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Maurício Bonatto Machado de Castilhos *Editor*

# Basic Protocols in Enology and Winemaking

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# Basic Protocols in Enology and Winemaking

Edited by

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# Preface

Welcome to the "Basic Protocols in Enology and Winemaking." This book provides valuable information regarding technical and analytical methods applied in wine analysis worldwide. The book presents 14 chapters and each one is dedicated to a punctual wine chemical property. Each chapter provides insights into traditional and advanced methods used for major and minor component wine analysis, the latter quantitated at trace levels. This book aims to facilitate wine analysis through a set of pre-established analyses that promote safe, accurate, and precise results. All methods are based on established literature and can be relied upon for any wine type.

The principal subject of this book is centered on methods using classical apparatus and mechanisms such as titration, distillation, spectrophotometry, and advanced methods applying high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MSn), gas chromatography coupled with mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR). It is a set of easy-to-read and easy-to-understand analyses, making it an ideal tool for analysts who need to perform more accurate and precise analyses for their projects and experiments. The central subject of each chapter is described as follows:

Chapter 1, "Total and Volatile Acidity: Traditional and Advanced Methods," written by Brazilian researchers, begins with a presentation of wine acidity and all the acids responsible for total and volatile acidity. This chapter brings methods using potentiometric titration, distillation, chemical indicators, and simple reagents.

Chapter 2, "Alcohol Content: Traditional and Advanced Methods," written by researchers from Portugal begins with an introduction regarding the alcohols found in wine, highlighting ethanol, which is the most relevant alcohol in the wine matrix. The authors reported methods using ebulliometry, gas chromatography, liquid chromatography, enzymatic assays, and infrared spectroscopy, among others.

Chapter 3, "Total and Reducing Sugars: Traditional and Advanced Methods," written by Brazilian researchers, presents methodologies using refractometry and hydrometer, chemical and enzymatic methods, liquid chromatography (HPLC), and information regarding other methods concerning the identification and quantitation of sugars in wines.

Chapter 4, "Total Phenolic Content: Traditional Methods," written by Brazilian researchers, presents information about the importance of phenolic compounds identification and quantitation in wines since these chemical substances provide health benefits to consumers. The chapter shows the classical spectrophotometric methods and their variations for comprehensive analysis.

Chapter 5, "Color Indexes: Traditional and Advanced Methods," written by researchers from Italy and Chile, contains a brief description of the importance of wine color for quality and sensory appeal, showing the modus operandi to identify and quantitate the wine color indexes using spectrophotometry and the well-known CIELab space methodology.

Chapter 6, "Anthocyanin Identification and Quantitation by High-Performance Liquid Chromatography Coupled with Mass Spectrometry (HPLC-$MS^n$)," written in partnership between researchers from Brazil and Spain, reports the importance of anthocyanins for red wines since these compounds to respond for wine appearance and antioxidant activity. The

chapter brings methods using the spectrophotometry approach and HPLC-MSn methodology with high precision and accuracy for identifying and quantitating these minor compounds that have crucial importance for wine analysis.

Chapter 7, "Flavonol Identification and Quantitation by High-Performance Liquid Chromatography Coupled with Mass Spectrometry (HPLC-$MS^n$)," written by Spanish researchers, provides information regarding the flavonols' chemical structure and their importance for wine color due to their copigmentation effect, also related with sensory properties such as bitterness, astringency and color intensity of young red wines. The authors report methods based on the HPLC-$MS^n$ approach, bringing detailed information concerning the application of this method.

Chapter 8, "Flavan-3-ol (Flavanol) Identification and Quantitation by High-Performance Liquid Chromatography Coupled with Mass Spectrometry (HPLC-$MS^n$)," written by Brazilian researchers, contains information regarding the chemical structure of flavan-3-ols and their contribution to wine antioxidant capacity and sensory properties such as bitterness and astringency. The authors reported detailed information regarding the use of HPLC coupled with mass spectrometry for accurate wine analysis.

Chapter 9, "Hydroxybenzoic and Hydroxycinnamic Acid Derivatives (HCAD) Identification and Quantitation by High-Performance Liquid Chromatography Coupled with Mass Spectrometry (HPLC-$MS^n$)," written by Spanish researchers, provides valuable information regarding the phenolic acids and their contribution to wine chemistry. The authors described, rich in detail, the protocol to perform an HPLC-$MS^n$ analysis for phenolic acids identification and quantitation.

Chapter 10, "Stilbene Identification and Quantitation by High-Performance Liquid Chromatography Coupled with Mass Spectrometry (HPLC-MS)," written by researchers from Uruguay, brings information regarding the stilbenes and their high antioxidant activity in wines. The authors informed details of HPLC-MS application for stilbenes identification and quantitation.

