Organic Acid Composition in Croatian Predicate Wines

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Summary

Continental Croatia wine region and especially Kutjevo vineyards are famous for their predicate wines production and quality. The most common grape varieties used there for different types of predicate wine are Welsch Riesling and Traminer. Ice wines, selected harvest wines and dry berry selection wines from different harvest years were examined by HPLC method to determine organic acids composition. The oldest sample was Traminer selected harvest from vintage year 1990, while the youngest wine was Traminer ice wine from harvest 2011. The dominant organic acids in all analyzed wines were tartaric, malic, citric and galactaric, ranged from 0.09 to 2.98 g/L. In most wines the difference in concentration of glucuronic, galacturonic and gluconic acids was established. The highest content of glucuronic acid was 58.4 mg/L in Traminer dry berry selection 2011. Galacturonic acid dominated in the same wine (924 mg/L), just like gluconic acid (141 mg/L).

Key words

predicate wines, Croatia, organic acids, Traminer, Welsch Riesling

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Introduction

Predicate wines can be produced only in rare years when climatic conditions are appropriate. Grapes are left on vine where they start with overripening, drying, freezing and noble rot infection. Production of certain categories of predicate wines, especially berry selection and dry berry selection depend on grapes infection with fungus *Botrytis cinerea* which occurs in the late berry development state (Dittrich, 1989). *Botrytis cinerea* metabolism affects chemical composition of grape must (Dittrich, 1977; Dittrich, 1989; Ribérau-Gayon, 1988; Dittrich et al., 1975; Sponholz, 1991).

Rarely, with special climatic conditions, *Botrytis cinerea* occurs in "noble rot" state (Tupajić, 2001). Noble rot infected grapes contain higher sugar concentration and slight higher or reduced acidity (Dittrich, 1989). Total acidity of grapes decreases and pH increases although synthesis of citric acid, gluconic acid, galactaric acid, oxo-gluconic acid, glucuronic acid occurs (Blouin, 2000). Dehydration of berry leads to slower growth of *Botrytis cinerea* and change in its metabolism. In addition, concentration of berry content occurs (Jackson, 1994), leading to an increase in internal osmotic pressure that causes the death of the fungus (Ribérau-Gayon et al., 2000a).

Present microorganisms on grapes (*Botrytis cinerea*, *Aspergillus niger*, *Gluconobacter oxydans*) are capable of enzymatic synthesis of gluconic acid in must and wine, while *Saccharomyces cerevisiae* cannot utilize gluconic acid in reductive conditions during alcoholic fermentation (Sponholz and Dittrich, 1985; Donèche, 1989; Peinado et al., 2003).

Gluconic acid is a product of enzymatic glucose oxidation with glucose oxidase (McCloskey, 1974). Gluconic acid in wine can be indicator of grape infection with fungi (McCloskey, 1974). Concentration of gluconic acid in botrytized musts can be over 3 g/L (Ribérau-Gayon et al., 2000a). The concentration ratio of glycerol to gluconic acid represents noble rot quality index. According to Ribérau-Gayon et al. (2000a), wines made from healthy grapes should contain less than 0.5 g/L, noble rot wines 1-5 g/L and grey rot wines more than 5 g/L of gluconic acid. De Smedt et al. (1979) have found 0-2000 mg/L of gluconic acid in different wines.

Botrytis cinerea penetrates through grape skin using exocellular enzymes (pectinolytic, cellulosic complex, protease and phospholipase enzymes). As a result of these degradation galacturonic acid occurs (Ribérau-Gayon et al., 2000a). Sponholz and Dittrich (1985) have found 116 to 1048 mg/L galacturonic acid in white wines. Galactaric acid (mucic acid) has also been observed to accumulate in botrytized must. It is a product of the oxidation of galacturonic acid. Galactaric acid forms salts with calcium ions and is capable of precipitating in wine (Ribérau-Gayon et al., 2000a).

Botrytis cinerea development causes degradation of organic acid in must, especially tartaric acid. Malic acid and citric acid are less degraded. Degradation of malic acid occurs at the end of *Botrytis* attack (Ribérau-Gayon et al., 2000a).

The lack of published predicate wines studies may be attributed to high costs of these wines. The aim of this research was to make a chemical composition overview of Croatian predicate wines, especially in main organic acids (tartaric, malic, citric, succinic, lactic, galactaric, glucuronic, galacturonic and gluconic acids) that can be found in these type of wines.

