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Department of Viticulture and Enology, University of California, Davis, California, U. S. A.

The relationships between total acidity, titratable acidity and pH in grape tissue

by

R. BOULTON

Les rapports entre l'acidité total, l'acidité titrable et le pH dans le tissu de raisin

Résumé — Les rapports fondamentaux permettant une prédiction de l'acidité titrable et du pH du tissu et du jus de raisin ont été étudiés. L'influence de quelques substances minérales, en particulier du potassium, sur l'acidité titrable et sur le pH est exposée. Un mécanisme possible de transport dans les baies est discuté. Les relations existantes sont vérifiées à l'aide des analyses déjà publiées réalisées sur baies des cépages Merlot et Cabernet Sauvignon. Des analyses de la pulpe, des pellicules et des rafles des cépages Semillon, Sauvignon blanc, Merlot et Cabernet Sauvignon confirment les rapports discutés. Les valeurs de l'acidité titrable et du pH trouvées pour les jus des cépages Riesling, Crouchen et Syrah sont comparées avec les chiffres déjà publiés pour démontrer le caractère universel des relations existantes.

Introduction

The importance of the organic acids in grapes has led to several studies of their accumulation (GENEVOIS 1938, AMERINE and WINKLER 1942, PEYNAUD and MAURIE 1953, 1956, DU PLESSIS 1968), the factors influencing their synthesis (KLIEWER 1964, DRAWERT and STEFFAN 1966, HARDY 1968, SAITO and KASAI 1968) and the pathways thought to be responsible (PEYNAUD and RIBÉREAU-GAYON 1971, SAITO and KASAI 1978). However, the measures of acidity most commonly used in viticulture and enology are the titratable acidity and pH. While numerous studies have been conducted which show the effects of cultivar, climate, cropping level, rootstock, irrigation and soil on these acidity measures, the fundamental relationship between the organic acid concentrations, the titratable acidity and the pH in grape tissue have not previously been elucidated.

The influence of minerals on the titratable acidity was shown in early studies when anion and cation balances were attempted for wines (TARANTOLA 1932, BREMOND 1937, PEYNAUD 1947) and for juices (PEYNAUD and MAURIE 1956). The influence of minerals on the pH of juices does not appear to have been considered until some time later (RANKINE *et al* 1971). The possible link between potassium concentration and pH was suggested by correlation studies of the composition of juices and wines of several cultivars in Germany (WEJNAR 1971) and of Australian red table wines (SOMERS 1977). However, their relationships are not of a general nature and provide no insight regarding the biological and physiological cause of this phenomenon.

This paper proposes that in general, potassium and all other monovalent metal cations are taken up by the vine and enter the tissues of the cluster and grape in a stoichiometric exchange for protons derived from endogenous plant acids. The uptake and eventual accumulation of these species in the fruit is considered to involve a

series of such exchanges, each associated with movement across cellular membranes. In such a process, the charge balance is maintained, but both titratable acidity and pH of the cellular sap is modified. In the vegetative and reproductive tissues of the vine, the principal acids are tartaric and malic acids (PEYNAUD and MAURIÉ 1958). The proposed exchange is thought to be accomplished by a membrane-bound enzyme with a very strong preference for potassium over other monovalent metal cations. There appears to be no previous report of this type of exchange in grapes or fruits in general, although evidence supporting the generality of such a mechanism is accumulating from studies of the role of membrane-bound enzymes in the regulation of mineral uptake by roots (LEONARD and HANSON 1972, LEONARD *et al.* 1973) and microorganisms (ROTHSTEIN 1972).

The test that led to this postulate was that the discrepancy between the number of protons expected from the acid anions and those found by titration is exactly equal to the number of monovalent metal cations in the tissue. A second, more vigorous test is that the pH of a grape juice should be that which results from a mixture of the grape acids with a number of protons, equal to the number of monovalent metal cations present in the juice removed.

Materials and methods

The relationships proposed in this study are based on proton balances in the titrated and natural tissue samples. The relationship between total acidity, as measured by the equivalence of the organic acid anions at the end point, titratable acidity and the monovalent metal cations is then:

$$\begin{array}{lclcl} \text{Protons expected from} & & \text{Protons measured} & & \text{Monovalent metal} \\ \text{organic acid anions} & = & \text{by titration} & + & \text{cations} \end{array} \quad (1)$$

where the last term represents the protons lost by the proposed exchange mechanism.

At natural juice pH, the corresponding balance resulting from the dissociation of the organic acids and partial removal of protons by the proposed exchange mechanism is:

$$\begin{array}{lclcl} \text{Protons expected from} & & \text{Protons in the} & & \text{Monovalent metal} \\ \text{the equilibrium ionization} & = & \text{free form} & + & \text{cations} \\ \text{of the organic acids} & & & & \end{array} \quad (2)$$

The protons expected from the equilibrium ionization of the organic acids can be estimated from the hydrolysis equations, the acid compositions and the juice pH. The first term on the right side is simply the equilibrium pH expressed in molar units.

