wines had the highest content of total amines, and also of histamine, putrescine, cadaverine, ethylamine and 1-methylhistamine. Among the white wines, those from Chardonnay had the highest content of total amines and also of histamine, tyramine, methylamine and 1-methylhistamine. It is suggested that longer ageing as well as Sur-lie fermentation (Chardonnay) can account, at least in part, for these findings.

1. Introduction

Biogenic amines are an important class of compounds with major functions in mammalian organisms (see Tabor and Tabor, 1984 for review) including growth regulation (spermine, spermidine and cadaverine), neural transmission (catecholamines and serotonin) and as mediators of inflammation (histamine and tyramine). They are endogenously synthesized from amino acid precursors by regulated metabolic pathways in mammalian cells that usually involve decarboxylation of the parent amino acid. Similarly, they can be generated exogenously in the intestinal tract by bacterial-induced decarboxylation of amino acids released by the enzymatic hydrolysis of dietary protein. Such reactions occur when protein-containing foodstuffs develop bacterial contamination, although in certain processes, notably maturation of cheeses, amine production is a desirable outcome (Smith, 1980).

As with other plants, biogenic amines are synthesized in *Vitis* in several parts of the vine, including berries and leaves (Adams et al., 1990; Geny et al., 1997). This is especially true of spermine, spermidine and cadaverine, which appear to have growth-regulating roles similar to their functions in higher organisms, but histamine, tyramine and 1-methylhistamine can be found in trace amounts, and the enzymes responsible for the synthesis of the first two by decarboxylation of their parent amino acids are present in many plants, including vines, although their functions are unclear (Radler and Fath, 1991). However, their presence in wines, particularly red wines, can also be a consequence of malolactic femen-tation or the action of yeasts in primary fermentation (Maga, 1978), although in one varietal this assertion has been challenged (Buteau et al., 1984a). This is significant in two respects: firstly, high concentrations may indicate bacterial contamination and secondly, they may be responsible for adverse effects of wine consumption, including headache and gastric discomfort (Lovenberg, 1974; Taylor, 1986).

Recent investigations into the chemistry of wines have been stimulated by their potent antioxidant (Frankel et al., 1995) and anticancer activities, as well as their inhibition of cellular reactions leading to atherosclerosis, coronary heart disease and occlusive stroke (see

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Goldberg et al., 1995a; Soleas et al., 1997a for review). Several of these beneficial constituents demonstrate regional and cultivar-related differences (Goldberg et al., 1998a; Soleas et al., 1997b). However, it is also important to ascertain whether biogenic amines, as undesirable components, are also related to cultivar and region as well as to enological techniques that are amenable to change and improvement. In this paper, we describe a modification of previous high performance liquid chromatographic (HPLC) methods to assay the content of biogenic amines in wine and its application to a range of varietal wines produced in the circumscribed viticultural region of Niagara, Ontario, whose products are regulated by a Vintners' Quality Alliance (VQA) certifying that wines so designated are vinted from grapes that are exclusively grown in that region.

2. Methods

The method that we developed, like that of Lehtonen et al. (1992), utilised pre-column derivatization followed by gradient elution on a C_{18} column. Major changes included the following: derivatization reagent; column elution solvents and gradient program; wavelengths of excitation and emission; use of 25 μl instead of 1 μl of wine sample, thus providing greater sensitivity and robustness as well as the potential to assay more constituents, without imposing any practical limitation on sample volume availability.

2.1. Instrumentation

All modules were from Waters Canada Ltd (Mississauga, Ontario, Canada), comprising the Model 510 HPLC with Millenium Software fitted with the 420 fluorescence detector set at 340 nm excitation and 420 nm emission. The column was a 3.9×150 mm Nova Pak C_{18} stainless steel cartridge (60 A particle size) equipped with a 20 nm Nova Pak C_{18} guard column. The system reported to a Hewlett-Packard Laser Printer-5.

2.2. Pre-column derivatization

The derivatizing reagent comprised 1 g orthophthaladehyde (OPA, Sigma, St Louis, MO, USA) per liter of 0.05 M sodium tetraborate (Caledon Laboratories, Geoergetown, Ontario, Canada) containing 2% (v/v) methanol and 0.2% (v/v) mercaptoethanol. A Waters WISP-712 Enhanced Performance Kit was installed to provide the autoaddition capability for the derivatizing agent. 25 μl of the OPA reagent was reacted with 25 μl of the sample for 99 s before 25 μl of the derivatized sample was injected onto the column. The total run time allowing for clean-up and equiliberation was 25.30 min.

2.3. Chromatographic conditions

Gradient elution was performed at a fixed oven temperature of 45°C with the following mobile phase:

A. 0.05 M sodium acetate buffer:tetrahydrofuran (96:4 v/v)

B. 100% methanol distilled in glass.

The gradient was constructed as described in Table 1. Excellent resolution of the various amines was obtained as illustrated in Fig. 1.

2.4. Standards

These were made up as described in Table 2. Extensive calibration curves established excellent linearity between peak area and amine concentration over a wide range when the standards were spiked into simulated wine solutions (Table 3). Routinely, a three-point calibration for each amine was performed with each set of unknown wine samples.

