J. Int. Sci. Vigne Vin, 1997, **31**, n°3, 139-150

ACIDS AND BUFFERS IN WINE

ACIDES ET SUBSTANCES RESPONSABLES DU POUVOIR TAMPON DES MOÛTS ET DES VINS

U. HÖCHLI

Palmenweg 7, 8820 Wädenswil (Switzerland)

Abstract : A complete set of physico-chemical equations governing the interaction between acids and buffers is established. Together with formulae describing transformations of acids by biochemical reactions, these equations are used to monitor acid and buffer content during vinification. Several data sets from different wineries are used to test the validity of the approach.

Résumé : Au cours de l'élaboration des vins, les transformations chimiques ou microbiologiques entraînent une évolution des acides et des substances responsables du pouvoir tampon des vins. En tenant compte des transformations entre les acides et les composés responsables de l'effet tampon et en fonction de leur présence dans le moût, on peut prévoir leur composition dans le vin.
Deux points sont traités.
1) les acides ne sont pas mesurés séparément, mais sous forme d'acidité totale (tit) et d'ions hydrogènes libres (pH). La transformation entre les données globales tit et pH et entre les acides tartrique et malique est donnée par des équations décrivant la loi d'action des masses, la neutralité de charge et le bilan des éléments présents (Equations 1 à 14).
2) pour un moût donné, on peut disposer de la composition en acides (détermination par HPLC ou par méthode enzymatique) et des valeurs du pH et de tit. Il s'avère alors que le nombre d'équations excède le nombre de variables. Dans ce cas, un calcul d'estimations (équations a1 à a14) peut déterminer les meilleures valeurs pour les acides. Si celles-ci diffèrent des valeurs mesurées d'un taux inférieur à la précision de la mesure (0,5 g/l environ), cette méthode donne une bonne précision.
Les exemples présentés montrent que l'estimation de l'acidité totale est fiable à 1 g/L et celle du pH à 0,1 unité. En cas de macération, en présence de pellicules, on doit tenir compte du transfert de matière dans le jus qui enrichit le jus en potassium et donc, diminue considérablement l'acidité. Pour ces moûts, en particulier en rouge, il est préférable d'établir les estimations à partir des données prises 24 heures après encuvage.
Un essai de comparaison des données HPLC avec les données globales (pH et tit) montrent que les résultats sont compatibles pour le moût et le vin, mais pas pour les stades intermédiaires, c'est-à-dire après les fermentations alcoolique et malolactique. A ces stades, le vin jeune n'est pas en équilibre thermodynamique.
L'application de la simulation numérique aide à choisir le meilleur moment pour la vendange et permet d'appliquer des corrections pour l'acidité présente dans le moût.

Key words : acids, buffers, wine, must

Mots clés : acides, effet tampon, vin, moût

INTRODUCTION

Usually, in a research environment, the chemical consequences of biological processes are monitored by high-pressure liquid chromatography (HPLC), (FLAK and PLUHAR, 1983, MAXA *et al.*, 1991) whereas the practical control of vinification rests on data taken by electrochemical methods. So far, the two data sets have not been convertible on the basis of ab-initio calculations. This precludes comparisons between research and wine makers' data. Since in most cases the titratable acidity does not correlate with the sum of all acids revealed by HPLC, it might even be suspected that the two data sets are incompatible. If this is the case, a gap needs to be closed to ensure the relevance of biochemical Research for practical vinification.

The purpose of this contribution is to develop the physico-chemical equations governing the ensemble of acids and buffers in all generality needed in enology. Their solution, obtained numerically, allows one data set to be transformed into another thus shedding light on the problem of compatibility. They furthermore allow one to apply results from biochemical research to monitor the development of the acid-buffer ensemble in actual wines. A selection of experimental results serves to illustrate the possibility of forecasting subsequent stages of wine in the vinification process.

©Vigne et Vin Publications Internationales (Bordeaux, France)

It has been recognized long ago that this variable transformation is of central importance in enology. In particular, extensive research has established the connection between 10 different single acids and a potassium (K) buffer from a practical as well as a modelistic view (USSEGLIO-TOMASSET and BOSIA, 1978 ; 1983). It did not address the question of superposition of several acids. An attempt was made to obtain this relation on the basis of a hydrogen-potassium exchange model (BOULTON, 1980a, 1980b). Although empirically reasonably successful, it ignored the actual composition of the wine and the form of the laws of mass action. An equation relating the hydrogen-ion concentration with several acids was published more recently for the case of a saturated solution of potassium tartrate in the presence of other acids (NAGEL and HERRICK, 1989). The equation is based on the laws of mass action and charge neutrality, as are the equations presented here. Its use was, however, restricted to the limiting case of saturated solutions and it was solved for titratable acidity but not for pH.

Attempts to connect total acidity with pH empirically are numerous: A study presenting a comprehensive statistics (CHAMPAGNOL, 1994) is perhaps most relevant. Many authors report data on pH and titratable acidity during vinification processes. Such data will gain relevance when evaluated within the framework of the physico-chemical equations to be presented.

This paper is organized as follows: In section 2, the model is presented of a liquid containing n acids with valency up to 3, buffered by monovalent cations. From the solutions, obtained numerically, a transformation between concentration variables and electrochemical variables like pH and titratable acidity is derived. The next two sections, 3 and 4, are devoted to a presentation of experimental results and their interpretation, respectively. This serves to assess the validity of the approach (section 5). Applications are considered in section 6 and conclusions drawn in section 7. In an appendix a method is described that serves to illuminate the delicate problem of compatibility between research-laboratory data and winemakers' data.

MODEL OF SUPERPOSITION OF ACIDS AND BUFFERS

The relations between the concentration of acids, including buffers, and electrochemical parameters, namely titratable acidity and hydrogen-ion content, are reviewed and extended (HÖCHLI, 1994) to acids with 3 ionization states.

It is most convenient to start with the acids, the concentration of which is denoted c_i and the hydrogen-ion concentration h as known variables and compute the titratable acid t and the buffer concentration denoted by its major contributor, potassium (K).