The first relationship can be used on either a per volume or per mass basis, while the second relationship is restricted to solutions and a per volume basis.

The relationships are confirmed using published data for Merlot and Cabernet Sauvignon berry and juice analyses from five chateaux over three harvests (PEYNAUD and MAURIÉ 1956) and for analyses of other tissues from the clusters of Semillon, Sauvignon blanc, Merlot and Cabernet Sauvignon (PEYNAUD and MAURIÉ 1953). In these calculations the principal monovalent metal cations are considered to be potassium and sodium and their concentrations have been estimated from the reported alkalinity of ash values. The correlation between these quantities was developed from published wine analyses (PEYNAUD 1950). The principal organic acids are considered

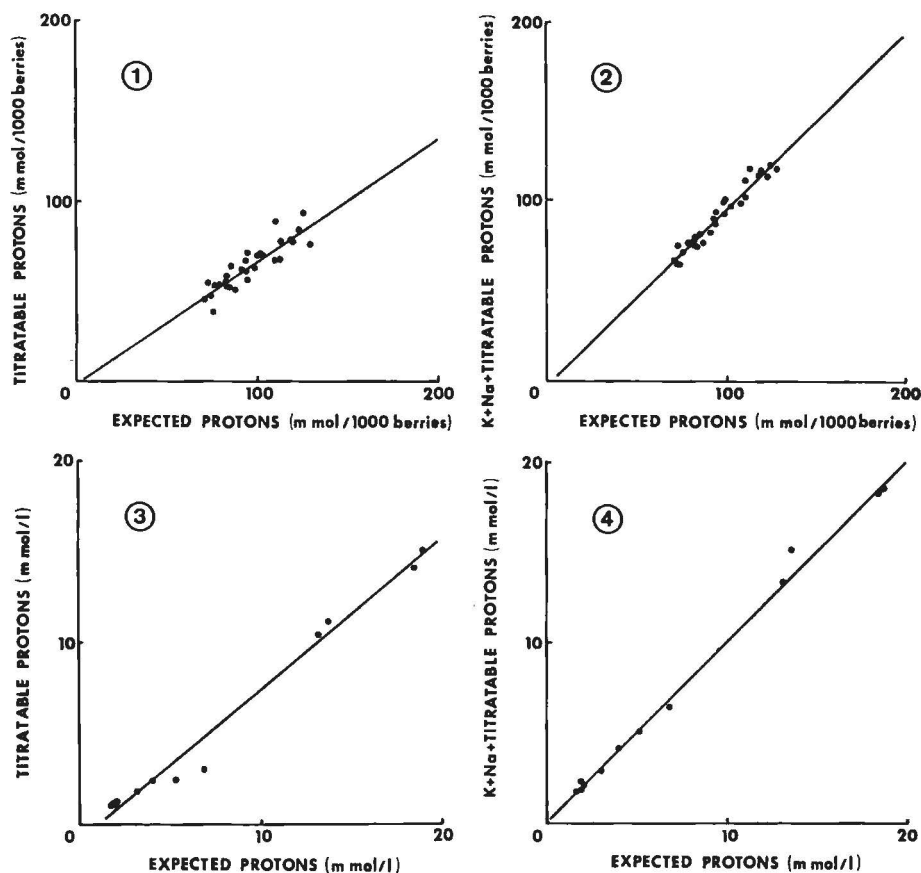


Fig. 1: The relationship between the titratable protons and the expected protons of whole berries of Merlot and Cabernet Sauvignon.

Fig. 2: The relationship between the sum of potassium, sodium and titratable protons and the expected protons of whole berries of Merlot and Cabernet Sauvignon.

Fig. 3: The relationship between the titratable protons and the expected protons of cluster tissue of Semillon, Sauvignon blanc, Merlot and Cabernet Sauvignon.

Fig. 4: The relationship between the sum of potassium, sodium and titratable protons and the expected protons of cluster tissue of Semillon, Sauvignon blanc, Merlot and Cabernet Sauvignon.

Fig. 1: Relations entre les protons titrables et les protons escomptés de baies entières de Merlot et de Cabernet Sauvignon.

Fig. 2: Relations entre la teneur totale en potassium, sodium et protons titrables et les protons escomptés de baies entières de Merlot et Cabernet Sauvignon.

Fig. 3: Relations entre les protons titrables et les protons escomptés dans le tissu de grappes de Semillon, Sauvignon blanc, Merlot et Cabernet Sauvignon.

Fig. 4: Relations entre la teneur totale en potassium, sodium et protons titrables et les protons escomptés dans le tissu de grappes de Semillon, Sauvignon blanc, Merlot et Cabernet Sauvignon.

to be tartaric and malic and the titratable acidity was taken to be 6 % higher due to the incomplete ionization of malic acid at the pH of 7.