2.5. Analytical characteristics

These are presented in Table 3. Recovery was calculated by adding three known amounts of each amine (low, medium and high) to each of six different wines whose amine content had been determined in quadriplicate. After addition, the concentration of each amine in each sample was measured in duplicate. The difference between the new and basal values was expressed as a percentage of the amount added, and for all 18 values the mean ± SD (standard deviation) was calculated as the overall recovery. All of the amines measured except methylamine, putrescine and cadaverine had a recovery of >90%. The highest detection limit was for histamine (0.12 mg l^{-1}). To calculate the reproducibility (imprecision) of the analyses, each biogenic amine was added at three concentrations (low, medium and high) to a simulated wine preparation. Six replicate assays were performed for each amine at each concentration level. Means and SDs were calculated; the latter was expressed as a percentage of the former

Table 1
Chromatographic conditions (gradient)

Run no.	Time (min)	Flow (ml min^{-1})	Solvent A %	Solvent B %	Curve
1	2.5	1.20	47.0	53.0	11
2	7.50	1.20	30.0	70.0	3
3	15.00	1.20	0.0	100.0	2
4	16.00	1.20	47.0	53.0	1
5	17.50	1.50	47.0	53.0	11
6	25.30	1.20	47.0	53.0	11

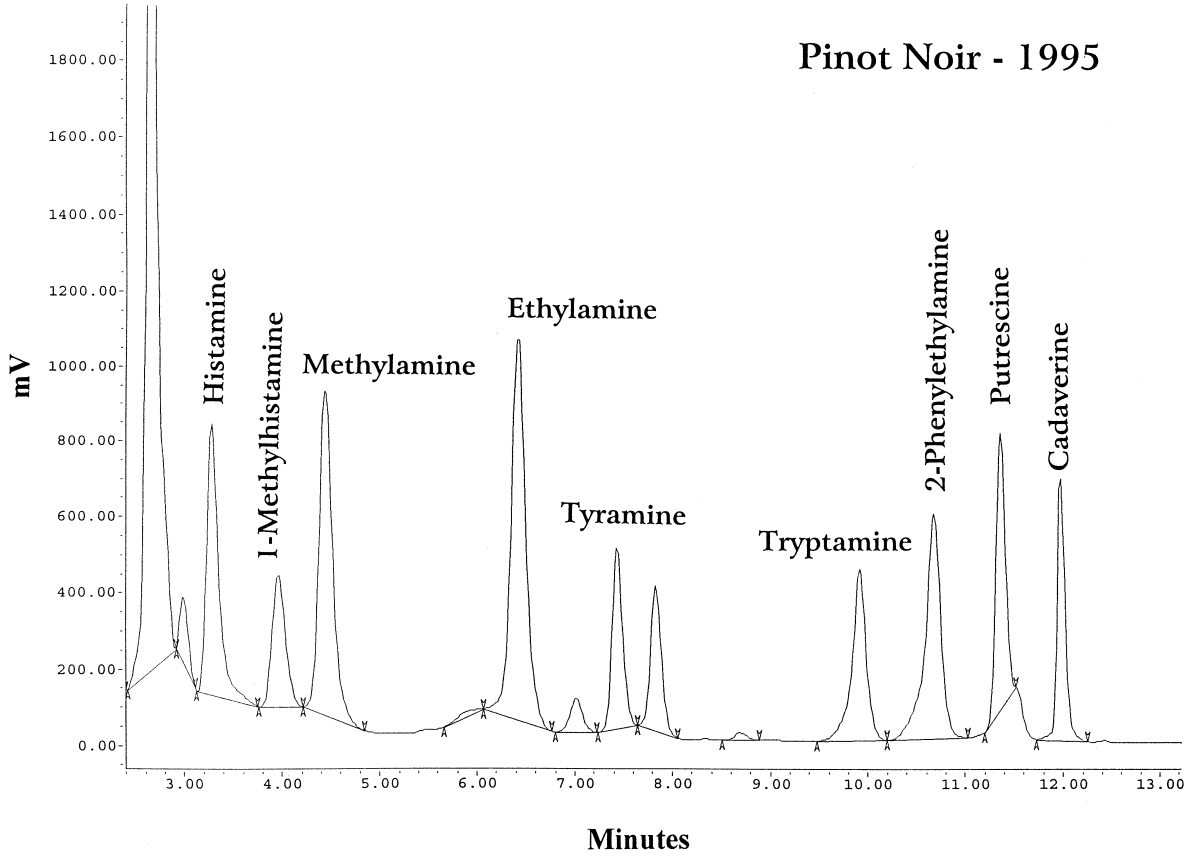

Fig. 1. Chromatographic separation of nine biogenic amines from a Pinot noir wine. The vertical axis displays the amplitude of the signal in millivolts.

(coefficient of variation, CV) and the results for the three concentrations were averaged. The imprecision was $<4.5\%$, apart from methylamine (8.8%).

2.6. Wines

A total of 34 red wines and 39 white wines from the 1995 vintage in the viticultural region of Niagara, Ontario, were analyzed. All were single varietal finished bottled wines for commercial consumption. Forty-three of the total (73) were produced by a single major winery. The numbers and the cultivars are given in Fig. 2. The analyses were completed within 48 h of opening the bottles, during which time no changes in amine concentrations were observed.