For each acid, the law of mass action (BUTLER, 1964 ; WÜRDIG and WOLLER, 1989) requires that

$$c_{in} * h = c_{i,n+1} * d_{i,n+1} \quad (1)$$

where c_{in} is concentration associated with the nth ionization state of acid i and d_{in} is the dissociation constant of order n for the ith acid (Handbook of Chemistry and Physics, 1967). These are n equations for the ionization states 0, 1,....n, i. e. for n+1 unknowns. A n+1th equation is provided if the total concentration of acid c_i is known,

$$\sum_n c_{in} = c_i \quad (2)$$

It requires straightforward linear algebra to solve the system for c_{in}: Omitting the constant index i one finds

$$c_0 = c * h^3 / (h^3 + h^2 * d_1 + h * d_1 * d_2 + d_1 * d_2 * d_3) \quad (3)$$

for the undissociated portion of c. The dissociated portions are given in the order of increasing charge :

$$c_1 = c_0 * d_1 / h \quad (4)$$

$$c_2 = c_0 * d_1 * d_2 / h^2 \quad (5)$$

$$c_3 = c_0 * d_1 * d_2 * d_3 / h^3 \quad (6)$$

Multiplying the concentration of each state n with its charge n, taking the sum over all states, and dividing the result by c_i allows one to express q as the charge dissociated from 1 mole of acid i :

$$q = \sum_n n * c_n = (h^2 * d_1 + 2 * h * d_1 * d_2 + 3 * d_1 * d_2 * d_3) / (h^3 + h^2 * d_1 + h * d_1 * d_2 + d_1 * d_2 * d_3) \quad (7)$$

Reintroducing index i and expressing the requirement of charge neutrality (5) in terms of concentrations yields

$$\sum_i c_i * q_i(h) = k + h \quad (8)$$

It is straightforward to solve equation 8 for k, if h is known as was supposed in the beginning. The equation does not, however, depend on the distinction between known and unknown variables. If, instead k is the known concentration of potassium, equation (8) can be solved for h by iteration on a computer. The solution can be formally expressed as the functional $h(c_i, k)$.

©Vigne et Vin Publications Internationales (Bordeaux, France)

A means has to be provided to prevent the computer from searching in the unreasonable range of 0 or negative h. For acids with a valence of less than 3, we have $d_3 = 0$ and for h = 0 the computer would evaluate equation (7) as q = 0 / 0 = 1 whereas the correct result would be 2. Of course, if $d_3 = 0$, equation (7) reduces to the familiar equation for 2-valent acids by dividing the numerator and the denominator by h (7). To extend the calculation to the range of very low hydrogen concentrations (not of interest in the present context) the OH concentration of the order of 10^{-14}/h should be subtracted from the right hand side of equation (8).

Titrating the superposition of acids and buffers (SAB) implies adding OH ions in the form of Na OH until the concentration of h reaches that of OH and disappears from the charge balance. At pH=7, the dissociated part of all acids concerned so far can be replaced by their valence, the error being below 1 p. cent, as was shown by simulating the titration of SAB (see also figure 6). Denoting the amount of Na added to the SAB by t (for titratable acidity), it is obvious that

$$t + k = \sum_i c_i * q(10^{-7}) = \sum_i c_i * v_i \qquad (9)$$

where v_1 is the valence of the ith acid. Eliminating k from equation (9) with the help of equation (8) yields

$$t = \sum_i (c_i * (v_i - q_i)) - h \qquad (10)$$

where is taken from equation (7).

Equations (8) and (10) thus transform the SAB variables into the set of variables used to monitor vinification (V variables). Evidently, h and t have a unique solution if the concentrations of all acids and buffers are known. It is equally obvious that the inverse is not true. To retransform the two V variables into the seven SAB variables, 5 additional pieces of information are required. Two of them can be provided by direct measurements readily done by the winemaker, the remaining three require more detailed consideration.

Must contains almost exclusively tartaric and malic acid. The tartaric acid can be determined by colorimetry (REBELEIN, 1973). Denoting the acids: malic, tartaric, lactic, succinic, acetic, and citric by c_i in the order i=1 to 6, c_2 is known and can be inserted into equation (10). Setting

$$c_3 = c_4 = c_5 = c_6 = 0 \qquad (11)$$

in equation (10) and solving for c_1 yields

$$c_1 = (t - h - c_2 (v_2 - q_2 (h)))/(v_1 - q_1 (h)) \qquad (12)$$

Knowing all values of c_i, the potassium concentration can be obtained from equation (8). An alternative approach is to estimate the ratio between malic and tartaric acid with the help of a thin-film or paper chromatogram. Denoting this ratio by $R = c_1/c_2$ yields

$$c_1 = (t + h / ((v_1 - q_1) + R * (v_2 - q_2)) \qquad (13)$$

This alternative faces the problem of calibration and provides thus only limited accuracy (HÖCHLI, 1996).

During fermentation, small amounts of succinic, acetic, and citric acids are produced. It is possible to forecast their final concentration by evaluating statistical results on yeast metabolites: In central Europe, yeast strains are known to consume 23 p. cent of the malic acid present before fermentation (DITTRICH, 1987) and to produce an average of 0.5 g/L succinic, 0.2 g/L acetic and 0.5 g/L citric acid (DITTRICH, 1987 ; PULVER *et al.*, 1991). *Leuconostoc oenos* converts malic acid into lactic acid at a rate of 2/3, and consumes most citric acid (Van WUUREN and DICKS, 1993). The amounts of succinic, acetic, and citric acid are used as stage-dependent default values throughout the rest of this paper. They are likely to depend on the strains of yeast and bacteria inoculated or procreated spontaneously as well as on the geographic location. The replacement of data taken by statistical averages, called defaults, leads to a modification of equation (13) :

$$c_1 = (t - h - \sum_{i=2}^{6} c_i * (v_i - q_i (h)) / (v_1 - q_1 (h)) \qquad (14)$$

where the summation omits the term with i=1.

If malolactic fermentation has taken place, the index 1 should be replaced by 3 wherever it occurs. Equation (14) allows one to convert observations relevant to the winemaker into a set of acid concentrations at all stages of vinification. As this procedure relies on default values for a series of concentrations, it is likely to lead to deviations from the observed behavior of wine. This issue is addressed in the following paragraph.

EXPERIMENTAL EVIDENCE

The traditional way to monitor acidity parameters during vinification is by taking electrochemical data such as pH and titration results. They are supplemented by colorimetric determinations and thin-film chromatography. Determination of acids by HPLC requires considerable instrumentation and technical skill. It is thus not only less widespread there also seems to be no consensus on the compatibility of HPLC and electrochemical data. In the following the analysis of acid reduction during vinification stages is performed on the basis of electrochemical and related variables. The

©Vigne et Vin Publications Internationales (Bordeaux, France)

question of whether these data are compatible with HPLC data is dealt with at the end of this paper.

Grapes (stage 0 of vinification) contain tartaric and malic acid but very little of other acids. Their major contribution to buffers is potassium. Small amounts of Na and Ca reinforce the buffering power of K and are considered incorporated in the potassium content (in milliequivalents /L), as long as no crystallization takes place. To determine these three variables, three observations are available, namely pH, t, and c_2. Under these conditions, the starting point for vinification, grape juice, can be determined unambiguously (the term « must » is reserved for the same item after skin contact, for white cultivars juice and must may be, but are not necessarily, identical). During the following stages of vinification, substantial amounts of other acids are formed and transformed, whereas the number of observables does not increase. As soon as fermentation has started, the number of observables by the winemaker is insufficient to calculate acid composition. On the other hand, microbiological research has provided signi-