Chapter 11, "Analysis of the Free and Bound Fraction of Volatile Compounds in Musts and Wines by GC/MS: Results Interpretation from the Sensory Point of View by OAV Technique," written by Spanish researchers, reports the importance of volatile compounds for wine aroma. They explained that the concentration of volatile compounds needs to be compared with the odor activity values (OAV) to observe which volatile compounds are responsible for wine aroma quality. The method using gas chromatography coupled with mass spectrometry (GC-MS) is the most used for this analysis.

Chapter 12, "Identification of Wine Compounds by Nuclear Magnetic Resonance," written by Brazilian researchers, gives valuable information about the wine components that can be identified and quantitated using the advanced method of nuclear magnetic resonance (NMR). The use of NMR for wine components is recent, and this chapter is extremely useful for analysts who have an interest in using the NMR approach to identify and quantitate all the wine components even those with lower concentrations.

Chapter 13, "Ethanol Suppression on Wine Analysis Using Nuclear Magnetic Resonance (NMR)," written by Brazilian researchers, is a continuation of Chap. 12 since ethanol suppression is mandatory for wine analysis. The ethanol must be suppressed for NMR wine analysis due to its higher concentration which provides a huge peak in the NMR spectra, hindering the identification of the other wine chemical compounds.

Chapter 14, "Methods to Determine Biogenic Amines in Wine by RP-HPLC," written by researchers from Portugal, reports the importance of identifying and quantitating biogenic amines in wines since they can cause beverage safety problems due to their high toxicity for humans. The authors reported an advanced method using RP-HPLC for identifying and quantitating these compounds with precision and accuracy.

The book "Basic Protocols in Enology and Winemaking" is considered an analytical guide for wine researchers and analysts to facilitate the laboratory routine and deliver simple and advanced methods that can provide results with high precision, accuracy, and repeatability.

I hope you appreciate the content of this book. Enjoy the moment and read without moderation!

Cheers!

*Frutal, Brazil* *Maurício Bonatto Machado de Castilhos*

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# Chapter 1

# Total and Volatile Acidity: Traditional and Advanced Methods

Lia Lucia Sabino and Maurício Bonatto Machado de Castilhos

## Abstract

One of the most relevant wine sensory attributes is acidity, which is also considered a parameter of the quality and microbiological stability of the beverage. The acidity is represented by different organic acids synthesized directly from the grape or resulted from the alcoholic fermentation process. The acidity assessment at various stages of winemaking is crucial to ensure a final product with high quality. Different procedures ranging from conventional titrations to alternative methods are applied for the acid profile determination. This chapter explains the principal methods to perform the total and volatile acidity in wines, describing the methodology and discussing their advantages.

**Key words** Total acidity, Volatile acidity, Organic acids, Titration, pH, HPLC, Microbial stability

## 1 Introduction

Considering the incessant search for obtaining the maximum quality results in wine production throughout the history of winemaking, innovative practices and technologies have been used [1], a premise used primarily regarding the determination of acidity since it is an attribute that plays a crucial role in the area of enology. Wine acidity is considered a parameter that affects the sensory properties and quality of wines, helping analyze the microbiological stability through the volatile acidity parameter [2].

Acidity is considered one of the most essential sensory attributes in wine, the sensation caused by this component derives primarily from the mixture of organic acids transferred from the grape pulp to the wine during the winemaking process [3], the acids formed during and after the alcoholic fermentation [4], and the acids resulting from the malolactic fermentation process that can occur spontaneously with the participation of lactic acid bacteria (LAB), naturally present in grapes, or be induced by commercial starter cultures [5]. The LAB degrades malic acid into lactic acid,

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metabolizing other substances, such as sugars, citric acid, and amino acids, into substances that may be undesirable, such as acetic acid [6].

All acids present in wines are classified into two categories: the fixed acidity, represented by tartaric, malic, lactic, succinic, and citric acids, with tartaric and malic acids representing about 90% of the wines' fixed acidity; and the volatile acidity represented by compounds such as acetic, formic, butyric, propionic, and fatty acids with chains longer than 12 carbons [7]. The total acidity is determined by the sum of the fixed and volatile acidity [8].

Tartaric acid, representative of fixed acidity, plays a crucial role in the acidity stability and the beverage's sensory quality, especially in the perception of astringency [3]. Acetic acid, the principal representative of the volatile acidity, works as an indicator of wine microbial stability and sanity, that is, high volatile acidity values are a wine microbial spoilage indicative, primarily from acetic acid bacteria, causing the off-flavor of acetic acid in wine. The production of acetic acid is also linked to the contamination of the fruit or must by acetic bacteria, resulting from the oxidation of the wine [8], and consequently its production is carefully monitored and controlled throughout the winemaking process [9].

The total and volatile acidity in wine can be determined using different procedures ranging from conventional titrations to alternative and advanced methods (Table 1).