2.7. Enological procedures

With few exceptions, the wines were produced by techniques that have become widely used throughout the Niagara viticultural region of Ontario and incorporated into the rules of the Vintners' Quality Alliance (VQA), which guarantees the quality and authenticity of wines from this region. The exceptional weather at harvest ensured that all grapes were fully ripe when picked: Seyval blanc, first week of September; Vidal blanc, Riesling, Chardonnay and Muscat Morio first two weeks of October; red cultivars, middle two weeks of October; Late Harvest Vidal, first two weeks of November. Skin contact was as follows: Gamay noir, 5–6 days; other reds, 14–21 days; Chardonnay, up to 8 h; Riesling and Vidal blanc, up to 4 h; Seyval blanc, Muscat Morio and Late Harvest Vidal, zero.

After the desired period of skin contact in the crushing room, the free run juice of the white cultivars was transferred to a stainless steel tank. The fully crushed red grapes, including skins and seeds with the juice, were similarly transferred. For most wines, Prise de Mousse was the yeast strain employed to initiate primary fermentation. Other strains of *Saccharomyces cerevesiae* occasionally utilised were Zymaflor CY 3079 (three Chardonnays), Zymaflor VL1 (two Rieslings and one Muscat Morio), Zymaflor 71B (two Gamays) and Zymaflor F10 (six miscellaneous red wines).

Chaptalization with high fructose corn syrup took place during the primary fermentation of all wines to raise the potential alcohol concentration to approximately 12% by volume. After the desired period, the skins of the red wines were pressed and the wine was transferred to a new stainless steel container. After completion of the primary

fermentation, malolactic fermentation was initiated in all red wines and Chardonnays by innoculating *Leuconostoc oenos*, inobacter strain at 200 mg l^{-1}.

On termination of secondary fermentation clarification took place with bentonite or diatomaceous earth. All wines were adjusted to a free sulfur concentration of 30–40 mg l^{-1} after clarification since much of the original, averaging 35 mg l^{-1}, became depleted during fermentation. Those wines not being transferred to oak barrels (all whites except most Chardonnay and some Gamay noir) were pumped into new stainless steel tanks and held until ready for bottling. Sorbic acid (180 mg l^{-1}) was added to all white wines immediately prior to bottling. All white wines were cold stabilised, or in two instances passed through an ion-exchange resin to remove potassium tartrate. Barrel ageing was for 3–6 months (Chardonnay), 0–6 months (Gamay) or 12–18 months (all other red wines). Before bottling, all wines

Table 2
Biogenic amine standards made up to an accurately known concentration approximately 1 g l^{-1} in methanol

Compound (chemical name)	MW	Supplier (catalogue no.)	Purity (%)
Cadaverine ($C_5H_{14}N_2$) (1,5-diaminopentane)	102.2	Sigma[a] (C 1541)	≈98
Ethylamine (C_2H_7N)	45.1	Sigma (E 3754)	70
Histamine ($C_5H_9N_3$) (2-[4-imidazolyl]ethylamine)	111.1	Sigma (H 7125)	99
Methylamine (CH_5N.HCl)	67.5	Sigma (M 0505)	>99
1-Methylhistamine ($C_6H_{11}N_3$.2HCl) (1-Methyl-4-[*b*-aminoethyl]imidazole	198.1	Sigma (M 4910)	>98
2-Phenylethylamine ($C_8H_{11}N$)	121.2	Lancaster[b] (2586)	99
Putrescine ($C_4H_{12}N_2$) (tetramethylenediamine)	88.2	Sigma (P 7630)	~98
Tryptamine ($C_{10}H_{12}N_2$) (3-[2-aminoethyl]indole)	160.2	Sigma (T 2891)	>99
Tyramine ($C_8H_{11}NO$) (4-hydroxyphenethylamine)	137.2	Sigma (T 7255)	99
Spermidine ($C_7H_{19}N_3$) (*N*-[3-aminopropyl]1,4-butanediamine)	145.2	Sigma (S 2626)	99
Spermine ($C_{10}H_{26}N_4$) (*N*,*N*′-bis[3-aminopropyl]-1,4-butanediamine	202.3	Sigma (S 3256)	~97

[a] Sigma = Aldrich Canada, Ltd, Mississauga, Ontario, Canada.
[b] Lancaster = Synthesis Inc., Windham, NH, USA.

Table 3
Analytical characteristics of method

	Overall recovery[a] (%)	Linearity (R^2)[b]	Linearity range (mg l^{-1})	Detection limit[c] (mg l^{-1})	Overall precision[a] (CV, %)
Histamine	106.2 ± 15.7	0.999	0.61–6.73	0.12	4.1
1-Methylhistamine	101.5 ± 13.2	1.000	0.50–6.00	0.05	3.0
Methylamine	86.9 ± 2.9	0.998	0.53–6.30	0.03	8.8
Ethylamine	90.8 ± 2.9	0.999	0.71–8.56	0.05	4.2
Tyramine	104.4 ± 9.6	0.999	0.51–6.13	0.06	3.6
Tryptamine	102.5 ± 6.2	0.999	0.52–6.22	0.09	4.4
2-Phenylethylamine	98.9 ± 2.8	0.999	0.58–6.96	0.06	3.3
Putrescine	83.3 ± 1.3	0.999	0.53–6.32	0.11	4.4
Cadaverine	79.8 ± 1.1	0.999	0.57–6.80	0.03	3.1

[a] Average of six replicates at each of three concentrations.
[b] Square of regression coefficient.
[c] Three times the noise level.