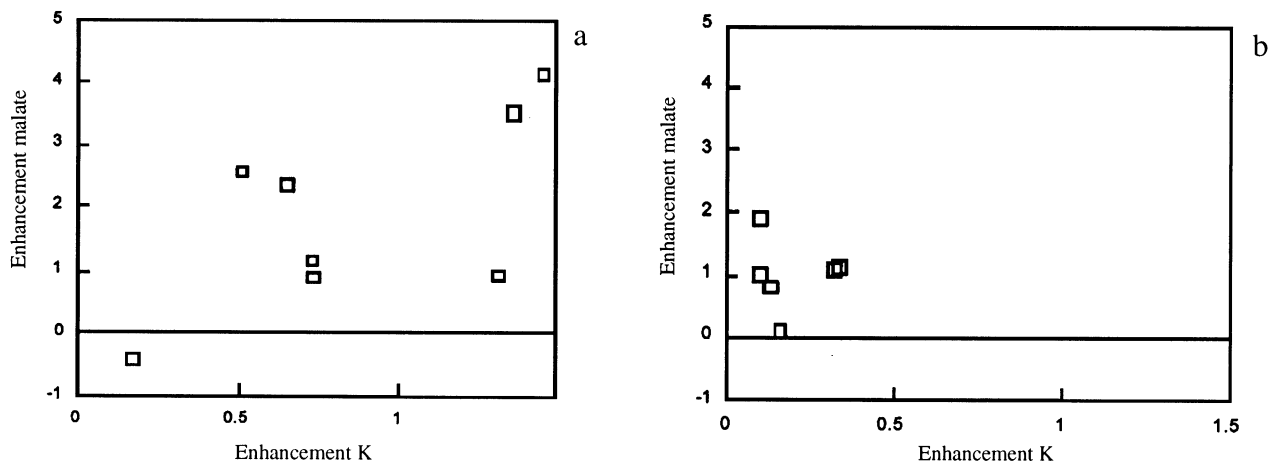


Fig. 1 — Enhancement of malate (y axis) and potassium (x axis) concentration after skin contact.
a : Data from Weinsberg, b : Data from Zürichsee.

Fig. 1 — Augmentation de la quantité de malate et de potassium après macération sur pellicule.
a : données de Weinsberg, b : données des côtes du Lac de Zürich.

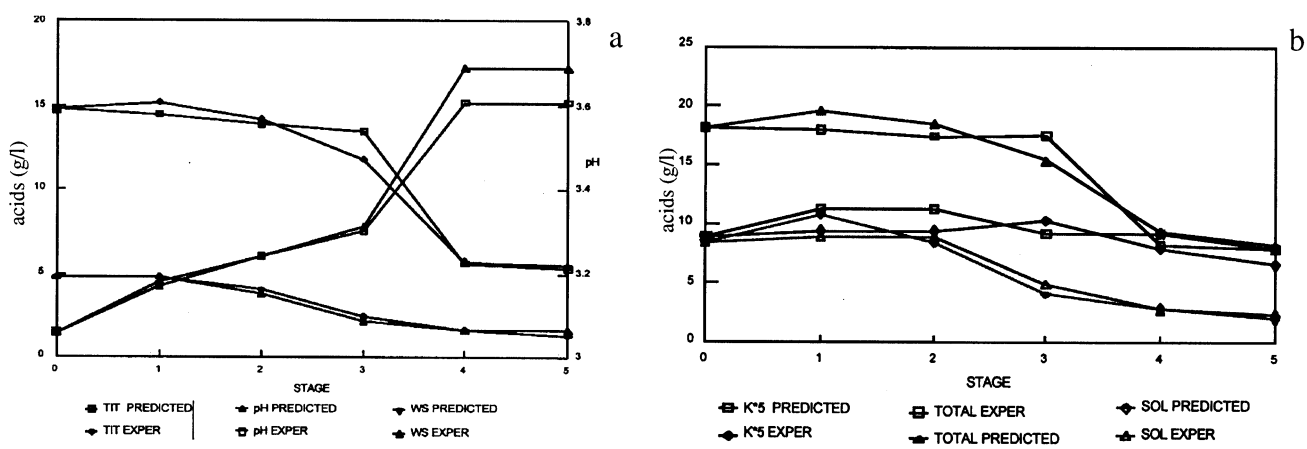


Fig 2 — Reduction of acids and buffers during vinification, model predictions vs. experimental results.
fig 2a: titratable acidity (expressed as tartaric acid), tartaric acid and pH.
fig 2b: Potassium (multiplied by 5), total acidity and solubility product (in $(g/L)^2$).
0 : juice ; 1: after skin contact ; 2: after deacidification by 1 g/L ; 3: after fermentation ;
4: after malolactic fermentation ; 5: after chilling wine

Fig 2 — Diminution des acides et des composés responsables du pouvoir tampon, au cours de l'élaboration d'un vin. Modèle prévisionnel d'après des données expérimentales
0 : jus ; 1 : après macération pelliculaire ; 2 : après désacidification de 1 g/l ; 3 : après fermentation alcoolique ;
4 : après fermentation malolactique ; 5 : après refroidissement

©Vigne et Vin Publications Internationales (Bordeaux, France)

ficant insight into acid transformations. It is the purpose of the rest of this section to assemble pertinent findings from microbiology, to parametrize these findings, and to use these quantized results to monitor acidity during vinification.

Interestingly enough, the first step of vinification, namely skin contact, has received little attention as far as its influence on acidity is concerned. When berries are pressed for test purposes, the juice yield is normally low. This raises the question whether the juice remaining in the skin, and transferring to the berry juice during contact, has the same properties as the test sample and whether any difference might influence the properties of the must. The juice of a series of samples was investigated right at harvest and compared with must pressed two days later. The white cultivars were separated from the skin right away, whereas the red cultivars and a Pinot gris were left in skin contact. Within experimental error, the acid composition of white must remained the same within two days, indicating that the possible onset of fermentation can be neglected. As shown in figure 1, the juice of red cultivars from Weinsberg (50 deg N) is enriched considerably by malic acid and potassium during two days of skin contact. The same is true, to a somewhat lesser degree, for samples from the region of the lake of Zürich (47 deg N). Included in the lake-of-Zürich data is a sample of Pinot gris, with very similar properties as the Pinot noir samples (fig 1b). It is the duration of contact, rather than the color of the skin that is crucial for malic-acid and potassium uptake. Uptake of K from the skin has been observed in other contexts (RYSER *et al.*, 1989 ; SCHALLER *et al.*, 1992), mainly in view of its influence on aromatic content. Crushed grapes stored at 5 °C for 10 days to prevent fermentation showed the same enhancement of malic acid and K, as did samples heated to 80 °C for a few minutes and then rapidly cooled.

Contrary to widespread belief, the first step in the winemaking process, skin contact, enhances not only potassium but also acidity. In figure 2a, pH, tartaric, and total acid labeled « experimental » are plotted for each development stage. In figure 2b, the parameters for potassium, total acidity, and the solubility product for potassium tartrate are plotted. These data are obtained by applying equations (12) and (9) to the data presented in figure 2a. The titratable acidity stays nearly constant between stages 0, juice, and stage 1, after skin contact. The large uptake of potassium appears to offset the uptake of malic acid as evidenced by a net increase of pH by about 0.2 units in 2 days. It is observed as well after heating the pomace to 80°C for a few minutes. Even storage of grape juice in contact with the skin in a refrigerator for a week has the same effect.