Materials and methods

Chemical and reagents

Malic, citric, lactic, succinic, galactaric, glucuronic, galacturonic and gluconic acids were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tartaric acid and 85% -orthophosphoric acid were obtained from Fluka (Buchs, Switzerland). Methanol was HPLC grade, provided from J.T. Baker (Derventer, Netherland). SAX cartridges (200 mg/3 mL) were obtained from Phenomenex, USA. Sodium hydroxide, 1 M was purchased from Kemika (Zagreb, Croatia).

Wine samples

Sixteen predicate commercial wines of four different grape cultivars were examined in this experiment: five Welsch Riesling wines (vintages 1993-2009), nine Traminer wines (vintages 1990-2011), Charodonnay wine (vintage 1995) and Kerner wine (vintage 2005). Predicate wines in experiment were from different categories, including selected harvest, ice wine and dry berry selection. All wines originate from continental Croatia region (Kutjevo winegrowing region).

All of the wine samples were in their original bottles. The content of galactaric, glucuronic, galacturonic, gluconic, citric, tartaric, malic and succinic acids was determinated by HPLC.

Sample preparation

Wines were filtered through 0.45 μm syringe CA filters (Phenomenex, USA) prior analysis of tartaric, malic, citric and lactic acids and directly injected to HPLC.

The SPE procedure was applied for analysis of gluconic, glucuronic, galactaric and galacturonic acids. SAX cartridges were conditioned with two consecutive volumes of methanol and two of distilled water. Five milliliter wine sample, previously adjusted to pH 9.0 with 1 M NaOH was applied to cartridge. Neutral compounds (sugars and alcohols) were washed away by 1.5 mL of water. Acidic compounds were eluted with 2 mL of mobile phase and injected to the chromatographic system.

HPLC analysis

An Agilent 1050 System (Agilent, Germany), equipped with autosampler, column thermostat and UV-Vis detector, was used. The separation of organic acids was performed on Aminex HPX-87H (Biorad, CA, USA) column (300 x 7.8 mm, 9 μm) at 65°C. Detection wavelength was 210 nm. Injected sample volume was 10 μL. Mobile phase was 0.065% *(v/v)* H_3PO_4 with isocratic elution at flow rate of 0.6 mL/min for determination of tartaric, malic, succinic, citric and lactic acids. For separation of gluconic, glucuronic, galactaric and galacturonic acids conditions were same as above, just the flow rate of mobile phase was 0.2 mL/min.

The linearity of the method was tested. Concentration range of acids, regression coefficients, limits of detection and quantifications are presented in Table 1.

Analytical procedure for SPE recovery

The recovery of the SPE method was determined by spiking wines in 10-mL volumetric flask with 2 mL of standard mixture containing 1 g/L of each acid. For comparison, additional

Table 1. Parameters of linearity, limits of detection and quantification

Organic acid	Linear range (g/L)	Correlation coefficient	Limit of detection (LOD) mg/L	Limit of quantification (LOQ) mg/L
Citric	0.05-1.5	0.9991	1.92	5.76
Tartaric	0.5-10	0.9978	5.38	16.14
Malic	0.2-8.0	0.9989	2.71	8.13
Lactic	0.1-5.4	0.9992	1.28	3.84
Succinic	0.2-2.0	0.9994	2.47	7.41
Galactaric	0.01-2	0.9992	1.38	4.14
Glucuronic	0.01-5	0.9995	2.56	7.68
Galacturonic	0.01-2	0.9986	3.87	11.61
Gluconic	0.01-3	0.9957	1.79	5.37

samples of each wine were diluted to the same extent by adding 2 mL of distilled water to the wine in a 10-mL volumetric flask. Recoveries were for all four acids (galactaric, glucuronic, galacturonic and gluconic) more than 90%.

Statistical analysis

An analysis of variance (one-way ANOVA) was applied to the experimental data. Results were considered significantly different if the associated *p* value was below 0.05. Tukey's test was applied for mean comparisons. A principal component analysis was applied to data. All statistical analysis was performed using the software SAS 9.3 (SAS Inc., Cary, USA).

Results and discussion

Concentrations of organic acids in Croatian predicate wines are shown in Table 2.

