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Polyphenol content and browning of Canadian icewines

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Abstract

The polyphenol composition of a range of Ontario icewines and late harvest wines was quantified. For 1999-2000 experimental wines, the FAUX icewines (harvested above -8°C and before 18 December) had high concentrations of hydroxycinnamates (10 to 37 mg/L in Riesling; 53 to 82 mg/L in Vidal) which dropped to concentrations below 10 mg/L for the REAL icewines (harvested at temperatures -8°C or below and after 28 December). However, in 2002 experimental icewines, high concentrations of hydroxycinnamates were seen in both REAL and FAUX wines (40 to 84 mg/L in Riesling; 106 to 129 mg/L in Vidal), but all of the grapes were harvested before 11 December. Low to moderate concentrations of total hydroxycinnamates were observed in commercial icewines (10 to 40 mg/L) and late harvest wines (5 to 60 mg/L). Freeze concentration during icewine making appears to have substantial impact upon polyphenol composition and concentration, but time of harvest and growing season appear to be equally important.

Key words: Polyphenols, icewine, browning, Vidal, Riesling.

Introduction

Icewine has become one of the most well-recognized, unique, and lucrative products to emerge from Canada's wine industry in the past 20 years. A large portion of Canada's wine export market, especially to Asia, has been based upon its sales. However, some products are sold in Asia as VQA icewine even though their origin and composition are dubious. This is a concern as it puts at risk the quality reputation of the authentic Canadian product. Other countries have also produced faux "icewine" using cryogenic extraction methods and have claimed that these cryogenically-produced wines, which sell at a fraction of the price of Canadian icewines, are of equivalent quality to those produced using grapes that were naturally frozen on the vine. There are presently no volatile or non-volatile chemical constituents unique to Canadian icewines identified that would distinguish authentic icewines from their faux counterparts. Moreover, there are no data that help describe the effects of harvest date or other factors on the chemical composition of icewines.

The polyphenol content of white wine grapes is important for the browning which occurs in juice or wine when exposed to oxygen. In icewines, these polyphenols are important in determining the color as well as certain mouthfeel components. The main white wine polyphenols are the hydroxycinnamic acids, of which caftaric acid is present in the highest amounts in juice, often exceeding 100 mg/L [1]. Other major grape hydroxycinnamates include fertaric and coutaric acids, while some flavanols (catechin and epicatechin), gallic acid and other polyphenols can be found[1].

During grape crushing the release of polyphenol oxidase enzymes brings about a rapid oxidation of the hydroxycinnamic acids, a process which is inhibited by the addition of SO_2. The initial quinone product from caftaric acid can react with available glutathione to form the "grape reaction product" (GRP), S-glutathionyl caftaric acid, which is less susceptible to enzymatic oxidation [2]. Otherwise, these quinones can react further with polyphenols, particularly with any flavanols present and also with the GRP to produce brown-colored pigments of unknown structure[3]. The tartrate esters of the hydroxycinnamates can also hydrolyze to some extent, releasing the free caffeic, ferulic and coumaric acids.

Riesling and the French-American hybrid Vidal are commonly used in Canada to produce icewines. In one study of table wines, the higher pH (3.29) and total phenols (244 mg/L) for the Vidal wines, versus a pH of 3.09 and total phenols of 212 mg/L for Riesling, was mentioned as a reason why the Vidal wines were browner after 3 months of storage at 37°C [4]. In a survey of 644 white wines from around the world, the highest flavan-3-ol concentrations (up to 10 mg/L of catechin) were observed in the French-American hybrids (Vidal and Seyval), with p-coumaric acid also high; Riesling had moderate catechin concentrations (4 to 5 mg/L), low epicatechin and high p-coumaric acid (1 to 2 mg/L) [5]. Correlations between flavan-3-ol content and browning have been reported [6]. In a further study on the polyphenol content of Ontario wines, nine Riesling, three Vidal and three late-harvest Vidal wines were included [7]. The late-harvest Vidal wines contained significantly higher concentrations of gentisic acid (1.07 mg/L), vanillic acid (0.38 mg/L), ferulic acid (6 mg/L – also high in Riesling at 4.42 mg/L), p-coumaric acid (3.21 mg/L), but a lower concentration of caffeic acid (2 mg/L) than regular Vidal wine (4.61 mg/L) which was also highest in gallic acid (2.8 mg/L), catechin and epicatechin; total resveratrols were less than 0.3 mg/L and no flavonols were detected. It was considered that since the late-harvest grapes are picked 3 to 4 weeks after grapes for table wines, considerable concentration could occur in certain compounds, while the lower concentration of catechin and epicatechin in the late-harvest Vidal wine could have been due to continuing UV exposure or over-maturation of the grapes. Overall, concentrations of polyphenols in wine will reflect both grape composition as well as the effect of different winemaking operations, particularly those which lead to higher rates of oxidation.

