1

Organic Acids in Wine

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1.1 INTRODUCTION

Organic acids make major contributions to the composition, stability and organoleptic qualities of wines, especially white wines (Ribéreau-Gayon *et al*., 1982); (Jackson, 1994). Their preservative properties also enhance wines' microbiological and physicochemical stability.

Thus, dry white wines not subjected to malolactic fermentation are more stable in terms of bitartrate (KTH) and tartrate (CaT) precipitation. Young white wines with high acidity generally also have greater aging potential.

Red wines are stable at lower acidity, due to the presence of phenols which enhance acidity and help to maintain stability throughout aging.

1.2 THE MAIN ORGANIC ACIDS

1.2.1 Steric Configuration of Organic Acids

Most organic acids in must and wine have one or more chiral centers. The absolute configuration of the asymmetrical carbons is deduced from that of the sugars from which they are directly

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Table 1.1. The main organic acids in grapes

L(+)-Tartaric acid	L(−)-Malic acid	Citric acid
D-Gluconic acid	2-keto D-Gluconic acid	Mucic acid
Coumaric acid ($R_1 = R_2 = H$) Caffeic acid ($R_1 = OH$; $R_2 = H$)	Coumaryl tartaric acid	

derived. This is especially true of tartaric and malic acids (Table 1.1). The absolute configuration of the asymmetrical carbons is established according to the Prelog rules (1953). Further reference to these rules will be made in the chapter on sugars, which are the reference molecules for stereoisomerism.

1.2.2 Organic Acids in Grapes

The main organic acids in grapes are described (Table 1.1) according to the conventional Fischer system. Besides tartaric acid, grapes also have a stereoisomer in which the absolute configuration of the two asymmetrical carbons is L, but whose optical activity in water, measured on a polarimeter, is d (or +). There is often confusion between these two notions. The first is theoretical and defines the relative positions of the substituents for the asymmetrical carbon, while the second is purely experimental and expresses the direction in which polarized light deviates from a plane when it passes through the acid in a given solvent.

Tartaric acid is one of the most prevalent acids in unripe grapes and must. Indeed, at the end of the vegetative growth phase, concentrations in unripe grapes may be as high as 15 g/l. In musts from northerly vineyards, concentrations are often over 6 g/l whereas, in the south, they may be as low as 2–3 g/l since combustion is more effective when the grape bunches are maintained at high temperatures.

Tartaric acid is not very widespread in nature, but is specific to grapes. For this reason, it is

called *Weinsäure* in German, or 'wine acid'. It is a relatively strong acid (see Table 1.3), giving wine a pH on the order of 3.0–3.5.

Tartrates originating from the wine industry are the main source of tartaric acid, widely used in the food and beverage industry (soft drinks, chocolates, cakes, canned foods, etc.). This acid is also used for medical purposes (as a laxative) and in dyeing (for mordanting fabric), as well as for tanning leather. Tartrazine, a diazoic derivative of tartaric acid, is the yellow coloring matter in wool and silk, but is also used as food coloring under the reference number E102.

L(−)-Malic acid is found in all living organisms. It is especially plentiful in green apples, which explains its German name *Äpfelsäure*, or 'apple acid'. It is also present in white and red currants, rhubarb and, of course, grapes. Indeed, the juice of green grapes, just before color change, may contain as much as 25 g/l. In the two weeks following the first signs of color change, the malic acid content drops by half, partly due to dilution as the grapes grow bigger, and also as a result of combustion. At maturity, musts from northerly regions still contain 4–6.5 g/l malic acid, whereas in southerly regions, concentrations are only 1–2 g/l.

Citric acid, a tri-acid, is very widespread in nature (e.g. lemons). Its very important biochemical and metabolic role (Krebs cycle) requires no further demonstration. Citric acid slows yeast growth but does not block it (Kalathenos *et al.*, 1995). It is used as an acidifying agent in the food and beverage industry (lemonade), while sodium (E331), potassium (E332), and calcium (E333) citrate have many uses in fields ranging from pharmaceuticals to photography. Concentrations in must and wine, prior to malolactic fermentation, are between 0.5 and 1 g/l.

In addition to these three acids, which account for the majority of the acidity in grapes, there are also phenol acids in the cinnamic series (e.g. coumaric acid), often esterified with an alcohol function of tartaric acid (e.g. coumaryltartaric acid).

Ascorbic acid (Figure 1.1) should also be mentioned in connection with these oxidizable phenol acids. It is naturally present in lactone form, i.e. a cyclic ester. Ascorbic acid also constitutes a Redox system in fruit juices, protecting the phenols from oxidation. In winemaking it is used as an adjuvant to sulfur dioxide (Volume 1, Section 9.5).

Must and wine from grapes affected by noble and/or gray rot have higher concentrations of acids produced by oxidation of the aldehyde function (e.g. aldose) or the primary alcohol function of carbon 1 of a ketose (e.g. fructose). Thus, gluconic acid, the compound corresponding to glucose, may reach concentrations of several grams per liter in juice from grapes affected by rot. This concentration is used to identify wines made from grapes affected by noble rot, as they contain less gluconic acid than those made from grapes affected by gray rot (Sections 10.6.4, 10.6.5 and 14.2.3). The compound corresponding to fructose is 2-keto gluconic acid (Table 1.1).

The calcium and iron salts of these acids are used in medicine to treat decalcification and hypochrome anemia, respectively.

Calcium gluconate is well known for its insolubility in wine and the turbidity it causes. Mucic acid, derived from galactose by oxidation, both of the aldehyde function of carbon 1 and the primary alcohol function of carbon 6, is just as undesirable. Also known as galactaric acid, it is therefore both

Fig. 1.1. Oxidation–reduction equilibrium of ascorbic acid

an onic and uronic acid. The presence of a plane of symmetry in its structure between carbons 3 and 4 makes it a meso-type stereoisomer. Mucic acid has no optical activity. Its presence has been observed in the crystalline deposits formed throughout the aging of sweet white wines made from grapes with noble rot.

1.2.3 Organic Acids from Fermentation

The main acids produced during fermentation are described in Table 1.2. The first to be described is pyruvic acid, due to its meeting function in the cell metabolism, although concentrations in wine are low, or even non-existent. Following reduction by a hydride H^- ion—from aluminum or sodium borohydride, or a co-enzyme (NADH) from L and D lactate dehydrogenases—pyruvic acid produces two stereoisomers of lactic acid, L and D. The first, 'clockwise', form is mainly of bacterial origin and the second, 'counter-clockwise', mainly originates from yeasts.

The activated, enolic form of the same acid, phosphoenol pyruvate (Figure 1.2), adds a nucleophile to carbon dioxide, producing oxaloacetic acid, a precursor by transamination of aspartic acid.

The enzymic decarboxylation of pyruvic acid, assisted by thiamin pyrophosphate (TPP) or vitamin B1, produces ethanal, which is reduced

Table 1.2. The main acids produced during fermentation

Structure	Name
$COOH-C(=O)-CH_3$	Pyruvic acid
$COOH-C(HO)(H)-CH_3$	L(+)-Lactic acid
$COOH-C(H)(OH)-CH_3$	D(−)-Lactic acid
$COOH-CH_2-CH_2-COOH$	Succinic acid
$COOH-CH_3$	Acetic acid
$COOH-C(CH_3)(OH)-CH_2-COOH$	Citramalic acid
$COOH-C(=O)-CH_2-COOH$	Oxaloacetic acid
$(H)(COOH)C=C(HOOC)(H)$	Fumaric acid

$$HO-\overset{O}{\overset{\|}{C}}-\underset{O-P}{C}{=}CH_2 + O{=}C{=}O \longrightarrow HO-\overset{O}{\overset{\|}{C}}-\underset{O}{\underset{\|}{C}}-CH_2-\overset{O}{\overset{\|}{C}}-OH + Pi$$

Fig. 1.2. Biosynthesis of oxaloacetic acid from phosphophenolpyruvic acid

Table 1.3. State of salification of the main inorganic and organic acids (Ribéreau-Gayon *et al.*, 1972)

Category	Name	pK_a	Form in wine
Strong inorganic acids	Hydrochloric	Less than 1	Completely dissociated salts
	Sulfuric 1	Approx. 1	
	Sulfuric 2	1.6	
	Sulfurous 1	1.77	Bisulfite acid
	Phosphoric 1	1.96	Phosphate acid
Strongest organic acids	Salicylic	2.97	Acid functions partly neutralized and partly free (not highly dissociated)
	Tartaric 1	3.01	
	Citric 1	3.09	
	Malic 1	3.46	
	Formic	3.69	
	Lactic	3.81	
	Tartaric 2	4.05	
Weakest organic acids	Benzoic	4.16	Free acid functions (very little dissociated)
	Succinic 1	4.18	
	Citric 2	4.39	
	Acetic	4.73	
	Butyric	4.82	
	Propionic	4.85	
	Malic 2	5.05	
	Succinic 2	5.23	
	Citric 3	5.74	
Weak inorganic acids	Phosphoric 2	6.70	Free acid functions (almost entirely non-dissociated)
	Carbonic 1	6.52	
	Sulfurous 2	7.00	
	Hydrogen sulfide 1	7.24	
	Carbonic 2	10.22	
	Phosphoric 3	12.44	
Phenols	Polyphenols (tannin and coloring)	7–10	Free (non-dissociated)

to form ethanol during alcoholic fermentation. Its enzymic, microbial or even chemical oxidation produces acetic acid.

Another acid that develops during fermentation due to the action of yeast is succinic or 1-4-butanedioic acid. Concentrations in wine average 1 g/l. This acid is produced by all living organisms and is involved in the lipid metabolism and the Krebs cycle, in conjunction with fumaric acid. It is a di-acid with a high pK_a (Table 1.3). Succinic acid has an intensely bitter, salty taste that causes salivation and accentuates a wine's flavor and vinous character (Peynaud and Blouin, 1996).

Like succinic acid, citramalic or α-methylmalic acid, confused with citric acid in chromatography for many years, is of yeast origin.

In conclusion, it is apparent from this description that, independently of their origins, most of the main organic acids in must and wine consist of poly-functional molecules, and many are hydroxy acids. These two radicals give these acids polar and hydrophilic characteristics. As a result, they are soluble in water, and even in dilute alcohol solutions, such as wine. Their polyfunctional character is also responsible for the chemical reactivity that enables them to develop over time as wine ages. In this connection, results obtained by monitoring ethyl lactate levels in Champagne for 2 years after malolactic fermentation are highly convincing. Indeed, after 2 years aging on the lees, concentrations reach 2 g/l and then decrease. The degree of acidity, indicated by their pK_a values,

controls the extent to which these acids are present in partial salt form in wine (Table 1.3).

A final property of the majority of organic acids in wine is that they have one or more asymmetrical carbons. This is characteristic of biologically significant molecules.

1.3 DIFFERENT TYPES OF ACIDITY

The fact that enologists need to distinguish between total acidity, pH and volatile acidity demonstrates the importance of the concept of acidity in wine. This is due to the different organoleptic effects of these three types of acidity. Indeed, in any professional tasting, the total acidity, pH and volatile acidity of the wine samples are always specified, together with the alcohol and residual sugar contents.

The importance of total acidity is obvious in connection with flavor balance:

sweet taste (sugars, alcohols) ⇌ acid taste (organic and inorganic acids) + bitter taste (phenols)

Looking at this balance, it is understandable that dry white wines have a higher total acidity than red wines, where phenols combine with acids to balance the sweet taste of the alcohols. Volatile acidity indicates possible microbial spoilage.

1.3.1 Total Acidity

Total acidity in must or wine, also known as 'titratable acidity', is determined by neutralization, using a sodium hydroxide solution of known normality. The end point of the assay is still often determined by means of a colored reagent, such as bromothymol blue, which changes color at pH 7, or phenolphthalein, which changes color at pH 9. Using one colored reagent to define the end point of the assay rather than the other is a matter of choice. It is also perfectly conventional to use a pH meter and stop the total acidity assay of a wine at pH 7, and, indeed, this is mandatory in official analyses. At this pH, the conversion into salts of the second acid function of the di-acids (malic and succinic) is not completed, while the neutralization of the phenol functions starts at pH 9.

The total acidity of must or wine takes into account all types of acids, i.e. inorganic acids such as phosphoric acid, organic acids including the main types described above, as well as amino acids whose contribution to titratable acidity is not very well known. The contribution of each type of acid to total acidity is determined by its strength, which defines its state of dissociation, as well as the degree to which it has combined to form salts. Among the organic acids, tartaric acid is mainly present in must and wine as monopotassium acid salt, which still contributes towards total acidity. It should, however, be noted that must (an aqueous medium) and wine (a dilute alcohol medium), with the same acid composition and thus the same total acidity, do not have the same titration curve and, consequently, their acid–alkaline buffer capacity is different.

Even using the latest techniques, it is difficult to predict the total acidity of a wine on the basis of the acidity of the must from which it is made, for a number of reasons.

Part of the original fruit acids may be consumed by yeasts and, especially, bacteria (see 'malolactic fermentation'). On the other hand, yeasts and bacteria produce acids, e.g. succinic and lactic acids. Furthermore, acid salts become less soluble as a result of the increase in alcohol content. This is the case, in particular, of the monopotassium form of tartaric acid, which causes a decrease in total acidity on crystallization, as potassium bitartrate still has a carboxylic acid function.

In calculating total acidity, a correction should be made to allow for the acidity contributed by sulfur dioxide and carbon dioxide. Sulfuric acid is much stronger ($pK_{a_1} = 1.77$) than carbonic acid ($pK_{a_1} = 6.6$).

In fact, high concentrations of carbon dioxide tend to lead to overestimation of total acidity, especially in slightly sparkling wines, and even more so in sparkling wines. This is also true

of young wines, which always have a high CO_2 content just after fermentation.

Wines must, therefore, be degassed prior to analyses of both total and volatile acidity.

1.3.2 Volatile Acidity

Volatile acidity in wine is considered to be a highly important physicochemical parameter, to be monitored by analysis throughout the winemaking process. Although it is an integral part of total acidity, volatile acidity is clearly considered separately, even if it only represents a small fraction in quantitative terms.

On the other hand, from a qualitative standpoint, this value has always been, quite justifiably, linked to quality. Indeed, when an enologist tastes a wine and decides there is excessive volatile acidity, this derogatory assessment has a negative effect on the wine's value. This organoleptic characteristic is related to an abnormally high concentration of acetic acid, in particular, as well as a few homologous carboxylic acids. These compounds are distilled when wine is evaporated. Those which, on the contrary, remain in the residue constitute fixed acidity.

Volatile acidity in wine consists of free and combined forms of volatile acids. This explains why the official assay method for volatile acidity, by steam distillation, requires combined fractions to be rendered free and volatile by acidifying the wine with tartaric acid (approximately 0.5 g per 20 ml). Tartaric acid is stronger than the volatile acids, so it displaces them from their salts.

In France, both total and volatile acidity are usually expressed in g/l of sulfuric acid. An *appellation d'origine contrôlée* wine is said to be 'of commercial quality' if volatile acidity does not exceed 0.9 g/l of H_2SO_4, 1.35 g/l of tartaric acid or 1.1 g/l of acetic acid. Acetic acid, the principal component of volatile acidity, is mainly formed during fermentation.

Alcoholic fermentation of grapes normally leads to the formation of 0.2–0.3 g/l of H_2SO_4 of volatile acidity in the corresponding wine. The presence of oxygen always promotes the formation of acetic acid. Thus, this acid is formed both at the beginning of alcoholic fermentation and towards the end, when the process slows down. In the same way, an increase in volatile acidity of 0.1–0.2 g/l of H_2SO_4 is observed during malolactic fermentation. Work by Chauvet and Brechot (1982) established that acetic acid was formed during malolactic fermentation due to the breakdown of citric acid by lactic bacteria.

Abnormally high volatile acidity levels, however, are due to the breakdown of residual sugars, tartaric acid and glycerol by anaerobic lactic bacteria. Aerobic acetic bacteria also produce acetic acid by oxidizing ethanol.

Finally, acescence in wine is linked to the presence of ethyl acetate, the ethyl ester of acetic acid, formed by the metabolism of aerobic acetic bacteria (Section 2.5.1).

1.3.3 Fixed Acidity

The fixed acidity content of a wine is obtained by subtracting volatile acidity from total acidity. Total acidity represents all of the free acid functions and volatile acidity includes the free and combined volatile acid functions. Strictly speaking, therefore, fixed acidity represents the free fixed acid functions plus the combined volatile acid functions.

When fixed acidity is analyzed, there is a legal obligation to correct for sulfur dioxide and carbon dioxide. In practice, these two molecules have a similar effect on total acidity and volatile acidity, so the difference between total acidity and volatile acidity is approximately the same, with or without correction (Ribéreau-Gayon *et al.*, 1982).

1.4 THE CONCEPT OF pH AND ITS APPLICATIONS

1.4.1 Definition

The concept of pH often appears to be an abstract, theoretical concept, defined mathematically as log subscript ten of the concentration of hydroxonium ions in an electrically conductive solution, such as must or wine:

$$pH = -\log_{10}[H_3O^+]$$

Furthermore, the expression of pH shows that it is an abstract measure with no units, i.e. with no apparent concrete physical significance.

The concepts of total or volatile acidity seem to be easier to understand, as they are measured in milliliters of sodium hydroxide and expressed in g/l of sulfuric or tartaric acid. This is rather paradoxical, as the total acidity in a wine is, in fact, a complex function with several variables, unlike pH which refers to only one variable, the true concentration of hydroxonium ions in must and wine.

The abstract character generally attributed to pH is even less justified as this physicochemical parameter is based on the dissociation equilibrium of the various acids, AH, in wine, at fixed temperature and pressure, as shown below:

$$AH + H_2O \rightleftharpoons A^- + H_3{}^+O$$

The emission of $H_3{}^+O$ ions defines the acidity of the AH molecule. Dissociation depends on the value of the equilibrium constant, K_a, of the acid:

$$K_a = \frac{[A^-][H_3{}^+O]}{[AH]} \tag{1.1}$$

To the credit of the concept of pH, otherwise known as true acidity, it should be added that its value fairly accurately matches the impressions due to acidity frequently described as 'freshness' or even 'greenness' and 'thinness', especially in white wines.

A wine's pH is measured using a pH meter equipped with a glass electrode after calibration with two buffer solutions. It is vital to check the temperature.

