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Ethanol and Education: Alcohol as a Theme for Teaching Chemistry

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Ethanol and Education: Alcohol as a Theme for Teaching Chemistry

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Foreword

The ACS Symposium Series was first published in 1974 to provide a mechanism for publishing symposia quickly in book form. The purpose of the series is to publish timely, comprehensive books developed from the ACS sponsored symposia based on current scientific research. Occasionally, books are developed from symposia sponsored by other organizations when the topic is of keen interest to the chemistry audience.

Before agreeing to publish a book, the proposed table of contents is reviewed for appropriate and comprehensive coverage and for interest to the audience. Some papers may be excluded to better focus the book; others may be added to provide comprehensiveness. When appropriate, overview or introductory chapters are added. Drafts of chapters are peer-reviewed prior to final acceptance or rejection, and manuscripts are prepared in camera-ready format.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previous published papers are not accepted.

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Preface

This book comes from the symposium "Chemistry of Fermented Beverages" at the Biennial Conference on Chemical Education (BCCE) that was held in Grand Rapids, Michigan in August of 2014. We have nonetheless cast a wide net, including several distinguished chemists who did not present in Grand Rapids. The symposium and book address some wonderful opportunities to engage students in a wide variety of chemistry classes, field experiences, study abroad, and other learning activities through examples involving alcoholic beverages. The topic lends itself to treatments that range from serious to quirky and light-hearted, depending on the audience and objectives. Every subdiscipline of chemistry can be addressed through alcoholic beverages. All levels, from introductory chemistry for non-science majors to advanced chemistry seminars and research can incorporate the science of alcoholic beverages. This volume will cover some of the possibilities through the lens of the experiences of chemistry faculty who teach about alcoholic beverages in a variety of settings, ranging from traditional chemistry programs to technical programs in the alcohol industry.

To some, this project may seem strange; we get a smattering of amused looks, raised eyebrows, and sly chuckles. But there is nothing strange about reaching students with chemistry that interests them. Whether we want to admit it or not, alcohol consumption is a big part of the lifestyle of many students, so it makes sense for faculty members to teach chemistry classes that revolve around alcohol, or that use alcoholic beverages to make some point in a chemistry class. Alcoholic fermentation may be the oldest chemical process mastered by humankind. The negative consequences of excessive drinking are well-known, yet numerous studies have pointed out the health benefits of mild to moderate regular alcohol consumption. Given the large percentage of students who regularly drink alcohol, often in violation of institutional prohibition and age restrictions, they should have access to the science behind the beverage, as well as some intelligent discussion about it.

Within this volume there are some very good chapters covering alcohol, its production, analysis, metabolism, as a source of livelihood, its role in the development of chemistry, and as a means of attracting student interest. The authors draw from experiences at every level from introductory chemistry for non-majors, through general and advanced chemistry major courses and projects, to specialized business/industrial applications. All of them show how some aspect of alcohol can be used as a model to enhance the learning process. This volume will provide resources for faculty who are considering, developing, or actively teaching:

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

- A course or sequence on alcohol production or service;
- A stand-alone course based on fermented beverage chemistry or biotechnology; or
- Alcohol-based lessons or examples for an existing chemistry or biochemistry course.

In addition, chemists who are interested in beer, wine, or spirits, and who would like to be able to field questions about alcoholic beverages and their chemistry, whether from students in a class, or from friends and colleagues in some less formal setting, will benefit from this book.

We extend heartfelt thanks to the many persons whose efforts greatly enhanced the quality of this book. These include ACS Books acquisitions editor Tim Marney, and Letitia Glozer and Jasmine Suarez of Technica Editorial. Marcy Barth (RB's wife) professionally redrew and redesigned several of the illustrations. Special thanks goes to more than thirty anonymous reviewers, whose outstanding work ensured the accuracy and clarity of each chapter. We thank our families and our universities whose constant support made our work possible.

We have approached the topic quite seriously, but have tried not to take ourselves too seriously in the process. And so, concerning what is within these pages, drink it all in!

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In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Editors' Biographies

Roger Barth

Roger Barth was born in New York City, attended public schools in Levittown, PA, and was awarded a bachelor's degree in chemistry from La Salle College in Philadelphia and a doctorate in physical chemistry from the Johns Hopkins University in Baltimore. He did post-doctoral work on heterogeneous catalysis at Drexel University and at University of Delaware. His current research is on beer chemistry. Barth has been a faculty member at West Chester University in Pennsylvania since 1985. His book, The Chemistry of Beer: The Science in the Suds, was published by Wiley in 2013.

Mark A. Benvenuto

Mark Benvenuto is a Professor of Chemistry at the University of Detroit Mercy, in the Department of Chemistry & Biochemistry. His research spans many subjects, including energy dispersive X-ray fluorescence spectroscopy to determine trace elemental compositions of aquatic and land-based plant matter, food and dietary supplements, and medieval and ancient artifacts. Benvenuto received a B.S. in chemistry from the Virginia Military Institute, and after several years in the Army, a Ph.D. in inorganic chemistry from the University of Virginia. After a post-doctoral fellowship at The Pennsylvania State University, he joined the faculty at the University of Detroit Mercy in 1993.

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In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
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Chapter 1

Overview

Ethanol and Education: Alcohol as a Theme for Teaching Chemistry

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Fermented beverages have been in use since early prehistory. Fermentation itself as well as the processes before and after it all have important chemical implications. The subdisciplines of chemistry play significant roles in the preparation, quality, and packaging of fermented beverages. Several key concepts in chemistry and related technical disciplines have origins in fermented beverage production.

Introduction

Although the alcoholic beverage industry cultivates an image of traditional craftsmanship passed down the generations, it is in fact a highly technical endeavor. Production processes are constantly refined for efficiency, quality, and consistency. Each step is rich in chemistry, engineering, and biotechnology. Each offers opportunities for students to learn about aspects of chemistry. Every subdiscipline of chemistry is heavily represented. Because of its relevance and its ability to arouse student interest, teaching about the chemistry of fermented beverages is becoming more widespread in higher education.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

History

Alcohol goes back to the origins of humanity. It seems likely that when modern humans crossed the Red Sea from Africa to Asia, they were already equipped with the skills to ferment sugary liquids to alcoholic beverages (*1*). Chemical evidence for the deliberate production of alcoholic beverages dates from 9000 years ago in Neolithic China (*2*). Evidence for grape wine is reported dating at least 7000 years ago in the northern Mesopotamian region (*3*), but since grapes originate at least 500 km to the north, it is likely that wine is actually older (*4*). Barley beer has been found dating from 5500 years ago, also in the Mesopotamian region (*5*). When the invention of writing opened the book on history 5000 years ago, beer was already distributed throughout the Mesopotamian society (*6*). Distillation came much later. It was mentioned by Abu Musa Jabir ibn Hayyan "Geber" (721-815), an ancient chemist from the Moslem golden age whose writings influenced the teaching and practice of chemistry for centuries. Distilled beverages came into regular use in Germany in the late 1400's (*7*).