In relation to the effects of freezing on grape polyphenols, it was noted 30 years ago that in the presence of oxygen, browning of berries occurred [8]; but, freezing of muscadine grapes for 9 months at –28°C increased juice yield and pigment concentration, did not give rise to excessive browning, nor was browning noted during frozen storage; it was nevertheless expected that freezing would disrupt cells leading to the release of cellular constituents[9]. In a study on the effect of freezing to –7°C, then thawing to +10°C, on Chardonnay, Chenin blanc and Riesling, frozen grapes had a brown appearance and the juice was seen to be browner; caftaric acid was present at an average of 101 mg/L in wines from fresh grapes, but at 65 mg/L in wines from grapes after freeze-thaw [10]. Again cellular disruption was expected to release polyphenol oxidases and freeze-thaw result in the extraction of catechins and proanthocyanidins from the skins which could in turn be oxidized. However, after settling, racking, fining and cold stabilization the wine had a similar appearance to that from non-frozen grapes. Pigments formed by enzymatic oxidation in the must can be removed during clarification procedures [6].

A recent study on Canadian icewines reported that the 420 nm absorbance was higher in 28 Vidal wines (0.596±0.22, pH 3.5±0.2) than in 14 Riesling wines (0.365±0.12, pH 3.2±0.2) [11], consistent with a previous study on Canadian icewines [12], while higher absorbance values were obtained for Ontario wines relative to those from British Columbia, and for wines in the 1995-1997 range than in the 1998-2000 range. While a higher polyphenol concentration and lower titratable acidity was expected in the Vidal wines, leading to the high absorbance value for the Vidal wines, the polyphenol concentration of icewines has not been previously determined.

The objective of this study was to investigate the polyphenol composition of a range of Canadian icewines, with particular reference to the effect of harvest date and freeze concentration upon the polyphenol concentration. Wines were available for analysis from two icewine trials undertaken from grapes harvested in 1999-2000 and in 2002, including both REAL icewines (grapes harvested and pressed at -8°C or less), and FAUX icewines (produced by harvesting grapes under warmer conditions and subsequently freezing the grapes). A comparison was made also with commercial icewines and late harvest wines.

Experimental

Wines: Experimental Riesling and Vidal icewines were available from grapes harvested in 1999-2000 and in 2002, used to produce both REAL icewines and FAUX icewines. The 1999-2000 wines were produced from Riesling and Vidal grapes harvested from Hillebrand Estates Winery in Niagara-on-the-Lake, Ontario. Harvest dates were: 22 November 1999 (5.9°C; FAUX); 18 December 1999 (-5.5°C; FAUX); 28 December 1999 (-14.0°C; REAL); and 14 January 2000 (-15.2°C; REAL). About 375 kg of grapes were harvested on each date for each cultivar. Grapes were picked into 20 kg-capacity plastic totes that were immediately enclosed in a polyethylene sleeve and thereafter stored at -40°C. Once grapes from all harvest dates were collected, they were pressed using a vertical screw press. Three press fractions of approximately 25 L were collected into food-grade plastic pails from each harvest date treatment, of which 19 L were transferred to 20 L-capacity glass carboys. Musts were then sulfited to 150 mg/L SO_2, allowed to reach room temperature and were inoculated with EC1116 (Lallemand Inc., Montreal, QB) at a rate of 80 g/hL. Fermentations proceeded at 18°C until residual sugar reached ca. 180 g/L. Wines were then cold stabilized at -2°C, filtered through 0.45 µm pads and a 0.2 µm cartridge filter, sulfited with an additional 100 mg/L SO_2, sorbated at 250 mg/L potassium sorbate, and bottled. Wines were stored at 11°C. The 2002 wines were made from Vidal and Riesling grapes harvested from Garphil Farms in St. Catharines, ON on 12 November (-4°C; FAUX) and 11 December 2002 (-11°C; REAL). Storage of grapes, pressing and fermentation were carried out as per the 1999-2000 vintage. Comparisons were also made among five commercial icewines and four late harvest wines (one a *Botrytis*-affected Riesling) as well as three dry experimental Rieslings produced from grapes from three different vintages obtained from Vineland Estates, Vineland, ON. Details of the wines are provided in Table 1.