The pH values of wines range from 2.8 to 4.0. It is surprising to find such low, non-physiological values in a biological, fermentation medium such as wine. Indeed, life is only possible thanks to enzymes in living cells, and the optimum activity of the vast majority of enzymes occurs at much higher intra-cellular pH values, close to neutral, rather than those prevailing in extra-cellular media, i.e. must and wine. This provides some insight into the role of cell membranes and their ATPases in regulating proton input and output.

On the other hand, it is a good thing that wines have such low pH values, as this enhances their microbiological and physicochemical stability. Low pH hinders the development of microorganisms, while increasing the antiseptic fraction of sulfur dioxide. The influence of pH on physicochemical stability is due to its effect on the solubility of tartrates, in particular potassium bitartrate but, above all, calcium tartrate and the double salt calcium tartromalate.

Ferric casse is also affected by pH. Indeed, iron has a degree of oxidation of three and produces soluble complexes with molecules such as citric acid. These complexes are destabilized by increasing pH to produce insoluble salts, such as ferric phosphates (see 'white casse') or even ferric hydroxide, $Fe(OH)_3$.

1.4.2 Expression of pH in Wine

Wines are mixtures of weak acids, combined to form salts to a greater or lesser extent according to their pK_a (Table 1.3). The proportion of salts also depends on geographical origin, grape variety, the way the vines are trained, and the types of winepress and winemaking methods used.

Due to their composition, musts and wines are acidobasic 'buffer' solutions, i.e. a modification in their chemical composition produces only a limited variation in pH. This explains the relatively small variations in the pH of must during alcoholic and malolactic fermentation.

The pH of a solution containing a weak monoprotic acid and its strong basic salt proves the Anderson Hasselbach equation:

$$\begin{aligned} pH &= pK_a + \log \frac{[\text{salt formed}]}{[\text{remaining acid}]} \\ &= pK_a + \log \frac{[A^-]}{[AH]} \end{aligned} \tag{1.2}$$

This equation is applicable to must and wine, where the strongest acids are di-acids. It is an approximation, assuming the additivity of the acidity contributed by each acid to the total. The application of Eqn (1.2) also makes the 'simplifying' assumption that the degree to which the acids are combined in salts is independent.

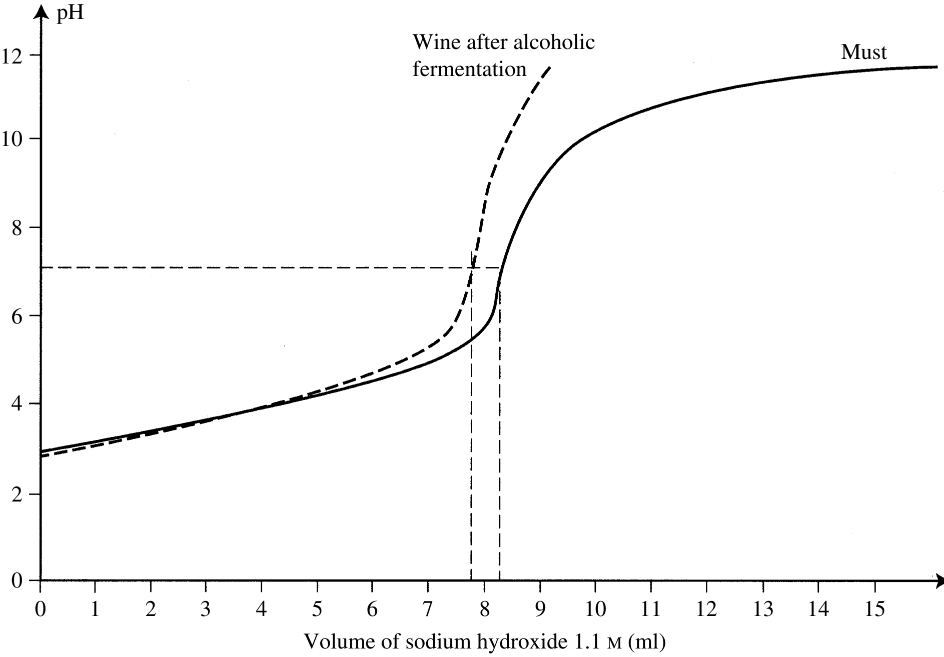


Fig. 1.3. Comparison of the titration curves of a must and the corresponding wine

These assumptions are currently being challenged. Indeed, recent research has shown that organic acids react among themselves, as well as with amino acids (Dartiguenave *et al.*, 2000).

Comparison (Table 1.3) of the pK_a of tartaric (3.01), malic (3.46), lactic (3.81) and succinic (4.18) acids leads to the conclusion that tartaric acid is the 'strongest', so it will take priority in forming salts, displacing, at least partially, the weaker acids. In reality, all of the acids interact. Experimental proof of this is given by the neutralization curve of a must, or the corresponding wine, obtained using sodium or potassium hydroxide (Figure 1.3). These curves have no inflection points corresponding to the pH of the pK of the various acids, as there is at least partial overlapping of the maximum 'buffer' zones (p$K_a \pm 1$). Thus, the neutralization curves are quasi-linear for pH values ranging from 10 to 90% neutralized acidity, so they indicate a constant buffer capacity in this zone. From a more quantitative standpoint, a comparison of the neutralization curves of must and the corresponding wine shows that the total acidity, assessed by the volume of sodium hydroxide added to obtain pH 7, differs by 0.55 meq. In the example described above, both must and wine samples contained 50 ml and the total acidity of the wine was 11 meq/l (0.54 g/l of H_2SO_4) lower than that of the must. This drop in total acidity in wine may be attributed to a slight consumption of malic acid by the yeast during alcoholic fermentation, as well as a partial precipitation of potassium bitartrate.

The slope of the linear segment of the two neutralization curves differs noticeably. The curve corresponding to the must has a gentler slope, showing that it has a greater buffer capacity than the wine.

The next paragraph gives an in-depth description of this important physicochemical parameter of wine.

1.4.3 The "Buffer" Capacity of Musts and Wines

Wines' acidobasic buffer capacity is largely responsible for their physicochemical and microbiological stability, as well as their flavor balance.

For example, the length of time a wine leaves a fresh impression on the palate is directly related to the salification of acids by alkaline proteins in saliva, i.e. the expression of the buffer phenomenon and its capacity. On the contrary, a wine that tastes "flat" has a low buffer capacity, but this does not necessarily mean that it has a low acidity level. At a given total acidity level, buffer capacity varies according to the composition and type of acids present. This point will be developed later in this chapter.

In a particular year, a must's total acidity and acid composition depend mainly on geography, soil conditions, and climate, including soil humidity and permeability, as well as rainfall patterns, and, above all, temperature. Temperature determines the respiration rate, i.e. the combustion of tartaric and, especially, malic acid in grape flesh cells. The predominance of malic acid in must from cool-climate vineyards is directly related to temperature, while malic acid is eliminated from grapes in hotter regions by combustion.

Independently of climate, grape growers and winemakers have some control over total acidity and even the acid composition of the grape juice during ripening. Leaf-thinning and trimming the vine shoots restrict biosynthesis and, above all, combustion, by reducing the greenhouse effect of the leaf canopy. Another way of controlling total acidity levels is by choosing the harvesting date. Grapes intended for champagne or other sparkling wines must be picked at the correct level of technological ripeness to produce must with a total acidity of 9–10 g/l H_2SO_4. This acidity level is necessary to maintain the wines' freshness and, especially, to minimize color leaching from the red-wine grape varieties, Pinot Noir and Pinot Meunier, used in champagne. At this stage in the ripening process, the grape skins are much less fragile than they are when completely ripe. The last method for controlling the total acidity of must is by taking great care in pressing the grapes and keeping the juice from each pressing separate (Volume 1, Section 14.3.2). In champagne, the *cuvée* corresponds to cell sap from the mid-part of the flesh, furthest from the skin and seeds, where it has the highest sugar and acidity levels.

Once the grapes have been pressed, winemakers have other means of raising or lowering the acidity of a must or wine. It may be necessary to acidify "flat" white wines by adding tartaric acid after malolactic fermentation in years when the grapes have a high malic acid content. This is mainly the case in cool-climate vineyards, where the malic acid is not consumed during ripening. The disadvantage is that it causes an imbalance in the remaining total acidity, which, then, consists exclusively of a di-acid, tartaric acid, and its monopotassium salt.

One method that is little-known, or at least rarely used to avoid this total acidity imbalance, consists of partially or completely eliminating the malic acid by chemical means, using a mixture of calcium tartrate and calcium carbonate. This method precipitates the double calcium salt, tartromalate, (Section 1.4.4, Figure 1.9) and is a very flexible process. When the malic acid is partially eliminated, the wine has a buffer capacity based on those of both tartaric and malic acids, and not just on that of the former. Tartrate buffer capacity is less stable over time, as it decreases due to the precipitation of monopotassium and calcium salts during aging, whereas the malic acid salts are much more soluble.

Another advantage of partial elimination of malic acid followed by the addition of tartrate over malolactic fermentation is that, due to the low acidification rate, it does not produce wines with too low a pH, which can be responsible for difficult or stuck second fermentation in the bottle during the champagne process, leaving residual sugar in the wine.

Standard acidification and deacidification methods are aimed solely at changing total acidity levels, with no concern for the impact on pH and even less for the buffer capacity of the wine, with all the unfortunate consequences this may have on flavor and aging potential.

This is certainly due to the lack of awareness of the importance of the acid-alkali buffer capacity in winemaking. Changes in the acid-alkaline characteristics of a wine require knowledge of not only its total acidity and real acidity (pH), but also of its buffer capacity. These three parameters

may be measured using a pH meter. Few articles in the literature deal with the buffer capacity of wine: Genevois and Ribéreau-Gayon, 1935; Vergnes, 1940; Hochli, 1997; and Dartiguenave *et al.*, 2000. This lack of knowledge is probably related to the fact that buffer capacity cannot be measured directly, but requires recordings of 4 or 5 points on a neutralization curve (Figure 1.3), and this is not one of the regular analyses carried out by winemakers.

It is now possible to automate plotting a neutralization curve, with access to the wine's initial pH and total acidity, so measuring buffer capacity at the main stages in winemaking should become a routine.

Mathematically and geometrically, buffer capacity, β, is deduced from the Henderson-Hasselbach equation [equation (1.2), (Section 1.4.2)]. Buffer capacity is defined by equation (1.3).

$$\beta = \frac{\Delta \mathrm{B}}{\delta \mathrm{pH}} \tag{1.3}$$

where ΔB is the strong base equivalent number that causes an increase in pH equal to ΔpH. Buffer capacity is a way of assessing buffer strength. For an organic acid alone, with its salt in solution, it may be defined as the pH interval in which the buffer effect is optimum [equation (1.4)].

$$\mathrm{pH} = \mathrm{pK}_a \pm 1 \tag{1.4}$$

Buffer capacity is normally defined in relation to a strong base, but it could clearly be defined in the same way in relation to a strong acid. In this case, the pH = f (strong acid) function decreases and its β differential is negative, i.e.:

$$\mathrm{B} = -\frac{\Delta(\text{acid})}{\Delta \mathrm{pH}}$$

Strictly speaking, buffer capacity is obtained from the differential of the Henderson-Hasselbach expression, i.e. from the following derived formula:

$$\mathrm{pH} = \mathrm{pK}_a + \frac{1}{2.303} \cdot \mathrm{Log_e}[\mathrm{A}^-] - \frac{1}{2.303} \cdot \mathrm{Log_e}[\mathrm{HA}]$$

as only the Naperian logarithm is geometrically significant, and provides access to the slope of the titration curve around its pK_a (Figure 1.4).

Both sides of the equation are then differentiated, as follows:

$$\mathrm{dpH} = \frac{1}{2.303} \cdot \frac{\mathrm{d}[\mathrm{A}^-]}{[\mathrm{A}^-]} - \frac{1}{2.303} \cdot \frac{\mathrm{d}[\mathrm{HA}]}{[\mathrm{HA}]}$$

Making the assumption that the quantity of strong base added, d[B], generates the same variation in acidity combined as salts, $\mathrm{d}[\mathrm{A}^-]$, and leads to an equal decrease in free acidity d[HA], per unit, now

$$\mathrm{d}[\mathrm{B}] = \mathrm{d}[\mathrm{A}^-] = \mathrm{d}[\mathrm{HA}]$$

the differential equation for pH is then:

$$\mathrm{dpH} = \frac{1}{2.303} \cdot \frac{\mathrm{d}[\mathrm{B}]}{[\mathrm{A}^-]} + \frac{1}{2.303} \cdot \frac{\mathrm{d}[\mathrm{B}]}{[\mathrm{HA}]} = \frac{1}{2.303} \cdot \mathrm{d}[\mathrm{B}] \left\{ \frac{1}{[\mathrm{A}^-]} + \frac{1}{[\mathrm{HA}]} \right\}$$

or,

$$\mathrm{dpH} = \frac{\mathrm{d}[\mathrm{B}]}{2.303} \cdot \left\{ \frac{[\mathrm{HA}] + [\mathrm{A}^-]}{[\mathrm{A}^-] \cdot [\mathrm{HA}]} \right\}$$

Dividing both sides of the equation by d[B] gives the reverse of equation (1.3), defining the buffer capacity. Equations (1.2) and (1.3) have been defined for monoproteic acids, but are also applicable as an initial approximation to di-acids, such as tartaric and malic acids.

Theoretically, variations ΔB and ΔpH must be infinitely small, as the value of the $\Delta\mathrm{B}/\Delta\mathrm{pH}$ ratio at a fixed pH corresponds geometrically to the tangent on each point on the titration curve (Figure 1.4). More practically, buffer capacity can be defined as the number of strong base equivalents required to cause an increase in pH of 1 unit per liter of must or wine. It is even more practical to calculate smaller pH variations in much smaller samples (e.g. 30 ml). Figure 1.4 clearly shows the difference in buffer capacity of a model solution between pH 3 and 4, as well as between pH 4 and 5.

This raises the issue of the pH and pK_a at which buffer capacity should be assessed. Champagnol (1986) suggested that pH should be taken as the mean of the pK_a of the organic acids in the must

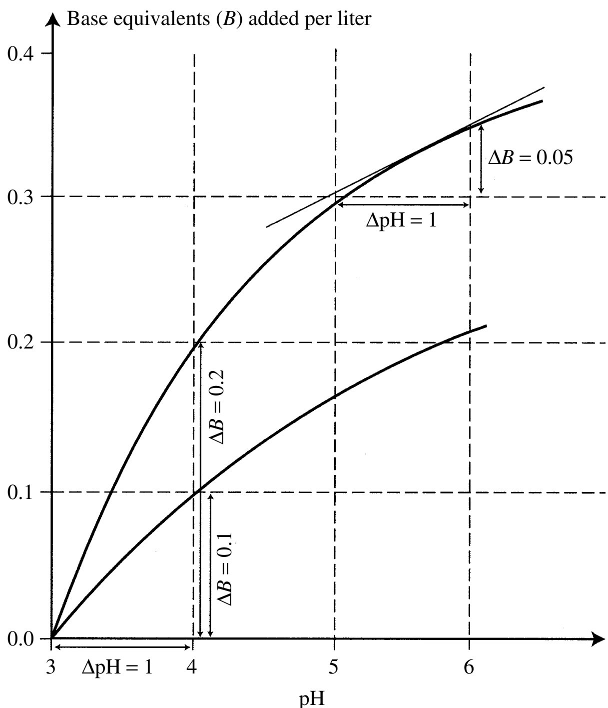


Fig. 1.4. Determining the buffer capacity β from the titration curves of two model buffer solutions

or wine, i.e. the mean pK_a of tartaric and malic acids in must and tartaric and lactic acids in wine that has completed malolactic fermentation.

This convention is justified by its convenience, provided that (Section 1.4.2) there are no sudden inflection points in the neutralization curve of the must or wine at the pK_a of the organic acids present, as their buffer capacities overlap, at least partially. In addition to these somewhat theoretical considerations, there are also some more practical issues. An aqueous solution of sodium hydroxide is used to determine the titration curve of a must or wine, in order to measure total acidity and buffer capacity. Sodium, rather than potassium, hydroxide is used as the sodium salts of tartaric acid are soluble, while potassium bitartrate would be likely to precipitate out during titration. It is, however, questionable to use the same aqueous sodium hydroxide solution, which is a dilute alcohol solution, for both must and wine.

Strictly speaking, a sodium hydroxide solution in dilute alcohol should be used for wine to avoid modifying the alcohol content and, consequently, the dielectric constant, and, thus, the dissociation of the acids in the solution during the assay procedure. It has recently been demonstrated (Dartiguenave *et al.*, 2000) that the buffer capacities of organic acids, singly (Table 1.4 and 1.5) or in binary (Table 1.6) and tertiary (Table 1.7) combinations, are different in water and 11% dilute alcohol solution. However, if the solvent containing the organic acids and the sodium hydroxide is the same, there is a close linear correlation between the buffer capacity and the acid concentrations (Table 1.4).

Table 1.5 shows the values (meq/l) calculated from the regression line of the buffer capacities for acid concentrations varying from 1–6 g/l in water and 11% dilute alcohol solution. The buffer capacity of each acid alone in dilute alcohol solution was lower than in water. Furthermore, the buffer capacity of a 4-carbon organic acid varied more as the number of alcohol functions increased (Table 1.8). Thus, the variation in buffer capacity of malic acid, a di-acid with one alcohol function,

Table 1.4. Equations for calculating buffer capacity (meq/l) depending on the concentration (mM/l) of the organic acid in water or dilute alcohol solution (11% vol.) between 0 and 40 mM/l. (Dartiguenave *et al*., 2000)

Solvent	Water	Dilute alcohol solution
Tartaric acid	$Y = 0.71\ x + 0.29$; $R^2 = 1$	$Y = 0.60\ x + 1.33$; $R^2 = 1$
Malic acid	$Y = 0.56\ x + 0.43$; $R = 0.998$	$Y = 0.47\ x + 0.33$; $R^2 = 0.987$
Succinic acid	$Y = 0.56\ x - 1.38.10^{-2}$; $R^2 = 0.993$	$Y = 0.53\ x + 0.52$; $R^2 = 0.995$
Citric acid	$Y = 0.57\ x + 0.73$; $R^2 = 1$	$Y = 0.51\ x + 0.62$; $R^2 = 1$

Table 1.5. Buffer capacity (meq/l) depending on the concentration (g/l) of organic acid in water and dilute alcohol solution. (Dartiguenave *et al*., 2000)

Acid concentration	and type of medium	Tartaric acid	Malic acid	Succinic acid	Citric acid
1 g/l	Water	5.0	4.6	4.7	3.7
	Dilute alcohol	5.3	3.8	4.0	3.5
2 g/l	Water	9.7	8.8	9.5	6.7
	Dilute alcohol	9.3	7.3	9.4	5.9
4 g/l	Water	16.4	17.1	19.0	12.6
	Dilute alcohol	14.9	14.3	17.5	11.3
6 g/l	Water	28.7	25.5	28.4	18.5
	Dilute alcohol	25.3	21.3	26.4	16.6

in a dilute alcohol medium, was 1.4 meq/l higher than that of succinic acid. When the hydroxyacid had two alcohol functions, the increase was as high as 5.3 meq/l (17.7%), e.g. between tartaric and malic acids, even if the buffer capacities of the three acids were lower than in water.