The production of alcoholic beverages has been a driving force for chemistry from its origins as a science. Antoine Lavoisier (1743-1794), in whose analytical balance chemistry took its modern form, described the fermentation reaction as grape must = carbonic acid + alcohol. Lavoisier is the author of the first modern chemistry textbook (*8*). The fascination with alcoholic fermentation and alcoholic beverages has not subsided; a search for "alcoholic beverage" on the Amazon® book site turns up 22,898 results.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch001

Classification of Alcoholic Beverages

All alcoholic beverages are produced by fermentation of simple sugars by microorganisms, most commonly a yeast, *Saccharomyces cerevisiae* or one of its close relatives, *S. uvarum* or *S. pastorianus*. A major classification is whether, the alcohol is significantly concentrated, either by distillation or freezing. A second major classification is the source of sugar for fermentation. The sugar can be provided by a natural source of sugar, such as fruit, honey, or sugar cane, or it can derive from the hydrolysis of starch. A myriad of secondary distinctions emerge when post-processes, such as addition of flavors or aging in barrels are considered.

Non-Concentrated Beverages

The key distinction in this class of beverages is the type of the carbohydrate, sugar or starch. Some examples of non-concentrated beverages derived from sugar are given in Table 1. There are many flavored and hybrid varieties, each with its own name, and often more than one name.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Table 1. Non-Concentrated Beverages from Sugar

Sugar source	*beverage*
Fruit	wine, cider (apples), perry (pears)
Sugar	*basi*
Honey	mead
Milk	*kumis*
Tree sap	toddy, palm wine, maple wine
Cactus	*pulque*

Distinctions among non-concentrated beverages from starch take into account both the source of the starch and that of the hydrolysis enzymes. Some examples are given in Table 2. Sometimes, especially for beer, additional sources of starch or sugar are added.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch001

Table 2. Non-Concentrated Beverages from Starch

Starch source	*hydrolysis enzymes*	*beverage*
Barely, wheat, rye	malt	beer
Rice	mold	*sake, huangjiu*
Maize (corn)	saliva or malt	*chicha*
Manioc	saliva	*masato*

One feature of beverages from starch is the importance of water. The character of the beverage can be significantly affected by the trace compounds in the water used to hydrolyze the starch and extract the sugar. Often the chemistry of the water, especially its hardness and alkalinity, determine the type of beverage that can be optimally produced. Water chemistry, especially acid-base chemistry, is a branch of inorganic/physical chemistry that is a universal part of the chemistry curriculum.

Concentrated Beverages

Some examples of concentrated beverages prepared from sugar sources are given in Table 3.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Table 3. Concentrated Beverages from Sugar

Sugar source	*beverage*
Sugar, molasses	rum
Fruit	brandy, aquavit
Apples, conc. by freezing	applejack
Cactus	tequila, mezcal

Concentrated beverages from starch are sometimes distinguished by preparation technique in addition to starch source. Some examples are given in Table 4.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch001

Table 4. Concentrated Beverages from Starch

Starch and other details	*beverage*
Barley, maize, rye, wheat	whiskey
Grain or potatoes, distillation or filtration regulated to remove flavor compounds	vodka
Rice	*baiju, shochu*

Preparation of Fermented Beverages

The details of preparation of fermented beverages vary widely, but a few broad generalizations can be made. A mixture of water and fermentable sugars is prepared. The mixture is allowed to come into contact with fermentation organisms, usually one of a few species of yeast. (*9*). The organism consumes the sugar and releases ethanol, carbon dioxide, and various side products that can be important to the flavor of the beverage. After fermentation, the beverage can be drunk directly out of the fermentation vessel, but more often there is post-fermentation processing of varying degrees of complexity.

Preparation of Fermentation Mixture

For sugar-based beverages, such as wine, mead, or rum, the preparation of a fermentable mixture is largely mechanical. The plant material often needs to be ground or crushed, and sometimes cooked. The sugar may need to be dissolved or diluted to provide a suitable concentration for the fermentation organism. In a few cases the sugar is concentrated by boiling or freezing. The determination of the amount and nature of the sugars can be important in the production process, and can serve as practical problems for students.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Starch-based beverages require more complex processing to effect the enzymatic hydrolysis of the starch to fermentable sugar. For many Western beverages the source of fermentation enzymes is malt. To make malt, seeds of grain are steeped in water to cause them to sprout. During sprouting, the seeds need moisture and oxygen. Cellular respiration releases heat which must be allowed to escape. During sprouting the embryo releases hormones that stimulate the production of amylolytic (starch hydrolyzing) and other enzymes. At an appropriate stage of sprouting the seeds are dried in a kiln, killing the embryo and stabilizing the malt. Malt can be stored for months before use (*10*). To make the fermentation solution (called wort) the malt is crushed and mixed with water, and possibly other sources of starch, at about 65-70° C. If beer is being prepared, the sugary liquid is separated from the spent grain and boiled, usually with hops, then chilled before fermentation. If a distilled beverage is being prepared, the wort may be fermented without separation.

For Eastern rice beverages, the rice is cooked, then seeded with *koji* (*Aspergillus oryzae*), a mold that releases amylolytic enzymes. Often, in the case of rice beverages, the hydrolysis and fermentation take place simultaneously. Additional cooked rice is inoculated with a mixture of *koji*-bearing rice and yeast. After the hydrolysis/fermentation process, the liquid is squeezed out through cloth (*11*).

A third method of starch hydrolysis is the exposure of ground grain to human saliva, which contains amylolytic enzymes. This is the traditional method of making *chicha* from maize. Although this process is sometimes called mastication, it does not involve chewing. The ground maize is dampened, put into the mouth to absorb saliva, then removed in the form of a wad, called *muko*, which is sun-dried for later use. When *chicha* is to be made, *muko* and other sources of starch are treated with hot water to hydrolyze the starch. After a separation process, the liquid is boiled, chilled and fermented (*12*).