The highest concentration of gluconic acid (141 mg/L) was found in Traminer dry berry selection wine from harvest 2011. Compared to other studies concentration of gluconic acid was relatively low. De Smedt et al. (1979) found over 2000 mg/L of gluconic acid in different wines and according to Suomalainen (1983) concentrations of 0.09 – 1.9 g/L were found in selected harvest wines. Same author notify concentrations from 2.2 – 3.8 g/L in dry berry selection wines. Ribérau-Gayon et al. (2000a) state that wines made from healthy grapes should contain less

Table 2. Concentrations of organic acids in Croatian predicate wines

Wine sample	Galactaric acid (g/L)	Glucuronic acid (mg/L)	Galacturonic acid (mg/L)	Gluconic acid (mg/L)	Citric acid (g/L)	Tartaric acid (g/L)	Malic acid (g/L)	Lactic acid (g/L)	Succinic acid (g/L)
Welsch Riesling s.h. 1993	0.23±0.01^{cd}	n.d.	n.d.	n.d.	0.97±0.06^{j}	0.80±0.04^{a}	1.7±0.02^{g}	n.d.	n.d.
Welsch Riesling s.h.1999	0.47±0.03^{g}	n.d.	726.10±0.52^{c}	n.d.	0.45±0.02^{d}	1.27±0.11^{c}	1.35±0.01^{f}	n.d.	n.d.
Welsch Riesling i.w. 2005	0.25±0.01^{de}	n.d.	837.04±0.43^{d}	n.d.	0.38±0.01^{cd}	1.48±0.14^{ef}	1.21±0.04^{de}	n.d.	0.20±0.01^{b}
Welsch Riesling i.w. 2008	0.26±0.02^{de}	29.08±1.02^{b}	n.d.	116.00±0.08^{d}	0.82±0.13^{hi}	1.26±0.10^{c}	0.99±0.01^{c}	n.d.	n.d.
Welsch Riesling i.w.2009	0.24±0.01^{cd}	23.14±1.09^{a}	n.d.	19.27±0.49^{a}	0.22±0.01^{a}	1.37±0.12^{d}	n.d.	1.02	0.47±0.02^{d}
Traminer s.h. 1990	0.23±0.02^{cd}	n.d.	n.d.	n.d.	0.68±0.04^{fg}	1.52±0.09^{f}	0.92±0.06^{c}	n.d.	0.27±0.01^{c}
Traminer s.h. 1991	0.46±0.03^{fg}	n.d.	533.00±0.08^{b}	n.d.	0.52±0.01^{e}	1.26±0.08^{c}	1.56±0.01^{g}	n.d.	0.66±0.03^{e}
Traminer s.h. 1997	0.12±0.01^{a}	n.d.	326.00±0.05^{a}	63.00±0.10^{c}	0.61±0.01^{f}	1.21±0.14^{c}	0.58±0.01^{a}	n.d.	0.26±0.01^{c}
Traminer s.h. 1999	0.11±0.01^{a}	n.d.	n.d.	n.d.	0.24±0.01^{a}	0.89±0.03^{ab}	0.71±0.01^{b}	n.d.	0.16±0.01^{ab}
Traminer s.h. 2000	0.28±0.02^{e}	n.d.	n.d.	n.d.	0.27±0.01^{bc}	1.42±0.01^{de}	0.75±0.00^{b}	n.d.	0.12±0.01^{a}
Traminer i.w. 2001	0.27±0.01^{e}	n.d.	n.d.	102.00±0.33^{d}	0.68±0.08^{fg}	1.02±0.02^{b}	0.92±0.02^{c}	n.d.	n.d.
Traminer i.w. 2003	0.20±0.01^{bc}	32.71±1.39^{b}	n.d.	38.53±0.14^{b}	0.25±0.01^{ab}	1.95±0.00^{g}	0.89±0.02^{bc}	n.d.	n.d.
Traminer i.w. 2011	0.19±0.02^{b}	n.d.	n.d.	137.00±0.25^{cf}	0.49±0.03^{de}	1.59±0.30^{f}	0.51±0.01^{a}	n.d.	0.68±0.03^{e}
Traminer d.b.s. 2011	0.43±0.01^{f}	58.41±1.78^{c}	924.00±1.25^{e}	141.00±0.10^{f}	0.79±0.02^{gh}	2.98± 0.05^{h}	1.13±0.02^{d}	n.d.	1.56±0.08^{f}
Chardonnay s.h. 1995	0.09±0.01^{a}	n.d.	n.d.	n.d.	0.48±0.01^{d}	1.99±0.04^{g}	1.24±0.01^{e}	n.d.	n.d.
Kerner s.h. 2005	0.13±0.02^{a}	n.d.	n.d.	n.d.	0.89±0.01^{i}	0.92±0.02^{b}	1.37±0.04^{f}	n.d.	n.d.