Standard must analysis was performed consistent with previously described methods [13]. Brix was determined using a temperature-corrected benchtop Abbé refractometer (model 10450; American Optical Corp., Buffalo, NY). Must titratable acidity (TA) was determined by titration of a 25 mL sample using a PC Titrate auto-titrator (Man-Tech Associates Ltd., Guelph, ON) utilizing 0.1 N NaOH. Berry/must pH was obtained using an Accumet model 25 pH/ion meter (Denver Instruments, Denver, CO). The wine absorbance at 420 nm was recorded using a Pharmacia Biotec Ultrospec UV/VIS spectrophotometer model 1000E (Biochrom Ltd., Cambridge, UK).

HPLC analysis: An HP1100 HPLC instrument was used with diode array detector set at wavelengths of 280 nm (for gallic acid and flavanols), 320 nm (for hydroxycinnamates) and 360 nm (for flavonols) using a method derived from previously published procedures [14, 15]. An Agilent Zorbax SB-C_{18} column, 3.5 mm packing, 4.6 mm x 50 mm, was used as the stationary phase. 10 mL of wine was directly injected after first filtering through 0.45 mm Millipore HV Durapore membrane filters. The flow rate was 1.0 mL/min, with a gradient elution comprised of A: 0.2% trifluoroacetic acid (Aldrich, 99+% spectral grade, 302031) in Milli-Q grade water, B: 0.2% trifluoroacetic acid in HPLC grade acetonitrile-190 (Caledon Laboratories Ltd., Georgetown, ON). The solvent composition was increased linearly from 5% B to 35% B after 15 minutes, to 100% B after 16 minutes where it was held until 25 minutes, then reduced to 5% B after 26 minutes.

Standards were obtained from Sigma-Aldrich for caffeic acid (CO625 – 99%), gallic acid (G7384), epicatechin (E1753), catechin (CO567), ferulic acid (128708 – 99%), p-coumaric acid (C9008), while quercetin was obtained from Extrasynthèse (HPLC, 1135 S). Stock solutions of standards were prepared in an aqueous 10% ethanol solution for gallic acid (24.6 mg in 25.0 mL), epicatechin (25.2 mg in 25.0 mL), caffeic acid (25.5 mg in 50.0 mL) and quercetin (1.0 mg in 10.0 mL); in each case the compounds were first dissolved in 5 mL of 50/50 water/ethanol (expect quercetin was dissolved in pure methanol) before making up to volume with the 10% ethanol

Table 1. Wine descriptions, must composition data, 420 nm absorbance, and peak areas for the 280 nm peak at 8.2 minutes.