The following steps are straightforward : A chemical deacidification by 1g tartrate reduces t and the total acidity at stage 2. After the second step, fermentation, which typically takes place in 300 L stainless-steel tanks, tartaric acid (figure 2a) and potassium contents (figure 2b) both decrease, leaving pH nearly invariant. All samples underwent malolactic fermentation, (stage 3) with the obvious consequence that malic acid practically disappeared and gave rise to an increase of lactic acid by about 2/3 of the malic-acid content expressed in g/L. A final chilling of the samples further reduced both tartaric acid and potassium by amounts in accordance with literature values (TROOST, 1988). Also plotted are the solubility products expressed in (g/L) squared, which are found to be slightly higher than model values, which is attributed to the presence of inhibitors (of tartrate crystallization) in the wine (DUNSFORD and BOULTON, 1991).

EVALUATION OF EXPERIMENTAL RESULTS

In this section an attempt is made to model subsequent vinification stages by introducing findings obtained from microbiological research.

From the observation that juice is enriched by malic acid and potassium it is obvious to conclude that the skin contains large amounts of malic acid and K. It has, in fact, been shown that uptake of malic acid preferentially takes place via skins , especially in the late stages of grape maturity, whereas the distribution of tartaric acid is rather uniform throughout the berry. The charge dissociated by these large amounts of acidity must be compensated by potassium which is taken up in large amounts (CURRLE *et al.*, 1983) to the extent that the liquid in the cytoplasmas is nearly neutral. This is a prerequisite for the functioning of enzymes and is achieved by ATP type proton pumps (JACKSON; 1993). The cytoplasma liquid is thus a supersaturated solution of tartrate and malate. Its property has been analyzed in detail (ILANI and COOMBE, 1988). By separating flesh from skin it was possible to determine both a malate and a potassium concentration in the skin which exceeded those of the flesh by one order of magnitude. Applying equations (8) and (10) to the results for mature berries (K=11 g/L, malate = 10 g/L, tartrate = 10 g/L, (15) one finds pH = 7.2, in agreement with the prerequisite for enzyme activity. The question remains whether this supersaturated solution is stabilized by inhibitors, as in wine, or by compartimentation in the cells. It follows from the same argument, that an admixture of 5 p. cent cytoplasma solution to a juice containing 5 g/L of tartrate and malate each, buffered by 1.2 g/L K, raises its pH from 3.06 to 3.28.

©Vigne et Vin Publications Internationales (Bordeaux, France)

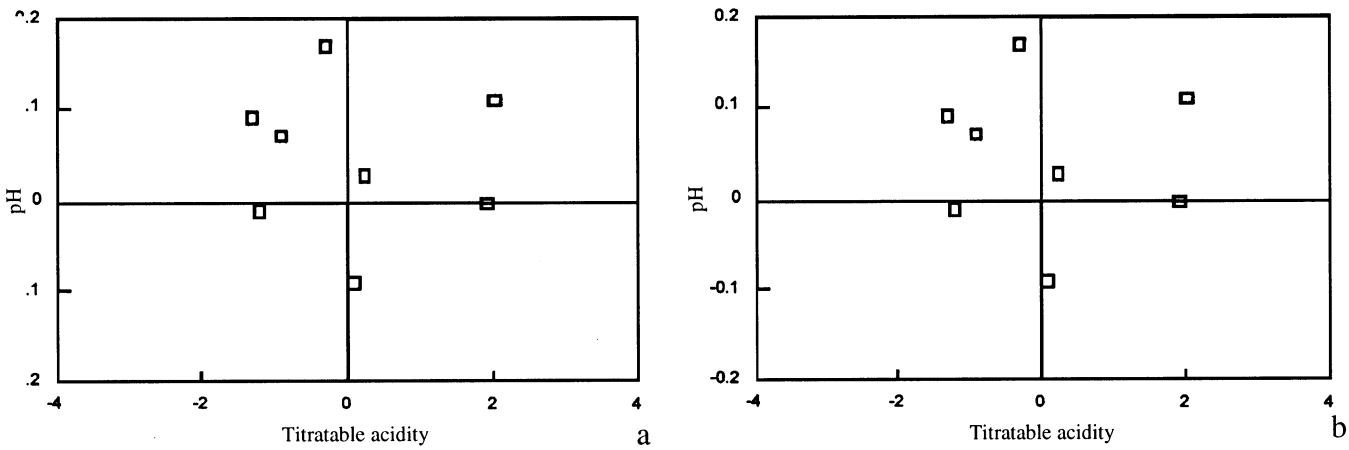


Fig. 3. — Difference between experimental values and predicted values for titratable acidity (x axis) and pH (y axis). Fig. 3a — before malolactic fermentation - Fig. 3b — after malolactic fermentation.

Fig. 3. — Différence entre les valeurs expérimentales et les valeurs calculées pour l'acidité totale (axe des abscisses) et le pH (axe des ordonnées) Fig. 3 a — avant fermentation malolactique - Fig. 3b — après fermentation malolactique

This is the enhancement typical for a large number of juices in contact with the skin (BAUMES *et al.*, 1989 ; BROUSSOUS and FERRARI, 1994 ; CHAMPAGNOL, 1994). An attempt to generalize these findings consists of postulating that the cytoplasma solution is neutral, that the content of tartaric acid is the same as in the flesh, and that the content of malic acid is twice that in the flesh, a view that is supported qualitatively by studies of acid metabolisms in *vitis* (RUFFNER, 1982), although the actual relationships depend on the state of maturity and on cultivar. Accepting this statement, allows to predict the enhancement of potassium and malate during skin contact. The actual relations between cytoplasma and juice composition is not critical: A reduction of pH from 7 to 5.6 changes the potassium content by 10 p. cent, which, after admixture to the juice amounts to 0.5 p. cent. Similarly, a misjudgment of malate content by 10 p. cent changes the final pH by 0.01 units, which goes unnoticed. Adding thus 5 p. cent of model cytoplasma liquid to the juice, we obtain a model value for the juice after skin contact. Its properties are plotted in figures 2a and b: The differences between stages 0 and 1 are of the order of 1 g/L for the malic and total acid and 0.5 g/L for potassium.

The next (possible) step of vinification is chemical deacidification which can take place at this stage, and/or later. Here it was performed by adding 660 mg of $CaCO_3$/L which reduced tartaric acid by 1 g/L. It is trivial to plot the corresponding reduced acidity (figure 2, stage 2). The reaction, however, is sluggish, and the expected changes appear to be partly masked by the rapidly developing fermentation.

Alcoholic fermentation has been analyzed in great detail (CURRLE, 1983). Yeast is known to consume about 23 p. cent of the malic acid present in the juice and to produce small amounts of succinic, acetic, and citric acid. For these values we take averages found in statistics as established by HPLC methods. The actual default values are 0.5, 0.2 and 0.5 g/L, respectively. The alcohol produced by the yeast reduces the solubility of potassium tartrate which therefore crystallizes partly (TROOST, 1988). Taking these processes into account allows us to propose a model description of stage 3, also plotted in figures 2a and 2b.