**Table 1**
**Conventional and alternative methods for the total and volatile acidity determination**

| | Conventional Methods | Alternative Methods |
|---|---|---|
| Total acidity | Titration using standard alkaline solution with bromothymol blue indicator (OIV-MA-AS313-01) [13] | FTIR spectroscopy—Interferometer WineScan FT 120 [10] |
| | Titration using standard alkaline solution and pH meter (IAL 235/IV) [14] | Sequential injection analysis (SIA) with spectrophotometric detection [4] |
| | | High-performed liquid chromatography (HPLC) [11] |
| Volatile acidity | Titration of wine distillate obtained from distillation process with alkaline solution (OIV-MA-AS313-02) [13] | Sequential injection analysis (SIA) with spectrophotometric detection [4] |
| | Titration of wine distillate obtained from steam distillation process with alkaline solution (IAL 236/IV) [14] | FTIR spectroscopy—Interferometer WineScan FT 120 [12] |

## 2 Methods

### *2.1 Total Acidity by International Organization of Vine and Wine (OIV-MA-AS313-01) [13]*

The wine total acidity is the sum of its titratable acidities when it is titrated to pH 7.0 using a standard alkaline solution. Carbon dioxide is disregarded in the total acidity analysis. The principle of this method is based on a potentiometric titration or titration with bromothymol blue as chemical indicator and comparison with an end-point color standard.

#### *2.1.1 Chemicals*

1. Buffer solution pH 7,0 (*see* **Note 1**).
2. Sodium hydroxide solution, NaOH, 0.1 mol/L.
3. Bromothymol blue indicator solution, 4 g/L (*see* **Note 2**).

#### *2.1.2 Apparatus*

1. Water vacuum pump.
2. Vacuum flask 500 mL.
3. Potentiometer with scale graduated in pH values and electrodes. The glass electrode must be kept in distilled water. The calomel/saturated potassium chloride electrode must be kept in a saturated potassium chloride solution.
4. Beakers.

#### *2.1.3 Procedure*

Sample Preparation

1. Elimination of the carbon dioxide (if existent): Place approximately 50 mL of wine in a vacuum flask and apply vacuum to the flask using a water pump for 1–2 min, while shaking continuously. Other $CO_2$ elimination systems may be used if the $CO_2$ elimination is guaranteed.

Potentiometric Titration

1. Calibration of the pH meter: The pH meter is calibrated for use at 20 °C, according to the manufacturer's instructions, with the pH 7.0 buffer solution at 20 °C.

Measurement: Into a beaker, introduce a volume of the sample, prepared as described in Subheading 2.1.3.1, equal to 10 mL in the case of wine and 50 mL in the case of rectified concentrated grape must. Add about 10 mL of distilled water and then add sodium hydroxide solution, 0.1 mol/L, from a burette until the pH is equal to 7.0 at 20 °C. The sodium hydroxide must be added slowly and the solution stirred continuously. Let $n$ mL be the volume of sodium hydroxide, 0.1 mol/L, added.

Titration with Indicator (Bromothymol Blue)

1. Preliminary test for end-point color determination: Into a beaker, place 25 mL of boiled distilled water, 1 mL of bromothymol blue solution, and a volume prepared as in Subheading 2.1.3.1 equal to 10 mL in the case of wine, and 50 mL in the case of rectified grape concentrated must. Add sodium hydroxide solution, 0.1 mol/L, until the color changes to blue-green. Then add 5 mL of the pH 7.0 buffer solution.

2. Measurement: Into a beaker place 30 mL of boiled distilled water, 1 mL of bromothymol blue solution, and a volume of the sample prepared as described in Subheading 2.1.3.1to 10 mL in the case of wine and 50 mL in the case of rectified grape concentrated must. Add sodium hydroxide solution, 0.1 mol/L, until the same color is obtained as in the preliminary test above. Let *n* mL be the volume of sodium hydroxide solution, 0.1 mol/L, added.

#### 2.1.4 Calculation

The total acidity expressed in milliequivalents per liter is given by:

1. A = 10 **n**. Data expressed with one decimal place.
2. The total acidity expressed in grams of tartaric acid per liter is given by: A' = 0.075 × A. Data expressed with two decimal places.
3. The total acidity expressed in grams of sulfuric acid per liter is given by: A' = 0.049 × A. Data expressed with two decimal places.

### 2.2 Total Acidity by Adolfo Lutz Institute (IAL 235/IV) [14]

This method is based on the neutralization of acids using titration with standardized alkali solution, using a phenolphthalein indicator for white and rosé wines or with the pH meter for red wines.

#### 2.2.1 Chemicals

1. Sodium hydroxide solution 0.1 N.
2. Phenolphthalein solution.

#### 2.2.2 Apparatus

1. pH meter.
2. Magnetic shaker.
3. Magnetic stirring bar.
4. Volumetric pipette 10 mL.
5. Erlenmeyer flask 250 mL.
6. Beaker 250 mL.
7. Burette 25 mL.
8. Graduated pipette 1 mL.