	Wine	Harvest date, press fraction, or supplier	°Brix or % alc. [b]	TA[b] g/L	pH	420 nm absorbance	Peak at 8.2 min
1	2002 Riesling	1st Harvest (F[c])	---	11.8	3.34	0.377	882
2	2002 Riesling	1st Harvest (F)	---	11.8	3.34	0.358	852
3	2002 Riesling	2nd Harvest (R[d])	---	11.8	3.34	0.318	792
4	2002 Riesling	2nd Harvest (R)	---	11.8	3.34	0.324	811
5	2002 Vidal	1st Harvest (F)	---	10.1	3.64	0.337	802
6	2002 Vidal	1st Harvest (F)	---	10.1	3.64	0.358	736
7	2002 Vidal	2nd Harvest (R)	---	10.1	3.64	0.312	1076
8	2002 Vidal	2nd Harvest (R)	---	10.1	3.64	0.364	1021
9	1999 Riesling	22 Nov 1999 (F) pf1[e]	37.4	7.4	3.05	0.410	12
10	1999 Riesling	22 Nov 1999 (F) pf2	41.7	8.2	3.04	0.402	11
11	1999 Riesling	18 Dec 1999 (F) pf1	34.1	5.3	3.20	0.303	8
12	1999 Riesling	18 Dec 1999 (F) pf2	33.9	5.4	3.21	0.251	3
13	1999 Riesling	18 Dec 1999 (F) pf3	35.7	5.4	3.19	0.213	4
14	1999 Riesling	28 Dec 1999 (R) pf1	29.4	4.5	3.25	0.239	5
15	1999 Riesling	28 Dec 1999 (R) pf2	33.4	5.2	3.21	0.232	4
16	1999 Riesling	28 Dec 1999 (R) pf3	35.1	5.3	3.16	0.289	5
17	1999 Riesling	14 Jan 2000 (R) pf3	31.9	4.9	3.25	0.248	4
18	1999 Vidal	22 Nov 1999 (F) pf1	39.3	9.1	3.21	0.335	15
19	1999 Vidal	22 Nov 1999 (F) pf2	31.9	7.5	3.31	0.379	8
20	1999 Vidal	22 Nov 1999 (F) pf3	29.1	6.6	3.37	0.265	7
21	1999 Vidal	18 Dec 1999 (F) pf1	37.5	6.9	3.45	0.400	9
22	1999 Vidal	18 Dec 1999 (F) pf2	37.8	7.0	3.45	0.468	12
23	1999 Vidal	18 Dec 1999 (F) pf3	36.7	6.8	3.45	0.474	16
24	1999 Vidal	28 Dec 1999 (R) pf1	29.0	4.9	3.54	0.149	4
25	1999 Vidal	28 Dec 1999 (R) pf2	36.6	6.0	3.02	0.326	12
26	1999 Vidal	28 Dec 1999 (R) pf3	29.7	4.7	3.49	0.237	6
27	1999 Vidal	14 Jan 2000 (R) pf2	37.1	5.7	3.50	0.525	15
28	1999 Vidal	14 Jan 2000 (R) pf3	25.8	4.1	3.58	0.235	7
29	1999 Riesling	Henry of Pelham	9.5 %	---	---	0.443	0
30	1999 Vidal	Konzelmann Estate	11 %	---	---	0.557	1177
31	1999 Riesling	Konzelmann Estate	11.5 %	---	---	0.464	1036
32	2003 Vidal	Peller Estates	11.5 %	---	---	0.357	0
33	2002 Riesling	Peller Estates	11.0 %	---	---	0.314	0
34	2000 Vidal	Henry of Pelham (LH[f])	10.5 %	---	---	0.414	12
35	2001 Riesling	Strewn (*Botrytis*-affected)	13.2 %	---	---	0.352	1360
36	1998 Chardonnay	Rief Estate (LH)	12.5 %	---	---	0.166	1014
37	1999 Vidal	Henry of Pelham (LH)	9.5 %	---	---	0.391	0
38	2002 Riesling	DRY	11.4%	9.1	3.02	0.137	3
39	2001 Riesling	DRY	10.9%	9.2	2.97	0.156	1
40	1999 Riesling	DRY	10.3%	8.6	3.09	0.194	4

[a] All 2002 icewine musts were 35 °Brix; [b] TA = titratable acidity; [c] F = Faux; [d] R = Real; [e] pf = press fraction; [f] LH = Late Harvest.

solution. Successive 2.5-fold dilutions were used to create five standard solutions (2.6 to 100 mg/L for gallic acid and epicatechin, 1.3 to 50 mg/L for caffeic acid, and 0.26 to 10 mg/L for quercetin), which were each injected in duplicate.

The significance of differences between mean values obtained was determined using a Student t-test ($p \leq 0.05$).

Results and Discussion

pH and absorbance: The mean pH for the 1999-2000 experimental Riesling wines at 3.17±0.08 was significantly lower than that for the Vidal wines at 3.37±0.16 ($p \leq 0.05$); the pH at bottling was also recorded as lower for the 2002 experimental Riesling (3.34) than for the Vidal (3.64). Regarding icewine color and the absorbance at 420 nm, the 13 experimental Rieslings had a lower mean value of 0.31±0.07 compared to the 15 experimental Vidal wines at 0.34±0.10 (Table 1), but this difference was not statistically significant (at $p \leq 0.05$), even when the five commercial icewines were included and the values became 0.32±0.08 for Riesling and 0.36±0.11 for Vidal. No correlation was observed between the concentration of total hydroxycinnamates and the absorbance at 420 nm. The 2002 experimental icewines had a 420 nm absorbance of 0.34±0.02 which was not statistically different from the 1999-2000 icewines at 0.32±0.10 ($p \leq 0.05$). Browning is still likely to develop gradually with age, and the higher value for the 2002 wines may reflect a larger initial polyphenol concentration for grapes which were harvested at an earlier date in the year (see below). This example only serves to illustrate the potential variability between years, both in the grapes themselves and in wine handling procedures.