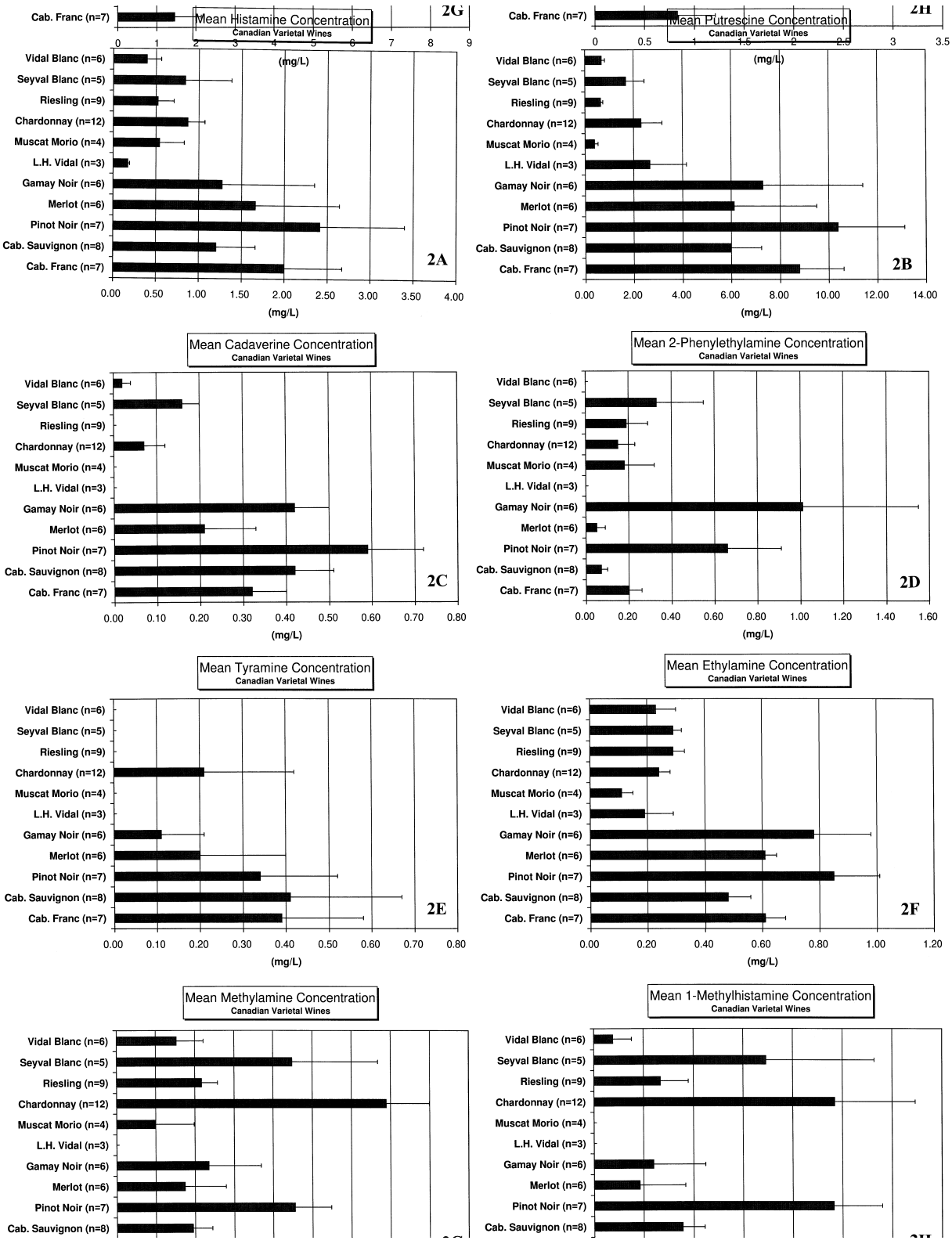

Fig. 2. Mean concentrations (+1 SEM) of eight biogenic amines (mg l^{-1}) in Canadian wines from individual cultivars. Cab. = Cabernet; L.H. = late harvest.

were subjected to membrane filtration to remove yeast and bacterial debris.

2.8. Statistical analyses

For each cultivar, the data for each individual amine were used to calculate the mean and standard error of the mean (SEM), presented in Fig. 2(A–H), and total biogenic amine content, shown in Fig. 3. Since the distribution showed departure from Gaussianity, the non-parametric Mann-Whitney U-test (Sokol and Rohlfs, 1981) was used to test for the significance of differences between groups. Initially, all red wines were compared with all white wines. If the former were significantly higher, the cultivar with the highest mean value was compared with all the others in the same category. If this proved to be significantly higher, the second highest was compared with the remaining wines, excluding the highest. Where there were two cultivars whose mean values were close and higher than all others in the same category, they were combined and compared with the remainder, e.g. the histamine content of Chardonnay and Seyval blanc (*vide infra*). In no instance was the same data set used more than once. In the Results section the p values rounded up to the orthodox intervals (0.05, 0.02, 0.01, 0.005 and 0.001) are listed where the differences were significant, and no value is provided where they were not.