However, the fact that the buffer capacities of binary (Table 1.6) or tertiary (Table 1.7) combinations of acids in a dilute alcohol medium were higher than those measured in water was certainly unexpected. This effect was particularly marked when citric acid was included, and reached spectacular proportions in a T.M.C. blend (Table 1.7), where the buffer capacity in dilute alcohol solution was 2.3 times higher than that in water.

These findings indicate that the acids interact among themselves and with alcohol, compensating for the decrease in buffer capacity of each individual acid when must (an aqueous solution) is converted into wine (a dilute alcohol solution). From a purely practical standpoint, the use of citric acid to acidify dosage liqueur for bottle-fermented sparkling wines has the doubly positive effect of enhancing the wine's aging potential, while maintaining its freshness on the palate.

Table 1.6. Demonstration of interactions between organic acids and the effect of alcohol on the buffer capacity of binary combinations (Dartiguenave *et al*., 2000)

Medium	Buffer capacity (meq/l)	Composition of equimolar mixes of 2 acids Total acid concentration (40 mM/l)		
		Tartaric acid Malic acid	Tartaric acid Succinic acid	Tartaric acid Citric acid
Water	Experimental value	21	20	23.5
	Calculated value	25.7	25.7	26.3
	Difference (Calc. – Exp.)	4.7	5.7	2.8
EtOH (11% vol.)	Experimental value	18.3	20.1	29
	Calculated value	24	23.3	24
	Difference (Calc. – Exp.)	5.7	3.2	−5
Effect of ethanol	(EtOH – H_2O) Exp.	−2.7	0.1	5.5

Table 1.7. Demonstration of interactions between organic acids and the effect of alcohol on the buffer capacity of tertiary combinations (Dartiguenave *et al*., 2000)

Medium	Buffer capacity (meq/l)	Composition of equimolar mixes of 3 acids (13.3 mM/l) Total acid concentration (40 mM/l)	
		Tartaric acid Malic acid Succinic acid	Tartaric acid Malic acid Citric acid
Water	Experimental value	9.4	11.6
	Calculated value	25.4	25.5
	Difference (Calc. − Exp.)	16.0	13.9
EtOH (11% vol.)	Experimental value	21.7	26.4
	Calculated value	22.8	23.2
	Difference (Calc. − Exp.)	1.1	−3.2
Effect of ethanol	(EtOH − H_2O) Exp.	12.3	14.8

Table 1.8. Effect of hydroxyl groups in the structure of the 4-carbon di-acid on buffer capacity (meq/l) (Dartiguenave *et al*., 2000)

Medium	1 hydroxyl group			2 hydroxyl groups		
	Malic acid	Succinic acid	Δ (Mal.− Suc.)	Tartaric acid	Malic acid	Δ (Tart.− Mal.)
Water	23.8	23.4	0.4	29	23.8	5.2
11% vol. dilute alcohol solution	22,0	20.6	1.4	25.9	22	3.9

Table 1.9. Changes in the buffer capacity of must from different pressings of Chardonnay grapes at various stages in the winemaking process. (Buffer capacity is expressed in meq/l). (Dartiguenave, 1998)

	Cuvée		Second pressing	
	1995	1996	1995	1996
Initial value of must	77.9	72.6	71.2	65.9
After alcoholic fermentation	60.7	63.6	57.5	ND
After malolactic fermentation	51.1	60.1	48.4	ND
After cold-stabilization	48.1	50.3	ND	42.4

Table 1.9 shows the changes in buffer capacity in successive pressings of a single batch of Chardonnay grapes from the 1995 and 1996 vintages, at the main stages in the winemaking process.

The demonstration of the effect of alcohol and interactions among organic acids (Table 1.6, 1.7, and 1.8) led researchers to investigate the precise contribution of each of the three main acids to a wine's buffer capacity, in order to determine whether other compounds were involved. The method consisted of completely deacidifying a wine by precipitating the double calcium tartromalate salt. After this deacidification, the champagne-base wine had a residual total acidity of only approximately 0.5 g/l H_2SO_4, whereas the buffer capacity was still 30% of the original value. This shows that organic acids are not the only compounds involved in buffer capacity, although they represent 90% of total acidity.

Among the many other compounds in must and wine, amino acids have been singled out for two reasons: (1) in champagne must and wine, the total concentration is always over 1 g/l and may even exceed 2 g/l, and (2) their at least bi-functional character gives them a double-buffer effect. They form salts with carboxylic acids via their ammonium group and can become associated with a non-dissociated acid function of an organic

acid via their carboxyl function, largely dissociated from wine pH, thus creating two buffer couples (Figure 1.5).

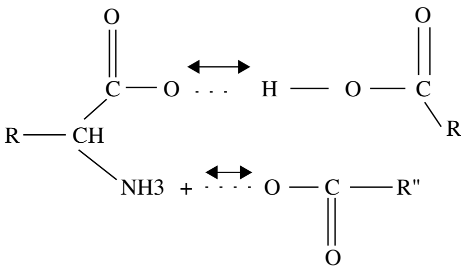


Fig. 1.5. Diagram of interactions between amino acids and organic acids that result in the buffer effect

An in-depth study of the interactions between amino acids and tartaric and malic acids focused on alanine, arginine, and proline, present in the highest concentrations in wine, as well as on amino acids with alcohol functions, i.e. serine and threonine (Dartiguenave *et al*., 2000).

The findings are presented in Figures 1.6 and 1.7. Hydrophobic amino acids like alanine were found to have only a minor effect, while amino acids with alcohol functions had a significant impact on the buffer capacity of an aqueous tartaric acid solution (40 mM/l). An increase of 0.6 meq/l was obtained by adding 6.7 mM/l alanine, while addition of as little as 1.9 mM/l produced an increase of 0.7 meq/l and addition of 4.1 mM/l resulted in a rise of 2.3 meq/l.

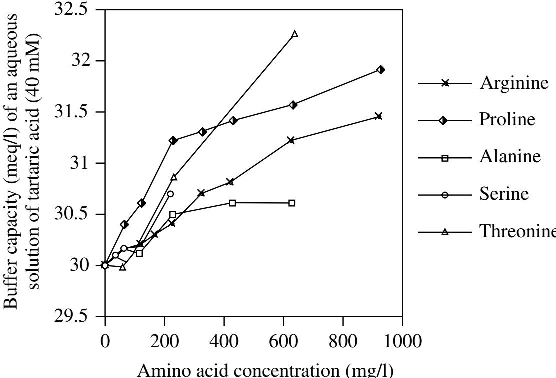


Fig. 1.6. Variations in the buffer capacity of an aqueous solution of tartaric acid (40 mM) in the presence of several amino acids. (Dartiguenave *et al*., 2000)

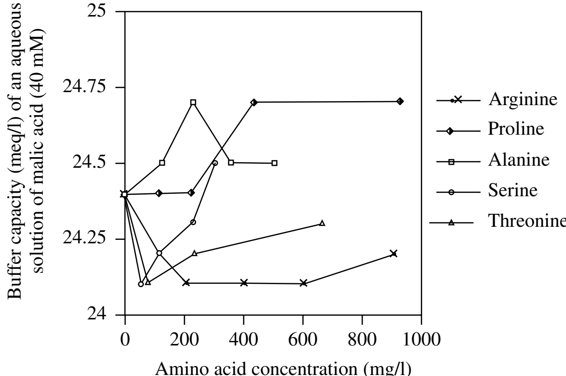


Fig. 1.7. Variations in the buffer capacity of an aqueous solution of malic acid (40 mM in the presence of several amino acids. (Dartiguenave *et al*., 2000))

The impact of amino acids with alcohol functions was even more spectacular in dilute alcohol solutions (11% by volume). With only 200 mg/l serine, there was a 1.8 meq/l increase in buffer capacity, compared to only 0.8 meq/l in water. It was also observed that adding 400 mg/l of each of the five amino acids led to a 10.4 meq/l (36.8%) increase in the buffer capacity of a dilute alcohol solution containing 40 mM/l tartaric acid.

It is surprising to note that, on the contrary, amino acids had no significant effect on the buffer capacity of a 40 mM/l malic acid solution (Figure 1.7).

All these observations highlight the role of the alcohol function, both in the solvent and the amino acids, in interactions with organic acids, particularly tartaric acid with its two alcohol functions.

The lack of interaction between amino acids and malic acid, both in water and dilute alcohol solution, can be interpreted as being due to the fact that it has one alcohol function, as compared to the two functions of tartaric acid. This factor is important for stabilizing interactions between organic acids and amino acids via hydrogen bonds (Figure 1.8).

1.4.4 Applying Buffer Capacity to the Acidification and Deacidification of Wine

The use of tartaric acid (known as 'tartrating') is permitted under European Community (EC) legislation, up to a maximum of 1.5 g/l in must and 2.5 g/l in wine. In the USA, acidification is permitted, using tartrates combined with gypsum ($CaSO_4$) (Gomez-Benitez, 1993). This practice seems justified if the buffer capacity expression (Eqn 1.3) is considered. The addition of tartaric acid (HA) increases the buffer capacity by increasing the numerator of Eqn (1.3) more than the denominator. However, the addition of $CaSO_4$ leads to the precipitation of calcium tartrate, as this salt is relatively insoluble. This reduces the buffer capacity and, as a result, ensures that acidification will be more effective.

Whenever tartrating is carried out, the effect on the pH of the medium must also be taken into account in calculating the desired increase in total acidity of the must or wine. Unfortunately, however, there is no simple relationship between total acidity and true acidity.

An increase in true acidity, i.e. a decrease in pH, may occur during bitartrate stabilization, in spite of the decrease in total acidity caused by this process. This may also occur when must and, in particular, wine is tartrated, due to the crystallization of potassium bitartrate, which becomes less soluble in the presence of alcohol.

The major difficulty in tartrating is predicting the decrease in pH of the must or wine. Indeed, it is important that this decrease in pH should not be incompatible with the wine's organoleptic qualities, or with a second alcoholic fermentation in the case of sparkling wines. To our knowledge, there is currently no reliable model capable of accurately predicting the drop in pH for a given level of tartrating. The problem is not simple, as it depends on a number of parameters. In order to achieve the required acidification of a wine, it is necessary to know the ratio of the initial concentrations of tartaric acid and potassium, i.e. crystallizable potassium bitartrate.

It is also necessary to know the wine's acido-basic buffer capacity. Thus, in the case of wines from northerly regions, initially containing 6 g/l of malic acid after malolactic fermentation, tartrating may be necessary to correct an impression of 'flatness' on the palate. Great care must be taken in acidifying this type of wine, otherwise it may have

Fig. 1.8. Assumed structure of interactions between tartaric acid and amino acids. (Dartiguenave *et al.*, 2000)

a final pH lower than 2.9, which certainly cures the 'flatness' but produces excessive dryness or even greenness. White wines made from red grape varieties may even take on some red color. The fact that wine has an acidobasic buffer capacity also makes deacidification possible.

Table 1.10 shows the values of the physicochemical parameters of the acidity in champagne-base wines, made from the *cuvée* or second pressing of Chardonnay grapes in the 1995 and 1996 vintages. They were acidified with 1 g/l and 1.5 g/l tartaric acid, respectively, after the must had been clarified.

Examination of the results shows that adding 100 g/hl to a *cuvée* must or wine only resulted in 10–15% acidification, corresponding to an increase in total acidity of approximately 0.5 g/l (H_2SO_4). Evaluating the acidification rate from the buffer capacity gave a similar result. The operation was even less effective when there was a high potassium level, and potassium bitartrate precipitated out when the tartaric acid was added.

Adding the maximum permitted dose of tartaric acid (150 g/hl) to second pressing must or wine was apparently more effective, as total acidity increased by 35% and pH decreased significantly (−0.14), producing a positive impact on wine stability and flavor. The effect on pH of acidifying *cuvée* wines shows the limitations of adding tartaric acid, and there may also be problems with the second fermentation in bottle, sometimes resulting in "hard" wines with a metallic mouth feel.

It would be possible to avoid these negative aspects of acidification by using L(-)lactic acid. This is listed as a food additive (E270) and meets the requirements of both the Food chemical Codex and the European Pharmacopoeia. Lactic acid is commonly used in the food and beverage industry, particularly as a substitute for citric acid in carbonated soft drinks, and is even added to some South African wines.

Its advantages compared to tartaric acid are the pK_a of 3.81 (tartaric acid: 3.01), and the fact that both its potassium and calcium salts are soluble. This enhances the acidification rate while minimizing the decrease in pH. Finally, lactic acid is microbiologically stable, unlike tartaric, malic, and citric acids. Until recently, one disadvantage of industrial lactic acid was a rather nauseating odor, which justified its prohibition in winemaking. The lactic acid now produced by fermenting sugar industry residues with selected bacteria no longer has this odor.

Current production quality, combined with low prices, should make it possible to allow experimentation in the near future, and, perhaps, even a lifting of the current ban on the use of lactic acid in winemaking.

The additives authorized for deacidifying wines are potassium bicarbonate ($KHCO_3$) and calcium carbonate ($CaCO_3$). They both form insoluble salts with tartaric acid and the corresponding acidity is eliminated in the form of carbonic acid (H_2CO_3) which breaks down into CO_2 and H_2O. A comparison of the molecular weights of these two salts and the stoichiometry of the neutralization reactions leads to the conclusion that, in general, one gram of $KHCO_3 (PM = 100)$ added to one liter of wine produces a drop in acidity of 0.49 g/l, expressed in grams of $H_2SO_4 (PM = 98)$. Adding one gram of $CaCO_3 (PM = 100)$ to a liter of wine produces a decrease in acidity equal to its own weight (exactly 0.98 g/l), expressed in grams of sulfuric acid.

In fact, this is a rather simplistic explanation, as it disregards the side-effects of the precipitation of insoluble potassium bitartrate salts and, especially, calcium tartrate, on total acidity as well as pH. These side-effects of deacidification are only fully expressed in wines with a pH of 3.6 or lower after cold stabilization to remove tartrates. It is obvious from the pH expression (Eqn 1.2) that, paradoxically, after removal of the precipitated tartrates, deacidification using $CaCO_3$ and, more particularly, $KHCO_3$ is found to have reduced the [salt]/[acid] ratio, i.e. increased true acidity. Fortunately, the increase in pH observed during neutralization is not totally reversed.

According to the results described by Usseglio-Tomasset (1989), a comparison of the deacidifying capacities of potassium bicarbonate and calcium carbonate shows that, in wine, the maximum deacidifying capacity of the calcium salt is only 85% of that of the potassium salt. Consequently, to bring a wine to the desired pH, a larger

Table 1.10. Composition of Chardonnay wines after tartaric stabilization, depending on the time of acidification (addition to must or wine after malolactic fermentation). *Cuvées* were acidified with 1 g/l tartaric acid and second pressings with 1.5 g/l. (Dartiguenave, 1998)

	Cuvée						Second pressing		
	1995			1996			1996		
	Control	Acidified must	Acidified wine	Control	Acidified must	Acidified wine	Control	Acidified must	Acidified wine
pH	3.06	2.97	2.97	3.06	2.99	2.97	3.18	3.04	3.00
Total acidity (g/l, H_2SO_4)	5.2	6.0	5.6	5.4	5.9	5.8	4.1	4.9	5.0
Tartaric acid (g/l)	3.6	4.0	4.3	4.4	5.2	5.0	3.4	4.6	4.8
Malic acid (g/l)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lactic acid (g/l)	4	4.3	4.4	4.2	4.1	4.1	3	3	2.7
Total nitrogen (mg/l)	274.7	221.9	271	251.6	280.3	289.8	245.9	250.4	254.4
Amino acids (mg/l)	1051.4	703.7	1322.6	1254.2	1422.7	1471.7	1177.5	1350.4	1145
Potassium (mg/l)	390	345	320	345	290	285	380	305	300
Calcium (mg/l)	71.5	90	79	60	64	61	50	55	48
Buffer capacity (NAOH, H_2O)	48.1	56.6	56.2	50.3	55.5	56.9	42.4	49.1	47.7
Buffer capacity (NAOH.EtOH 11% vol)	55.6	59.2	55.9	47.1	51.9	50.2	37.9	44.3	42

Fig. 1.9. Formation of insoluble calcium tartromalate when calcium tartrate reacts with malic acid in the presence of calcium carbonate

quantity of $CaCO_3$ than $KHCO_3$ must be used, as compared to the theoretical value. On the other hand, $CaCO_3$ has a more immediate effect on pH, as the crystallization of CaT is more complete than that of KTH, a more soluble salt.

Another side-effect of deacidification using calcium carbonate, and especially potassium bitartrate, is a decrease in the alkalinity of the ash.

Finally, deacidification with these two carbonic acid salts only affects tartaric acid. This accentuates the tartromalic imbalance in the total acidity in wines that have not completed malolactic fermentation, as the potassium and calcium salts of malic acid are soluble.

There is a way of deacidifying these wines while maintaining the ratio of tartaric acid to malic acid. The idea is to take advantage of the insolubility of calcium tartromalate, discovered by Ordonneau (1891). Wurdig and Muller (1980) used malic acid's property of displacing tartaric acid from its calcium salt, but at pHs above 4.5 (higher than the pK_{a_2} of tartaric acid), in a reaction (Figure 1.9) producing calcium tartromalate.

The technology used to implement this deacidification known as the DICALCIC process (Vialatte and Thomas, 1982) consists of adding volume V, calculated from the following equation, of wine to be treated, to obtain the desired deacidification of the total volume (V_T):

$$V = V_T \frac{A_i - A_f}{A_i - 1} \qquad (1.5)$$

In Eqn (1.5), A_i and A_f represent initial and final acidity, respectively, expressed in g/l of H_2SO_4, of the total volume V_T. The volume V of wine to be deacidified by crystallization and elimination of the calcium tartromalate must be poured over an alkaline mixture consisting, for example, of calcium carbonate (1 part) and calcium tartrate (2 parts). Its residual acidity will then be very close to 1 g/l of H_2SO_4.