Fermentation

The alcohol in alcoholic beverages is produced by fermentation of simple sugars by microorganisms, most commonly a yeast, *Saccharomyces cerevisiae* or one of its close relatives, *S. uvarum* or *S. pastorianus*. Other yeasts and even bacteria are occasionally used (*13*). In addition to ethanol and water, fermentation produces a variety of minor products that greatly influence the character of the beverage. Fermentation temperature is the most important variable that needs to be controlled. Sugar concentration, alcohol concentration, pH, hydrostatic pressure, and other factors play significant roles. For some beverages, including cider and some wines, a malo-lactic fermentation may take place subsequent to alcoholic fermentation. In this step, bacteria decarboxylate malic acid (hydroxybutanedioic acid) to the less sour lactic acid (2-hydroxypropanoic acid) (*14*).

Alcoholic fermentation is a classic example of the glycolysis pathway of biochemistry. Some form of glycolysis occurs in every living cell. Alcoholic fermentation in the teaching laboratory is inexpensive, easy to implement, and provides samples for several analytical techniques as the process proceeds over a period of several days.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch001

Post-Fermentation Processing

Carbonation

Nearly all beer, cider, and a few varieties of wine are carbonated. Carbon dioxide is dissolved in the beverage under pressure. Most beverages are carbonated by the direct addition of food-grade carbon dioxide during packaging. Some are carbonated by the controlled addition of sugar before the package is sealed. The added sugar undergoes alcoholic fermentation, producing the dissolved carbon dioxide. Beverages carbonated in this way have yeast in the container. When present, carbon dioxide is a primary flavor compound (*15*). Carbonation provides a practical example of Henry's law and of the van't Hoff equation.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch001

Aging/Conditioning

Most alcoholic beverages undergo some sort of maturation process. Often clarifiers are added followed by filtration or sedimentation. Maturation can involve interaction with yeast, bacteria, or with wooden containers. It can be a matter of a few days to decades. Maturation for more than a few days becomes a dominant factor in the cost of the beverage. Lager beer is an interesting case. During low temperature fermentation, the yeast produces butanedione (diacetyl), and 2,3-pentanedione, which contribute a buttery off-flavor to the beer. Three weeks or more of conditioning in the presence of live yeast under refrigeration are needed for the yeast to convert these compounds to nearly flavorless alcohols. The brewery thus needs chilled maturation tanks for three weeks of production. This is a huge capital outlay for a relatively inexpensive product. Finding ways to reduce the conditioning time is a significant preoccupation of beer chemists. Some wines are aged for months or years. The aging reduces sourness and astringency, to some extent as a result of esterification of acids and precipitation of phenolic compounds. Distilled beverages are often aged in wooden barrels which, in addition to contributing flavors from the wood, also absorb undesired flavor compounds, such as higher alcohols.

Distillation

Distillation is a critical factor in the character of beverages that use it. The process typically involves two runs through the still (or two stills). In the first run, the distillation is run until all volatile components have distilled over. The distillate is distilled again (more than once in some cases). The low boiling fraction (foreshots) and the high boiling fraction (feints) of the second distillation are discarded or reprocessed. The boiling point range for the accepted fraction determines the flavor components in the beverage.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Distillation is an exercise in vapor-liquid equilibrium, an important topic in physical chemistry. The effect of the configuration of the still on the extent of reflux of the distillate can explain the effect of different configurations on the character of the beverage.

Packaging

Except in the case of brewpubs, alcoholic beverages are packaged in some way. Carbonated beverages must be packaged under pressure. In all cases the beverage must be sealed to prevent exposure to oxygen and microbes in the air and to prevent loss from evaporation. Hopped beer must be protected from light, which causes a photochemical reaction giving a skunky odor. In addition to protecting the product, commercial beverages must be packaged in ways that appeal to potential consumers. Glass, despite its high cost and weight, is preferred by many consumers. Wine packaged in anything else is perceived to be of low quality. Quaint packaging materials, like natural cork, which can be a significant threat to the quality of the wine, are still in use. Most beer is packaged in epoxy-lined aluminum cans, but the more expensive craft beers go into bottles.

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Conclusion

Fermented beverages involve every area of chemistry, including the history of chemistry. Many key issues in chemistry are readily addressed in the context of fermented beverages. Several familiar methods or concepts in chemistry originated with alcoholic beverage production. These include Kjeldahl's method for proteins, the statistical Student's T, and the concept of pH (*16*).

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In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
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Chapter 2

Fermentation Science in a Global Society with a Study Abroad Flavor

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We present here a quarter-format course on fermentation science with a study abroad component. Unlike other reported study abroad courses, we teach course content on campus during half of a semester and then travel abroad for 7–10 days when university classes are not in session. This format is generally more accessible to students due to the lower cost and a shorter time abroad commitment than traditional study abroad options and has been well received over the past nine years. The enrollment is weighted to juniors (29%) and seniors (53%), although first and second year students have been successful in the course. Additionally, enrollment is generally evenly distributed between males and females. This chapter highlights the basic course content, examples of international components, our strategies for the course, and our experiences abroad. Course content includes, but is not limited to, the chemistry and biology of beer, wine, distilled spirits, cheese, and fermented vegetables, as well as the language, history, and culture of the destination. Course destinations have been Belgium and the Netherlands, Czech Republic, Germany, and Scotland. Students are assessed and their performances evaluated based on homework, exams, and journal assignments

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
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We report here on a quarter-format course on fermentation science with a study abroad component, which we have taught eight times since 2006. Unlike previously reported study abroad courses (*1–3*), our format teaches course content on campus during half of a semester, and then travels abroad for 7–10 days when university classes are not in session. Previous authors have highlighted the chemistry and biochemistry of beer and wine (*4–8*), and more recently authors have reported methods for developing study abroad courses involving chemistry (*1–3*). Even though an entire semester abroad might be desired for every student, in many cases students are limited by budget and time constraints as well as course offerings. Our format is generally more accessible to students due to the lower cost and a shorter time abroad commitment than traditional study abroad options. These quarter-format courses were developed on the Oswego campus approximately twelve years ago and were largely focused on the arts and humanities. As the popularity of these courses grew, requests were made for new options. You can imagine the response when we answered, "We could teach a course about beer and whisky and go to Belgium and Scotland." After much laughter initially and many side comments with much teasing since, we have developed and refined a successful study abroad course. The course explores the impact of fermentation and distillation science on the global society. It also builds upon students' understanding of basic science principles in order to develop their understanding of the interdisciplinary nature of science and allows students to make connections to the history, art, and culture of a global society. The overall goals of this course are for the students to gain an understanding of the scientific principles involved in fermentation and to develop an appreciation of the impact of fermentation on the global society.

