

Jean L. Jacobson

# Introduction to Wine Laboratory Practices and Procedures

With 50 Figures, Including 2 Color Plates

 Springer

# Preface

In the beginning, for me, winemaking was a romanticized notion of putting grape juice into a barrel and allowing time to perform its magic as you sat on the veranda watching the sunset on a Tuscan landscape. For some small wineries, this notion might still ring true, but for the majority of wineries commercially producing quality wines, the reality of winemaking is far more complex.

The persistent evolution of the wine industry demands continual advancements in technology and education to sustain and promote quality winemaking. The sciences of viticulture, enology, and wine chemistry are becoming more intricate and sophisticated each year. Wine laboratories have become an integral part of the winemaking process, necessitating a knowledgeable staff possessing a multitude of skills. Science incorporates the tools that new-age winemakers are utilizing to produce some of the best wines ever made in this multibillion dollar trade.

A novice to enology and wine chemistry can find these subjects daunting and intimidating. Whether you are a home winemaker, a new winemaker, an enology student, or a beginning-to-intermediate laboratory technician, putting all the pieces together can take time. As a winemaker friend once told me, “winemaking is a moving target.”

*Introduction to Wine Laboratory Practices and Procedures* was written for the multitude of people entering the wine industry and those that wish to learn about wine chemistry and enology. It is a guide to understanding basic enology, wine chemistry, safety, quality control, wine history, winemaking processes, bottling, and analytical procedures. Areas of study are applicable to the majority of wine laboratories in the world. The reader will gain a firm basic working knowledge of the wine laboratory and enology upon which to build a greater understanding of more advanced theory and technical information.

Make wine with honor.

Healdsburg, California

Jean L. Jacobson

# Acknowledgments

With great appreciation, I would like to thank the people who laboriously read chapters and supplied feedback: Marcia Manix, Sharon Dougherty, Cathy Jacobson, Dr. Les Nouget, Joyce Nouget, Dr. Will Brogdon, Christine Brogdon, Jeralyn Heath, Joanne Foote-Lynch, and Galen McCorkle.

Thank you to Kendall-Jackson Wine Estates for your cooperation in this project. I would also like to thank the manufacturers, governmental agencies, and wineries that provided photographs and information used in this book.

# Contents

<b>Preface</b>	<b>vii</b>
<b>Color Insert</b>	<b>facing page 46</b>
<b>1 Chemistry to Remember</b>	<b>1</b>
1.1 Introduction . . . . .	1
1.2 Metric System . . . . .	1
1.3 Density and Specific Gravity . . . . .	1
1.4 Liquids, Solids, and Gases . . . . .	3
1.4.1 Pressure and Temperature . . . . .	4
1.4.2 Liquids . . . . .	4
1.4.3 Gases . . . . .	5
1.5 Chemistry Fundamentals . . . . .	5
1.5.1 Matter . . . . .	5
1.5.2 Structure . . . . .	6
1.5.3 Periodic Table . . . . .	6
1.5.4 Compounds . . . . .	6
1.5.5 Solutions . . . . .	7
1.5.6 Electrolyte Solutions . . . . .	11
1.5.7 pH . . . . .	12
1.5.8 Oxidation: Reduction and Electrolysis . . . . .	12
1.5.9 Halogens . . . . .	13
1.5.10 Sulfur . . . . .	13
1.6 Organic Chemistry . . . . .	13
<b>2 Safety First</b>	<b>18</b>
2.1 Introduction . . . . .	18
2.2 Regulatory Agencies and Acts . . . . .	18
2.3 Laboratory and Winery Hazards . . . . .	20
2.4 Chemical Hazards . . . . .	21
2.4.1 Toxic Chemicals . . . . .	21
2.4.1.1 Dose, Duration, Frequency, and Routes of Exposure . . . . .	21
2.4.1.2 Classification of Toxins . . . . .	23
2.4.2 Reactive Chemical Hazards . . . . .	24
2.4.3 Chemical Handling and Storage . . . . .	25
2.4.3.1 Ordering and Receiving . . . . .	27
2.4.3.2 Proper Labeling . . . . .	28
2.4.3.3 Proper Storage . . . . .	29
2.4.4 Chemical Waste . . . . .	31
2.4.4.1 Classification . . . . .	31
2.4.4.2 Waste Disposal . . . . .	32
2.4.5 Personal Protection Equipment . . . . .	35
2.4.6 Chemical Accidents and Emergencies . . . . .	37