It is important that the wine should really neutralize the $CaCO_3$/CaT mixture and not the reverse, as the formation of the stable, crystallizable, double tartromalate salt is only possible above pH 4.5. Below this pH, precipitation of the endogenous calcium tartrate occurs, promoted by homogeneous induced nucleation with the added calcium tartrate, as well as precipitation of the potassium bitartrate by heterogeneous induced nucleation (Robillard *et al.*, 1994).

The addition of calcium tartrate is necessary to ensure that the tartaric acid content in the wine does not restrict the desired elimination of malic acid by crystallization of the double tartromalic salt, but also to maintain a balance between the remaining malic and tartaric acid.

1.5 TARTRATE PRECIPITATION MECHANISM AND PREDICTING ITS EFFECTS

1.5.1 Principle

At the pH of wine, and in view of the inevitable presence of K^+ and Ca^{2+} cations, tartaric acid is mainly salified in the following five forms, according to its two dissociation balances:

potassium bitartrate (KTH)
potassium tartrate (K_2T)

calcium tartrate (CaT) with the formula $CaC_4H_4O_6 \cdot 4H_2O$
potassium calcium tartrate
calcium tartromalate

In wine, simple salts are dissociated into TH^- and T^{2-} ions. The last two tartrates (Figure 1.10) share the property of forming and remaining stable at a pH of over 4.5. On the other hand, in terms of solubility, they differ in that potassium calcium tartrate is highly soluble, whereas the tartromalate is relatively insoluble and crystallizes in needles. The properties of this mixed salt may be used to eliminate malic acid, either partially or totally. Table 1.11 shows the solubility, in water at 20°C, of tartaric acid and the salts that cause the most problems in terms of crystalline deposits in wine.

```
    O                O
    ‖                ‖
    C—Ō···Ca⁺⁺···Ō—C
    |                |
    CHOH             CHOH
    |                |
    CHOH             CHOH
    |                |
    C—Ō···K⁺K⁺···Ō—C
    ‖                ‖
a   O                O

    O                O
    ‖                ‖
    C—Ō···Ca²⁺···Ō—C
    |                |
    CHOH             CHOH
    |                |
    CHOH             CH₂
    |                |
    C—Ō···Ca²⁺···Ō—C
    ‖                ‖
b   O                O
```

Fig. 1.10. Structure of (a) double potassium calcium tartrate and (b) calcium tartromalate

Table 1.11. Solubility in water at 20°C in g/l of L-tartaric acid and the main salts present in wine

Tartaric acid	Potassium bitartrate	Neutral calcium tartrate
L(+)-$C_4H_6O_6$	$KHC_4H_4O_6$	$CaC_4H_4O_6 \cdot 4H_2O$
4.9 g/l	5.7 g/l	0.53 g/l

While potassium bitartrate is perfectly soluble in water, it is relatively insoluble in alcohol. Thus, in a dilute alcohol solution at 10% v/v and 20°C, its solubility (S) is only 2.9 g/l.

The potassium concentration in wine is frequently as high as 780 mg/l or 20 meq/l, i.e. 3.76 g/l of potassium bitartrate. Therefore, the concentration (C) of the salt is greater than its solubility (S). It follows that the product CP of the real concentrations (r)

$$CP = [TH^-]_r[K^+]_r \qquad (1.6)$$

is greater than the solubility product SP defined by

$$SP = [TH^-]_e[K^+]_e \qquad (1.7)$$

according to the solubility balance:

$$\underset{\text{solid}}{\text{KTH}} \underset{}{\overset{K_e}{\rightleftharpoons}} \underset{\text{in solution}}{TH_e^- + K_e^+} \qquad (1.8)$$

In this equation, the concentrations (e) of TH^- anions and K^+ cations are theoretically obtained at the thermodynamic equilibrium of the solid KTH/dissolved KTH system, under the temperature and pressure conditions in wine.

The diagram (Figure 1.11) presenting the states of potassium bitartrate in a system correlating the temperature/concentration axes with conductivity shows three fields of states, 1, 2 and 3, with borders defined by the solubility (A) and hypersolubility (B) exponential curves. The exponential solubility curve (A) is obtained by adding 4 g/l of crystallized KTH to a wine. The increase in the wine's electrical conductivity according to temperature is then recorded. This corresponds to the dissolving and ionization of tartrates. As explained in Section 1.6.4, conductivity values correspond to saturation temperatures (T_{Sat}), since wine is capable of dissolving increasing amounts of KTH as the temperature rises. The exponential solubility curve represents the boundary between two possible states of KTH in a wine according to temperature. Thus, at a constant concentration (or conductivity), when the temperature of the wine rises, KTH changes from state 2, where it is supersaturated and surfused, to state 1, i.e. dissolved, where

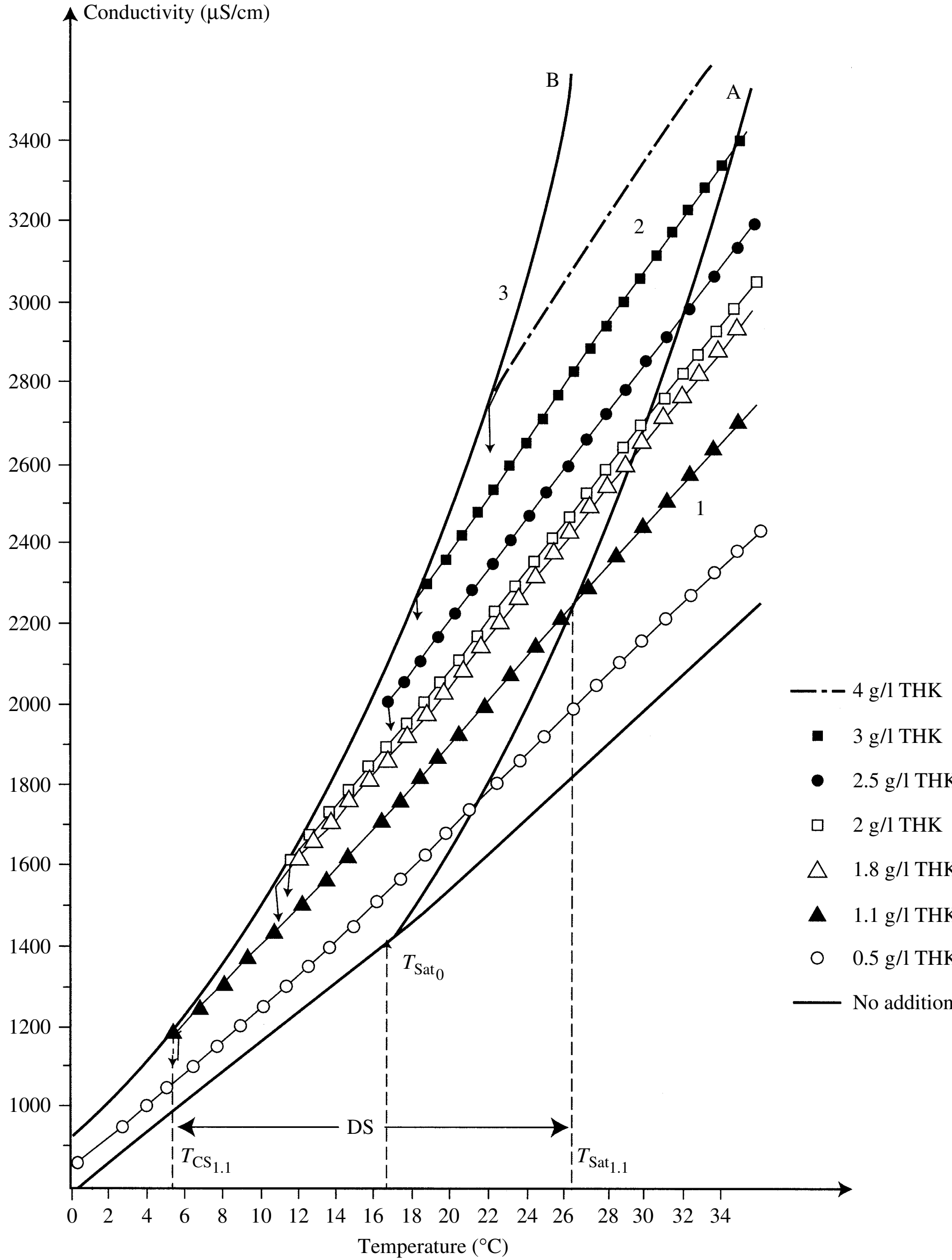


Fig. 1.11. Determining the solubility (A) and hypersolubility (B) exponential curves of potassium bitartrate in a wine. Defining the hyper-saturation and instability fields according to the KTH content (Maujean *et al.*, 1985). DS = saturation field; 1, dissolved KTH; 2, supersaturated, surfused KTH; 3, crystallized KTH; $T_{CS_{1.1}}$, spontaneous crystallization temperature when 1.1 g/l KTH is added; $T_{Sat_{1.1}}$, saturation temperature of a wine in which 1.1 g/l KTH have been dissolved

its concentration product CP is lower than its solubility product SP.

The exponential hypersolubility curve (B) is obtained experimentally and geometrically from the envelope linking the spontaneous crystallization temperature (TCS_i) points of a wine brought to various states of supersaturation by completely dissolving added KTH and then reducing the temperature of the wine until crystallization is observed. The exponential hypersolubility curve represents the boundary between state 2, where potassium bitartrate is in a state of supersaturation ($C - S$) and surfusion, and state 3, where it is crystallized.

Once the solubility (A) and hypersolubility (B) exponential curves have been defined, it is possible to determine the state of a wine at a known temperature with considerable accuracy. Indeed, any wine with a KTH concentration, or conductivity, above that defined by the intersection of the vertical line drawn upwards from the temperature of the wine and the exponential solubility curve (A) is in a supersaturated state so, theoretically, there is a probability of spontaneous crystallization. The crystallization phenomenon will, in fact, be observed at the intersection of the same vertical line and the exponential hypersolubility curve (B). It appears, therefore, that supersaturation is necessary, but not sufficient, for primary nucleation phenomena and spontaneous crystallization to occur in a wine.

The delay in crystallization of a salt in relation to its solubilization, which is partially responsible for the supersaturated state in superfused form, is due to lack of energy.

The formation of a small crystal, known as a nucleus, in a liquid phase corresponds to the creation of an interface between two phases. This requires a great deal of energy, known as interfacial surface energy. In a wine, the width DS of the supersaturation field (Figure 1.7), expressed in degrees Celsius, is increased by the presence of macromolecules that inhibit the growth of nuclei and crystallization of the KTH. These macromolecules, known as 'protective colloids', include proteins and condensed tannins, and also glucide polymers, such as pectins and gums, i.e. neutral polysaccharides. Besides these chemical macromolecules, there are also more complex polymers, such as glycoproteins, e.g. mannoproteins of yeast origin (Lubbers *et al*., 1993).

The impact of the protective colloid effect on the bitartrate stabilization of a wine varies according to the winemaking methods used. Red wines have a higher phenol content than white wines, and their condensed tannins have a strong inhibiting effect.

In its natural state, wine is always supersaturated and therefore unstable. This situation may be more or less durable, depending on the reorganization of the colloids that occurs during aging. Storage temperatures may be decisive in triggering bitartrate crystallization.

It is certainly true that spontaneous crystallization, under natural conditions, is an unreliable, unpredictable phenomenon. This is why the production process for many red and white wines includes artificial cold stabilization before bottling. This type of treatment is justified, especially as consumers will not tolerate the presence of crystals, even if they do not affect quality.

Furthermore, artificial cold stabilization is indispensable for sparkling wines. Indeed, microcavities in the surface of the glass or in solid particles in suspension, especially microcrystals of potassium bitartrate, may lead to the formation of too many bubbles when the bottle is opened, causing excessive effervescence known as 'spraying'. This is sometimes responsible for the loss of large quantities of wine during disgorging, or when bottles are opened by consumers (Volume 1, Section 14.3.4). The origin of this effervescence and spraying is given by the repetitive bubble formation model (Casey, 1988) (Figure 1.12). This bubble degassing model is based on the phenomenon of heterogeneous induced nucleation.

However, nucleation may be induced and the microcavities are efficient only if they have a radius R_1 greater than a critical radius R_c defined by Laplace's law. Indeed, below this value, the excess pressure in the bubble is such that carbon dioxide passes from the gas phase to the liquid phase and so the bubble disappears.

On the other hand, if R_1 is greater than R_c, carbon dioxide diffusion occurs in the opposite

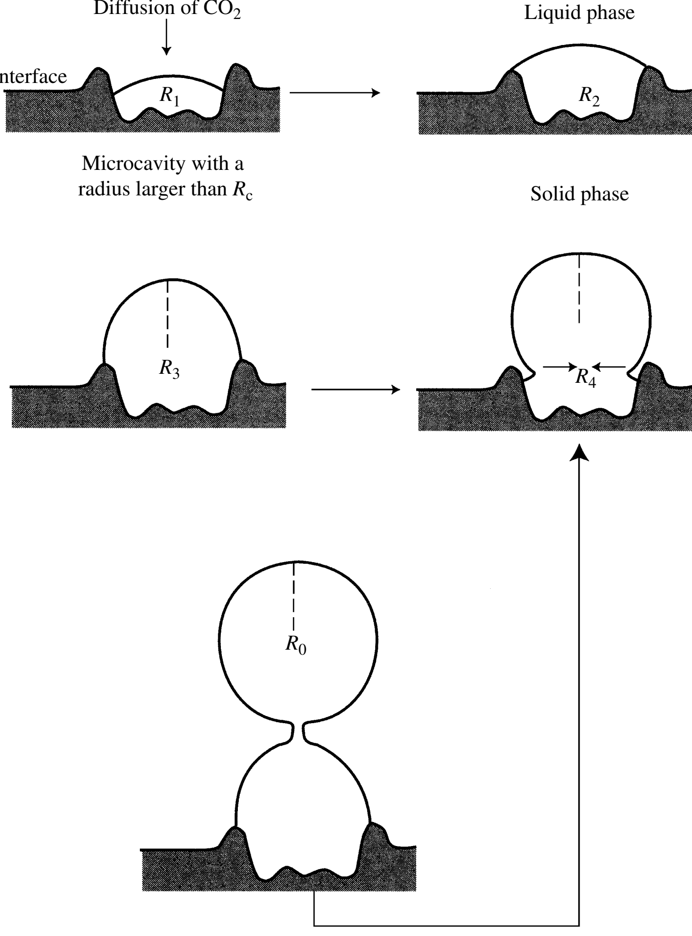


Fig. 1.12. Repetitive bubble formation on a microcavity in a tartrate microcrystal in a sparkling wine. Heterogeneous induced nucleation, according to the Casey model (1988)

direction and the bubble increases in size, reaching the values R_2, R_3 and R_4. At this last stage, the bubble is subjected to the laws of gravity and starts to rise when its radius reaches the value R_0, leaving behind a new bubble that has started to form. This is how the phenomenon of durable effervescence is achieved.

The fact that the phenomenon of effervescence may be exacerbated due to a large number of microcavities in tartrate microcrystals is an additional reason for ensuring the thorough tartrate stabilization of still wine intended for sparkling wine production. Treatment parameters at this stage must take into account the destabilizing effect of the increase in alcohol content following the second alcoholic fermentation in vat or in bottle.

There are two main types of must and wine treatment technologies for preventing bitartrate instability based on the phenomenon of low-temperature crystallization. The first uses traditional slow stabilization technology (Section 1.7.2), as opposed to the more recent Müller-Späth rapid contact stabilization process (1979), where the wine is seeded with cream of tartar crystals. There are two variants of the short process, one static and the other dynamic, known as 'continuous treatment'.

Besides these two systems, a new separation technique, electrodialysis, is also applied to the bitartrate stabilization of wine (Section 12.5). The use of ion-exchange resins is also permitted in certain countries, including the USA (Section 12.4.3). Finally, it is possible to prevent the precipitation of these salts by adding crystallization inhibitors, such as metatartaric acid or yeast mannoprotein extracts (Section 1.7.7), or carboxymethylcellulose (Section 1.7.8).

1.5.2 Tartrate Crystallization and Precipitation

The two artificial cold stabilization technologies described elsewhere (Sections 1.7.1. and 1.7.2) do not use the same crystallization mechanism. The traditional stabilization process involves spontaneous, primary nucleation, a long process that produces large crystals because the nuclei grow slowly. In rapid stabilization processes, the awkward stage of primary nucleation is replaced by a fast, homogeneous secondary nucleation. This is induced by adding massive quantities of small exogeneous tartrate crystals, which also considerably boost supersaturation ($C - S$).

Furthermore, in this technique, the temperature of the wine is reduced abruptly, promoting the formation of small endogeneous tartrate nuclei, i.e. significantly increasing the surface area (A) of the liquid/solid interface by maximizing the diffusion of bitartrate aggregates with pre-crystalline structures, thus ensuring faster growth of the nuclei (Figure 1.13).

It has been experimentally verified (Maujean *et al.*, 1986) that the crystallization rate, monitored by measuring the electrical conductivity of wine, is directly proportional to the surface area of the liquid/solid interface represented by the nuclei. This result is consistent with the following equation, proposed by Dunsford and Boulton (1981), defining the mass velocity at which the precrystalline aggregates of potassium bitartrate diffuse towards the surface (A) of the adsorption interface:

$$\frac{dm}{dt} = k_d(A)(C - C_i) \tag{1.9}$$

where C is the concentration of the solution and C_i is the concentration of the interface.