We developed this course to satisfy the SUNY General Education Requirements. The pre-requisites for the course are the equivalent of high school biology and chemistry. As a result, we have had a broad range of majors taking the course, which is summarized in Figure 1. Although the majority of the students are from the sciences, significant numbers of students from many other majors enroll. This diverse range of majors creates challenges to teaching the course; however, we have found that the different perspectives and opinions that contribute to the conversation far outweigh the challenges. The course has been fairly evenly enrolled by females and males (44%:56%) and is more heavily weighted toward seniors (1st year: 1%, 2nd year: 16%, 3rd year: 29%, 4th year: 53%, other: 1%).

We intend for the on-campus component to provide the foundation for students to experience the cultural and social implications of fermentation in an international setting first-hand. The on-campus component of the course presents the science and practice of fermentation in a lecture setting. This introduces topics that the students will experience during the international component. Students can then focus on the impacts of fermentation in an international setting without needing to simultaneously learn the science.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch002

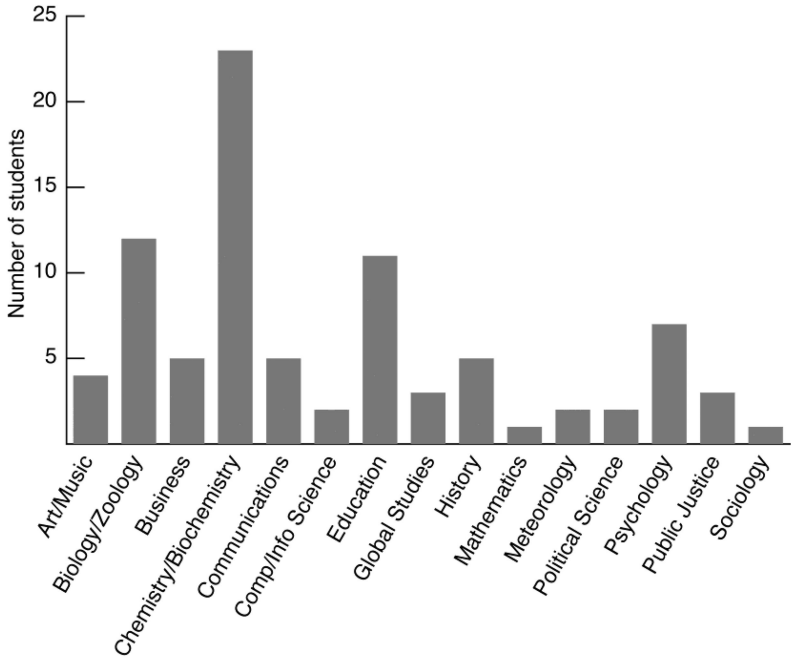

Figure 1. The distribution of major areas of students enrolled in GLS 316.

A typical course outline includes:

I. Introduction to the language of chemistry
II. History of yeast/fermentation
III. Science of fermentation
IV. Examples of fermented items
V. Beer and Whisk(e)y

    a. History, Styles
    b. Components: Water, Malt, Hops
    c. Process (mashing)

VI. Cheese

    a. History, Styles
    b. Components: Milk, Rennet, Cultures
    c. Processing and aging

VII. Yogurt, bread, other fermented products, and distillation, depending on the study abroad location.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

VIII. Alcohol and people

a. Economics and taxation
b. Temperance and prohibition
c. Effect on the body (*e.g.* metabolism of ethanol, blood alcohol content, and hangovers)

IX. Customs and language of travel destination

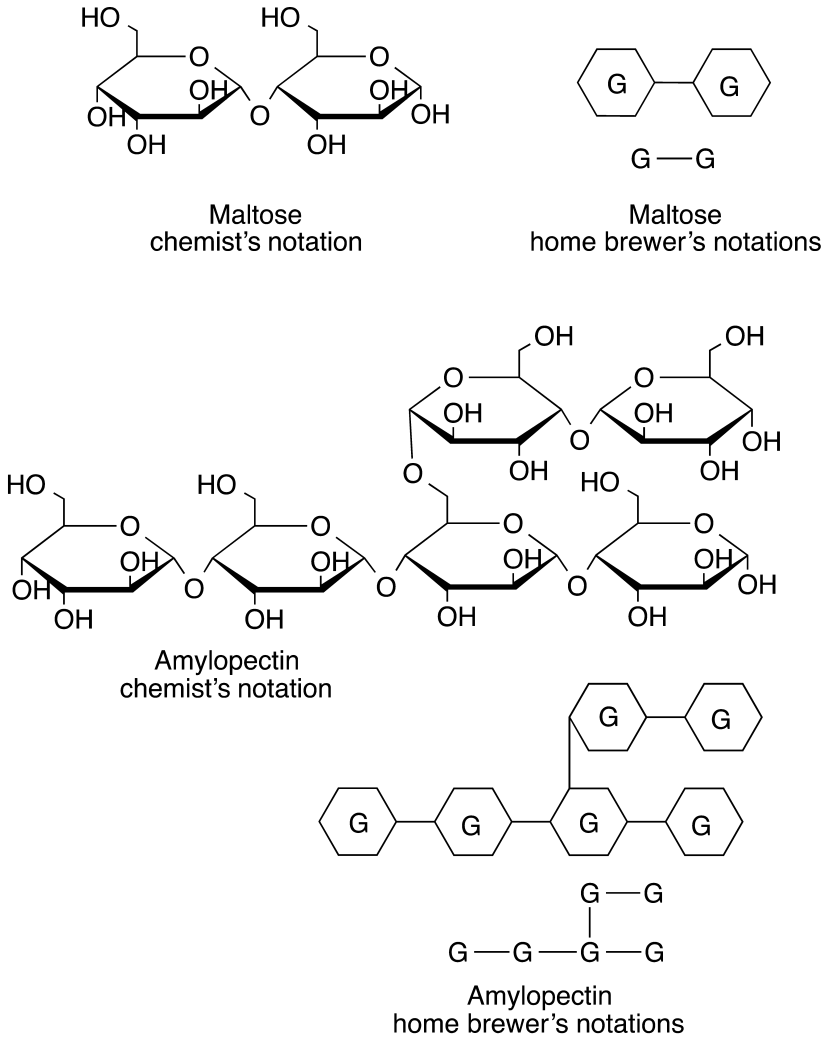

Figure 2. Examples of the different shorthand notations that we introduce to the students in the course. The amylopectin fragment provides an example of both 1–4 and 1–6 linkages.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch002