2.4.6.1	Spills . . . . .	37
2.4.6.2	Accidental Inhalation or Ingestion . . . . .	39
2.4.6.3	Fire . . . . .	40
2.4.6.4	Gas Cylinder Leaks . . . . .	41
2.5	Physical Hazards . . . . .	42
2.5.1	Physical Hazards Associated with Laboratory Work . . . . .	42
2.5.1.1	Equipment Hazards . . . . .	42
2.5.1.2	Compressed and Liquid Gas Hazards . . . . .	44
2.5.1.3	Vacuum Hazards . . . . .	45
2.5.1.4	Glassware Hazards . . . . .	46
2.5.1.5	Slip, Trip, and Fall Hazards . . . . .	46
2.5.1.6	Ergonomic Hazards . . . . .	47
2.5.2	Physical Hazards in the Cellar . . . . .	49
2.5.3	Damage Control . . . . .	52
<b>3</b>	<b>What's Your Number?</b>	<b>54</b>
3.1	Introduction . . . . .	54
3.2	Quality . . . . .	56
3.2.1	Setting up a QA Program . . . . .	56
3.2.2	Laboratory Staff . . . . .	57
3.2.3	Analytical Methods . . . . .	58
3.2.3.1	Method Validation . . . . .	59
3.2.3.2	Reagents, Standards, and Controls . . . . .	64
3.2.4	Analytical Techniques . . . . .	66
3.2.4.1	Calculations . . . . .	66
3.2.4.2	Proper Measurement . . . . .	70
3.2.4.3	Sample Quality . . . . .	77
3.2.4.4	Preventative Maintenance . . . . .	78
3.2.5	Records . . . . .	80
3.2.6	Inadvertent Error . . . . .	82
<b>4</b>	<b>Berry to Bottle</b>	<b>83</b>
4.1	Introduction . . . . .	83
4.2	Wine History . . . . .	83
4.3	Grape Varieties . . . . .	87
4.4	Appellations . . . . .	88
4.4.1	France . . . . .	89
4.4.1.1	Burgundy . . . . .	89
4.4.1.2	Bordeaux . . . . .	91
4.4.1.3	Rhône . . . . .	92
4.4.1.4	Loire . . . . .	93
4.4.1.5	Champagne . . . . .	93
4.4.1.6	Languedoc-Roussillon . . . . .	93
4.4.1.7	Provence . . . . .	93
4.4.2	Italy . . . . .	94
4.4.2.1	Piedmont . . . . .	94
4.4.2.2	Tuscany . . . . .	94
4.4.2.3	Emilia Romagna-Veneto . . . . .	96
4.4.3	Spain . . . . .	96
4.4.3.1	Rioja-Penedés-Cava . . . . .	96
4.4.3.2	Jerez . . . . .	97
4.4.4	Portugal . . . . .	97
4.4.4.1	Douro . . . . .	97
4.4.4.2	Vinhos Verdes . . . . .	98

4.4.4.3	Madeira . . . . .	98
4.4.5	Germany . . . . .	98
4.4.6	South Africa . . . . .	99
4.4.7	Chile . . . . .	101
4.4.8	Argentina . . . . .	101
4.4.9	Australia . . . . .	101
4.4.10	New Zealand . . . . .	102
4.4.11	United States . . . . .	102
4.4.11.1	New York . . . . .	103
4.4.11.2	Washington . . . . .	103
4.4.11.3	Oregon . . . . .	105
4.4.11.4	California . . . . .	105
4.5	Wine Production Overview . . . . .	105
4.5.1	Vineyard . . . . .	106
4.5.2	Harvest . . . . .	106
4.5.3	Fermentation . . . . .	108
4.5.4	Maturation . . . . .	112
4.5.5	Blending, Fining, and Stabilization . . . . .	113
4.5.6	Filtration and Bottling . . . . .	113
4.5.7	Sparkling Wine . . . . .	114
4.5.8	Money Matters . . . . .	117
<b>5</b>	<b>Vineyard to Harvest</b>	<b>118</b>
5.1	Introduction . . . . .	118
5.2	The Grape . . . . .	118
5.2.1	Mature Grape Physiology . . . . .	119
5.2.1.1	Organic Acids . . . . .	119
5.2.1.2	Alkaline Metals . . . . .	120
5.2.1.3	Nitrogen Compounds . . . . .	120
5.2.1.4	Phenolic Compounds . . . . .	120
5.2.1.5	Aromatic Substances . . . . .	121
5.2.1.6	Sugar . . . . .	122
5.2.2	Grape Structure . . . . .	123
5.2.2.1	Seeds . . . . .	123
5.2.2.2	Skin . . . . .	123
5.2.2.3	Pulp . . . . .	124
5.3	Preharvest . . . . .	124
5.3.1	Vineyard Sampling . . . . .	125
5.3.2	Sugar Determinations . . . . .	126
5.3.2.1	°Oechsle . . . . .	129
5.3.2.2	Baumé . . . . .	129
5.3.2.3	°Balling and °Brix . . . . .	130
5.3.3	Glucose and Fructose Determinations . . . . .	131
5.3.3.1	Enzymatic Method . . . . .	132
5.3.3.2	Other Residual Reducing Sugar Methods . . . . .	133
5.3.4	Potential Alcohol (Ethanol) Determinations . . . . .	134
5.3.5	Alcohol (Ethanol) Determinations . . . . .	137
5.3.5.1	Ebulliometry Method . . . . .	138
5.3.5.2	Other Alcohol Determination Methods . . . . .	140
5.3.6	pH and Acidity Determinations . . . . .	141
5.3.6.1	pH . . . . .	142
5.3.6.2	Titratable Acidity . . . . .	143
5.3.7	Potassium, Calcium, and Other Alkaline Metals Determinations . . . . .	145
5.3.8	Total Phenols and Anthocyanin Determinations . . . . .	146