HPLC analysis, compounds identified: Representative chromatograms at 320 nm for a 2002 experimental Vidal and Riesling icewine are presented in Fig. 1. Retention times were very consistent between injections made over a 3-day period and were usually within 0.05 min. Using commercial standards the following retention times were obtained: 280 nm: gallic acid (1.2 min.), catechin (4.3 min.), epicatechin (5.7 min.); 320 nm: caffeic acid (4.9 min.), p-coumaric acid (6.7 min.), ferulic acid (7.5 min.); 360 nm: quercetin (12.4 min.). Various unidentified peaks were seen in the icewine samples at 280 nm, but no peaks were observed which could be assigned to the flavanols catechin or epicatechin. However, overlap with hydroxycinnamate peaks was seen to occur at these retention times. A peak for gallic acid was observed in each of the wine samples, reaching a maximum of 6 mg/L. The size of the 280 nm peaks (values not shown) was quite variable even between wines of the same treatment group, and they were much

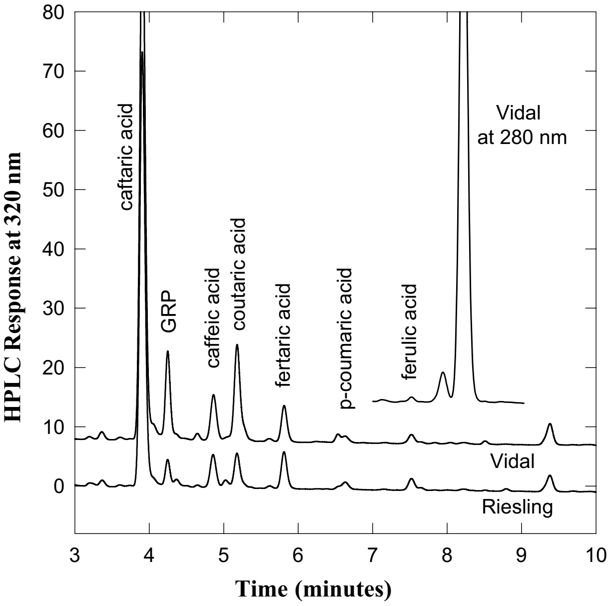

Figure 1. HPLC trace at 320 nm for 2002 experimental Vidal and Riesling icewines.

lower in the three dry wines tested compared to the icewines and late harvest wines. A major, but as yet unidentified peak at 8.2 minutes, observed in some icewines and late harvest wines, but not others, was a striking feature of the chromatograms (Table 1 and Fig. 1). It appeared in all of the 2002 experimental icewines, but in none of the 1999 experimental icewines.

Three of the peaks observed at 320 nm were assigned using commercial standards, while the major peak at 3.9 min. was expected to be due to caftaric acid; the peak at 4.25 min was assigned to the grape reaction product (GRP), S-glutathionyl caftaric acid, consistent with previous HPLC studies where this compound was found to elute shortly after caftaric acid [16]. Considerable similarity in the detail of the UV spectra was seen between the peaks at 5.2 min and 6.7 min. (coumaric acid) (both have a peak absorbance at 305 to 315 nm and minimal absorbance at 360 nm), and between peaks at 5.8 and 7.5 min. (ferulic acid) (maximum absorbance at 322 to 330 nm), leading to the tentative assignment of the peak at 5.2 min to coutaric acid and that at 5.8 min to fertaric acid, the respective tartrate esters. Further unknown compounds with an absorbance maximum around 320 nm were seen at 9.4 and 10.9 minutes. These compounds were included in determining a measure of the total hydroxycinnamates in terms of caffeic acid equivalents.

The peaks obtained at 360 nm were generally smaller versions of peaks seen at 320 nm, consistent with the smaller absorbance at 360 nm of the hydroxycinnamates, which showed absorbance maxima in the 310 to 330 nm range. In some wines (more often in Vidal) a small peak assigned to quercetin was observed at 12.4 min., but this never exceeded 0.4 mg/L. None of the wines exhibited aromas indicative of over-oxidation or premature aging.