3. Results

3.1. Histamine

This was higher in all red wines than white wines (Fig. 2(A); $p < 0.01$). Among the former, Pinot noir wines had the highest mean concentration ($p < 0.05$) followed by Cabernet Franc, whereas with the latter Chardonnay and Seyval blanc wines had the highest mean histamine concentrations ($p < 0.01$, combined).

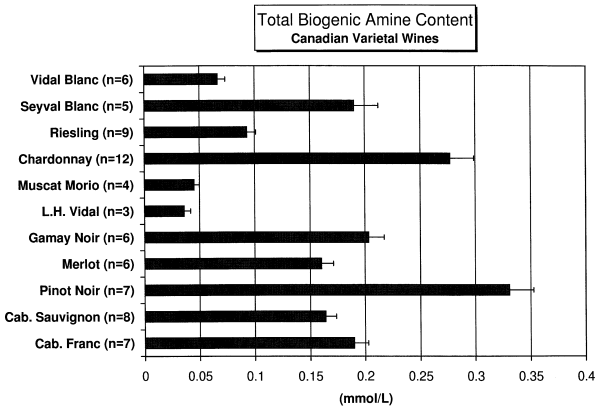

Fig. 3. Mean concentrations (+1 SEM) of total biogenic amines (mmoll^{-1}) in Canadian wines from 11 individual cultivars. Cab. = Cabernet; L.H. = late harvest.

3.2. Putrescine

The concentrations of this amine were much higher than those of histamine ($p < 0.001$) but, like the latter, higher concentrations were present in red than in white wines, with Pinot noir and Cabernet Franc wines again having the highest concentrations among the former (Fig. 2(B); $p < 0.051$, combined). Although Chardonnay and Seyval blanc wines had higher putrescine concentrations than most of the other white wines, their concentrations were surpassed by those of Late Harvest Vidal.

3.3. Cadaverine

Although the concentrations of this amine were much lower than those of the first two compounds ($p < 0.001$ for both), all red wines had mean values surpassing those of the whites ($p < 0.001$), with Pinot noir wines highest, followed by Gamay noir and Cabernet Sauvignon (Fig. 2(C)). Only Chardonnay and Seyval blanc wines had consistently detectable values among the whites, with the latter having the higher mean value.

3.4. 2-phenylethylamine

The pattern for this amine was quite different from that of the first three (Fig. 2(D)). The mean concentrations of several of the white wines surpassed those of some red wines, notably Merlot and Cabernet Sauvignon, although overall the wines from Gamay noir had the highest mean 2-phenylethylamine concentration ($p < 0.02$), followed by those from Pinot noir ($p < 0.02$).

3.5. Tyramine

Of the white wines surveyed, only those from Chardonnay had measurable concentrations that overlapped those of the Gamay noir and Merlot wines (Fig. 2(E)). Wines from Cabernet Sauvignon and Cabernet Franc as well as Pinot noir had higher concentrations than Merlot and Gamay noir ($p < 0.05$ for all three combined).

3.6. Ethylamine

All of the red wines had higher mean concentrations than the whites ($p < 0.02$); those from Riesling and Seyval blanc had marginally the highest levels (Fig. 2(F)). Pinot noir, followed by Gamay noir, had the highest mean ethylamine concentrations of the red wines.

3.7. Methylamine

Concentrations of this amine were much higher than for most of the other compounds (histamine, $p < 0.02$; 2-phenylethylamine, $p < 0.01$; ethylamine, cadaverine and

tyramine, $p < 0.001$) and the concentrations of red and white wines showed almost complete overlap (Fig. 2(G)). Indeed, Chardonnay wines had the highest mean concentration of all the cultivars ($p < 0.01$). Next highest were Seyval blanc and Pinot noir ($p < 0.02$, combined). Surprisingly, Late Harvest Vidal had negligible concentrations although significant concentrations were present in wines from regular Vidal blanc grapes.

3.8. 1-Methylhistamine

The pattern for this amine was similar to the previous one with overlapping values between the red and white wines (Fig. 2(H)). Wines from Chardonnay and Pinot noir had the highest mean concentrations ($p < 0.02$, combined), followed by wines from Seyval blanc ($p < 0.02$). This amine could not be detected in wines from Muscat Mario and Late Harvest Vidal, although wines from regular grapes of the latter cultivar had consistent but low levels.

3.9. Tryptamine

This amine was detectable in very few of the Ontario wines examined in this survey, and there were insufficient data to conduct a proper analysis.

3.10. Total biogenic amines

The relative concentrations of biogenic amines ($mg\,l^{-1}$) approximated the following order: putrescine > methylamine > histamine > ethylamine > 1-methylhistamine > 2-phenylethylamine = tyramine = cadaverine. To calculate the total amine content, each amine concentration was converted from $mg\,l^{-1}$ to $\mu mol\,l^{-1}$ and the sum of the molar concentrations of all the amines in each wine was derived as $mmol\,l^{-1}$ and averaged for the different cultivars. The data (Fig. 3), not unexpectedly, demonstrate that wines from Pinot noir had the highest mean total amine content ($p < 0.05$); there was little difference between the other four red cultivars. Chardonnay wines had the highest mean total amine content among the white wines ($p < 0.05$) with Seyval blanc wines next in line ($p < 0.02$). Late Harvest Vidal wines had the lowest content, some way behind the regular wines from this cultivar.