Because we commonly have students with non-science backgrounds, we spend time in the course going over some of the language of chemistry, specifically how to draw molecules in the shorthand line notation popular in organic chemistry. Although this is commonly presented over multiple lectures in a typical organic chemistry course, we only want to briefly introduce the concept, so that we can use it as we discuss the science of fermentation. Many times the non-science students feel overwhelmed by the idea, while the science majors cannot believe that we are distilling several lectures from a typical organic chemistry course into one. We take this shorthand notation one step further in discussing polysaccharides and carbohydrates as presented in many of the homebrewing publications. Examples for maltose, starch, and amylopectin are given in Figure 2.

This becomes important as we want to talk about the processes of malting and mashing without needing to get overly complicated in the structures we draw. We typically ask the students to identify different types of sugars, complex carbohydrates, and other biologically active molecules. We also ask students to identify sources of some of the common molecules that come out of the brewing process particularly related to beer, including components from hops (α-acids, β-acids, iso-α-acids, and hop oils and resins). Throughout the rest of the course, we continue to draw on the shorthand notation and introduce other chemistry-related topics as they apply directly to that stage of the fermentation process.

We then briefly review the history of fermentation (*9–12*):

- Some of the evidence for the earliest application of fermentation dates back over 9000 years with multiple origin sites (Mesopotamia, China, Egypt).
- Theories flourish about the happy accidents that resulted in the discovery of early fermented products.
- The methods used to create early fermented products were passed from generation to generation with little or no knowledge of the scientific basis of the techniques.
- Scientific investigations into alcoholic fermentation did not begin until the late 1700s.
- Antoine Lavoisier (1789) and Joseph Louis Gay-Lussac (1810) defined fermentation as the conversion of sugar into ethanol and characterized the proportions of the reaction.
- As others began to expand on these investigations, a rather large divide developed between the fields of chemistry and biology. In the 1830s, Charles Cagniard de la Tour and Theodor A. H. Schwann proposed that fermentation was caused by a living organism. However, J.J. Berzelius, Justus von Liebig, and Friedrich Wöhler proposed that fermentation was just a chemical reaction. They even went so far as to publish a mocking description of the labware as "animals" involved in fermentation (*13*).

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch002

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

- Throughout the rest of the 1800s, scientists such as Louis Pasteur, Moritz Traube, Marcelin Berthelot, and Wilhelm Kühne refined the basis of fermentation as the result of living organisms that used enzymes to perform the chemical reactions.

We present fermentation as the conversion of sugar to either ethanol or lactic acid under anaerobic conditions using the abbreviated glycolysis and metabolism scheme in Figure 3. We also introduce the general microorganisms that are involved: bacteria (gram negative *Acetobacter* and gram positive *Lactobacillus*) and fungi (gram negative *Penicillium* and gram positive *Saccharomyces*). While discussing the general metabolic pathway we compare the respiration pathway to the two fermentation pathways. We introduce the concept of energy storage with ATP and the breakdown of glucose to two pyruvate (pyruvic acid) molecules and two equivalents of ATP. The pyruvate has different fates depending on the organism and the conditions. Under aerobic conditions the respiration pathway produces 36 equivalents of ATP per glucose molecule, while under anaerobic conditions the fermentation pathways do not produce any additional equivalents of ATP. We then pose the students the question, "Why does the organism convert pyruvate to ethanol or lactic acid?" This allows us to introduce the idea of a catalytic cycle and the need to convert the NADH formed during glycolysis back to NAD^+ to complete the loop.

At this point in the course, we ask students to list fermented products that they know and to try to identify the source of sugar in each. Some of the cases are quite easy, such as the fermentation of honey to make mead, the production of yogurt from milk, or the creation of wine from grape juice. However, we challenge the students when they discuss making bread and beer from grains. We ask them "Does flour taste sweet? If not, where are the sugars coming from to feed the yeast?" This provides a transition into the processes involved in the production of beer, bread, and many distilled spirits.

This leads directly into the discussion of preparing grains for use in fermentation. We examine what occurs during malting of grains, such as barley and wheat, and then study the chemical processes of mashing to create a sweet wort. This also provides a good opportunity to explore which carbohydrate sources are used, and we typically will consider the treatment of rice with molds in sake production (*14*) and the conversion of corn starches with human saliva to produce chicha (*15*). We can also examine how malting technologies resulted in new beer styles, such as in Bohemia where the ability to dry malt at low temperatures led to a very pale pilsner/pilsener beer.

Water plays a key role in these processes and is an important component of most alcoholic beverages. In this portion of the class, we introduce a few more key chemical topics: pH, alkalinity, and hardness. These three factors have large impacts on the resulting product. The water chemistry of a specific locality is directly related to the types of beer that historically developed (*16–20*). As an example, the ales that developed in Burton-on-Trent, England are quite bitter. The perceived bitterness is accentuated by the high sulfate concentrations (>300 ppm) present in the water supply and the relatively high hardness and alkalinity. The unusually high sulfate concentrations are the result of rainwater percolating

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch002

through gypsum deposits on the way to the groundwater aquifier (*17*). A second example is the classic dry stout, most often associated with Guinness and Dublin, Ireland. This style resulted from the porter style, which was popular in London in the 1700s. However, Dublin's water has a higher calcium carbonate content and required a much more roasted malt to obtain an appropriate pH for mashing and brewing. The result was a drier, more roasted version of a porter, which became known as dry stout (*16*).