Malolactic fermentation is standard practice in Switzerland but less so in France and Germany. Modeling poses no problems: malic acid is replaced by lactic acid with a yield coefficient of 2/3. Furthermore, citric acid is generally metabolized, and was known to do so for the bacteria (viniflora, a *leuconostoc oenos* strain) used in this experiment (PULVER *et al.*, 1991). A possible reduction of the solubility of potassium tartrate due to chilling has to be taken into account. The modeled results are also plotted in figures 2 and 3, stage 4. These model calculations were applied to a number of juices developing into wine. The results of the forecasts are plotted in terms of differences between experimental values and model predictions of titratable acidity and pH. For the example shown in figures 2 and 3 the model predicts the titratable acidity reasonably well and overestimates the pH by 0.08 units. Figures 3a and 3b show the results in a diagram representing shots on a target. The coordinate 0,0 would be a precise forecast. It appears that precision is better after malolactic fermentation (figure 3b) than before (figure 3a). Deviations are generally of the order of 1 g/L for tit and 0.1 for pH.

©Vigne et Vin Publications Internationales (Bordeaux, France)

MERITS AND LIMITS OF THE SAB MODEL

In this section the question is raised whether the acid-buffer model is a reasonable approximation of the processes that take place in real wine. The assumption was made that the transformation of must into wine could be expressed in terms of a sequence of equilibrium states separated by dynamic biochemical processes. The equilibrium states, called stages of vinification, were described by physico-chemical equations reflecting the conservation of energy and charge. The result of biochemical processes were converted into operators, which transformed one set of acidity components into another. To test the reliability of the model two questions should be raised: Are the experimental data internally consistent and can the biochemical processes be described adequately by net formulae?

Knowledge of biochemical processes is usually subject to test by HPLC measurements of the status before and after the process under investigation. On the other hand, the development of the maturation of wine is controlled by electrochemical data. Are these data sets compatible?

This is a delicate question of fundamental importance for which no study appears to be published in the literature. The most obvious question, namely whether the sum of all acids evaluated by HPLC is in agreement with results from titration, appears to be left unanswered.

At first glance, the answer is no, for titratable acidities usually turn out to be lower than the sum of HPLC-determined acids, whereas, if interpreted as total acidity, they should be equal or larger, since in HPLC experiments certain acids may be masked by unidentified compounds. If titration is, however , interpreted as the addition of charge to a buffered solution until pH=7 is reached, then it follows that

$$t = T - K = \sum_i c_i - K \qquad (11)$$

and accordingly T should be larger than t and larger than the sum of all HPLC-determined acids. A preliminary test of a data set (HÖCHLI, 1994) turned out to be encouraging : Electrochemical data taken on Pinot-noir must were indeed compatible with corresponding HPLC data (WOLF and KAMBER, 1996). In fact, the titratable acidity turned out to be only slightly lower when evaluated from HPLC data than from direct electrochemical measurements. This finding was readily, and perhaps prematurely, interpreted in terms of compatibility, and the slight differences were attributed to

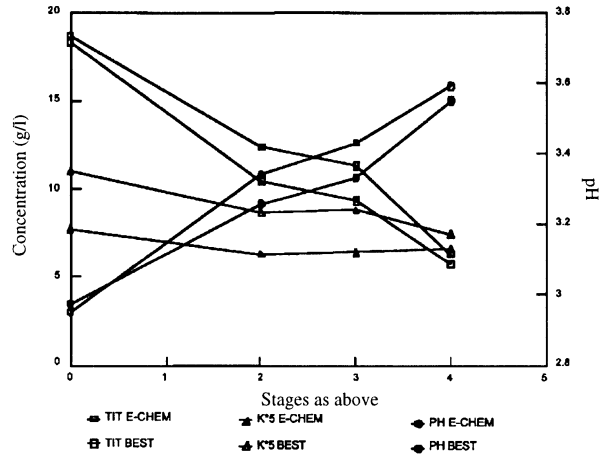


Fig. 4 — Test for compatibility between electrochemical and HPLC data : Electrochemically determined values for titratable acidity, potassium and pH data and least-square fit from a combined HPLC/ electrochemical investigation.

Fig. 4 — Essai de compatibilité entre les valeurs électrochimiques et les valeurs obtenues par HPLC : acidité totale, potassium et pH sont obtenus par méthode électrochimique et par combinaison entre méthode électrochimique et HPLC

possible failure to detect a masked low-concentration acid by HPLC (HÖCHLI, 1994). Subsequent stages of vinification show, however, severely incompatible results. The standard procedure to reconcile contradicting results is to require that the error function (defined as the sum of differences between data squared) be a minimum. Performing this calculation, which is tedious and will be referred to in appendix 1, yields a set of « best values » which are intermediate between the conflicting data sets. On the basis of the calculus performed in the appendix, « best values » are derived from a set consisting of both HPLC and electrochemical data (WOLF and KAMBER, 1996).

TABLE I

Possible error in the assessment of a process and reason for failure

Tableau I — Erreur des prévisions en fonction des méthodologies d'élaboration

Process	Reason for failure
skin contact :	inhomogeneous sample, duration of contact, temperature, handling
alcoholic fermentation	supersaturation of potassium tartrate
malolactic fermentation	inoculation before end of alcoholic fermentation
chemical deacidificaction	supersaturation of calcium tartrate

©Vigne et Vin Publications Internationales (Bordeaux, France)

In figure 5 the « best values » and the measured values are plotted for the original and subsequent stages of the developing must. Obviously, the discrepancies in the intermediate stages are larger than the internationally accepted errors for analogous data (OTTENEDER and MARX, 19??). The stage after malolactic fermentation was again turn described in terms of both data sets within a tolerable error. No data are available for chilled samples.

The experimental K values are consistently lower than the measured values. This reflects the fact that atomic absorption is selective for potassium only, whereas « best values » stand for buffer content, which is typically 25 p. cent higher in view of the contribution of Na and Ca. The problem of incompatible results appears to be most severe for intermediate states. In the following, an analysis is presented of possible uncertainties: Possible origins of errors are related to incomplete knowledge of the appropriate vinification process.

Obviously the final state should be in thermodynamical and chemical equilibrium, a prerequisite for bottling.

It is evident that insufficient statistical evidence may make it difficult to estimate how much acid is reduced or produced by biological processes. It has become standard practice to inoculate must with *saccharomyces cerevisiae* and *leuconostoc oenos*. For a given strain,

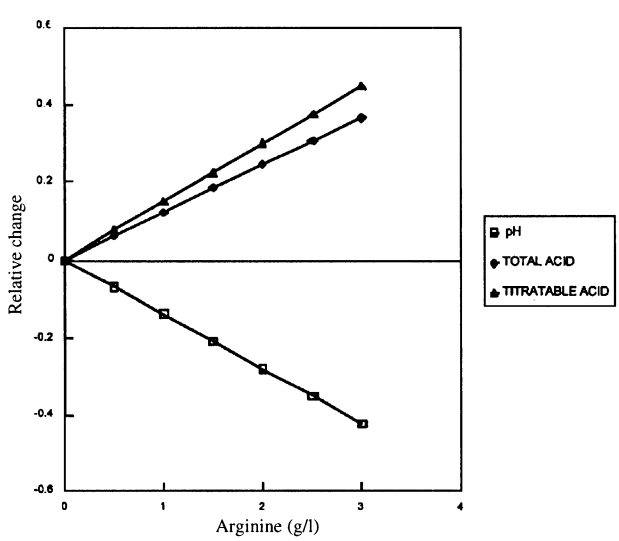


Fig. 5 — Changes of pH (in standard units) of the total and the titratable acidity (in g/L) due to addition of arginine.