One practical application of these theoretical results is that producers and distributors have been

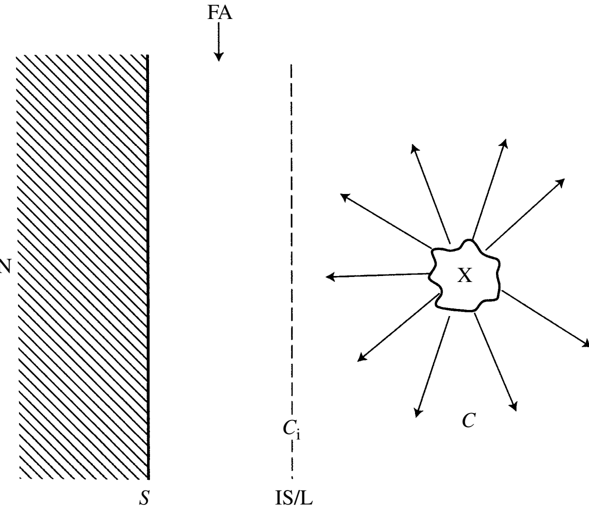


Fig. 1.13. Diagram illustrating the importance of the diffusion speed of THK aggregates towards the solid/liquid adsorption interface for the growth of nuclei: FA, adsorption film; X, molecular aggregate of THK diffusing towards the interface; IS/L, solid/liquid interface; N, nuclei; C, THK concentration in the liquid phase; C_i, THK concentration at the solid/liquid interface; S, theoretical solubility of THK; $C - S$, supersaturation of the wine; $C > C_i > S$

obliged to ensure that their cream of tartar particles have a radius of less than 40 μm. This parameter is also important as nuclei with a radius greater than 200 μm grow much more slowly than smaller nuclei.

This confirms the findings of Devraine (1969), who also concluded that large nuclei stop growing as they release 'fines', i.e. 'daughter' nuclei. This observation explains the continued effectiveness in stabilizing white wines of cream of tartar that has been recycled five times, provided that the particles were initially very small. On the other hand, it is not possible to recycle cream of tartar so many times in red wines due to the affinity between tartaric acid and phenols, known to be powerful crystallization inhibitors.

Another advantage of the contact process is that seeding with small cream of tartar particles enhances the state of supersaturation $(C - C_i)$. This is important as the crystallization rate is not only proportional to the interface value (A), but also to the state of supersaturation $(C - C_i)$ (Eqn 1.9).

The added cream of tartar must be maintained in suspension homogeneously, throughout the vat by appropriate agitation, so that the nuclei provide a maximum contact interface with the aggregates of endogeneous tartrate. As soon as the cream of tartar is added, the crystallization rate depends solely on the interface factor (A), as $(C - C_i)$ is so large that it may, at least in the first hour of contact, be considered constant. It may therefore be stated that, during the first hour, the crystallization rate depends solely on the rate of diffusion of the aggregates (Eqn 1.9).

After this initial contact time, the nuclei have grown but, more importantly, $(C - C_i)$ has decreased, as the very high crystallization rate has consumed large quantities of exogeneous tartrate. In other words, A, i.e. the diffusion rate, is no longer the limiting factor, but rather the state of supersaturation $(C - C_i)$. As C tends towards C_i, the situation in the wine approaches the theoretical solubility (S) of tartrate under these treatment conditions. Therefore, by the end of the treatment process, the crystallization rate is controlled more by thermodynamics than kinetics.

These theoretical considerations, applied to a short treatment involving seeding with tartrate crystals, show that great care and strict supervision is required to ensure the effectiveness of artificial cold stabilization. The following factors need to be closely monitored: the wine's initial state of supersaturation, the particle size of the added tartrates, the seeding rate, the effectiveness of agitation at maintaining the crystals in suspension, treatment temperature and, finally, contact time.

1.5.3 Using Electrical Conductivity to Monitor Tartrate Precipitation

Wurdig and Muller (1980) were the first to make use of the capacity of must and wine to act as electrolytes, i.e. solutions conducting electricity, to monitor tartrate precipitation. Indeed, during precipitation, potassium bitartrate passes from the dissolved, ionized state, when it is an electrical conductor, to a crystalline state, when it precipitates and is no longer involved in electrical conductivity:

$$HT^- + K^+ \longrightarrow KTH \downarrow$$

The principle of measuring conductivity consists of making the wine into an 'electrical conductor', defined geometrically by the distance l separating two platinum electrodes with S-shaped cross-sections. The resistance R (in ohms) of the conductor is defined by the relation:

$$R = \rho \frac{l}{S}$$

In this equation, ρ is the resistivity. Its inverse (γ) is the conductivity expressed in siemens per meter (S/m) or microsiemens per centimeter (μS/cm $= 10^{-4}$ S/m).

The expression of resistivity $\rho = RS/l$ involves the term S/l, known as the cell 'k' constant. This constant is particular to each cell, according to its geometry, and may also vary with use, due to gradual deterioration of the electrodes or the effect of small impacts.

It is therefore necessary to check this constant regularly and to determine it at a conductivity close

Table 1.12. Resistivity and conductivity of a KCl (0.02 M) solution according to temperature (in °C)

Temperature (°C)	15	16	17	18	19	20	21	22	23	24	25
Resistivity (Ω/cm)	446	436	426	417	408	400	392	384	376	369	362
Conductivity (μS/cm)	2242	2293	2347	2398	2451	2500	2551	2604	2659	2710	2769

to that of wine. In practice, a 0.02 M KCl solution is used. The temperature of the KCl (0.02 M) solution must be taken into account in checking the cell constant. The resistivity and conductivity values of this solution according to temperature are specified in Table 1.12.

The conductivity meter cell is subjected to an alternating current. The frequency is set at 1 kHz for the standardized solution (KCl = 0.02 M) and wine, to avoid polarizing the electrodes. A conductivity meter is used for continuous monitoring of tartrate precipitation in wine (see Section 1.6.4, Figure 1.16).

1.6 TESTS FOR PREDICTING WINE STABILITY IN RELATION TO CRYSTAL PRECIPITATION AND MONITORING THE EFFECTIVENESS OF ARTIFICIAL COLD STABILIZATION TREATMENT

1.6.1 The Refrigerator Test

This traditional test is somewhat empirical. A sample (approximately 100 ml) of wine, taken before or after artificial cold stabilization, is stored in a refrigerator for 4–6 days at 0°C and then inspected for crystals. In the case of wines intended for a second fermentation, alcohol may be added to increase the alcohol content by 1.3–1.5% v/v. This simulates the effects of the second fermentation and makes it possible to assess the bitartrate stability of the finished sparkling wine.

The advantages of this test are that it is simple and practical, and requires no special equipment. On the other hand, it is mainly qualitative, and does not provide an accurate indication of the wine's degree of instability. Its major disadvantage is that it takes a long time and is incompatible with short contact stabilization technologies, where rapid results are essential to assess the treatment's effectiveness in real time.

Finally, this test is neither reliable, nor easily repeatable, as it is based on the phenomenon of spontaneous, non-induced crystallization—a slow, undependable process.

1.6.2 The 'Mini-contact' Test

A sample of wine with 4 g/l added potassium bitartrate is maintained at a temperature of 0°C for 2 hours, and constantly agitated. The wine sample is cold-filtered and the weight increase of the tartrate collected (exogeneous tartrate + wine tartrate) is assessed. It is also possible to dissolve the precipitate in a known volume of hot water and measure the increase in acidity as compared to that of the 4 g/l exogeneous potassium bitartrate added to the wine.

The mini-contact test is based on homogeneous induced nucleation, which is faster than primary nucleation. However, this test does not take into account the particle size of the seed tartrate, although the importance of its effect on the crystallization rate is well known. The operative factor in this test is the surface area of the liquid/solid contact interface. Furthermore, this test defines the stability of the wine at 0°C and in its colloidal state at the time of testing. In other words, it makes no allowance for colloidal reorganization in wine, especially red wine, during aging.

It is normal to find potassium bitartrate crystals, associated with precipitated condensed coloring matter, in wine with several years' aging potential. When phenols condense, they become bulky, precipitate and are no longer able to express their 'protective colloid' effect.

It should be noted that mini-contact test results tend to overestimate a wine's stability and therefore the effectiveness of prior treatment. This statement is based on work by Boulton (1982). After 2 hours' contact, only 60–70% of the endogeneous tartrate has crystallized and therefore the increase in weight of the crystal precipitate is minimized. These results are interpreted to mean that the treatment was more effective, or the wine more stable, than was actually the case. In order to make the mini-contact test faster, more reliable and compatible with the dynamic contact process, the Martin Vialatte Company proposed the following variant in 1984: seeding a wine sample with 10 g/l of cream of tartar and measuring the drop in conductivity at 0°C.

The rules governing stability under the extreme supersaturation conditions prevailing in wine are as follows:

1. If, in the 5–10 min after seeding, the drop in conductivity is no more than 5% of the wine's initial conductivity (measured before adding potassium bitartrate), the wine may be considered to be properly treated and stabilized.
2. If the drop in conductivity is over 5%, the wine is considered unstable.

As this test is based on measuring the wine's electrical conductivity, it has the tremendous advantage that there is no need to collect the precipitate by filtration and determine the increase in weight. This new mini-contact test, measuring conductivity, is much faster (5–10 min instead of 2 h). Furthermore, by comparison with the first variant of the mini-contact test, as the contact surface (A) and, consequently, the state of supersaturation of the wine are multiplied by 2.5 (adding 10 g/l of KTH instead of 4 g/l), it gives a more accurate assessment of a wine's stability.

In spite of these improvements, this test remains open to criticism and its reliability is limited. Indeed, as is the case with the preceding test, it does not always take into consideration the effect of particle size, and is based on excessively small variations in conductivity and too short a contact time. The results in Tables 1.13, 1.14 and 1.15 corroborate this point of view. In Table 1.13, results indicate that a variation of over 5 units in the concentration product PC_K (see samples A and D) only caused a decrease of 1% from the wine's initial conductivity. In this instance, a white wine with a PC_K close to 13 was considered unstable, but this assessment was not confirmed by the percentage conductivity.

Table 1.13. Values of the concentration products of wines and the corresponding percentage drop in conductivity produced by the mini-contact test

Samples	$PC_K \times 10^5$	Drop in conductivity at 0°C (%)
A	7.28	0.5
B	11.62	1.0
C	11.84	0.0
D	12.96	1.5

The unreliability of this result is confirmed by the experiment described in Table 1.14, involving a wine with an initial PC_K of 9.17×10^5, maintained at 30°C, in which increasing concentrations of commercial cream of tartar were dissolved. It was observed that, when the PC_K of a wine was doubled (e.g. wine +0.2 g/l of dissolved KTH and wine +1 g/l of dissolved KTH) the percentage drop in conductivity was the same, although there was obviously a difference in stability.

Table 1.8 shows that the effects of variations in cream of tartar particle size and contact time in the same wine were capable of causing a difference of 5% in the drop in initial conductivity, which is the benchmark for deciding whether a wine is stable or not.

In practice, a rapid-response test is required for monitoring the effectiveness of artificial cold stabilization. The preceding results show quite clearly that the tests based on induced crystallization are relatively unreliable for predicting the stability of a wine at 0°C.

1.6.3 The Wurdig Test and the Concept of Saturation Temperature in Wine

Wurdig *et al.* (1982) started with the idea that the more KTH a wine is capable of dissolving at low

Table 1.14. Demonstrating the limitations of the reliability of the mini-contact test in assessing the stability of a wine by adding increasing quantities of potassium bitartrate and measuring the percentage drop in conductivity

Samples	pH	K^+ (mg/l)	$PC_K \times 10^5$	Drop in initial conductivity (%)
Control	3	390	9.17	1.5
Wine + 0.2 g/l KTH	3	420	10.85	11.5
Wine + 0.5 g/l KTH	3.03	469	13.33	7.5
Wine + 0.7 g/l KTH	3.05	513	15.26	12.5
Wine + 1 g/l KTH	3.06	637	21.16	11.5

Table 1.15. Influence of tartrate particle size and mini-contact test time on the percentage drop in conductivity of the wine

Drop in conductivity (%)	Commercial KTH	KTH: particle size greater than 100 μm	KTH: particle size smaller than 63 μm
After 10 min	12	9	14
After 20 min	13	11	16

temperatures, the less supersaturated it is with this salt and, therefore, the more stable it should be in terms of bitartrate precipitation. The authors defined the concept of saturation temperature (T_{Sat}) in a wine on the basis of this approach.

The saturation temperature of a wine is the lowest temperature at which it is capable of dissolving potassium bitartrate. In this test, temperature is used as a means of estimating the bitartrate stability of a wine, on the basis of the solubilization of a salt.

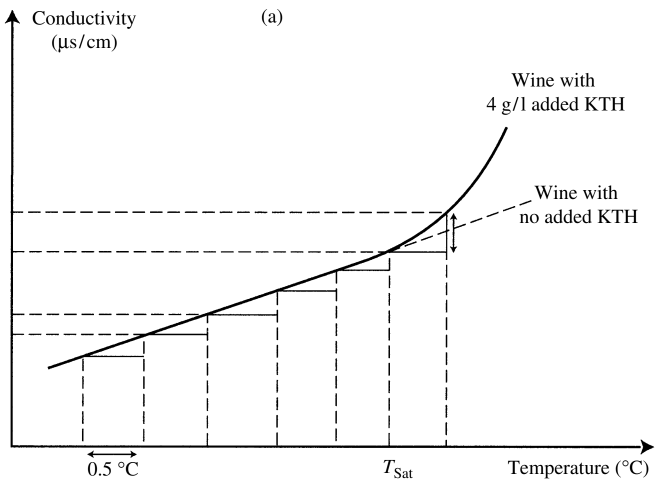
In comparison with the previously described tests, based on crystallization, this feature seems very convincing. Indeed, the solubilization of a salt is a spontaneous, fast, repeatable phenomenon, much less dependent on the particle size of the added tartrate crystals. The solubilization of KTH is also much less affected by the colloidal state of the wine at the time of testing. It has been observed that 'protective colloids' act as crystallization inhibitors, but do not affect the solubilization of salts. Consequently, estimating the bitartrate stability of a wine by testing the solubilization of KTH, i.e. saturation temperature, is a more reliable measurement in the long term as it is independent of any colloidal reorganization during storage and aging.

The saturation temperature of a wine was determined by measuring electrical conductivity (Figure 1.14) in a two-stage experiment.

In the first experiment, the wine was brought to a temperature of approximately 0°C in a thermostat-controlled bath equipped with sources of heat and cold. The temperature was then raised to 20°C in 0.5°C increments and the wine's conductivity measured after each temperature change. In this way, it was observed that the variation in conductivity according to the temperature of a wine containing no KTH crystals was represented by a roughly straight line.

In the second experiment, a volume (100 ml) of the same wine was brought to a temperature close to 0°C, 4 g/l of KTH crystals were added and the temperature was once again raised to 20°C in 0.5°C increments. The wine was agitated constantly and its conductivity measured after each temperature change. Two patterns were observed:

1. Subsequent to the addition of 4 g/l of KTH, the wine (Figure 1.14a) showed a linear variation in conductivity at low temperatures that could almost be superimposed on that of the wine without crystals until a temperature T_{Sat}, where the conductivity left the straight line and followed the exponential solubility curve.



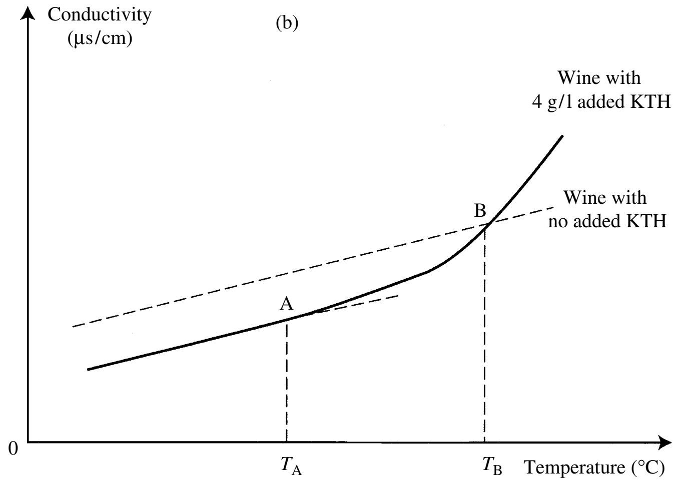


Fig. 1.14. Experimental determination of the saturation temperature of a wine by the temperature gradient method (Wurdig *et al.*, 1982). (a) Example of a wine that is not highly supersaturated, in which no induced crystallization occurs after the addition of tartrate crystals at low temperature. (b) Example of a highly supersaturated wine, in which induced crystallization occurs immediately after the addition of calcium potassium tartrate crystals

2. Following the addition of 4 g/l of KTH, the wine's conductivity (Figure 1.14b) at temperatures around 0°C was below that of the wine alone. This meant that low-temperature induced crystallization had occurred, revealing a state of supersaturation with high endogeneous KTH levels in the wine. Its conductivity then increased in a linear manner until temperature T_A; then the KTH started to dissolve and the conductivity followed the exponential solubility curve. At temperature T_B, the exponential solubility curve crossed the straight line showing the conductivity of the wine alone. This intersection corresponds to the wine's true saturation temperature. The temperature T_A corresponds to that of the same wine after a 'contact', leading

to desaturation caused by induced crystallization. It is therefore normal that, following desaturation, the wine should solubilize more KTH, at a temperature lower than its true saturation temperature, T_B.

On a production scale, where rapid stabilization technologies are used, experimental determination of the saturation temperature by the temperature gradient method is incompatible with the rapid response required to monitor the effectiveness of ongoing treatment.

On the basis of statistical studies of several hundred wines, Wurdig *et al.* (1982) established a linear correlation defined by:

$$T_{Sat} = 20 - \frac{(\Delta L)_{20^\circ C}}{29.3} \quad (1.10)$$

This straight-line correlation (Figure 1.15) between the variation in conductivity of a wine at 20°C before and after the addition of 4 g/l of potassium bitartrate (ΔL) and the saturation temperature has only been verified for wines where the solubilization temperature of KTH is between 7 and 20°C. The practical advantage of using this equation is that the saturation temperature of a wine may be determined in just a few minutes, using only two measurements.

In some wines, crystallization may be induced by adding cream of tartar at 20°C. This means that they have a lower conductivity after the addition of tartrate, i.e. a saturation temperature above 20°C. This is most common in rosé and red wines. In order to determine their precise saturation temperature, the samples are heated to 30°C. Cream of tartar is added and the increase in conductivity at this temperature is measured. The saturation temperature is deduced from (Maujean *et al.*, 1985):

$$T_{Sat} = 29.91 - \frac{(\Delta L)_{30^\circ C}}{58.30} \quad (1.11)$$

Calculating the saturation temperature of a wine prior to cold stabilization provides information on the optimum seeding rate for that wine. Indeed, it is not necessary to seed at 400 g/hl, as often recommended, if 40 g/hl are sufficient.