Effects of harvest date: There was a marked difference in the concentration of hydroxycinnamic acids between REAL and FAUX icewines for the 1999 experimental wines (Table 2). All of the FAUX icewines had high concentrations of total hydroxycinnamates (10 to 37 mg/L in Riesling; 53 to 82 mg/L in Vidal) which dropped to very low concentrations for the REAL icewines (3.3 to 5.1 mg/L in Riesling; 3.8 to 11 mg/L in Vidal). However, in the 2002 experimental icewines, high concentrations of hydroxycinnamates were seen in both REAL and FAUX wines (40 to 84 mg/L in Riesling; 106 to 129 mg/L in Vidal). Low to moderate concentrations of total hydroxycinnamates were observed in the commercial icewines (10 to 40 mg/L) and late harvest wines (5 to 60 mg/L), while values in the three dry Riesling wines ranged from 23 to 60 mg/L. The reason for the lower concentrations of hydroxycinnamates in the REAL 1999

Table 2. Concentrations of the main hydroxycinnamic acids (in mg/L caffeic acid equivalents).

	Wine	Caftaric	GRP[a]	Caffeic	Coutaric	Fertaric	Coumaric	Ferulic
1	2002 Riesling (F[b])	60	2.4	4.9	5.3	5.4	1.3	1.9
2	2002 Riesling (F)	58	2.5	4.7	5.1	5.4	1.2	1.9
3	2002 Riesling (R[c])	41	1.5	3.3	3.3	4.6	1.2	2.1
4	2002 Riesling (R)	26	0.5	2.6	2.5	3.8	1.3	1.9
5	2002 Vidal (F)	75	9.8	6.5	14.5	5.3	1.0	1.9
6	2002 Vidal (F)	81	10.8	6.8	15.9	5.4	1.1	1.9
7	2002 Vidal (R)	68	7.7	7.5	15.5	5.1	1.4	1.5
8	2002 Vidal (R)	65	7.1	7.1	15.3	4.9	1.2	1.6
9	1999 Riesling (F)	17	0.5	2.4	2.4	2.5	0.7	1.0
10	1999 Riesling (F)	24	0.6	3.2	2.3	3.1	0.9	1.1
11	1999 Riesling (F)	10.5	0.2	1.7	1.1	2.2	0.8	1.0
12	1999 Riesling (F)	4.5	0.1	1.2	0.8	1.7	0.8	0.9
13	1999 Riesling (F)	4.7	0	1.0	0.6	1.8	0.8	0.9
14	1999 Riesling (R)	1.7	0	0.6	0.2	1.1	0.6	0.8
15	1999 Riesling (R)	0.7	0	0.5	0.2	1.0	0.6	0.9
16	1999 Riesling (R)	0.7	0	0.3	0.2	1.0	0.5	0.9
17	1999 Riesling (R)	0.6	0	0.4	0.2	0.8	0.5	0.8
18	1999 Vidal (F)	47	7.3	8.7	3.8	4.7	3.5	1.1
19	1999 Vidal (F)	49	6.2	5.4	11.1	5.5	1.0	1.2
20	1999 Vidal (F)	38	5.4	6.9	4.9	5.3	2.9	1.3
21	1999 Vidal (F)	35	3.2	4.6	6.0	4.3	1.3	1.3
22	1999 Vidal (F)	35	2.2	4.7	5.6	4.2	1.6	1.4
23	1999 Vidal (F)	33	1.9	4.5	5.5	4.2	1.1	1.3
24	1999 Vidal (R)	2.0	0.5	0.7	0.4	2.2	0.9	0.9
25	1999 Vidal (R)	3.5	0.7	1.1	1.2	2.7	0.6	1.2
26	1999 Vidal (R)	1.7	0.4	0.7	0.4	2.4	0.7	1.0
27	1999 Vidal (R)	0.5	0	0.5	0.1	0.8	0.6	1.1
28	1999 Vidal (R)	0.4	0	0.7	0.2	0.8	1.0	0.9
29	1999 Riesling	2.5	0	2.2	0.5	1.4	1.2	2.1
30	1999 Vidal	1.2	0.4	4.1	0.4	1.2	1.5	2.1
31	1999 Riesling	6.7	0	2.5	0.2	1.7	1.7	1.2
32	2003 Vidal	4.7	1.2	8.2	0.5	2.1	2.0	1.6
33	2002 Riesling	12.0	2.7	12.9	1.4	4.0	4.4	1.6
34	2000 Vidal	31	5.9	4.2	8.6	5.3	1.7	1.0
35	2001 Riesling	0.6	0	0.8	0.2	0.7	1.5	0.8
36	1998 Chardonnay	5.3	0.5	4.7	2.7	1.1	2.9	0.4
37	1999 Vidal	4.8	1.0	2.5	1.4	2.1	0.8	1.2
38	2002 Riesling	37	0.8	6.0	7.1	3.5	1.6	0.9
39	2001 Riesling	24	0.6	3.6	6.8	3.4	3.1	0.9
40	1999 Riesling	8.4	0.3	3.4	6.3	1.1	2.2	0.4