The predictions of juice pH were obtained by the iterative solution of ionization equations for the acids concerned using a Tektronix 4051 Computer (BOULTON and WARD, unpublished).

Results

The proposed acidity relationship

1. Berries. — The relationship between the titratable acidity and the protons expected from the acid anions of Merlot and Cabernet Sauvignon berries from five chateaux and over three harvests (PEYNAUD and MAURIÉ 1956) is shown in Fig. 1. The proposed relationship which incorporates the potassium and sodium contents is shown in Fig. 2. The statistical parameters of these regressions are presented in Table 1.

2. Other tissues. — The relationship between the titratable acidity and the expected protons in the skin, pulp and stems of Semillon, Sauvignon blanc, Merlot and Cabernet Sauvignon (PEYNAUD and MAURIÉ 1953) is shown in Fig. 3. The proposed relationship, including the monovalent metal cations, for these tissue samples is shown in Fig. 4 and the statistical parameters of these regressions are presented in Table 2.

The proposed pH relationship

The pH values predicted by two methods are compared with the reported values for the juices of the Merlot berries in Table 3. The first method uses values of the monovalent metal cation content estimated from the alkalinity of ash values, while the second method uses an estimate from the alkalinity of ash values, while the

Table 1

Linear regression parameters of acidity relationships in Merlot and Cabernet Sauvignon berries

Paramètres de la régression linéaire des relations d'acidité dans des baies de Merlot et de Cabernet Sauvignon

	Titratable protons vs. expected protons (Fig. 1)	Sum of monovalent metal cations and titratable protons vs. expected protons (Fig. 2)
Regression coefficient	0.679 ± 0.065 ¹⁾	0.977 ± 0.048
Intercept ²⁾	-2.345 ± 6.254	-3.597 ± 4.693
Correlation coefficient	0.891 ³⁾	0.966 ^{**}
F ratio for slope	108.67	399.44

¹⁾ Standard error estimate.

²⁾ m mol/1000 berries.

³⁾ p < 0.001.

Table 2

Linear regression parameters of acidity relationships in skin, pulp and stem tissue of Semillon, Sauvignon blanc, Merlot and Cabernet Sauvignon grapes

Paramètres de la régression linéaire des relations d'acidité dans le tissu des pellicules, de la pulpe et des pédoncules de baies de Semillon, Sauvignon blanc, Merlot et Cabernet Sauvignon

	Titrateable protons vs. expected protons (Fig. 3)	Sum of monovalent metal cations and titrateable protons vs. expected protons (Fig. 4)
Regression coefficient	$0.834 \pm 0.035^1)$	1.005 ± 0.024
Intercept ²⁾	-1.075 ± 0.351	-0.072 ± 0.242
Correlation coefficient	$0.990^{***3)}$	0.997^{***}
F ratio of slope	546.265	1664.549

¹⁾ Standard error estimate.

²⁾ m mol/100 berries.

³⁾ $p < 0.001$.

second method uses an estimate from the total and titrateable acidities according to equation (1).

The prediction of titrateable acidity and pH

In Table 4 the predicted titrateable acidity and pH values in Riesling, Crouchen and Syrah juices are compared with those reported by RANKINE *et al.* (1971).

Discussion

The biosynthesis of organic acids requires that the acid be produced rather than its mineral salts. Mineral elements can only be transported across cellular membranes by one of four mechanisms. These are bulk flow, diffusion, active transport and enzymatic exchange. If minerals enter the cell by any of the first three mechanisms, the protons from the acids should be retained and fully recovered on titration to the appropriate endpoint. The titrateable protons would be equal to those expected from the acid anion concentrations and in grape tissue the pH would be in the range 2.1—2.2. The data presented in Tables 3 and 4 show that this is clearly not the case. In whole grapes only about 68 % of the expected protons are found, as indicated by the slope of the regression line in Fig. 1. The missing protons can be accounted for by postulating an ion exchange process in the cell membranes which interchanges ions such as potassium and sodium for protons. Thus, as shown in Fig. 2, 98 % of the expected protons are accounted for by substitution of the levels of the monovalent metal cations of sodium and potassium. Of particular significance is the fact that the correlation coefficient is improved and the standard error estimates are reduced in Fig. 2 compared with Fig. 1, even though the former includes two additional variables, the potassium and sodium contents. This suggests that there is indeed a special relationship between these variables.