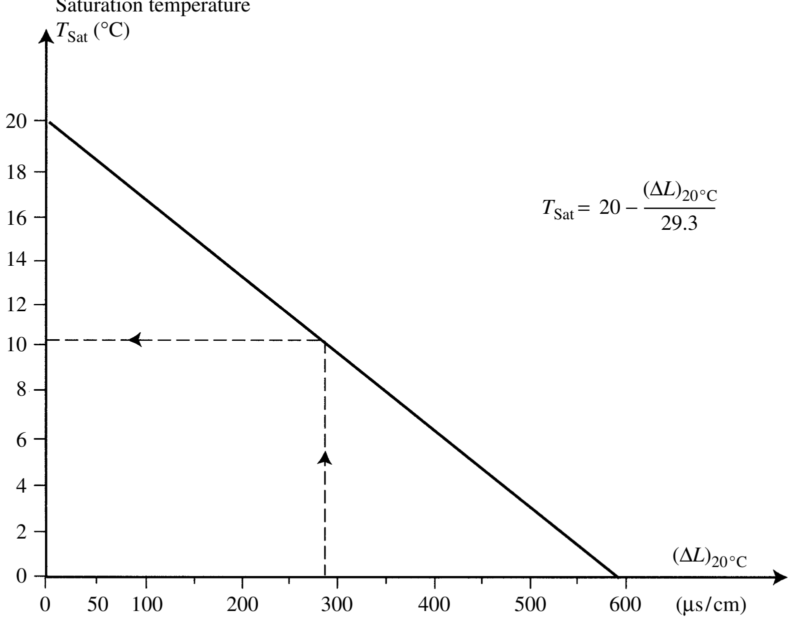


Fig. 1.15. Determining the saturation temperature of a wine according to the variation (ΔL) in conductivity at 20°C before and after the addition of potassium bitartrate (KHT) (Wurdig *et al.*, 1982)

1.6.4 Relationship Between Saturation Temperature and Stabilization Temperature

The temperature at which a wine becomes capable of dissolving bitartrate is a useful indication of its state of supersaturation. However, in practice, enologists prefer to know the temperature below which there is a risk of tartrate instability. Maujean *et al.* (1985, 1986) tried to determine the relationship between saturation temperature and stability temperature.

The equations for the solubility (A) and hypersolubility (B) curves (Section 1.5.1, Figure 1.11) were established for this purpose by measuring electrical conductivity. They follow an exponential law of the following type: $C = a\ e^{bt}$, where C is the conductivity, t is the temperature and a and b are constants.

The experiment to obtain the exponential hypersolubility curve (B) consisted of completely dissolving added cream of tartar in a wine at 35°C and then recording the conductivity as the temperature dropped. This produced an array of straight-line segments (Figure 1.11) whose intersections with the exponential solubility curve (A) corresponded to the saturation temperatures (T_{Sat_i}) of a wine in which an added quantity i of KTH had been dissolved. The left-hand ends of these straight-line segments corresponded to the spontaneous crystallization temperatures (T_{CS_i}). For example, if 3 g/l of KTH is dissolved in wine, the straight line representing its linear decrease in conductivity stops at a temperature of 18°C, i.e. the temperature where spontaneous crystallization occurs (T_{CS_3}).

Of course, if only 1.1 g/l of KTH is dissolved in the same wine, crystallization occurs at a lower temperature, as the wine is less supersaturated ($T_{\text{CS}_{1.1}} = 4.5$°C). It is therefore possible to obtain a set of spontaneous crystallization temperatures based on the addition of various quantities i of KTH (Figure 1.11).

The envelope covering this set of spontaneous crystallization temperatures (T_{CS_i}) defines the exponential hypersolubility curve (B). The exponential solubility and hypersolubility curves, representing the boundaries of the supersaturation field, are parallel. This property, first observed in champagne-base wines, is used to deduce the spontaneous crystallization temperature of the initial wine.

Indeed, projecting from the intersections between the straight lines indicating conductivity and the two exponentials (A) and (B) to the temperature axis, produces temperatures T_{Sat_i} and T_{CS_i}, respectively. The difference, $T_{\text{Sat}_i} - T_{\text{CS}_i}$, defines the width of the supersaturation field of the wine in which i added KTH has been dissolved, expressed in degrees Celsius. The width of the supersaturation field is independent of the addition value i, as exponents (A) and (B) are roughly parallel. Thus, in the example described (Figure 1.11), the width of the supersaturation field is close to 21°C, whether 1.1 g/l ($T_{\text{Sat}_{1.1}} - T_{\text{CS}_{1.1}} = 25.2 - 4.5 = 20.7$°C) or 1.8 g/l ($T_{\text{Sat}_{1.8}} - T_{\text{CS}_{1.8}} = 30.2 - 10.4 = 20.8$°C) of KTH is added. If 21°C is subtracted from the true saturation temperature of the wine (T_{Sat_0}), i.e. no added KTH ($i = 0$), it may be deduced that spontaneous crystallization is likely to occur in this wine at temperature $T_{\text{CS}_0} = T_{\text{Sat}_0} - 21 = -5$°C.

The experimental method for finding the width of the supersaturation field has just been described, and the relationship between the saturation temperature and the temperature below which there is a risk of crystallization has been deduced. The width of the supersaturation field, corresponding to the delay in crystallization, must be linked, at least partially, to the phenomenon of surfusion (the effect of alcohol), as well as the presence of macromolecules in the wine which inhibit the growth of the nuclei. These macromolecules include carbohydrate, protein and phenol colloids. It seems interesting, from a theoretical standpoint, to define the contribution of these protective colloids to the width of the supersaturation field. It also has a practical significance, and should be taken into account in preparing wines for tartrate stabilization. For this purpose, aliquots of the same white wine at 11% v/v alcohol were subjected to various treatments and fining (Table 1.16). At the same time, a model dilute alcohol solution was prepared: 11% v/v buffered at pH 3, containing 4 g/l of

Table 1.16. Influence of pre-treatment on the physicochemical parameters of a cold-stabilized white wine. Wines treated with slow cold-stabilization (10 days at −4°C). Assessment of protective effects (Maujean *et al.*, 1985)

Samples		Total acidity (g/l H_2SO_4)	pH	Potassium (mg/l)	Tartaric acid (g/l H_2SO_4)	PCK × 10^5	T_{Sat} measured (°C)	T_{Sat} calculated (Wurdig) (°C)	T_{CS} calculated (°C)	$T_{Sat} - T_{CS}$ measured (°C)[a]
	Before cold	7.03	3.13	970	1.46	19.67	18.19	17.85	−2.60	20.8
Control	After cold	7	3.05	730	0.98	9.21	9.55	11.06	−12.7	22.25
Bentonite	Before cold	7.29	3.09	985	1.59	20.97	17.05	17.14	−1.15	18.2
(30 g/hl)	After cold	6.97	3.04	740	0.77	7.26	9.6	9.77	−9.4	19
Charcoal decolorant	Before cold	7.21	3.1	940	1.59	20.97	17.05	17.2	−2.7	19.75
(30 g/hl)	After cold	6.89	3.1	750	1.01	10.24	9.1	10.33	−11.3	20.4
Gum arabic	Before cold	7.31	3.08	940	1.45	18.07	16.8	16.98	−3.8	20.6
(3 g/hl)	After cold	7.04	3.03	730	0.91	8.37	11	11.32	−10.95	21.95
Tanin (6 g/hl) and	Before cold	7.25	3.08	970	1.42	18.26	18	17.97	−4.9	22.9
Gelatin (3 g/hl)	After cold	7.2	3.08	970	1.32	17.46	16	16.16	−5.5	21.05
Metatartaric acid	Before cold	7.19	3.01	975	1.23	20.35	19.25	18.91	<−3.75	>23
(5 g/100 bottles)	After cold	7.26	3.09	975	0.23	16.06	18.65	18.61	−6.09	24.7
Filtered	Before cold	6.51	3.08	955	1.25	15.83	16.9	16.54	2.85	14.05
membrane 10^3 Da	After cold	5.67	3.01	535	0.3	2.24	1.8	0.63	−12.8	14.6
Filtered	Before cold	7.22	3.08	970	1.54	19.8	17	17.06	−3.65	20.65
membrane 0.22 μm	After cold	7	3.03	970	0.94	9.08	11.6	11.21	−8.5	20.1

[a]The differences, $T_{Sat} - T_{CS}$, were determined by dissolving 1 and 2 g/l of THK in the wine. Conductivity was then recorded at decreasing temperatures until crystallization occurred; the T_{CS} values were deduced.

KTH, with a saturation temperature of 22.35°C. The spontaneous crystallization temperature of the same solution was also determined after 1.4 g/l of KTH had been dissolved in it, $T_{CS_{1.4}} = 7.4°C$. It was thus possible to find the width of the supersaturation field, i.e. 15°C.

The spontaneous crystallization temperature of each sample of treated wine (Table 1.16) was also determined using the same procedure. Examination of the results shows that a wine filtered on a 10^3 Da Millipore membrane, i.e. a wine from which all the colloids have been removed, has the lowest value for the supersaturation field ($T_{Sat} - T_{CS_0}$), closest to that of the model dilute alcohol solution. Therefore, the difference between the results for this sample and the higher values of the supersaturation fields of 'fined' samples define the effect of the protective colloids. It is interesting to note that the sample treated with metatartaric acid had the widest supersaturation field, and cold stabilization was completely ineffective in this case. This clearly demonstrates the inhibiting effect this polymer has on crystallization and, therefore, its stabilizing effect on wine (Section 1.7.6). Stabilization by this method, however, is not permanent.

On the basis of these results evaluating the protective effects of colloids and saturation temperatures before and after cold stabilization, it is possible to determine the most efficient way to prepare a white wine for bitartrate stabilization. It would appear that tannin–gelatin fining should not be used on white wines, while bentonite treatment is the most advisable. The effect of tannin–gelatin fining bears out the findings of Lubbers *et al.* (1993), highlighting the inhibiting effect of yeast-wall mannoproteins on tartrate precipitation.

There are quite tangible differences in the performance of slow stabilization when wines have no protective colloids (cf. wine filtered on a membrane retaining any molecule with a molecular weight above 1000 Da). These effects ought to be even more spectacular in the case of rapid stabilization technologies. Indeed, the results presented in Figure 1.16 show the impact of prior preparation on the effectiveness of the contact process.

It was observed that the crystallization rate during the first hour of contact, measured by the slope of the lines representing the drop in conductivity of the wine in μS/cm per unit time, was highest for the wine sample filtered on a 10^3 Da membrane, i.e. a wine containing no protective colloid macromolecules. On the contrary, the addition of metatartaric acid (7 g/hl) completely inhibited the crystallization of potassium bitartrate, even after four hours. In production, bentonite and charcoal decolorant are the best additives for preparing wine for tartrate stabilization using the contact process.

1.6.5 Applying the Relationship between Saturation Temperature (T_{Sat}) and Stabilization Temperature (T_{CS}) to Wine in Full-scale Production

In practice, the saturation temperature is obtained simply by two electrical conductivity measurements, at 20°C for white wines and 30°C for red wines. The first is measured on the wine alone, the other after the addition of 4 g/l of KHT crystals. Equations (1.10) and (1.11) are used to calculate T_{Sat} for white wines and for red wines, respectively. The relationship between saturation temperature T_{Sat} and true stability temperature in various types of wine is yet to be established.

In order to define a rule that would be reliable over time, i.e. independent of the colloidal reorganizations in white wine during aging, Maujean *et al.* (1985, 1986) proposed the following equation:

$$T_{CS} = T_{Sat} - 15°C$$

Note that this equation totally ignores protective colloids, and is valid for a wine with an alcohol content of 11% v/v. For white wines with an alcohol content of 12.5% v/v, or those destined for a second fermentation that will increase alcohol content by 1.5% v/v, the equation becomes:

$$T_{CS} = T_{Sat} - 12°C$$

Thus, if stability is required at −4°C, the saturation temperature should not exceed 8°C. The stability normally required in Champagne corresponds to the temperature of −4°C used in

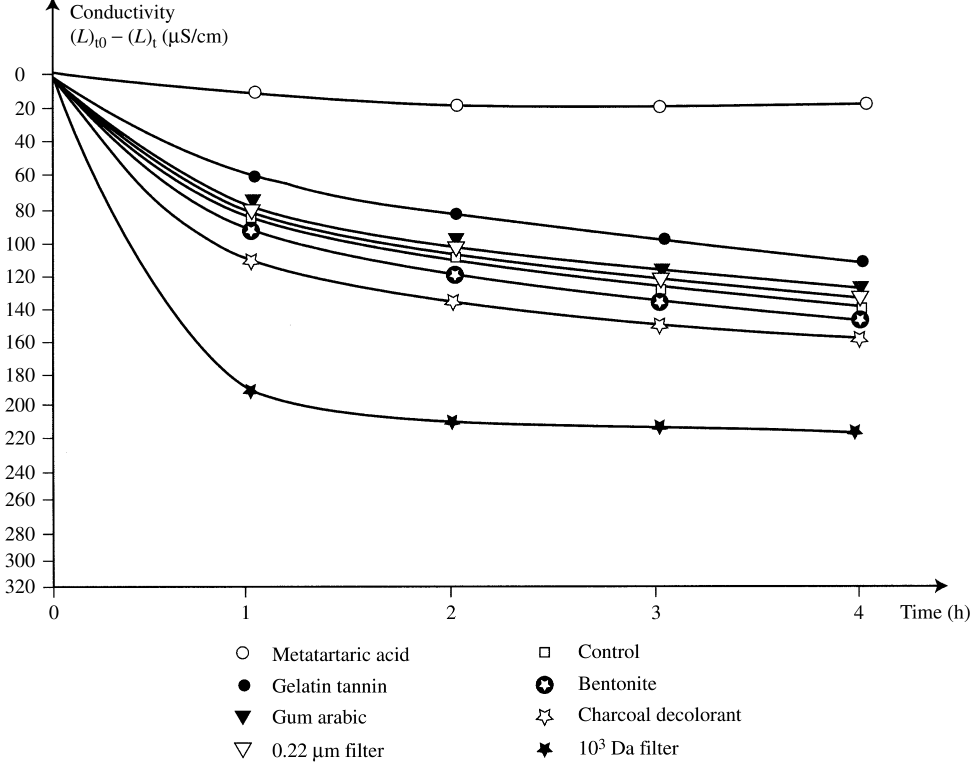


Fig. 1.16. Crystallization kinetics of potassium bitartrate analyzed by measuring the drop in conductivity of a wine according to the type of treatment or fining. Samples were stored at 2°C, seeded with 5 g/l of KTH and subjected to the static contact process for four hours (Maujean *et al.*, 1986)

the slow artificial cold stabilization process. It is questionable whether such a low temperature is necessary to minimize the probability of tartrate crystallization.

In the case of a rosé champagne-base wine, the equation is as follows:

$$T_{CS} = T_{Sat} - 15^{\circ}C$$

This equation shows that, if stability is required at $-4^{\circ}C$, the saturation temperature must be 11°C or lower.

In the case of red wines, it is possible to be less demanding, due to the presence of phenols. To simplify matters, Gaillard and Ratsimba (1990) relate the tartrate stability of wines uniquely to saturation temperature. They estimate that stability is achieved if:

1. In white wines, $T_{Sat} < 12.5^{\circ}C$.
2. In red wines, $T_{Sat} < (10.81 + 0.297\ \text{IPT})^{\circ}C$, where IPT represents the total polyphenol number.

These methods, based on the solubilization of KHT, independent of the medium's composition, are applicable to monitoring cold stabilization treatments.

1.6.6 Using Mextar® Calculation Software

This is a completely different approach to forecasting tartrate instability, still one of the main problems in winemaking.

By transposing methods used for crystallization in solution, Devatine *et al.* (2002) developed

Mextar®, a software program that offers a reliable measure of the stability or degree of instability of a wine, by means of calculations using analysis data on the constituents of the wine's acidity. It is, thus, theoretically possible to obtain an accurate assessment of the need to subject a wine to stabilization treatment. The calculation also predicts changes in chemical composition during spontaneous or induced transformations. Finally, Mextar® can be used to model changes in a wine's acidity, by simulating acidification and deacidification operations, as well as malolactic fermentation, and predicting the pH and total acidity values following these processes.

It will be interesting to monitor the development of this system and its application to different types of wine.

1.7 PREVENTING TARTRATE PRECIPITATION

1.7.1 Introduction

This section will describe the main bitartrate stabilization technologies used for wine (see also Section 12.3.2).

Whatever the technology used, and regardless of any treatment used preparatory to bitartrate stabilization, wine treated with artificial cold must be clean, i.e. not excessively contaminated with yeast or bacteria, as is often the case with wines stored in large vats. These wines should, therefore, be filtered on a simple continuous earth filter. Another advantage of filtration is the elimination of part of the protective colloids. Fine filtration is not useful at this stage, and is certainly not recommended, as there is a risk of eliminating microcrystals likely to act as crystallization nuclei.

1.7.2 Slow Cold Stabilization, Without Tartrate Crystal Seeding

This is the traditional technology for the bitartrate stabilization of wine. Before wineries were equipped with refrigeration and air-conditioning systems, wines were simply exposed to natural cold by opening the vat room doors during the coldest winter weather.

The temperature may decrease at varying rates. It is gradual if the wine is chilled by means of a submerged refrigerating rod in the vat. It may be much faster in a normal installation (Section 12.3.4, Figure 12.1) including a plate heat exchanger to recover energy from the treated wine and reduce the temperature of wine to −4°C more rapidly prior to treatment. It is known (Section 12.3.4) that faster cooling promotes more complete precipitation of the tartrate in the form of small crystals.

Heat-insulated vat rooms, equipped with heating/cooling systems, are also used. The wines are stored in uninsulated vats with a high heat-transfer coefficient, such as stainless steel. The entire room is maintained at the desired temperature, keeping the wine at a negative temperature for 8–10 days (white wines) or up to several weeks, in the case of red wines (Blouin, 1982).

The treatment temperature is generally defined by the following rule:

$$\text{Treatment temperature} = -\frac{\text{Alcohol content}}{2} - 1 \quad (1.12)$$

This rule is deduced from the equation defining the freezing temperature of wine according to its alcohol content:

$$\text{Freezing temperature} = -\frac{\text{Alcohol content} - 1}{2} \quad (1.13)$$

Slow stabilization is tending to evolve towards pseudo-contact technology by seeding with 30–40 g/hl of cream of tartar, agitating for 36 hours and ensuring that the wine does not oxidize. Paddle agitators with variable-speed motors are the most efficient, also ensuring that only a minimal amount of oxygen is dissolved in the wine. There is a significant risk of excessive oxidation as gases dissolve more readily at low temperatures. It is recommended that the agitation rate is monitored by measuring the optical density at 420 nm. In a white wine that has not suffered oxidation, this value decreases by 10% during cold stabilization. Seeding with 20–40 g/hl of KTH should be envisaged if, for example,

natural chilling of the wine has produced some crystallization, so that it is in a less-saturated state.