[a] GRP = grape reaction product; [b] F = Faux; [c] R = Real

icewines can rather be found through an examination of the grape harvest date (Fig. 2). Grapes used to make FAUX icewines were harvested on 22 November and 18 December, and by the second harvest date a lower concentration of these polyphenols was obtained. However, for REAL wines made from grapes harvested on 28 December, and more so for the 14 January harvest, significantly lower concentrations were obtained ($p \leq 0.05$) pointing to a decline occurring within the grape. Changes brought about by weather (freeze-thaw cycles) or sheer length of time on the vine, need to be considered for the loss of hydroxycinnamates. We can also note that the REAL icewines for the 2002 experimental wines were harvested at an earlier date of 11 December and contained substantial concentrations of hydroxycinnamates, but not as high as for the FAUX wines harvested on 12 November.

The decline in caftaric acid alone, or of total caffeic acids (when the GRP and caffeic acid are included – the three compounds with an easily oxidizable catechol group), was even more pronounced in the 1999-2000 experimental icewines (Fig. 2). The percentage of total caffeic acids of the total hydroxycinnamates went from 56 to 81% in the FAUX icewines to 24 to 48% in the REAL icewines; by contrast the combined concentrations of fertaric and ferulic acids went from 7 to 26% of the total hydroxycinnamates in the FAUX icewines to 36 to 55% in the REAL icewines. In the 2002 wines the range of total caffeic acids remained between 75 and 80%, and in the nine commercial wines went from 31% (the *Botrytis*-affected wine) to 70%. Both caftaric and coutaric acids are known to be excellent substrates for polyphenol oxidase activity in grapes, generating the same quinone product [17]. On the other hand, fertaric acid is known to be not susceptible to enzymatic oxidation and thus appears in relatively higher levels in later press fractions when caftaric acids have already been oxidized away [18].

Looking at the absolute concentrations in the 1999-2000 icewines, the level of caftaric and coutaric acids was only 6 and 11%, respectively, of their values in the FAUX icewines. On the other hand, the concentrations of fertaric acid in the REAL icewines remained at an average of 40% of the concentrations in the FAUX icewines, and for ferulic acid the concentrations were nearly as high in both REAL and FAUX icewines.

Effect of press fraction: Regarding the different press fractions for the 1999-2000 experimental icewines, no clear trends were observed in TA, pH, 420 nm absorbances or the concentrations of hydroxycinnamates (Tables 1 and 2). However, the two replicate samples for each treatment for the 2002 experimental icewines gave very similar hydroxycinnamate concentrations, with the REAL 2002 Riesling pair showing the greatest difference (Samples 3 and 4 in Table 2).

Effects of grape cultivar: Regarding grape cultivar, higher concentrations of hydroxycinnamates, including caftaric acid, fertaric acid, caffeic acid and the GRP were observed in Vidal versus Riesling for the experimental icewines (Table 2). For example, the concentration of total hydroxycinnamates in the six 1999 FAUX Vidal icewines at $65(\pm 12)$ mg/L was significantly higher than that of the five 1999 FAUX Riesling icewines at $21(\pm 12)$ mg/L ($p \leq 0.05$). The commercial wines were more variable, reflecting differences in harvest and winemaking procedures. When Riesling wines were considered alone, the concentrations of hydroxycinnamates in the three dry Rieslings lay between the low values seen in the 1999 experimental icewines and the higher values in the 2002 experimental icewines. While the concentrations of polyphenol components may have occurred through the removal of water as ice during the production of REAL icewines, the concentrations obtained in the icewines were not dramatically high (at least looking at the 2002 wines – the effect of 5 years in the bottle on the 1999 icewines remains unknown).