The end point of an arginine titration is at pH=12. Standard titration to pH=7 will not detect arginine. See also figure 6.

Fig. 5 — Variation du pH et de l'acidité totale en fonction de l'addition d'arginine (en g/l).

Le point final du dosage d'arginine est à pH = 12. Le dosage standard à pH = 7 ne détecte par l'arginine. Voir également figure 6.

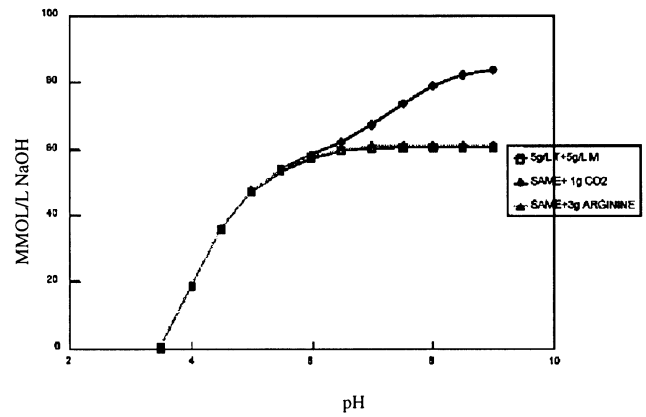


Fig. 6 — Simulated titration of a solution containing 5 g/L tartaric acid and 5 g/L malic acid.

Same after addition of 3 g/L arginine (practically identical with the former curve) and after addition of 1 g/L CO_2 (which leads to a 25 p. cent enhancement of the titratable acidity).

Fig. 6 — Simulation d'une titration d'une solution contenant 5 g/l d'acide tartrique et 5 g/l d'acide malique

Même chose après utilisation de 3 g/l d'arginine (pratiquement même courbe que la courbe précédente) et après addition de 1 g/l de CO_2 (qui permet d'augmenter de 25 p. cent l'acidité totale).

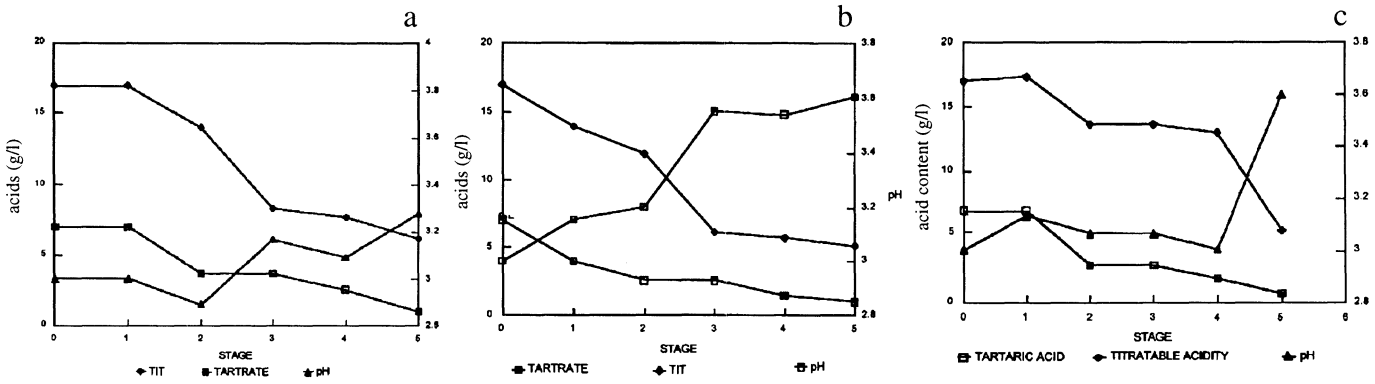
variances should be reduced (DITTRICH, 1995) and, after determination of the standard yield, forecasts should be improved.

In some cases there may be compensation : The citric acid produced by certain strains of yeast is later metabolized by *Leuconostoc oenos*.

Another objection might be that the list of acidities is (always) incomplete. Additional acids that are present but omitted in the analysis may lead to erroneous conclusions. A test performed for arginine (LEHTONEN PEKKA, 1996) gives the following result. Unrecognized arginine in a concentration of 1 g/L in a must containing 5 g/L Tartrate and 5 g/L Malate and having a pH of 3, leads to a misjudgment of the potassium content by 10 p. cent . Adding arginine to the same must changes the pH and t as shown in figure 7. Again the value for pH is altered most sensitively. Since Arginine is partly dissociated at all pH levels relevant for must and wine, it has a major influence on charge-sensitive parameters such as K and pH. It appears to have its maximum concentration during fermentation and to have little influence on the initial and final stages of vinification. It is thus suspected to contribute to the discrepancies found to be maximum at intermediate stages.

CO_2 is volatile. Its contribution to titration is shown in figure 8. One g/L of CO_2 may enhance t by 10 p. cent. It is, however, easily removed by heating the sample to some 60 °C. Arginine has practically no impact on titration at a level of 3 g/L if the end point is chosen below pH = 9.

©Vigne et Vin Publications Internationales (Bordeaux, France)



Stades : 0 : juice ; 1 : no deac ; 2 : fermentation ; 3 : malo ; 4 : chill ; 5 : deacidifi. wine

Stades : 0 : juice ; 1 : deacid must ; 2 : fermentation ; 3 : malo ; 4 : chill ; 5 : possible deac wine

Stades : 0 : juice ; 1 : skin contact two days 2 : fermentation ; 3 : malo ; 4 : chill ; 5 : ds deacid

Fig. 7 — Result of a deacidification at a late stage (wine, fig. 7a) and at an early stage (must, fig. 7b) and double-salt desacidification (fig. 7c)

Fig. 7 — Résultats d'une désacidification du vin (fig. 7 a) et du moût (fig. 7b) et d'une désacidification par précipitation au sel double (fig. 7c)

These examples show how the pH value changes when acids are added. Obviously, the form of the titration curve depends markedly on the sample composition. It is, in fact, possible to determine n acids and one buffer by taking n+1 data points of a titration curve, at least in a mathematical analysis. An attempt to perform a real analysis on this basis showed that the precision necessary to determine the pH level must be better than 0.001 and 10 mg for the titration agent (NaOH), thus barring practical application.