Further evidence of the proposed acidity relationship can be found in the analyses of skin, pulp and stem tissue. Approximately 83 % of the expected protons are recovered by titration (Fig. 3, Table 2). The proposed relationship (Fig. 4) accounts

Table 3
Predicted and reported pH values of Merlot juices
Valeurs du pH de jus de Merlot prévues et rapportées

Absence of exchange mechanism	Minerals estimated from alkalinity of ash	Minerals estimated from acid anions and titratable acid	Reported value
2.17	3.67	3.34	3.52
2.15	3.22	3.37	3.30
2.10	3.12	2.97	3.15
2.17	3.74	3.46	3.60
2.14	3.30	3.28	3.35
2.12	3.14	2.99	3.29
2.15	3.16	3.26	3.30
2.15	3.42	3.27	3.50
2.11	2.98	2.95	3.20
2.12	3.52	3.22	3.40
2.14	3.43	3.22	3.38
2.11	3.13	2.99	3.15
2.16	3.51	3.17	3.61
2.14	3.46	3.27	3.30
2.13	3.10	3.08	3.25

Table 4
Comparison of predicted titratable acidity and pH values for Riesling, Crouchen and Syrah juices
Comparaison de l'acidité titrable prévue avec les valeurs du pH de jus de Riesling, Crouchen et Syrah

	Predicted		Reported	
	TA (m mol/l)	pH	TA (m mol/l)	pH
Riesling				
1959	118	3.18	117	3.12
1960	120	3.12	116	3.12
1961	109	3.27	109	3.28
1962	111	3.16	109	3.21
1963	107	3.29	103	3.34
Crouchen				
1959	88	3.44	90.7	3.39
1960	81	3.43	84.0	3.43
1961	78	3.72	84.0	3.57
1962	86	3.49	88.0	3.47
1963	96	3.55	96.0	3.53
Syrah				
1959	85	3.49	86.7	3.44
1960	88	3.44	89.3	3.43
1961	80	3.63	78.6	3.59
1962	77	3.64	81.3	3.58
1963	92	3.58	92.0	3.56

for the missing protons, and again lower standard error estimates are obtained even though additional variables have been included.

The prediction of juice pH has been accomplished by the simultaneous solution of the ionization equations for malic and tartaric acids. The solution is advanced by removing protons from the equilibrium in numbers equal to the potassium and sodium levels. In typical juice samples, the free proton pool is generally between 0.001 and 0.0001 mol/l while the concentration of the potassium and sodium ions is in the range of 0.02–0.05 mol/l. The total acid concentration is generally in the range 0.05–0.10 mol/l. As a result, the pH value is more sensitive to changes in potassium and sodium concentrations than it is to similar molar changes of tartaric and malic acids. The predicted pH values in Table 3 show the variation caused by uncertainty in the estimate of potassium and sodium concentrations. At typical juice pH values a change in 10 % in potassium and sodium concentration leads to a shift of approximately 0.1 pH units, with higher levels leading to higher pH values. The predictions based on the alkalinity of ash determination would be expected to have less accumulated error and these are in closer agreement with the reported values. The estimates of monovalent cations from equation (1) contains the errors due to each of the determinations, namely tartaric, malic and titratable acid, as well as those due to neglecting the trace acids and minerals. These acids would be a range of carboxylic acids, amino acids and phenolic compounds which would appear in the titratable acidity determination, but which would contribute in a minor way to pH. The minerals, other than potassium and sodium would be rubidium, and, to a lesser extent, caesium and lithium; these however, are rarely reported in grape juice analyses. Mineral anions of polyprotic acids such as phosphoric play a secondary role in determining the titratable acidity. The most probable form in which phosphorus enters the vine is the dihydrogen phosphate ion. This is the most predominant form in the pH range 3.0–6.0, and would be the form most available in soils. While this ion would not generally interfere with the pH, approximately 90 % of this species would be deprotonated to the monohydrogen phosphate ion by titration to a pH endpoint of 8.2. The only influence on pH would occur at low pH values when approximately 15 % would become protonated to phosphoric acid, these protons coming from the organic acid equilibrium, thus tending to raise the pH slightly.

The predictions of juice pH and titratable acidity from organic acid and mineral analyses (Table 4) have been corrected for an addition of sulfur dioxide at 80 ppm and the titration of dihydrogen phosphate ions. The predicted titratable acidities are generally within 5–7 % at the reported values. The predicted pH values are generally within 1/10 of a unit of the reported values. It should be noted that the reported analyses are the average of three growing regions and that average pH values do not truly reflect the individual pH values.

That the relationships described in this paper have a direct bearing on similar acidity measures in wines has been demonstrated in a previous study (BOULTON 1979).

Summary

The relationships between the total acidity, titratable acidity and pH in grape cluster tissue have been proposed and confirmed. Published data for seven cultivars, eight vineyard locations and eight seasons have been used to demonstrate the general nature of these relationships. A mechanism for the uptake of monovalent metal cations is proposed and its effect on the acidity measures in grapes is shown.

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R. BOULTON
Department of Viticulture
and Enology
University of California
Davis, California 95616
U. S. A.