5.4	Harvest . . . . .	148
5.4.1	Nitrogen and Amino Acid Determinations . . . . .	150
5.4.2	Insoluble Solids Determinations . . . . .	151
5.4.3	Total, Free, and Bound Sulfur Dioxide Determinations . . . . .	152
5.4.3.1	Forms of Sulfur Dioxide . . . . .	153
5.4.3.2	Maintaining SO <sub>2</sub> Levels . . . . .	155
5.4.3.3	Aeration–Oxidation Method . . . . .	157
5.4.3.4	Ripper Method . . . . .	158
5.4.3.5	Other Methods . . . . .	160
5.5	Summary . . . . .	161
<b>6</b>	<b>Fermentation × 2</b> . . . . .	<b>162</b>
6.1	Introduction . . . . .	162
6.2	Preparation for Fermentation . . . . .	163
6.2.1	Sugar Adjustments . . . . .	164
6.2.2	Acid Adjustments . . . . .	165
6.2.2.1	Acid Additions . . . . .	165
6.2.2.2	Deacidification . . . . .	166
6.3	Baseline Analyses . . . . .	166
6.3.1	Volatile Acidity and Acetic Acid Determinations . . . . .	167
6.3.1.1	Acetic Acid Enzymatic Method . . . . .	168
6.3.1.2	Volatile Acidity Distillation Method . . . . .	169
6.4	Primary Alcohol Fermentation . . . . .	173
6.4.1	Yeast Metabolism . . . . .	174
6.4.2	Cultured Yeast and Inoculation . . . . .	177
6.4.3	Monitoring Fermentation . . . . .	180
6.4.3.1	Soluble Solids (Apparent °Brix) via Hydrometer . . . . .	181
6.4.3.2	Soluble Solids (Apparent °Brix) via Density Meter . . . . .	186
6.4.3.3	Verification of Dryness . . . . .	187
6.5	Secondary Malolactic Fermentation . . . . .	188
6.5.1	Taxonomy and Morphology of Wine Microorganisms . . . . .	190
6.5.1.1	Lactic Acid Bacteria . . . . .	190
6.5.1.2	Yeasts . . . . .	191
6.5.1.3	Spoilage Bacterium . . . . .	192
6.5.1.4	Other Methods of Identification . . . . .	192
6.5.2	Prefermentation Analyses . . . . .	193
6.5.3	<i>Oenococcus oeni</i> Culture Preparation . . . . .	193
6.5.3.1	Culture Buildup . . . . .	194
6.5.4	Monitoring MLF . . . . .	196
6.5.5	L-Malic Acid Determinations . . . . .	196
6.5.5.1	Paper Chromatography . . . . .	197
6.5.5.2	Thin-Layer Chromatography . . . . .	197
6.5.5.3	Capillary Electrophoresis . . . . .	197
6.5.5.4	Enzymatic Method . . . . .	198
6.5.6	Cessation of Fermentation . . . . .	198
<b>7</b>	<b>Maturation Matters</b> . . . . .	<b>200</b>
7.1	Introduction . . . . .	200
7.2	Maturation and Aging . . . . .	201
7.2.1	Advantages of Maturation . . . . .	201
7.3	Removal of Substances . . . . .	202
7.4	Storage Containers . . . . .	203
7.4.1	Cooperage . . . . .	203