Selected harvest (s.h.), ice wine (i.w.), dry berry selection (d.b.s.); Results are mean values from three determinations; Values not sharing the same latter within the vertical line are different according to the Tukey's test (p<0.05);

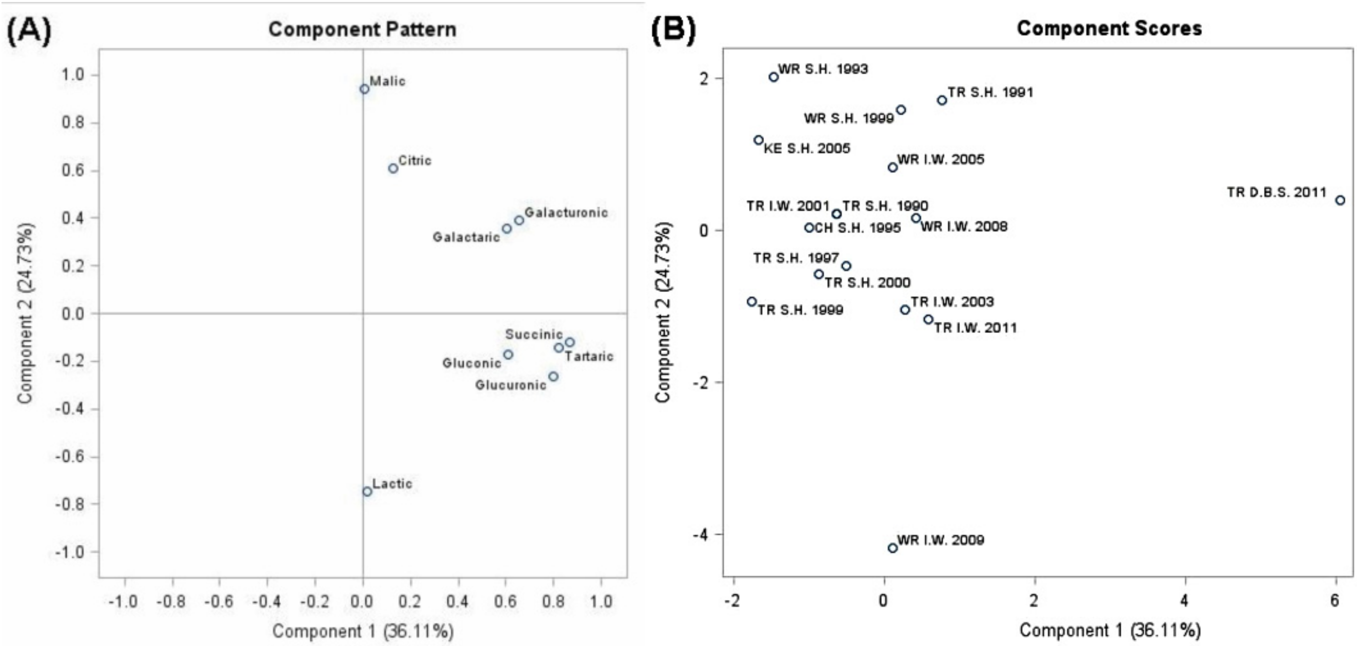

Figure 1. Principal component analysis: projection of variables and wines in the space defined by the first and second principal components. (A) Variables: organic acids. (B) Wines: Welsch Riesling (WR), Traminer (TR), Chardonnay (CH), Kerner (KE); selected harvest (S.H.), ice wine (I.W.), dry berry selection (D.B.S.).

than 0.5 g/L of gluconic acid. Since high concentration of gluconic acid (more than 5 g/L) indicates poor rot evolution and occurs much later in berry over ripening process, it seems that examined wines were made from grapes where *Botrytis* was in noble phase. In nine wines, mainly selected harvest, gluconic acid was not detected. It can be indication of low microbial activity on grapes since microorganisms infecting grape berries have the enzymatic pool to produce gluconic acid (Sponholz and Dittrich, 1985; Donèche, 1989; Peinado et al., 2003).

The origin of glucuronic acid is also associated with grape infection by *Botrytis cinerea*. Sponholz and Dittrich (1985) found concentrations of glucuronic acid from 0 – 35 mg/L in white wines and 0 – 33 mg/L in red wines. This is in agreement with our research where concentrations from 23.14 – 58.41 mg/L were in four wines (Welsch Riesling ice wines 2008, 2009; Traminer ice wine 2003, and Traminer dry berry selection 2011). Suomalainen (1983) reported 0.08 – 0.45 g/L of glucuronic acid, concentrations that are much higher compared to our results. The highest concentration was again determined in Traminer dry berry selection wine from harvest 2011. The absence of glucuronic acid in other wines was probably connected with lower microbial activity on grapes.