Slow stabilization often causes loss of color (OD at 520 nm) in both red and white wines. It is therefore recommended that the length of treatment is reduced by adding small particles of cream of tartar, which are easier to maintain in a homogeneous suspension. Another advantage of seeding is that the wine may be maintained at a less cold temperature (−2°C instead of −4°C).

It has been demonstrated on a production scale (360 hl vats) that the stabilization time for a white wine treated with 30 g/hl of bentonite, maintained at −2°C and seeded with 30 g/hl of cream of tartar, may be reduced to 62 hours (including 24 hours without agitation before filtration), instead of 6 days for the standard treatment. Under these conditions, the wine was found to be perfectly stabilized ($T_{Sat} = 7°C$).

1.7.3 Rapid Cold Stabilization: Static Contact Process

This technique has the major advantage of reducing the artificial cold treatment of wine to 4 hours, and sometimes less for white wines. Furthermore, the wine no longer has to be maintained at negative temperatures, but only at 0°C, which minimizes not only energy consumption but also frost accumulation on the equipment. A heat-insulated, conical-bottomed vat known as a crystallizer is used. It is equipped with a drain to remove excess crystals at the end of the cycle.

Such high-performance levels can only be achieved with this type of rapid stabilization treatment by seeding with large quantities of cream of tartar (400 g/hl). This large mass of crystals, with a small initial particle size, must absolutely be maintained in suspension by an agitator, taking care to avoid any unwanted aeration (Section 1.5.2). It is also advisable to blanket the wine with inert gas, or at least use an airtight crystallizer.

Treatment effectiveness is monitored by the rapid response analysis technique described in Section 1.6.4. If the results are satisfactory, agitation is stopped to allow most of the tartrate to settle in the conical bottom of the crystallizer. Complete clarification is not easy to obtain. Great care must be taken in using centrifugation as the crystals are highly abrasive. Good results are obtained with horizontal plate filters, using the crystals themselves as the filter layer. Of course, all these operations must be carried out at 0°C.

Table 1.17. Changes in the physicochemical parameters of cold-stabilized wine when the contact tartrate was recycled (Maujean *et al.*, 1986)

Number of times used	K^+ (mg/l)	Total acidity (g/l H_2SO_4)	Tartaric acid (g/l H_2SO_4)	pH	pC × 10^5
1	315	4.93	1.59	3.11	6.83
2	325	4.92	1.54	3.12	6.88
3	320	4.90	1.59	3.11	6.84
4	300	4.98	1.83	3.09	7.35
5	320	4.94	1.55	3.08	6.57

The static contact process is a very flexible system. It is possible to run 2–3 cycles per day with volumes of 50–100 hl in each batch. This technology is advisable for small and medium-sized wineries. The weak point of this system is the price of cream of tartar, but costs may be reduced by recycling tartrate.

In the case of white champagne-base wines, it has proved possible to recycle the tartrate four times, with almost constant treatment effectiveness (Table 1.17). The continued effectiveness of the treatment, even when the tartrate has been recycled four times, has been explained (Maujean *et al.*, 1986). They showed that the smallest particle size after treatment (<50 µm) was larger than the initial size in the commercial product.

Of course, recycling is not possible when red wines are treated, as the crystals become coated with phenols and coloring matter and rapidly lose their effectiveness.

1.7.4 Rapid Cold Stabilization: Dynamic Continuous Contact Process

Unlike the preceding 'batch' technology, the process described in Figure 1.17 is a continuous

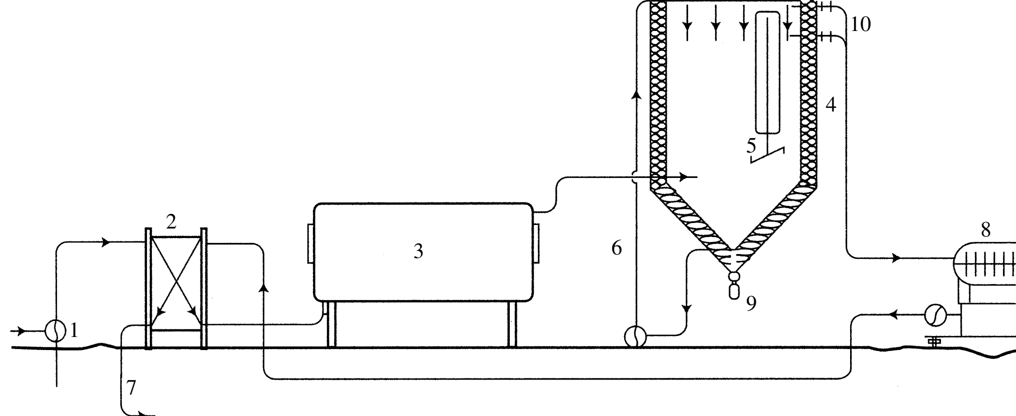


Fig. 1.17. Schematic diagram of a continuous cold stabilization system: 1, intake of wine to be treated; 2, heat exchanger; 3, refrigeration system (with compressor, condenser, etc.); 4, insulation; 5, mechanical agitator; 6, recycling circuit (optional); 7, outlet of treated wine; 8, filter (earth); 9, drain; 10, overflow

bitartrate stabilization process, where the length of time the crystals are in contact with the wine, i.e. the treatment time, is defined by the throughput in relation to the volume of the crystallizer. Thus, for example, if the throughput is 60 hl/h and the volume of the crystallizer is 90 hl, the average time the wine spends in the system is 1 h 30 min.

This emphasizes the need for a method of monitoring effectiveness with a very short response time. There is, of course, a system for recycling wine through the crystallizer if the treatment is insufficiently effective, but the results must be determined very rapidly, as the energy required to treat these quantities of wine is expensive, and unnecessary extra treatment will by no means improve quality.

Continuous treatment is understandably more demanding than the other processes, because it requires close monitoring, but it is also more efficient. For example, the particle size of the contact tartrate and the level in the crystallizer must be monitored by sampling after a few hours, using the drain system.

Agitation is partly provided by a tangential input of wine into the crystallizer. This creates turbulence in the mass of the liquid and maintains at least the smallest crystals in suspension. The wine may also be mechanically agitated.

The throughput, i.e. the average time in the crystallizer, is defined according to the wine's initial state of supersaturation, as well as the type of preparatory treatment (fining, bentonite, etc.) the wine received prior to artificial cold stabilization. The importance of preparation has already been mentioned (Section 1.6.4).

The effectiveness of the three processes described above is generally satisfactory, although results depend on the type of wine (white or red), its alcohol content and any previous treatment or fining.

It is true that, in contact treatments involving large-scale seeding, the wine's background is less important. Indeed, enologists do not always have this information if the wine has been purchased from another winery. In any event, wine must be well prepared and, above all, properly clarified, to ensure the effectiveness of rapid artificial cold stabilization treatments.

1.7.5 Preventing Calcium Tartrate Problems

Calcium tartrate is a relatively insoluble salt, ten times less soluble than potassium bitartrate (see 1.5.1, Table 1.11). Independently of any accidental contamination, calcium added in the form of calcium bentonite for treating must or wine,

calcium carbonate for deacidification purposes, or even as a contaminant in saccharose used for chaptalization, may cause an increase in the calcium tartrate content of wine. Combined with an increase in pH, this may put the wine into a state of supersaturation for this salt, leading to crystal deposits. Robillard *et al.* (1994) reported that crystallization of TCa was even observed in champagne-base wines with a particularly low pH. There is considered (Ribéreau-Gayon *et al.*, 1977) to be a real risk of tartrate deposits in the bottle when the calcium content is over 60 mg/l in red wine and 80 mg/l in white wine.

Stabilizing wines to prevent precipitation of calcium tartrate is not easy, as the crystallization of potassium bitartrate does not induce that of calcium tartrate, despite the fact that these two salts should logically syncrystallize as they have the same crystal systems. On the contrary, crystallization of TCa may induce that of KTH. The prevention of calcium tartrate precipitation is further complicated by the fact that the solubility of TCa (Postel, 1983) is not very temperature-sensitive. Thus, TCa is hardly three times more soluble at 20°C than at −4°C.

Furthermore, according to Abgueguen and Boulton (1993), although the crystallization kinetics of TCa should be higher than those of KTH, the time required for spontaneous nucleation of TCa is much longer. It is therefore easier to understand why calcium tartrate precipitation generally occurs in wine after several years' aging.

On the basis of research into potassium bitartrate (Figure 1.7), Vallée (1995) used measurements of electrical conductivity to define the width of the supersaturation field expressed in degrees Celsius, as well as the calcium tartrate saturation temperature of various types of wines. The low solubility of calcium tartrate indicates that saturation temperatures are likely to be much higher than those of potassium bitartrate.

In order to avoid the risk of calcium tartrate precipitation, the saturation temperature of white, rosé and *vins doux naturels* must be lower than 26°C to ensure that calcium tartrate deposits will not be formed if the wine is kept at 2°C for one month. The calcium tartrate saturation temperature for red wines must be below 35°C.

According to Postel (1983), the addition of 100 mg/l of metatartaric acid is capable of stabilizing a wine stored at 4°C for several months, so that it does not suffer from crystalline deposits of TCa. Furthermore, the use of racemic acid (D-L-tartaric acid) or left-calcium tartrate has been suggested for eliminating excess calcium (Ribéreau-Gayon *et al.*, 1977). In both cases, the precipitation of calcium racemate, a highly insoluble salt, totally eliminates the cation. The treatment's effectiveness depends on the colloid content of the wine, as it hinders precipitation of the salt. These treatments are used to varying degrees in different wine regions according to the types of wines produced.

Finally, ion exchange (Section 12.4.3) and electrodialysis (Section 12.5) are also processes for preventing calcium tartrate deposits.

1.7.6 The Use of Metatartaric Acid

In the processes described above, tartrate precipitations are prevented by eliminating the corresponding salts. It is also possible to envisage the addition of crystallization inhibitors.

The first positive results were obtained with hexametaphosphate, which certainly proved to be effective (Ribéreau-Gayon *et al.*, 1977). However, very high doses were necessary in certain wines and, above all, the increase in phosphate content led to the formation of a ferric complex that caused instability on contact with air (phosphatoferric casse).

Metatartaric acid is currently the product most widely used for this purpose. Carboxymethylcellulose (Section 1.7.8) and mannoproteins extracted from yeast (Section 1.7.7) have also been suggested as stabilizers.

The use of carboxymethylcelluloses has also been suggested. These are a group of complex, poorly-defined products with various properties. Their effectiveness seems to vary according to the type of wine, but especially in relation to the presence of protective colloids. Carboxymethylcelluloses modify a wine's viscosity. They have not as yet been developed on an industrial scale.

The possibility of using mannoproteins extracted from yeast seems worth considering, since this product is both effective and stable (Section 1.7.7).

Metatartaric acid is a polyester resulting from the inter-molecular esterification of tartaric acid at a legally imposed minimum rate of 40%. It may be used at doses up to a maximum of 10 g/hl to prevent tartrate precipitation (potassium bitartrate and calcium tartrate) (Ribéreau-Gayon *et al.*, 1977).

When tartaric acid is heated, possibly at low pressure, a loss of acidity occurs and water is released. A polymerized substance is formed by an esterification reaction between an acid function of one molecule and a secondary alcohol function of another molecule. Tartaric acid may be formed again if the metatartaric acid is subjected to hydrolysis. In reality, however, not all of the acid functions react (Figure 1.18).

Metatartaric acid is not a single compound, but rather a dispersed polymer, i.e. a mixture of polymers with different molecular weights. There are many metatartaric acid preparations with different anti-crystallizing properties, depending on the average esterification rate of their acid functions. It is possible to obtain an esterification rate higher than the theoretical equilibrium rate (33% for a secondary alcohol) by heating tartaric acid

CH[OH] −COOH
|
CHOH −COO[H]
CH[OH] −COOH
|
CHOH −COO[H]
CH[OH] −COOH
|
CHOH −COO[H]
CH[OH] −COOH
|
CHOH −COO[H]

Fig. 1.18. Metatartaric acid polyesterification reaction

to 160°C in a partial vacuum. Under these conditions, the thermodynamic esterification equilibrium is shifted by eliminating water.

The esterification number of different metatartaric acid preparations may be determined by acidimetric assay, before and after saponification. Table 1.18 shows the importance of the preparation conditions in determining this value.

Metatartaric acid is by no means a pure product: solutions are slightly colored and oxidizable. They may contain oxaloacetic acid, but the main impurity is pyruvic acid, representing 1–6% by weight of the metatartaric acid, according to the preparation conditions (Table 1.18). It is, therefore, important to correct the esterification number to compensate for this impurity. The formation of

Table 1.18. Detailed analysis of various metatartaric acid preparations (Peynaud and Guimberteau, 1961)

Preparation method	For 1 g of chemical			Esterification number (%)	Pyruvic acid (%)	Corrected esterification number (%)
	Acidity (meq)	Esters (meq)	Acidity+ esters (meq)			
Reduced pressure, 160°C						
15 min	10.67	3.13	13.80	22.6	0.9	22.8
40 min	8.77	5.14	13.91	36.9	4.2	37.5
45 min	8.63	5.57	14.20	39.2	4.4	40.6
50 min	8.48	5.70	14.18	40.2	4.1	41.5
55 min	8.32	5.74	14.06	40.8	5.6	42.7
Normal pressure, 175°C						
20 min	9.91	3.65	13.46	27.1	5.2	28.3
90 min	9.56	3.76	13.32	28.2	2.3	28.7
105 min	9.11	4.58	13.69	33.4	5.4	35.0

Oxaloacetic acid

Pyruvic acid

Fig. 1.19. Impurities in metatartaric acid

these two acids results from the intra-molecular dehydration of a tartaric acid molecule, followed by decarboxylation (Figure 1.19).

There are many laboratory tests for assessing the effectiveness of a metatartaric acid preparation. Table 1.19 presents an example of a procedure where a saturated potassium bitartrate solution is placed in 10 ml test tubes and increasing quantities of metatartaric acid preparations with different esterification numbers are added. This inhibits the precipitation of potassium bitartrate induced by adding 1 ml EtOH at 96% vol and leaving the preparation overnight at 0°C. Only 1.6 mg of a preparation with an esterification number of 10 is required to inhibit crystallization, while 4.0 mg are necessary if the preparation has an esterification number of 26.6.

Metatartaric acid acts by opposing the growth of the submicroscopic nuclei around which crystals are formed. The large uncrystallizable molecules of metatartaric acid are in the way during the tartrate crystal building process, blocking the 'feeding' phenomenon, i.e. crystal growth. If the dose is too low, inhibition is only partial, and anomalies and unevenness are observed in the shape of the crystals.

The fact that metatartaric acid solutions are unstable has a major impact on their use in winemaking. They deteriorate fairly rapidly and are also sensitive to temperature. Hydrolysis of the ester functions occurs, accompanied by an increase in acidity. After 20 days at 18–20°C, there is a considerable decrease in the esterification number (Figure 1.20). Under experimental conditions, total hydrolysis of a 2% metatartaric acid solution took three months at 23°C and 10 months at 5°C. Consequently, it is necessary to ensure that metatartaric acid solutions for treating wine are prepared just prior to use.

Furthermore, the same phenomenon occurs in wine and is detrimental to the treatment's effectiveness. Ribéreau-Gayon *et al.* (1977) demonstrated that stability in terms of tartrate precipitations may be considered effective for the following lengths of time, depending on temperature:

Several years at 0°C
Over two years at 10–12°C

Table 1.19. Inhibition of potassium bitartrate precipitation by various metatartaric acids (Peynaud and Guimberteau, 1961)

Number	Esterification number	Metatartaric acid added in each tube (in mg)					
		0.4	0.8	1.6	2.4	3.2	4.0
1	40.8	12.0	15.8	17.2	17.2	17.2	17.2
2	38.2	12.0	15.6	17.2	17.2	17.2	17.2
3	37.3	12.0	15.3	17.2	17.2	17.2	17.2
4	33.4	9.6	12.0	16.3	17.0	17.2	17.2
5	31.5	8.6	11.0	15.3	15.9	16.5	17.2
6	26.6	7.9	10.5	12.7	15.0	16.0	17.2
7	22.9	6.4	7.6	11.2	13.6	15.6	16.8

Potassium remaining in solution (in mg) in each tube containing 10 ml of a saturated potassium bitartrate solution. The original amount was 17.2 mg. Only 5 mg of potassium was left in the tube without metatartaric acid.

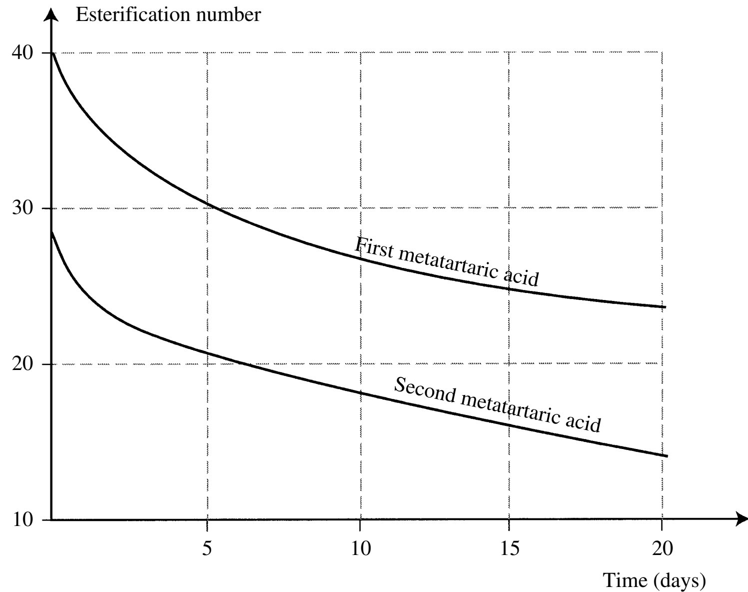


Fig. 1.20. Hydrolysis rate of two qualities of metatartaric acid in 2% solution ($t = 18-20°C$), followed by a decrease in the esterification number (Ribéreau-Gayon *et al.*, 1977)

One year to eighteen months at temperatures varying between 10°C in winter and 18°C in summer
Three months at 20°C
One month at 25°C
One week at 30°C
A few hours between 35 and 40°C

Metatartaric acid instability accounts for initially surprising observations concerning wines treated in this way. One sample, stored at 0°C in a refrigerator, had no precipitation, while calcium tartrate precipitation occurred in another sample stored at 20–25°C when it was no longer protected due to hydrolysis of the metatartaric acid.