7.4.1.1	Wood	204
7.4.1.2	Barrel Construction	204
7.5	Protecting the Wine	205
7.5.1	Oxidation	206
7.5.1.1	Oxygen and Wine Color	207
7.5.1.2	Formation of Acetaldehyde	208
7.5.2	Microbial Spoilage	208
7.5.2.1	Acetic Acid Bacterial Spoilage	208
7.5.2.2	Lactic Acid Bacterial Spoilage	209
7.5.2.3	Yeast Spoilage	210
7.5.2.4	Conclusion	212
7.6	Maintenance of Maturing Wines	212
7.6.1	Barrel Storage	213
7.6.1.1	Topping	213
7.6.1.2	Racking	213
7.6.1.3	Cellar Temperature	214
7.7	Organoleptic Evaluation	214
7.7.1	Organoleptic Terminology	215
7.7.2	Wine Sensory	216
7.7.2.1	Flavor Identification	216
7.8	Fining	216
7.8.1	Bentonite Fining	220
7.8.2	Albumen Fining	222
7.8.3	Gelatin and Isinglass Fining	222
7.8.4	Copper Sulfate Fining	223
7.9	Wine Stability	224
7.9.1	Freeze Test	225
7.9.2	Cold Conductivity	226
7.10	Filtration	227
7.11	Blending	229
7.11.1	Fractional Blending	230

## **8 Bottling Basics 231**

8.1	Introduction	231
8.2	Packaging	231
8.2.1	Labels	232
8.2.2	Bottles	233
8.2.3	Cork	233
8.2.3.1	Cork Quality Control	235
8.2.3.2	TCA	237
8.3	Bottling Lines	238
8.4	Preparing Wines for Bottling	239
8.4.1	Required Analyses	240
8.4.1.1	Sulfur Dioxide	240
8.4.1.2	EtOH	241
8.4.1.3	Acidity	241
8.4.1.4	Microbial Growth	242
8.4.1.5	Carbon Dioxide	242
8.4.1.6	Oxygen and Wine Temperature	243
8.4.1.7	Wine Density	243
8.5	Prebottling Activities	244
8.5.1	Bottling Line Sanitation	244
8.5.2	Membrane Filter Integrity Test	245
8.6	Bottling	246



8.6.1	Bottle Queue . . . . .	246
8.6.2	Membrane Filter . . . . .	247
8.6.3	Filler . . . . .	247
8.6.4	Corking Machine. . . . .	248
8.6.5	Quality Control . . . . .	249
8.6.6	Capsules, Wax, Labeling, and Storage . . . . .	249
8.7	Microbiological Monitoring . . . . .	251
8.7.1	Plating Media . . . . .	251
8.7.2	Plate Inoculation, Incubation, and Identification . . . . .	252
8.8	Required Analysis for International Trade . . . . .	255
8.9	Bottle Aging . . . . .	257
<b>9</b>	<b>Analytical Procedures</b>	<b>258</b>
9.1	Introduction . . . . .	258
9.2	Procedures . . . . .	259
9.2.1	Spectrometric Color and Phenolic Measurement . . . . .	259
9.2.2	Density Measurement, Direct Method . . . . .	262
9.2.3	Specific Gravity Measurement . . . . .	263
9.2.4	Soluble Solids Measurement via Hydrometer . . . . .	265
9.2.5	Density, Specific Gravity, and °Brix Measurements via Density Meter . . . . .	267
9.2.6	Soluble Solids Measurement via Refractometer. . . . .	269
9.2.7	pH Measurement. . . . .	271
9.2.8	Titrateable Acids Assay, Manual. . . . .	273
9.2.9	Titrateable Acids and pH Assay via Automated Titration . . . . .	275
9.2.10	Fluoride (F) Assay via ISE . . . . .	277
9.2.11	Ammonia (NH <sub>3</sub> ) Assay via ISE . . . . .	279
9.2.12	Free and Total Sulfur Dioxide (SO <sub>2</sub> ) Assay via Ripper . . . . .	280
9.2.13	Free and Total Sulfur Dioxide (SO <sub>2</sub> ) Assay via Aeration–Oxidation . . . . .	282
9.2.14	Reducing Sugar and Residual Reducing Sugar (RS) Enzymatic Assay . . . . .	285
9.2.15	L-Malic Acid (LMA) Enzymatic Assay . . . . .	287
9.2.16	Acetic Acid (AC) Enzymatic Assay . . . . .	289
9.2.17	Volatile Acidity Assay via Distillation . . . . .	292
9.2.18	Insoluble Solids (% Solids) Measurement . . . . .	295
9.2.19	% v/v Alcohol Measurement via Ebulliometer. . . . .	296
9.2.20	Yeast and Bacteria Viability and Cell Counts . . . . .	299
9.2.21	Cold Stability Assay for the Verification of Bitartrate Stability via Freeze Test . . . . .	300
9.2.22	Cold Stability Assay for the Verification of Bitartrate Stability via Conductivity . . . . .	302
9.2.23	Heat Stability Assay for Verification of Protein Stability. . . . .	304
9.2.24	Carbon Dioxide (CO <sub>2</sub> ) Measurement via Carbodoseur . . . . .	305
9.2.25	Acidulation Trial . . . . .	306
9.2.26	Deacidulation Trial: Potassium Bicarbonate KHCO <sub>3</sub> (or K <sub>2</sub> CO <sub>3</sub> ) . . . . .	308
9.2.27	Gelatin Fining Trial. . . . .	309
9.2.28	Isinglass Fining Trial . . . . .	311
9.2.29	Egg White Fining Trial . . . . .	312
9.2.30	Bentonite Fining Trial . . . . .	314
9.2.31	Copper Sulfate (CuSO <sub>4</sub> ) Trial . . . . .	315