4. Discussion

4.1. Analytical aspects

A very useful review has been published by Lehtonen (1996) in which he has summarized the features of 16 methods for measuring biogenic amines in wine. Nine of these employed derivatization with OPA, but in only one (Lehtonen et al., 1992) was pre-column (as opposed to post-column) derivatization used together with automation of the derivatization procedure. While dansyl chloride and fluorescamine have also been used, a detailed comparison performed by Buteau et al. (1984b) has clearly established OPA as the optimal derivatizing agent. Apart from two early reports in which isocratic elution was employed (Subden et al., 1978; Cilliers and Van Wyk, 1985) all other authors have utilized gradient elution to separate the OPA adducts of the biogenic amines. Only Mayer and Pause (1984) and Crespo and Lasa (1994) used an acetate:methanol gradient in their elution procedure although they differ in detail from that which we employed.

Few authors have provided a full description of the analytical characteristics of the methods they have developed or used, as we have done in the present report (Table 3). Cilliers and Van Wyk (1985) reported similar imprecision for histamine and tyramine as we experienced; their recovery for histamine was 99% and for tyramine 84%, a lower value than we found. Yen and Chandra (1988) described good linearity and excellent recoveries (range 95 to 101.3%) for six biogenic amines (not including 1-methylhistamine, methylamine and ethylamine) that they measured in a variety of alcoholic beverages, not including wine, using pre-column derivatization with dansyl chloride. Vidal-Carou et al. (1991), using a spectrofluorometric method to measure histamine and tyramine, obtained imprecision around 6.4% (CV) and recoveries around 93% for both compounds.

Lehtonen et al. (1992) provided a fairly complete critique of their method which allowed the determination of six biogenic amines (not including 1-methylhistamine, methylamine, ethylamine and tryptamine). They reported excellent reproducibility with the CV ranging from 0.9% (histamine) to 3.6% (putrescine). The recoveries they obtained were also very satisfactory, being in the range 95–100% for all constituents. However, their detection limits, ranging from 0.5 to $1.0\,mg\,l^{-1}$, were very much higher than ours and although they did not give details of linearity experiments, it may be assumed because of its inferior sensitivity that their method gives a narrower range over which a linear response may he expected.

Crespo and Lasa (1994) also described many features of their method, which enabled seven biogenic amines to be measured, all of which could also be assayed by our method. Their CVs were much higher than ours, ranging from 10% (ethylamine) to 21% (putrescine). The detection limits they reported were also higher than our own, ranging from $0.1\,mg\,l^{-1}$ (2-phenethylamine) to $0.5\,mg\,l^{-1}$ (putrescine). Data for recovery and linearity were not included in their report.

Taking into account the number of amines that can be measured, the assay time, the degree of automation

and its analytical characteristics, the present method ranks among the most versatile and optimal published to date.

4.2. Biogenic amine content of wines

Vidal-Carou et al. (1990a) described changes in histamine and tyramine content of five Spanish wines over periods ranging up to 297 days from initiation of fermentation to bottling. The final content of the former was 0.25 and 0.30 mg l^{-1} in one rosé and one white wine, respectively and in three red wines the values ranged from 0.35 to 8.50 mg l^{-1}. The corresponding data for tyramine were 0.60, 0.90 and 0.75–8.30 mg l^{-1}. The cultivars fermented were not described. Changes in temperature, in volatile acidity and in total sulfur dioxide content of unsealed bottles did not affect amine concentrations over a period of 121 days (Vidal-Carou et al., 1990b, Vidal-Carou et al., 1991).

Lehtonen et al. (1992) reported data on a total of 21 red and white wines from 11 countries and found the following range of concentrations: histamine 0.1–15.1 mg l^{-1}; tyramine 0.5–12.8 mg l^{-1}; putrescine 1.6–72 mg l^{-1}; cadaverine 0.1–1.1 mg l^{-1}; β-phenethylamine 0.2–2.7 mg l^{-1}. No cultivars were identified in this publication.

Subden et al. (1979) observed that the histamine content of Canadian wines ranged from 0.051 to 6.11 mg l^{-1}. The cultivar of origin was identified for all of the 22 wines and *Vinifera* were found to have a higher mean content than American hybrids or French–American hybrids. This is somewhat at variance with the report of Baucom et al. (1986), who found higher mean values for histamine in hybrids (5.6 mg l^{-1} for reds and 3.5 mg l^{-1} for whites) compared with *Vinifera* (2.0 mg l^{-1} for reds and 1.1 mg l^{-1} for whites). Tyramine concentrations were extremely low in all wines, but mean putrescine concentrations were 1.1 and 2.7 mg l^{-1} for red and white hybrids, respectively, and 2.6 and 2.4 mg l^{-1} for red and white *Vinifera*; mean cadeverine concentrations were 21.8 and 37.3 mg l^{-1} for red and white hybrids and 17.5 and 17.6 mg l^{-1} for red and white *Vinifera*, respectively.