The conditions for using metatartaric acid depend on its properties. A concentrated solution, at 200 g/l, should be prepared in cold water at the time of use. As metatartaric acid is strongly hygroscopic, it must be stored in a dry place.

Metatartaric acid is added after fining, as there is a risk of partial elimination due to flocculation. It is particularly affected by bentonite and potassium ferrocyanide treatments. Although there was some cause for concern that high-temperature bottling would reduce the effectiveness of metatartaric acid, in fact, under the actual conditions where it is used, this technique has little or no negative impact (Section 12.2.4). Incidentally, a slight opalescence may be observed after a wine has been treated, especially when the most efficient products, with high esterification numbers, have been used. It is therefore recommended that metatartaric acid be added before the final clarification.

1.7.7 Using Yeast Mannoproteins

It is well known that wine, especially red wine, naturally contains macromolecules that act as protective colloids (Section 9.4.2). At concentrations present in wine, these substances tend to hinder tartrate crystallization, but do not completely inhibit it (Section 3.6.5). Little research has been done into isolating these crystallization inhibitors in wine and making use of their stabilizing properties. On the contrary, for many years, major efforts were made to eliminate these colloids, by drastic fining and filtration, as they reduce the effectiveness

of physical stabilization treatments, especially cold stabilization.

It is known, however, that the traditional practice of barrel-aging white wines on yeast lees for several months often gives them a high level of tartrate stability, so that cold stabilization is unnecessary (Section 12.3.2). Although, in practice, this phenomenon is very widespread, very little mention of it has been made until now in enology theory. Thus, in Bordeaux, most dry white wines aged on the lees are not stable in March after their first winter, but become stable by June or July without any further treatment. When the same wines are not aged on the lees, they must be systematically cold-stabilized to protect them from tartrate crystallization. As it was known that white wines are enriched with mannoproteins released by the yeast during aging on the lees, it was reasonable to suppose that these macromolecules contributed to the tartrate stabilization of wine.

Yeast mannoproteins were first found to have a certain inhibiting effect on tartrate crystallization in a model medium by Lubbers *et al.* (1993). However, these experiments used mannoproteins extracted by heat in alkaline buffers, under very different conditions from those accompanying the spontaneous enzymic release of mannoproteins during aging on the lees. Furthermore, the effectiveness of mannoproteins extracted by physical processes in preventing tartrate precipitation has not been established in most wines, despite demonstrations in a model medium.

The discovery of the crystallization-inhibiting effect of mannoproteins extracted by the enzymic treatment of yeast walls (Dubourdieu and Moine-Ledoux, 1994) adds a new dimension to this subject. The mannoprotein preparations are obtained by digesting yeast walls with an industrial preparation of β-(1–3)- and β-(1–6)-glucanases (Glucanex™), permitted in winemaking as a clarifying enzyme for improving the filtrability of wines made from botrytized grapes (Sections 3.7.2 and 11.5.2). These preparations inhibit tartrate crystallization in white, red and rosé wines, whereas the same dose (25 g/hl) of heat-extracted mannoproteins does not have this stabilizing effect (Moine-Ledoux and Dubourdieu, 1995).

The inhibiting effect of mannoproteins extracted from yeast on tartrate crystallization is not due to compound MP32, the invertase fragment responsible for protein stabilization in wine (Section 5.6.4) (Dubourdieu and Moine-Ledoux, 1996). The mannoproteins in question are more highly glycosylated, with an average molecular weight of approximately 40 kDa. They have been purified (Moine-Ledoux *et al.*, 1997) from the same mannoprotein preparations, obtained by the enzymic treatment of yeast walls.

Furthermore, it has been demonstrated that these mannoproteins share covalent bonds with glucane (Moine-Ledoux and Dubourdieu, 1999). They remain in the cell walls treated simultaneously with sodium dodecyl sulfate (SDS) (which cuts the hydrogen bonds) and β-mercaptoethanol (Figure 1.21), which do not affect osidic bonds.

The presence of peak 2, corresponding to elution of the mannoprotein responsible for tartrate stabilization, confirms that the bond is covalent. Some of the mannoproteins that share covalent bonds with glucane also have a special type of glycosylation, leading to a glycosyl-phosphatidyl-inositol (GPI). The use of a mutant strain (FBYII), deficient in GPI-anchored mannoproteins when cultured at 37°C (FBYII-37), showed that the mannoproteins responsible for tartrate stabilization had this type of glycosylation. Two types of mannoprotein extracts were obtained by enzyme hydrolysis of yeast cell walls (FBYII), cultured at 24°C or 37°C.

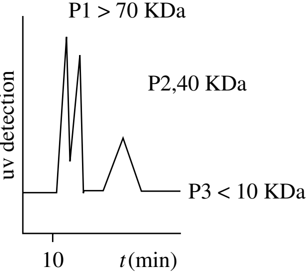


Fig. 1.21. HPLC analysis of molecular-screened mannoprotein extract obtained by enzyme digestion of cell walls treated simultaneously with SDS and β-mercaptoethanol

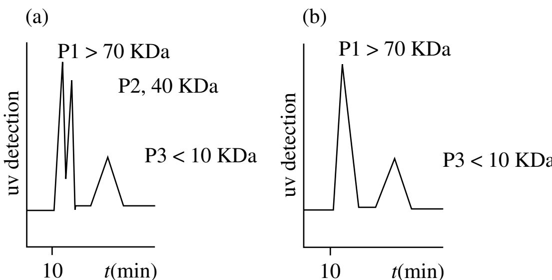


Fig. 1.22. HPLC analysis of molecular-screened mannoprotein extract obtained by enzyme digestion of (a) FBYII-24 and (b) FBYII-37 yeast cell walls, cultured at 24°C and 37°C, respectively

HPLC analysis of these two extracts (Figure 1.22) showed that peak 2 was absent when the cell walls came from yeast cultured at 37°C, i.e. deficient in GPI-anchored mannoproteins. These results: (1) show that the mannoproteins responsible for tartrate stabilization are GPI-anchored and (2) explain why they are only extractible by enzyme digestion.

An industrial preparation (Mannostab™) has been purified from yeast-wall mannoprotein. It is a perfectly soluble, odorless, flavorless, white powder. This product has been quite effective (Table 1.20) in preventing tartrate precipitation in white wine samples taken before the normal cold stabilization prior to bottling. Initial results show that Mannostab™ inhibits potassium bitartrate crystallization at doses between 15 and 25 g/hl. However, in certain wines in Table 1.13 (1996 white Bordeaux and 1996 white Graves), larger quantities apparently reduced the stabilizing effect. A similar phenomenon has been reported with a protective colloid used to prevent protein precipitation (Pellerin *et al.*, 1994). The dose of Mannostab™ necessary to stabilize a wine must be determined by preliminary testing. It is very clear that the use of excess amounts of this additive is inefficient.

The addition of this product could replace current stabilization methods (Moine-Ledoux *et al.*, 1997). With this in mind, its effectiveness has been compared to that of two other tartrate stabilization methods: continuous contact cold stabilization and the addition of metatartaric acid (Table 1.21). This comparison was carried out by measuring spontaneous crystallization after the addition of KHT (Section 1.6.4). The values obtained indicate the effectiveness of protective colloids, even if they do not necessarily correspond to the instability temperatures. The addition of 15 g/hl of Mannostab™ to wine 2 and 25 g/hl

Table 1.20. Tartrate stabilization of various wines by adding Mannostab™. Visual observation of potassium crystallization after 6 days at −4°C (Moine-Ledoux *et al.*, 1997)

Wines		Mannostab™ (g/hl)				
		0	15	20	25	30
1996 Blanc de Blanc	Visual test	[a]	0	0	0	0
	$\Delta(K^+)$ (mg/l)	52	72	17	0	0
White *vin de table*	Visual test	[a]	0	0	0	0
	$\Delta(K^+)$ (mg/l)	104	53	33	0	0
1996 white Bordeaux	Visual test	[a]	0	0	0	0
	$\Delta(K^+)$ (mg/l)	62	21	0	0	21
1996 white Graves	Visual test	[a]	[a]	0	0	0
	$\Delta(K^+)$ (mg/l)	155	52	0	0	62
1996 white Bordeaux	Visual test	[a]	0	0	0	0
	$\Delta(K^+)$ (mg/l)	51	0	0	0	0
1996 Entre Deux Mers	Visual test	0	0	0	0	0
	$\Delta(K^+)$ (mg/l)	52	0	0	0	11

[a] precipitation; 0, no precipitation.

Table 1.21. Effect of different treatments on the spontaneous crystallization temperature of various wines (Moine-Ledoux *et al.*, 1997)

Stabilization treatments	Wine 1	Wine 2
Control	−10°C	−11°C
Mannostab™ (15 g/hl)	−21°C	−18°C
Mannostab™ (25 g/hl)	−31°C	−13°C
Continuous contact cold	−28°C	−17°C
Metatartaric acid (10 g/hl)	−40°C	−40°C

Wine 1, 1996 Entre Deux Mers; Wine 2, 1996 white Bordeaux.

to wine 1 produced the same spontaneous crystallization temperature, i.e. a stability comparable to that obtained by continuous cold stabilization (Table 1.21). The addition of metatartaric acid, however, considerably reduced the crystallization temperature.

However, metatartaric acid is hydrolyzed in wine, and loses its effectiveness, while adding tartaric acid may even facilitate potassium bitartrate crystallization. Under the same conditions, mannoproteins are stable and have a durable protective effect on tartrate crystallization. To demonstrate this difference, white wines treated with metatartaric acid or Mannostab™ and kept at 30°C for 10 weeks were then subjected to a cold test. Crystallization occurred in the sample treated with metatartaric acid, while the Mannostab™ sample remained stable (Table 1.22).

This new treatment process to protect wines from tartrate precipitation has been used experimentally in France since 1997 (Moine-Ledoux and Dubourdieu, 2002). Mannoprotein preparation treatment of white wine is registered in the OIV

Table 1.22. Influence of keeping a white wine supplemented with metatartaric acid or Mannostab™ at 30°C for 10 weeks on the tartrate stability, estimated by the decrease in potassium concentration after 6 days at −4°C (Moine-Ledoux *et al.*, 1997)

	$\Delta(K^+)$ mg/l, after 6 days at −4°C
Control	200
Metatartaric acid (10 g/hl)	260
Mannostab™ (25 g/hl)	0

International Code of Oenological Practice. Their findings are likely to lead to the authorization of this type of treatment in the near future.

1.7.8 The Use of Carboxymethylcellulose

Carboxymethylcellulose (CMC) is a polysaccharide. Like metatartaric acid and mannoproteins, its polymer structure gives it "protective colloid" characteristics. It is obtained by priority etherification of the primary alcohol functions of the glucopyranose units (Figure 1.23) linked by β-type stereochemical 1–4 etheroxide bonds. A CMC is, therefore, characterized partly by the degree of etherification of its alcohol functions, known as the degree of substitution (DS), and partly by its degree of polymerization (DP), i.e. the average number of glucopyranose units per polymer molecule. This mean number indicates that a given CMC, such as metatartaric acid, is a polymer with a dispersed molecular weight.

A DS of 0.65 means that, out of 100 glucopyranose units, 65 have been etherified by sodium

Fig. 1.23. Structure of a carboxymethylcellulose (CMC) chain

$$R - \text{cellulose}\ (OH)_3 + 2Cl - CH_2 - COONa \xrightarrow{2NaOH} R - (OH) - (OCH_2 - COONa)_2 + 2\ NaCl + 2H_2O$$

Fig. 1.24. Formula for the etherification of celluloses (R-$[OH]_3$) by sodium chloroacetate

chloroacetate in an alkaline medium, as shown in the reaction diagram (Figure 1.24).

The DP determines the viscosity of a CMC and increases with molecular weight. The viscosity of a CMC also varies according to the cation—divalent cations (calcium, magnesium, iron, etc.) reduce viscosity. The DP determines the molecular weight, which may vary from 17,000 to 1,500,000 Daltons.

For a CMC with a given DP, the higher its DS, the more cation anchor sites it has, and the more effective it is as a protective colloid (Lubbers *et al.*, 1993).

In the past, CMCs were poorly-defined compounds, with relatively heterogeneous DPs. Their viscosity was unreliable, to the extent that they could modify the viscosity of a wine. The CMCs currently on the market have much more clearly-defined characteristics, and quality control is more effective, resulting in purer products. Minimum purity is 99.5%, with a sodium content between 7 and 8.9%. Viscosity varies from 25,000–50,000 mPa at 25°C, depending on the type of CMC selected, and cannot, therefore, alter the viscosity of the finished beverage.

The production and use of CMCs as a gelatin substitute dates back to the 1940s to 1950s. They are now used in the food and beverage industry (code: E466), at levels up to 10 g/l or 10 g/kg, as well as in cosmetics and pharmaceuticals. The CMC content of alcoholic and non-alcoholic beverages may be as high as 500 mg/l.

Water solubility of CMCs is variable, depending on their degree of substitution and polymerization. They owe their hydrophilic qualities to their highly hydric carbohydrate character. CMCs used in very sweet beverages are less viscous, probably due to the formation of hydrogen bonds between the sugar and the gum. CMC-saccharose interactions depend on the order in which the products are added: if the sugar is dissolved in the water first, its hydrophilic character reduces the solubility of CMC (Federson and Thorp, 1993). This should be taken into account in preparing the concentrated CMC solutions (20–40 g/l) used to treat beverages, such as wine, that require a restricted addition of water (0.05–0.1 l/l).

CMCs are also reputed to promote solubilization of proteins and stabilize solutions containing them (Federson and Thorp, 1993). This property is useful in winemaking for the purpose of preventing protein casse. These CMC–protein interactions may be compared to the carbohydrate–protein association in glycoproteins and yeast mannoproteins.

CMCs are available in the form of powder or white granules. As these absorb humidity from the air, they must be stored in a dry place. They are not yet authorized for use in winemaking in the EU but an application is pending. Recent results, indicating that low-viscosity CMCs are effective in preventing tartrate crystallization at doses 12–250 times lower than those currently used in the food industry (Crachereau *et al.*, 2001), should lead to an authorization in the near future. A dose of 2 g/hl is often ineffective, but good results have been obtained in wines supersaturated with potassium bitartrate without exceeding 4 g/hl. Details of the results are given in Table 1.23 and Figure 1.25.

These results demonstrate comparable effectiveness for metatartaric acid (10 g/hl) and CMC (4 g/hl). Furthermore, a comparison of the stability and effectiveness of these two additives, following prolonged heat treatment at 55–60°C for 5–30 days and one month at −4°C, showed that CMC was perfectly stable. It was still perfectly effective, whereas wine treated with metatartaric acid became totally unstable after only 5 days at 55–60°C (Peynaud and Guimberteau, 1961; Ribéreau-Gayon *et al.*, 1977).

The effectiveness of CMC is due to its property of significantly reducing the growth rate of crystals: a dose of 2 mg/l reduces crystal growth by a

Table 1.23. Treating various wines with CMC (Results after 1 month at −4°C; see Figure 1.25)

Wine treated	Dose of CMC used	Comments
Red A.O.C. Bordeaux	2 g/hl	Unfiltered
Red A.O.C. Buzet	4 g/hl	Filtered prior to treatment
White A.O.C. Bordeaux	4 g/hl	Fined, treated with CMC, then filtered
White *vin de pays* (Gers)	4 g/hl	Fined, treated with CMC, then filtered
White *vin de pays* (Loire)	4 g/hl	Fined, treated with CMC, then filtered
Sparkling wine (Gers)	4 g/hl	Treated prior to second fermentation

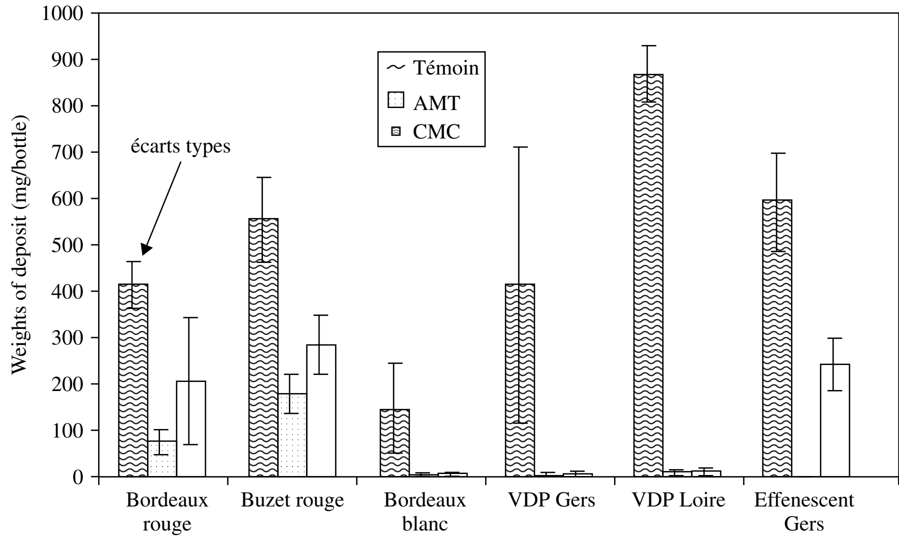


Fig. 1.25. Comparison of the effectiveness of metatartaric acid and carboxymethylcellulose on turbidity due to tartrate crystals (Crachereau *et al.*, 2001) (See Table 1.23 for treatment conditions)

ratio of 7 (Gerbaud, 1996). CMC also modifies the shape of potassium bitartrate crystals.

In the case of wines destined for a second fermentation, three different CMCs produced more stable, persistent bead. Only the CMC with the highest molecular weight caused a slight increase in bubble size. A similar inhibition of crystallization has also been observed in champagne-base wines (Maujean, 1997).

All these positive results, combined with the fact that they are easy to use, relatively inexpensive, and do not require special investments, should lead to their authorization for use in winemaking in the very near future, as is already the case in the food and beverage industry. Further research is required to assess the effectiveness in different types of wine, especially tannic red wines, which have a particularly complex colloidal structure.

(See Table 1.23 for treatment conditions)

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