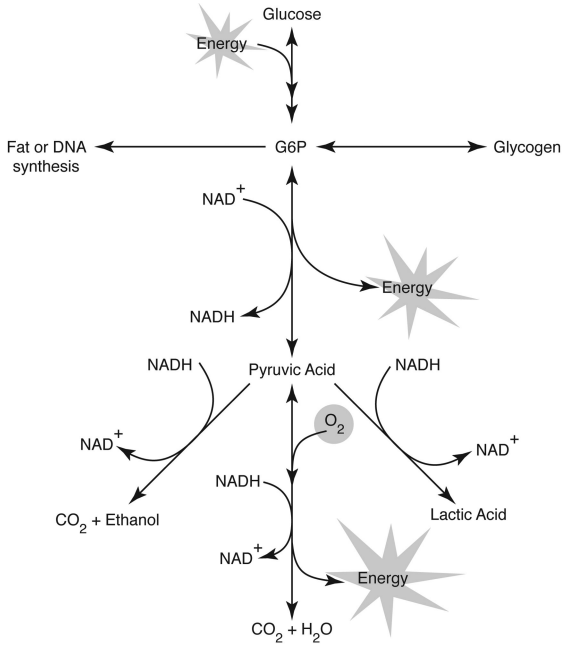

Figure 3. A simplifed scheme for the use of glucose by organisms. The regeneration of NAD^+ is an important component of the fermentation and respiration pathways.

Through the development of fermented beverages, people attempted to modify the flavors and to create a more stable product by adding many other items. These included herbs, spices, seaweed, heather blossoms, spruce boughs, and fruits. It was soon determined that hops extended the shelf life of beer and the resulting bitterness was an acceptable addition to the flavor profile in most cases. One example where the preservative benefit of hops is exploited without the associated bitterness is in the production of lambic beer. This Belgian beer style is prepared using old hops for the preservative benefit without adding any significant flavoring (*21*).

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch002

Although hops are used primarily as a source of bitterness from the isomerization of α-acids during boiling, there are many other flavors and aromas that are derived from compounds in the oils and resins in hops. We talk about the fate of these latter compounds during the boiling process and about the best time(s) to add hops. This provides an opportunity to discuss properties such as volatility (*22*).

We also talk about the development of the two main types of yeast used in brewing beer: *Saccharomyces cerevisiae* (top fermenting/ale) and *Saccharomyces uvarum/carlsbergensis* (bottom fermenting/lager) (*23*). In addition, some beers are still produced with wild yeasts. One example is lambic, which is only produced within about 20 km of Lembeek, Belgium (*21*, *24*).

When the course destination is Scotland, we spend some time discussing the distillation process and the differences among various distilled spirits, such as whisk(e)y and bourbon. Most students are surprised to hear that whisk(e)y results from the distillation of unhopped beer and that brandy comes from the distillation of wine. Part of the discussion related to whisky includes the differences in character resulting from the shape of a still pot. For example, tall, thin still necks tend to produce light and delicate whiskies, because there are more theoretical plates, thus improving the efficiency of the distillation. Short, thick necks tend to produce heavy, oily whiskies. We then go on to discuss the importance and origin of the various chemical components of whisky, the maturation process, and regional differences (*25–27*).

A few less-traditional course events are an introduction to sensory evaluation and a demonstration day. To get students thinking about using their senses when consuming food, we usually have at least one comparative tasting session in class. Past examples include: a) Coke with high fructose corn syrup, Coke with cane sugar, and Diet Coke; b) tonic water, club soda, and flavored carbonated water. During the demonstration day, usually planned in conjunction with the American Homebrewers Association's Big Brew Day (*28*) students participate in the process of brewing beer. We have also used this opportunity to prepare and sample other fermented products, including bread (using dough that was mixed in class earlier in the week), yogurt, and cheese. Students are generally quite surprised how much work is involved in the brewing process.

Throughout the course, we present and discuss the role of fermented products in society and history. One alcohol-related example that we have used is related to the debauchery of London in the mid-1700s due to gin. This was largely caused by the higher alcohol content in gin relative to beer, the lack of a need for a license to produce and sell gin, and gin's very low price due to a lack of taxation on both finished gin and its starting materials. Many people shifted away from beer and ale due to higher costs from taxation on malt (beginning in 1614), taxation on hops (1711), and excises on the finished product. Additionally, by the early 1700s, laws were in place to limit hours of operation and attendance in public houses and breweries.

We also discuss the history of regulation of alcoholic beverages dating back to the ale-conners in 1064 and the Reinheitsgebot of 1516 (*29*). We also discuss the impact of the European Union's many new universal standards for production on small, historical producers. These interfere with traditional methods and some

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch002

of these small, local producers face costly (monetary and quality loss) changes or going out of business. As residents of the United States looking to experience a different viewpoint, covering the history of temperance and prohibition is a must.

We discuss the fate of alcohol in the body, including the results of consumption of wood alcohol (methanol). Students are amazed as they realize that when alcohol dehydrogenase uses methanol as a substrate, formaldehyde is produced. We ask the students to perform blood alcohol content (*i.e.* BAC) calculations and discuss the impact of binge drinking (*30*).

The customs and language component totals 1–2 hours of content and is distributed throughout the course. For non-English speaking locations, we introduce the students to the language(s) they will encounter and stress that it's all right to draw pictures or write numbers on a piece of paper if that's what is required to communicate. We also stress the positive impact of a smile. We review food menus and ensure that students know three important phrases: "Please", "Thank you", and "Where is the toilet?"

Although we only meet for two hours a week for seven weeks, we do include several short homework assignments and we give midterm and final exams. Some of the homework assigments stress the identification of the types or sources of molecules drawn in shorthand or water chemistry calculations, while other assignments have the students discuss specific steps in the brewing and fermentation process or related different processes.

During the study abroad component, we require students to keep a daily journal of their experiences. Students are encouraged to use these journals to chronicle their personal experiences, as well as to complete their assignments. In general, we assign 5–7 specific questions or topics that the students must address. These questions vary with the destination and the exact course content in each semester, but are motivated by the location of the international component. Samples of these journal assignments are:

- Belgium: Discuss the development of the Rodenbach (or Cantillon) Brewery and how the beer produced is unique to the brewery and region.
- Scotland: Discuss your observations of the fermentation and distillation process at the Oban Distillery. How were the principles discussed in class applied? What has changed or modified the process over time?
- Germany: What role did monasteries play in the brewing industry?
- Czech Republic: Discuss how the water quality in the Czech Republic, particularly in Bohemia, contributed to the style of beer in the region. What was the historical impact of this beer style?
- All locations: Visit a café, order something, and address the following: What did you order? Why? Did you enjoy it? What were other patrons doing?

The overseas destination for the course changes on a regular basis. As a result, the content and the student experiences vary. However, this portion of the course strives to stimulate thoughts on the impact of fermentation on other cultures and to develop a global perspective. This is achieved through visits to fermentation related sites, discussions with people involved in fermentation, and observation of

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch002

local people. Furthermore, in order to help the students gain a better appreciation of a global society, students visit and tour other cultural and historical locations.