In a survey of South African wines (117 red, 62 white and 5 rosé of unstated origin), the mean histamine content of the reds was 4.8 mg l^{-1} (< 10 mg l^{-1} in around 90%), and of the whites 0.1 mg l^{-1}, whereas mean tyramine concentrations were 0.5 mg l^{-1} for the reds with very few white wines exceeding 0.1 mg l^{-1} (Cilliers and Van Wyk, 1985). Malolactic fermentation increased the concentrations of both amines.

Surprisingly, by far the most comprehensive data on wine amines were published as far back as 1983 by Zee et al., 1983 who measured their content in 230 wines from seven countries, most being from France and Canada. The Canadian red wines had mean values for histamine, tyramine, putrescine and cadaverine that were 3.7, 4.3, 2.2 and 0.3 mg l^{-1}, respectively, but no information about the grape origin was provided; indeed at that time many Canadian wines were made from fermented imported juices, as well as French hybrids and *V. labrusca* grapes. The highest concentrations were present in French red wines and although no information on cultivar of origin was given, the wines were classified as to region of origin. Interestingly, the highest values were present in Burgundy wines that are vinted exclusively from Pinot noir, in line with our own findings. Very high tyramine concentrations were present in all these wines irrespective of country of origin, and the content in white wines approached that of red wines, notably in those from France and Portugal.

From this survey of the literature, it is clear that our knowledge of the intrinsic concentrations of amines in wine is incomplete and the data, particularly for tyramine, show wide discrepancies that might be owing to differences in assay technique, to country of origin and to the cultivar used in the fermentation process. Variations in some enological procedures, e.g. temperature and use of sulfur, appear to have little influence (Vidal Carou et al. 1990b), whereas other procedures, such as malolactic fermentation, seem to play a greater role. However, these studies involve such small numbers, with lack of identification of the cultivar, that the role of the cultivar cannot be excluded. There are many other enological variables that remain to be examined. Among these, mention may be made of the following:

1. *Length of skin fermentation*. This could explain, at least in part, the higher concentrations of total amines in Pinot noir wines (Fig. 3) since this grape, because of its lighter colour, usually receives the longest skin contact. Taking all the red wines in this study together, however, there was no correlation between length of skin contact and concentration of any of the biogenic amines measured.
2. *Length of barrel ageing and Sur-lie fermentation*. The majority of Canadian winemakers utilize these processes in addition to malolactic fermentation in the production of Chardonnay wines. These had the highest total amine content of all the white wines surveyed (Fig. 3). However, all red wines received malolactic fermentation and there was no correlation between amine content and length of barrel ageing within the 0–18-month period employed for these wines. In particular, wines from Gamay noir (shortest or no ageing) had a higher content of individual or total amines than many receiving long barrel ageing.
3. *Growth of bacteria and yeasts in the must*. These factors can only be examined by sampling the must at different stages prior to racking, filtering and bottling. Such an investigation could only be

performed at the time of harvest following crushing of the grapes and initiation of fermentation. Since one yeast strain was used for the majority of these wines, it is unlikely that the exogenous yeast had any real influence. Nor are there any grounds for suspecting that some cultivars are more susceptible to contamination by yeast and bacteria than others.

Novel observations stemming from this work include the quantitative importance of methylamine, ethylamine and 1-methyl histamine, which were among the five highest of the eight amines assayed. Other new findings presented in this report emphasize the extent of intercultivar differences in wines from a very delimited viticultural region utilizing consistent enological techniques produced in a single vintage under identical climatic conditions. These suggest that there is a need to examine this factor in wines from many other regions and to determine whether their expression of amines is modulated by soil, sunlight and climatic conditions, as occurs with trihydroxystilbenes (Goldberg et al., 1995b, Goldberg et al., 1996), flavan-3-ols (Goldberg et al., 1998a) and flavonoids (Goldberg et al., 1998b), or whether cultivar-related differences play the major role, with or without influence from the region-specific features mentioned above.

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References

Adams, D. O., Franke, K. E. and Christensen, L. P. (1990) Elevated putrescine levels in grapevine leaves that display symptoms of potassium deficiency. *American Journal of Enology and Viticulture* **41**, 121–125.

Baucom, T. L., Tabacchi, M. H., Cotrell, T. H. E. and Richmond, B. S. (1986) Biogenic amines content of New York State wines. *Journal of Food Science* **51**, 1376–1377.

Buteau, C., Duitschaever, C. L. and Ashton, G. C. (1984a) A study of the biogenesis of amines in a Villard Noir wine. *American Journal of Enology and Viticulture* **35**, 228–236.

Buteau, C., Duitschaever, C. L. and Ashton, G. C. (1984b) High-perfomance liquid chromatographic detection and quantitation of amines in must and wine. *Journal of Chromatography* **284**, 201–210.

Cilliers, M. I. and Van Wyk, C. J. (1985) Histamine and tyramine content in South African wines. *South African Joural of Enology and Viticulture* **6**, 35–40.

Crespo, M. I. and Lasa, B. V. (1994) Determination of biogenic amines and other amines in wine by an optimized HPLC method with polarity gradient elution. *American Journal of Enology and Viticulture* **45**, 460–463.

Frankel, E. N., Waterhouse, A. L. and Teissedre, P. L. (1995) Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *Journal of Agricultural and Food Chemistry* **43**, 890–894.