APPLICATION

On the basis of samples taken in the vineyard, the values for acidity and buffer in the wine can be predicted. This forecast can help one to choose the optimum harvest date. It can also indicate whether the acidity will be sufficient for a stable wine or how much citric or tartaric acid should be added. Climatic conditions in central Europe rarely call for early harvest or acidification, but often for the opposite : To omit excessive acidity, prolonged skin contact is effective but may have undesirable side effects (RAPP *et al.*, 1985). Chemical deacidification may be performed in different ways at different stages of vinification. According to TROOST (1988), it is preferable to deacidify must rather than wine. The earlier the treatment, the less is its impact on the bouquet. Early treatment requires, however a means to predict its result.

To test the outcome of deacidification according to TROOST a comparison is made between the various ways to treat a must with pH = 3, tartaric acid = 7 g/L, and a titratable acidity of 17 g/L. The aim is to produce a wine with pH = 3.5 and t = 5.5 g/L leaving the tartaric-acid content at the minimum level of 1 g/L. If this is attempted by deacidifying the wine before bottling, removal of tartaric acid by 1.2 g/L is allowed. It is shown in figure 7a that this late deacidification fails both to raise the pH and to reduce t sufficiently. If, instead, must is to be deacidified, it turns out that 2.3 g/L tartaric acid can be removed, which leaves 1 g/L in the ready-made wine. Moreover, the malolactic fermentation starts at pH = 3.2 and the wine has the desired parameters (figure 7b). For the sake of completeness, figure 7c shows that a double-salt deacidification may also lead to the desired result.

Skin contact has been applied to enhance the sensorial complexity of wine. Since it buffers the must at the same time, the influence of the skin on the sensorial properties may well be, in part, indirect in that it enhances the pH level. In any case, skin contact may thus be used to buffer the must, and thus the wine deliberately.

It appears that the buffer content plays a crucial role in the regression formula between the titratable acidity and pH which has been established for a large amount of experimental data (CHAMPAGNOL, 1994). For the simple case of a solution of tartaric acid and potassium only, it follows from equations 8 and 9 to a good approximation, in particular if h<<K, that

$$t = T * (1 - (h * d1 + 2 * d1 * d2) / (h^2 + h * d1 + d1 * d2)) = T * f (h)$$

A plot of the function f (h) vs. t should thus yield a straight line without scatter. A plot of h vs. t should give a curved line, but with no scatter. The same is true, if the ratio between acids is the same in all samples. If scatter is observed, as it is in actual representations of h vs. t, it is to be attributed to acid composition varying among of the samples.

©Vigne et Vin Publications Internationales (Bordeaux, France)

CONCLUSIONS

Must, and wine, are solutions that contain variable amounts of six major acids and potassium. In some stages of vinification these solutions are in thermal and chemical equilibrium. If such a stage is reached it is legitimate to apply the laws of mass action and the condition of charge neutrality. The corresponding set of equations relates electrochemical parameters, pH, and titratable acidity to the concentration of acids and buffer.

It allows one to verify the cases in which acid concentrations obtained by HPLC methods or enzymatic reactions are compatible with pH and titratable acidity obtained electrochemically. It is shown for the few instances where both data sets were available that a self-consistent data set is obtained for the first and last stages of vinification , fresh juice and wine ready to be bottled, but not for intermediate stages.

Using statistical averages concerning the impact of biochemical processes on acid content, a prediction can be made for subsequent stages. In particular, based on our own experiments, it is found that mere skin contact for 2 days enhances the potassium and malic-acid concentration by significant amounts. Yeast consumes malic acid and produces succinic, acetic, and citric acid instead. *Leuconostoc* converts malic acid into lactic acid and consumes citric acid. Finally temperature changes and alcohol content limit the solubility product for potassium and tartrate.

All these effects are incorporated in the computation and lead to a forecast of the acid content in wine on the basis of a sample of juice at harvest. The test for the final state turns out to be more reliable (about ± 0.1 for pH, ± 1 g/L for the titratable acidity) than for intermediate stages. Both the variety of metabolite production by yeast, and the nonequilibrium character of intermediate stages could be the reason for the insufficient precision of model predictions for intermediate stages. Both phenomena preclude the application of the law of mass action.

The model does, however, predict the final stage with sufficient precision, provided 3 data points are available for the initial state, juice, or must before fermentation. The forecast allows application of (de-)acidification at an early stage, thus minimizing the harm to wine. It also assists in determining the optimum harvest date.

APPENDIX : BEST VALUES FOR VARIABLES DESCRIBING ACIDITY

Of the 9 variables generally applied to describe wine, namely 6 acids, pH, Tit, and K, only 7 are independent. Complete data sets containing 9 determined quantities overdetermines the system of equation. There is a conflict inherent in such a determination: If the concentrations of all acids are known from HPLC data, and that of K from atomic absorption, then pH and t are determined. These values of pH and K are usually different from the values determined directly by electrochemistry.

A simple example serves to illustrate how such a conflict may be reconciled. Assume that a HPLC chart indicates 5 g/L tartaric acid and 5 g/L malic acid. whereas the titration indicates (after correction) that the total acidity is 11 g/L. Neglecting for the purpose of simplicity the conversion factor for malic acid, one finds that the results for the total acidity are in conflict by 1 g/L. Either set of measurements may contain an error. To find a set of data that minimizes the error function E, one postulates that

$$E = (x - x_0)^2 + (y - y_0)^2 = \min \qquad (a1)$$

where x and y are the « best » values and the indexed variables are the measured values. From (a1) it follows that

$$\partial E / \partial x = \partial E / \partial y = 0 \qquad (a2)$$

thus

$$(x-5)^2 + (y-5)^2 + (x+y-11)^2 \text{ minimum} \qquad (a3)$$

Accordingly, the differentials with respect to x and y must vanish:

$$2*(x-5) + 2*(x+y+11) = 0 \qquad (a4)$$

$$2*(y-5) + 2*(x+y+11) = 0 \qquad (a5)$$

The solution of equation (a4) and (a5) reads

$$x = y = 5.333 \qquad (a6)$$

and implies that the values for HPLC data are somewhat higher than those indicated by the measurement, whereas those for titration are somewhat lower. If the differences between best and measured values are lower than the accepted error for the method, then the data are said to be internally consistent, otherwise a search for a hidden error is appropriate.

The calculus of balance with 7 independent and 2 dependent variables is performed according to the same scheme. Since not all data are measured with the same precision, each contribution to the error function

©Vigne et Vin Publications Internationales (Bordeaux, France)

is multiplied by a weight proportional to R^{-2} where R is the accepted error for the particular method. Expressing the derivatives of the error function in terms of the seven variables = 0 yields 7 equations to be solved numerically.