<b>10 Reagents</b>	<b>318</b>
10.1 Introduction	318
10.2 Reagents	319
10.2.1 Acetaldehyde (C <sub>2</sub> H <sub>4</sub> O) 10% w/v Solution	320
10.2.2 Acetic Acid (C <sub>2</sub> H <sub>4</sub> O <sub>2</sub> ) 1% w/v Standard	320
10.2.3 Ammonia (NH <sub>3</sub> ) 0.1% w/v Standard	321
10.2.4 Bentonite 5% w/v Slurry	322
10.2.5 20 °Brix Sucrose (C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ) Standard	323
10.2.6 Copigmentation Buffer	323
10.2.7 Copper Sulfate (CuSO <sub>4</sub> ) 100 mg/liter Solution	323
10.2.8 Isinglass 0.5% w/v Solution	324
10.2.9 Egg White 10% w/v Solution	325
10.2.10 Ethanol (EtOH) Solutions and Standards	325
10.2.11 Gelatin 1% w/v Solution	326
10.2.12 Hydrochloric Acid (HCl) Solutions	327
10.2.13 Hydrogen Peroxide (H <sub>2</sub> O <sub>2</sub> ) 0.3% v/v Solution	327
10.2.14 Indicator Solution	328
10.2.15 Iodine (I <sub>2</sub> ) 0.02 <i>N</i> Solution	328
10.2.16 L-Malic Acid (C <sub>4</sub> H <sub>6</sub> O <sub>5</sub> ) 0.2 g/L Standard	328
10.2.17 Malic Acid (C <sub>4</sub> H <sub>6</sub> O <sub>5</sub> ) 10% w/v Solution	329
10.2.18 Phosphoric Acid (H <sub>3</sub> PO <sub>4</sub> ) Solutions	329
10.2.19 Potassium Bicarbonate (KHCO <sub>3</sub> ) 4.5 % w/v Solution	330
10.2.20 Sodium Hydroxide (NaOH) Solutions	331
10.2.21 Starch 1% w/v Solution	331
10.2.22 Sulfur Dioxide (SO <sub>2</sub> ) 200 mg/L Solution	332
10.2.23 Sulfuric Acid (H <sub>2</sub> SO <sub>4</sub> ) Solutions	333
10.2.24 Tartaric Acid (C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> ) 100 g/L Solution	333
10.2.25 Thiosulfate (S <sub>2</sub> O <sub>3</sub> ) Solution	334
10.3 Standardization	335
10.3.1 Standardization of Acid Solutions	335
10.3.2 Standardization of Base Solutions	336
10.3.3 Standardization of 0.02 <i>N</i> Iodine Solution	336
<b>Appendix Laboratory Chemical Safety Summaries</b>	<b>337</b>
<b>Bibliography</b>	<b>359</b>
<b>Index</b>	<b>365</b>