Geny, L., Broquedis, M., Martin-Tanguy, J. and Bouard, J. (1997) Free, conjugated, and wall-bound polyamines in various organs of fruiting cuttings of *Vitis vinifera* L. CV. Cabernet Sauvignon. *American Journal of Enology and Viticulture* **48**, 80–84.

Goldberg, D. M., Hahn, S. E. and Parkes, J. G. (1995a) Beyond alcohol: beverage consumption and cardiovascular mortality. *Clinica Chimica Acta* **237**, 155–187.

Goldberg, D. M., Yan, J., Ng, E., Diamandis, E. P., Karumanchiri, A., Soleas, G. J. and Waterhouse, A. (1995b) A global survey of trans-resveratrol concentrations in commercial wines. *American Journal of Enology and Viticulture* **46**, 159–165.

Goldberg, D. M., Ng, E., Karumanchiri, A., Diamandis, E. P. and Soleas, G. J. (1996) Resveratrol glucosides are important components of commercial wines. *American Journal of Enology and Viticulture* **47**, 415–420.

Goldberg, D. M., Karumanchiri, A., Tsang, E. and Soleas, G. (1998a) Catechin and epicatechin concentrations of red wines: regional and cultivar-related differences. *American Journal of Enology and Viticulture*, **49**, 23–34.

Goldberg, D. M., Tsang, E., Karumanchiri, A. and Soleas, G. J. (1998b) Quercetin and *p*-coumaric acid concentrations in commercial wines. *American Journal of Enology and Viticulture*, **49**, 142–151.

Lehtonen, P. (1996) Determination of amines and amino acids in wine — a review. *American Journal of Enology and Viticulture* **47**, 127–133.

Lehtonen, P., Saarinen, M., Vesanto, M. and Riekkola, M.-L. (1992) Determination of wine amines by HPLC using automated precolumn derivatization with phthalaldehyde and fluorescence detection. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung* **194**, 434–437.

Lovenberg, W. (1974) Psycho- and vasoactive compounds in food substances. *Journal of Agriculture and Food Chemistry* **22**, 23–26.

Maga, J. A. (1978) Amines in foods. *CRC Critical Reviews in Food Science and Nutrition* **10**, 373–403.

Mayer, K. and Pause, G. (1984) Bestimmung von biogenen Aminen in Wein mit Hochleistungsflussigkeitschromatographie. *Lebensmitteluntersuch–Weinbereitung Technologie* **17**, 177–179.

Radler, F. and Fath, K.-P. (1991) Histamine and other biogenic amines. In *International Symposium on Nitrogen in Grapes and Wine*, ed. J. A. Rantz, pp. 185–194. The American Society of Enology and Viticulture, Davis.

Smith, T. A. (1980) Amines in food. *Food Chemistry* **6**, 169–200.

Sokol, R. R. and Rohlfs, F. J. (1981) *Biometry. The Principles and Practice of Statistics in Biological Research*, 2nd edn. Freeman, San Francisco.

Soleas, G. J., Diamandis, E. P. and Goldberg, D. M. (1997a) Wine as a biological fluid: history, production and its role in disease prevention. *Journal of Clinical Laboratory Analysis* **11**, 287–313.

Soleas, G. J., Dam, J., Carey, M. and Goldberg, D. M. (1997b) Toward the chemical fingerprinting of wines: cultivar-related patterns of polyphenolic constituents in Ontario wines. *Journal of Agriculture and Food Chemistry*, **45**, 3871–3880.

Subden, R. E., Brown, R. G. and Noble, A. C. (1978) Determination of histamine in wines and musts by reversed-phase high performance liquid chromatography. *Journal of Chromatography* **166**, 310–312.

Subden, R. E., Duitschaever, C., Kaiser, K. and Noble, A. C. (1979) Histamine content of Canadian wines determined by reverse phase

high performance liquid chromatography. *American Journal of Enology and Viticulture*. **30**, 19–21.

Tabor, C. W. and Tabor, H. (1984) Polyamines. *Annual Reviews of Biochemistry* **53**, 749–790.

Taylor, S. L. (1986) Histamine food poisoning: toxicology and clinical aspects. *CRC Critical Reviews in Toxicology*. **17**, 91–128.

Vidal-Carou, M. C., Ambatlle-Espunyes, A., Ulla-Ulla, M. C. and Marine-Font, A. (1990a) Histamine and tyramine in Spanish wines: their formation during the winemaking process. *American Journal of Enology and Viticulture* **41**, 160–167.

Vidal-Carou, M. C., Codony-Salcedo, R. and Marine-Font, A. (1990b) Histamine and tyramine in Spanish wines: relationships with total sulfur dioxide level, volatile acidity and malo-lactic fermentation intensity. *Food Chemistry* **35**, 217–227.

Vidal-Carou, M. C., Codony-Salcedo, R. and Marine-Font, A. (1991) Changes in the concentration of histamine and tyramine during wine spoilage at various temperatures. *American Journal of Enology and Viticulture* **42**, 145–149.

Yen, G. -C. and Chandra, T. (1988) Biogenic amines in alcoholic beverages produced in Taiwan. *Journal of Science Food and Agriculture* **44**, 273–280.

Zee, J. A., Simard, R. E., Heureux, L. L. and Tremblay, J. (1983) Biogenic amines in wines. *American Journal of Enology and Viticulture* **34**, 6–9.