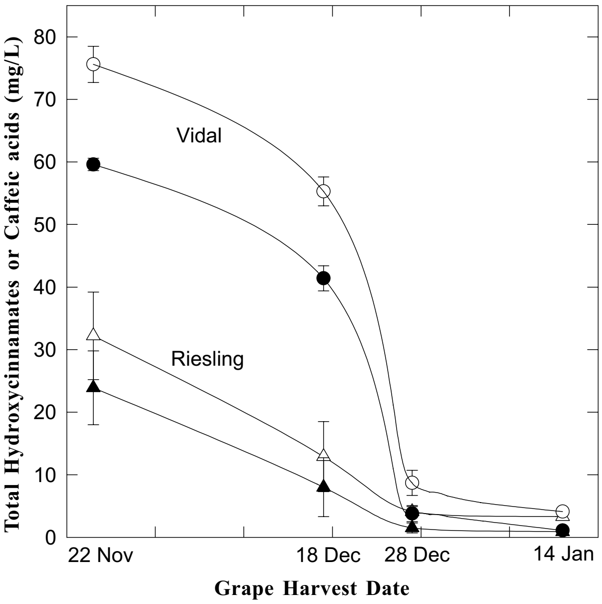

Figure 2. Icewine total hydroxycinnamates (open symbols) and total caffeic acids (filled symbols), for Vidal (circles) and Riesling (triangles) wines made from grapes harvested in 1999-2000.

Relevance with respect to browning potential: Wine browning potential can be related to the concentration and type of polyphenols present, as well as exposure to oxygen and the presence of compounds such as glutathione. Some browning through polyphenol oxidation no doubt occurs in the grapes with time, and possibly following freeze-thaw events. Some of these compounds will be retained in the wine, but others may be removed through fining and filtering (should they adsorb to particulate matter). The subsequent browning potential of an icewine with very low residual polyphenols would not be expected to be large, since the major substrates of wine oxidation have been largely removed. Sufficient concentrations of polyphenols remain, however, to react with any dissolved oxygen in preference to the oxidation of other wine components. From this point of view REAL icewines from grapes harvested late in the year (although somewhat golden to start) may share something in common with white wines exposed to “hyperoxidation” as a technique to oxygenate a wine, oxidize the polyphenols then remove them prior to bottling, producing a wine with a low subsequent browning potential [19]. However, while hyperoxidation of a dry wine can lead to a loss of varietal aroma compounds [17], the effects of freeze-concentration in icewines instead serve to intensify many aromas.

In future work, the compromise between the concentrating effect on wine components from the icewine production, versus degradation with time (and weather conditions) needs to be examined in more detail. In this study every effort was made to keep the winemaking as uniform as possible, but future research will need to include an examination of levels of polyphenols in the grapes leading up to harvest, along with changes which occur during the operations needed to produce icewines.

Conclusions

The polyphenol concentration of a range of Ontario icewines and late harvest wines was quantified. Experimental Riesling icewines had lower pH values than Vidal. There was a substantial difference in the concentration of hydroxycinnamic acids between REAL (harvested at temperatures -8°C or below) and FAUX icewines (harvested above -8°C) for 1999-2000 experimental wines, whereby all of the FAUX icewines had up to 11-fold and 21-fold higher concentrations, respectively, of total hydroxycinnamates in Riesling and Vidal compared to REAL icewines. In 2002 experimental icewines, relatively high concentrations of hydroxycinnamates were found in both REAL and FAUX wines, matching the earlier harvest dates (by up to a month) in that year. In 1999-2000, the concentration of caftaric and coutaric acids in the REAL icewines was only 6 and 11%, respectively, of their values in the FAUX icewines. The concentrations of fertaric acid, not susceptible to enzymatic oxidation, in the REAL icewines remained at about 40% of the concentrations in the FAUX icewines, while for ferulic acid the concentrations were nearly as high in both REAL and FAUX icewines. Higher concentrations of hydroxycinnamates, including caftaric acid, fertaric acid, caffeic acid and the grape reaction product (GRP) were seen in Vidal versus Riesling for the experimental icewines.

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