#### 2.2.3 Procedure

With the graduated pipette, take 10 mL of the decarbonated wine into a 250 mL Erlenmeyer flask containing 100 mL of water. Add 0.5 mL of phenolphthalein and titrate with standardized sodium hydroxide solution until persistent pink coloration (for white and rosé wines) or transfer the sample to a beaker and titrate it to the turning point (pH 8.2–8.4) using a pH meter (for red wines).

#### 2.2.4 Calculation

The total acidity expressed in milliequivalents per liter is given by:

$$\text{Total acidity}\left(\frac{\text{mEq}}{\text{L}}\right) = \frac{n.f.N.1000}{V}$$

$n$: volume in mL of sodium hydroxide solution spent in the titration.

$f$: correction factor (standardization) of the sodium hydroxide solution.

$N$: concentration of the sodium hydroxide solution

$V$: sample volume

### 2.3 Volatile Acidity by International Organization of Vine and Wine (OIV-MA-AS313-02) [13]

The volatile acidity is determined by the volatile acids of the acetic series present in wine in the free state and combined as salts. Carbon dioxide is first removed from the wine. Volatile acids are separated from the wine by steam distillation and titrated using standard sodium hydroxide. The acidity of free and combined sulfur dioxide distilled under these conditions should be subtracted from the acidity of the distillate. The acidity of any sorbic acid, which may have been added to the wine, must also be subtracted (*see* **Note 3**).

#### 2.3.1 Chemicals

1. Tartaric acid, crystalline.
2. Sodium hydroxide solution 0.1 M.
3. Phenolphthalein solution 1% in neutral alcohol 96% (m/v).
4. Hydrochloric acid (U20 = 1.18–1.19 g/mL) diluted 1/4 with distilled water.
5. Iodine solution 0.005 M.
6. Potassium iodide crystalline.
7. Starch solution 5 g/L (*see* **Note 4**).
8. Saturated solution of sodium tetraborate, Na2B4O7.10H2O about 55 g/L at 20 °C.
9. Acetic acid 0.1 M.
10. Lactic acid solution 0.1 M (*see* **Note 5**).

#### 2.3.2 Apparatus

1. Steam distillation apparatus consisting of: a steam generator (the steam must be free of carbon dioxide), a flask with steam pipe, a distillation column, a condenser (*see* **Note 6**).
2. Water aspirator vacuum pump.
3. Vacuum flask.

#### 2.3.3 Procedure

1. Eliminate the sample carbon dioxide placing about 50 mL of wine in a vacuum flask.
2. Apply vacuum to the flask with the water pump for 1–2 min while shaking continuously. Other $CO_2$ elimination systems may be used if the $CO_2$ elimination is guaranteed.

3. Place 20 mL of wine, free from carbon dioxide, into the flask. Add about 0.5 g of tartaric acid. Collect at least 250 mL of the distillate.
4. Titrate with the sodium hydroxide solution using two drops of phenolphthalein as indicator. Let $n$ mL be the volume of sodium hydroxide used.
5. Add four drops of the dilute hydrochloric acid, 2 mL starch solution and a few crystals of potassium iodide.
6. Titrate the free sulfur dioxide with the iodine solution 0.005 M.
7. Let $n^*$ mL be the volume used.
8. Add the saturated sodium tetraborate solution until the pink coloration reappears.
9. Titrate the combined sulfur dioxide with the iodine solution 0.005 M. Let $n^{**}$ mL be the volume used.

#### 2.3.4 Calculation

The volatile acidity, expressed in milliequivalents per liter, is given by (with one decimal place):

$$5\ (n - 0.1\ n^* - 0.05\ n^{**})$$

The volatile acidity, expressed in grams of sulfuric acid per liter, is given by (with two decimal places):

$$0.245\ (n - 0.1\ n^* - 0.05\ n^{**})$$

The volatile acidity, expressed in grams of acetic acid per liter, is given by (with two decimal places):

$$0.300\ (n - 0.1\ n^* - 0.05\ n^{**})$$

### 2.4 Volatile Acidity by Adolfo Lutz Institute (IAL 236/IV) [14]

This method determines the volatile titratable acidity of wines and other fermented beverages by volumetry after steam distillation.

#### 2.4.1 Chemicals

The chemicals used in this method are the same as in Subheading 2.2.

#### 2.4.2 Apparatus

1. Electric hotplate.
2. 10 mL volumetric pipette.
3. Cazenave-Ferré distillation apparatus or similar assembly.
4. Steam generator.
5. Erlenmeyer flasks 250 and 500 mL.
6. Liebig's or serpentine condenser.
7. Burette 10 mL.
8. Pipette 1 mL.