Galacturonic acid was found in five examined wines. Again, the highest concentration was found in Traminer dry berry selection wine from harvest 2011. Suomalainen (1983) reported 0.3 – 1.44 g/L in Italian white wines and 0.99 – 1.81 g/L of galacturonic acid in Italian red wines. Sponholz and Dittrich (1985) found 116 – 1048 mg/L in white wines and 381 – 1200 mg/L in red wines. So our results are in agreement with these studies. Galacturonic acid mainly originates from degradation of pectic compounds. Degradation of pectin was noticed in two Welsch Riesling wines (selected harvest 1999, ice wine 2005) and three Traminer wines (selected harvest 1991, 1997, dry berry selection 2011).

In contrast to other sugar acids examined in our study, galactaric acid (mucic acid) was found in all wines. Significantly the lowest concentration was determined in Chardonnay selected harvest wine from vintage year 1995 and the highest in Welsch Riesling selected harvest wine from 1999. According to Wuerdig (1977), wines with more than 0.1 g/L of galactaric acid are considered unstable and formation of calcium salts can occur. All our wines had concentrations of galactaric acid above 0.1 g/L except Chardonnay selected harvest 1995. Therefore, only Chardonnay seems to be stable with respect to calcium salts precipitation but also in no other wines precipitation was detected.

Main organic acids, including tartaric, citric, malic, lactic and succinic, were also determined. Depletion of the principal grape acids is connected with *Botritys cinerea* development, especially degradation of tartaric acid (Ribérau-Gayon et al., 2000a). This can be seen in our results where tartaric acid concentration is low, from 0.8 g/L to 2.98 g/L. Ribérau-Gayon et al. (2000a) reported over 6 g/L in musts from northern vineyards. According to same authors, concentrations of malic acid detected in examined wines are also low (0.5 g/L in Traminer ice wine 2011 to 1.7 g/L in Welsch Riesling selected harvest 1993). Ribérau-Gayon et al. (2000a) reported between 4 and 6.5 g/L of malic acid, and 0.5 to 1 g/L of citric acid in must and wine what is in accordance with our results. Lactic and succinic acids are derived from alcoholic or malolactic fermentation. Concentrations of succinic acid in wine is 1 g/L in average (Ribérau-Gayon et al., 2000b), therefore, only Traminer dry berry selection 2011 was above that value. Lactic acid was determined only in Welsch Riesling ice wine 2009 what indicates bacterial activity. In the same wine malic acid was not found, which suggested degradation of that acid by malolactic bacteria.

A principal component analysis (PCA) was applied to all wine samples. The first two principal components (PCs) explain 60.84% of the total variance, with the PC1 explaining 36.11% and PC2 explaining 24.74%. The presentation of nine variables (tartaric, malic, citric, succinic, lactic, galactaric, glucuronic, galacturonic and gluconic acids) and wine samples, using the first two PCs, is shown in Figure 1. The PC1 is positively correlated with succinic, tartaric, gluconic, glucuronic, galactaric and galacturonic acids. The PC2 is positively related to malic and citric acids, and negatively related to lactic acid. It can be seen that Traminer dry berry selection 2011 (positively related to PC1) and Welsch Riesling ice wine 2009 (negatively related to PC2) went outside the grouping. Traminer dry berry selection 2011 is located on right side of coordinate system because of high concentrations of overall organic acids found in that wine. Welsch Riesling ice wine 2009 is located in negative part of coordinate system and it is closly related with lactic acid content. Other wines can neither be clearly separated by the two first principal components nor by cultivar or year of harvest.

Conclusion

The results of our study showed that dominant organic acids in all analyzed wines were tartaric, malic, citric and galactaric acids.

Glucuronic acid concentration in Traminer predicate wines varied from below detection limit up to 58.41 mg/L, while in Welsch Riesling wines concentration ranged from below detection limit to 29.08 mg/L. Galacturonic acid concentration varied from below detection limit to 924 mg/L in Traminer dry berry selection wine 2011, while the amount of gluconic acid ranged from below detection limit to 141 mg/L in the same wine. Relatively low concentrations of gluconic acid were found compared to other published results. All wines had concentrations of galactaric acid above 0.1 g/L (except Chardonnay selected harvest 1995) and therefore are probably unstable with respect to calcium salts precipitation during prolonged storage.

Traminer dry berry selection wine from harvest 2011 had the highest concentrations of all examined organic acids in this study. For the first time organic acids content of Croatian predicate wines was presented. Moreover, the lack of organic acid content data in literature will be amended.

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