We work with the Office of International Education and Programs on our campus, which helps coordinate all of the study abroad opportunities. This office has witnessed everything from a third party planning all of the abroad components to the individual faculty member doing all of the planning. We have found that the experiences are better when the faculty actively plan and coordinate the study abroad components. Students are assessed an additional course fee, that covers all course activities (transportation, tours, events), lodging, and a couple of meals. Although a recent publication (*3*) provides a good foundation to developing a study abroad course, we would like to stress that it is very important for the faculty leading the course to visit the proposed locations prior to taking students. This does not require the faculty member to go to every possible destination or site, but we have discovered it allows us to provide better guidance to students and to handle the unexpected. In light of the latter, we believe that ample scheduled free time for the students is a must. We understand that there is a concern of student mischief during unorganized time, but we believe that it accomplishes several things for us and the students. First, it provides a buffer for when things don't go as planned. We have adjusted schedules and activities due to late flights, rainy days, unexpected closures, and even spur-of-the-moment opportunities. We also believe it minimizes the feeling of running from one site to the next and provides the students (and us) a chance to relax and refresh. Building on that concept, it also emphasizes a difference between the United States and much of Europe, *i.e.*, taking time out to relax or socialize. Students discover that sitting down for a bite to eat or a drink is not a rushed activity, and they rarely witness people eating a meal while walking. Lastly, these students are adults and we believe giving them the responsibility to find their own way is an invaluable experience. This unstructured time gives them an opportunity to observe a different culture and society on their own terms. We always ask students not to travel alone and to stay in pairs or small groups. We also ask to know approximately where they are going and when they expect to be back. This has become less crucial with the advances in cellphones for the nine years we've been teaching the course. In order to give the students a sense of place, we generally stay at least three nights in any one location. The first day we tend to do structured group activities and provide the students the "lay of the land". We then plan free time later in the stay, when students feel more comfortable with the location.

Whenever possible we use public transportation, as that is what much of Europe uses and it generally proves cost-effective. We have found that it is generally cheaper to purchase passes and tickets in-country than to purchase passes in the U.S. for Europe. For courses staying in Belgium, we have purchased each student a Belgian Rail ID and a week-long national rail pass. This can also include a supplement for regional public transportation. We attempt to stay near the city centers and in small, non-chain hotels. Many of these will provide group lodging discounts when we talk with them. In many cases, we have stayed in the hotels on previous scouting trips. We strive to put two students in each room, but have had everything from singles to five in a room. We've also rented small apartments and the students appreciated the opportunity to keep items cold in

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch002

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

a refrigerator. We also plan the room distribution for same-gender roommates, but we allow the students to divide themselves however they feel comfortable. Many of the lodgings provide breakfast, but students are responsible for the rest of their food. However, we typically schedule at least one group meal, which is sometimes negotiated in conjunction with a special group rate at the hotel. We have also found that it is helpful to spend the last night in Europe at a hotel near the airport if the public transportation between the city center and the airport is limited.

We can provide two specific examples where previous knowledge of a location was (or would have been) helpful.

- One year in Belgium, we intended to take the train to the airport for our departure. On arriving at the ticket window to purchase fares, we were told that we just missed the last train. It was 6:30 AM, but the conductors had staged an unannounced strike and it was not known how long it would last. (In Europe, most strikes are announced ahead of time, so this was unusual.) We quickly regrouped and headed to a ticket window for the local buses, as we knew there was a route that served the airport. That agent told us it would be faster to take the train, and he just rolled his eyes when we told him of the strike. We purchased the bus tickets and headed across Brussels to catch the correct bus. We all arrived at the airport with ample time, but had we not been aware of the other easy options it might not have worked out.
- The second time we offered the course was our first trip to Scotland, and we did not scout ahead for this location. We planned the travel with the help of a third party. Upon arrival in Scotland we knew we were in for an interesting trip when the charter bus driver asked "Where are we going?" When we said the hotel name his response was "Yeah, I know that, but where is it?" A few years later, on our second trip to Scotland, we had done much of the planning ourselves, but used a third party to arrange transportation from the airport to our initial hotel. This time, the charter bus driver said he knew where the hotel was, but gave us an ample tour of the city streets as he drove in circles, trying to figure out how to get there. These, along with other events, ensured that we always visit the locations in advance and we do as much of the planning as we possibly can.

An additional area that we needed to address for our course was the concerns of some of the administrators. We clarified with the campus attorney that we did not need an age requirement for the course, as no other international education course offered by Oswego imposed such a limit. We were instructed that the laws of the host country were the laws to be followed, as is the case with any other study abroad course. We stressed to the concerned individuals that this course was about the science of fermentation and that we would be focusing on the appreciation of alcoholic beverages and not their mass consumption. We received significant support from the directors of our international programs,

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch002

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Walter Opello and Joshua McKeown. After multiple offerings of this course, these directors were pleased to report that we had zero issues, while other study abroad opportunities did have issues (largely minor) involving alcohol. We believe it is becuase we educate the students on the appreciation of alcoholic beverages and that the course is designed for students to witness appropriate behaviors involving alcohol. Our experiences involving students who are under the age of 21 have been fairly consistent. The first 24–36 hours in Europe, it is obvious these students want to buy a drink everywhere—why not?—as it's the first time that they can do so legally. After this initial period, the novelty wears off and students have observed how others are treating alcohol. Several of the students that went to Belgium discovered that it is possible to respect a beer and only have one or two while chatting with friends. They also realized that no one was slamming beers or doing shots. One of the greatest realizations was that university students in Belgium did not know any drinking games, which drove home the idea of responsible and social uses of alcohol. Although there are places within the United States (including Oswego, NY) where students might witness similar reasonable appreciation of alcoholic beverages, placing the students in a completely foreign environment makes a more dramatic impact, as the students are outside their normal realm of experiences and are generally more cognizant of their surroundings.

Examples of the international content are listed below in Table 1 for each general location that we visit. We do not necessarily visit all of the sites in a given year's course, but this provides a summary of locations and we hope it provides some insight for others to develop similar courses.