An outline of the tedious procedure given for only 3 independent variables may suffice:

If x_{10} is the ith data point with weight $g_1 = R^{-2}$, x_1 the best value, F_{j0} the jth data point for dependent variables, and Fj (x_{i0}) the value obtained after applying the jth function to the set of measured independent variables, then with

$$z_i \equiv x_i - x_{i0} \qquad \text{(a7)}$$

and

$$\Delta F_j = F_j(x_{j0}) - F_{j0} \qquad \text{(a8)}$$

the error function becomes

$$\sum_i (g_i * z_i^{\ 2} + \sum_j (f_j * (\Delta F_j + \sum_i F_{ji} * z_i)^2 \qquad \text{(a9)}$$

where

$$F_{ji} = \partial F_j / \partial z_i \qquad \text{(a10)}$$

From the conditions

$$\partial E / \partial z_i = 0 \qquad \text{(a11)}$$

a linear relation can be derived which reads in matrix form

$$Gz = \Delta F \qquad \text{(a12)}$$

where the diagonal elements of G read

$$G_{ii} = g_i + f_i^2 \qquad \text{(a13)}$$

and the off-diagonal elements read

$$G_{ij} = f_i * f_j \qquad \text{(a14)}$$

Formally, the solution is $z = \Delta F/G$, which denotes the differences between « best » and measured values of the independent variables. It is evaluated numerically and inserted into figure 5 for the conditions mentioned in the figure caption.

Acknowlegments : It is my pleasure to thank H. LAY for sending his results on skin contact, F. CHAMPAGNOL for correspondence, E. KÜMIN for providing samples, and K. SCHALLER, W. KOBLET and their collaborators for illuminating discussions.

LITERATURE

BAUMES R. L., BAYANOVE C.L., BARILLIÈRE J.M., SAMSON A. et CORDONNIER R.E., 1989. La macération pelliculaire dans la vinification en blanc. Incidence sur la composante volatile des vins. *Vitis*, **28**, 31.

BOULTON R., 1980. The relationships between total acidity, titratable acidity and pH in wine. *Am J. Enol. Vitic.*, **31**, 76.

BOULTON R., 1980. The relationships between total acidity, titratable acidity and pH in grape tissue. *Vitis*, **19**, 113.

BROUSSOUS P. and G. FERRARI, 1994. Application de la macération pelliculaire aux cépages blancs méridionaux. *Rev Fr. Œnol.*, **145**, 41.

BUTLER, 1964. Ionic equilibrium, a mathematical approach. Addison Wesley. Menlo Park 1964

CHAMPAGNOL F., 1994. Facteurs agronomiques de l'acidité des moûts et des vins. *Progrès agric. vitic.*, **111**, 469.

CURRLE O. et al., 1983. *Biologie der Rebe*, Meininger. Neustadt p 192.

DITTRICH H.H., 1995. Bildung und Abbau organischer Säuren durch Mikroorganismen in Most und Wein. *Vitic. Enol. Sci.*, **50**, 50.

DITTRICH H.H., 1987. *Mikrobiologie des Weines*, Ulmer Stuttgart, p 231.

DUNSFORD P. and R. BOULTON, 1991. Kinetics of potassium tartrate crystallization from table wine. *Am. J. Enol. Vitic.*, **32**, 100-106.

FLAK W. and G. PLUHAR, 1983. Bestimmung der Säurehauptkomponenten mit einer modifizierten hochdruckflüssigkeitschromatographischen Methode. *Mitt. Klosterneuburg*, **33**, 60.

Handbook of Chemistry and Physics, 48th ed. (1967), p. 91.

HÖCHLI U., 1994. Numerische Simulation der Säurereduktion im Most und Wein während des Ausbaus. *Enol. Sci.*, **49**, 160.

HÖCHLI U., 1996. Säuregehalt in Weinen des Jahrgangs 1994, Voraussage und experimenteller Befund. *Vitic. Enol. Sci.*, 51, 19.

ILAND P.G. and B.G. COOMBE, 1988. Malate, Tartrate, Potassium , and Sodium in flesh and skin of shiraz grapes... *Am J. Enol. Vitic.*, **39**, 71.

JACKSON R.S., 1983. *Wine Science*, Academic San Diego.

LEHTONEN PEKKA, Determination of amines and amino acids in wine. *Am. J. Enol. Vitic.*, **47**, 127.

MAXA E., BRANDES W. and R. DANIEL, 1991. Modifizierte HPLC-Methode zu Quantifizierung der wichtigsten organischen Säuren in Wein... *Mitt. Klosterneuburg*, **41**, 233.

©Vigne et Vin Publications Internationales (Bordeaux, France)

NAGEL C. W. and I.W. HERRICK, 1989. The effect of Malate or Lactate Content on the pH-TA relationship of potassium bitartrate saturated alcohol-water solutions. *Am. J. Enol. Vitic.*. **40**, 81.

OTTENEDER H. and R. MARX, 1995. Ringversuch zur Bestimmung von Glucose, Fructose, Glycerin und Ethanol in Wein mittels Ionenaustausch-chromatographie und RI-Detektion. *Vitic. Enol. Sci.*, **50**, 66.

POSSNER D. R. E. and W. M. KLIEWER, 1985. The localization of acids, sugars, potassium and calcium in developing grape berries. *Vitis*, **24**, 229.

PULVER D., J. GAFNER, P. HOFFMANN and S. ALBISSER, 1991. Säureabbau-Starterkulturen im Vergleichstest. *Schweiz. Zeitschrift für Obst- Weinbau*, **129**, 685.

RAPP A. , M. GÜNTERT and W. RIETH, 1985. Einfluss der Maischestandzeit auf die Aromazusammensetzung des Traubenmostes und Weines. *Deutsche Lebensmittel-Rundschau*, **81**, 69.

REBELEIN H., 1973. Verfahren zur genauen Bestimmung der Wein- und Milchsäure in Wein. *Chem. Mikrobiol. Technol. Lebensm.*, **2**, 33.

RUFFNER H.P., 1982. *Metabolism of tartaric and malic acids in Vitis. Vitis*, **21**, 346.

RYSER J.-P et AERNI J. et MURISIER F., 1989. Fumure potassique de la vigne et acidité du vin. *Rev. Suisse Vitic.*, **21**, 319.

SCHALLER K., LÖHNERTZ O. and CHIKKASUBBANNA V., 1992. Uptake of potassium by the grape berries of different cultivars during growth and development. *Vitic. Enol. Sci.*, **47**, 36.

TROOST G., 1988. *Technologie des Weines*. Ulmer, Stuttgart, p 516.

USSEGLIO-TOMASSET L. and P.D. BOSIA, 1988. Determinazione delle costanti di dissociazione dei principali acidi del vino... *Riv. Enol.*, **31**, 380.

USSEGLIO-TOMASSET L. and P.D. BOSIA, 1983. Osservazioni sulla deacidificazione e sull' acidificazione dei vini. *Riv. Enol.*, **36**, 71.

Van VUUREN H.J.J. and M.L. DICKS, 1993. *Leuconostoc oenos*, a review. *Am. J. Enol. Vitic.*, **44**, 99.

WOLF I. and KAMBER S., 1996. Herstellung von süssen und alkoholreichen Weinen mittels Traubentrocknung. Schweiz. *Z. Obst-Weinbau*, **132**, 468.

WÜRDIG G. and R. WOLLER, 1989. *Chemie des Weines*, Ulmer Stuttgart.

Manuscrit reçu le 25 mars 1997 ;
accepté pour publication 22 septembre 1997.

©Vigne et Vin Publications Internationales (Bordeaux, France)