We include a few specifc student outcomes from the previous eight offerings of this course. However, one of the general outcomes that we observe is a much greater appreciation of alcoholic beverages and decreased desire for binge drinking. We have also observed a maturation in the majority of the students over the 7–10 days we spend abroad, which continues after our return to the United States. The latter is particularly evident in the non-graduating students who return to the campus the following year. One former student began working for a beverage manufacturing company and was asked to participate in the employee tasting panel. After his first session, he was quizzed on where he had learned his sensory analysis skills. Another student (a business major) was offered a position with a regional microbrewery and is now the head distiller for another local brewery/distillery. Both students reported to us that this study abroad course directly impacted their later lives and careers. Another pair of students were asked by the campus alumni magazine to submit their journals to create an article about the experiences in the course.

We believe that this course provides a good introduction to the science of fermentation and an appreciation of and a respect for alcoholic beverages.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch002

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Table 1. Summary of Locales and Their Associated Activites for This Course over the Years

Belgium & Netherlands (2006, 2009, 2011)	**Czech Republic** (2008)
Gent: Sint-Bavo's Cathedral, which also houses the Gent Altarpiece / Adoration of the Mystic Lamb, by Jan van Eyck **Brugge:** De Halve Maan Brewery, Procession of the Holy Blood on Ascension Day, chocolate, city architecture from the 1300 and 1400s. **Roeselare:** Rodenbach Brewery **Ieper:** In Flanders Field museum, Menin Gate, WWI battlefield tour, visit to café at Abbey of Sint-Sixtus of Westvleteren **Brussels:** Cantillon Brewery, Grand Place (UNESCO), Brewer's Guild, chocolate **Amsterdam:** Heineken Brewery, Het IJ Brewery, Van Gogh Museum, Rijksmuseum, House of Bols museum	**Brno:** Starobrno Brewery, Pegas Microbrewery, Černá Hora, Research Institute of Brewing and Malting, St. Thomas Abbey, Gregor Mendel Museum, Villa Tugendhat (UNESCO), Ignis Brunensis fireworks festival, Spilberk Castle **Valtice/Lednice:** National Wine Salon, Lednice Chateau (UNESCO) **Prague:** Prague Castle, Old Town Square, Charles Bridge We coordinated this trip with Masaryk University, so we spent most of our time in and around Brno.
Germany (2013)	**Scotland** (2007, 2010, 2014)
Munich: Hacker-Pschorr Micro-brewery, City Beer Tour with local guide, Dachau Concentration Camp Memorial, Englischer Garden **Hersching:** Kloster Andechs Monastery and Brewery **Füssen:** Hohenschwangau and Neuschwanstein Castles **Kelheim:** Schneider Weissbier Brewery	**Edinburgh:** International Center for Brewing and Distilling at Heriot-Watt University, BrewDog Brewpub, Edinburgh Castle **Glasgow:** West Brewery, Auchentoshan Distillery **Oban:** Oban Distillery, Tobermory Distillery (Isle of Mull), castle and castle ruin visits **Dufftown:** Glenfiddich Distillery, Balvenie Castle

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Chapter 3

The Role of Alcoholic Fermentation in the Rise of Biochemistry

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A brief history of modern biochemistry is presented from the point of view of alcoholic fermentation beginning with the first isolation of an enzyme in 1833 to the explanation of the role of ATP as energy currency in 1941. The focus is on glycolysis, the first biochemical pathway to be elucidated. The coverage emphasizes the evidence upon which our modern understanding of biochemistry is based.

Introduction

This chapter provides insight into the scientific background to glycolysis, the first biochemical pathway to be understood. Students often get the impression that textbooks are the primary source of key chemical concepts. This hardly encourages the intellectual independence and creativity that we value in scientists. Educators do not need a review article with references to all the original papers. They need a brief, readable account to help them explain how we know what we know. This brief history is intended as a resource to help introduce students to the evidence upon which the science of biochemistry is built.

During the period from 1833 to 1941, biochemistry became one of the main subdisciplines of chemistry, joining analytical, inorganic, organic, and physical chemistry. More recently it has in some ways attained the status of its own discipline. All of this resulted from discoveries of the molecular nature of the life processes. These discoveries started with metabolism, and specifically with fermentation. The work started with alcoholic fermentation in yeast and was

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

soon joined by lactic fermentation in muscle. Hundreds of brilliant scientists made essential contributions, mainly in Europe in the shadow of warfare, brutal repression, and forced migration. This era left a lasting mark on the face of biochemistry. The resulting systems, methods, and insights are pillars of modern medicine as well as of the global pharmaceutical and agricultural industries.

Origins of Modern Biochemistry

Chemistry and medicine have always been concerned about the nature of life. Two doctrines competed with one another, often within the same minds. *Vitalism* held that living systems had a special property that could not be understood by methods that had been successful in studying dead matter. *Mechanism* held that all matter obeyed the same laws of chemistry and physics and could, in principle, be understood by ordinary experimentation. The rise of biochemistry is often presented as the slow triumph during the nineteenth century of the mechanists (good guys) over the vitalists (bad guys). One problem with this approach is that the majority of the researchers at the time had both vitalistic and mechanistic outlooks simultaneously. Many concepts that are routine today, like catalysis and enzymes, have their roots in mysterious forces that were called up to fill in the gaps in the ability of the scientists to explain their observations. It may be best to think of doctrines like mechanism and vitalism not as right or wrong, but as more useful or less useful. In this sense mechanism, which holds that life is ultimately knowable, is the more optimistic and hence more useful basis for scientific progress.

The modern period of biochemistry can be said to start as early as Paracelsus (1493-1541) who may have been the first to apply chemical theories (some of them indistinguishable from magic) to the treatment of human disease (*1*). A large step in the direction of understanding carbohydrates was made by Gottlieb Sigismund Constantin Kirchhof (1764-1833) who hydrolyzed starch to sugar using sulfuric acid in 1811 (*2*). Some put the modern period starting as late as Eduard Buchner (1860-1917), who proved in 1897 that alcoholic fermentation could be accomplished in a cell-free extract of brewers' yeast (*3*). This history will start in 1833 with the first recognition and partial isolation of an enzyme by Anselme Payen (1795-1871) and Jean Persoz (1805-1868). The enzyme, recovered by precipitation with alcohol from an extract of barley malt, was named *diastase* by its discoverers and is now identified as a mixture of different types of amylase (*4*). The –ase ending has since been applied generally to identify enzymes. The next enzyme to be discovered, pepsin, was extracted from stomach tissue in 1836 by Theodor Schwann (1810-1882), a physiologist and the developer of the cell theory. Diastase catalyzes the hydrolysis of starch; pepsin that of protein. The discovery of enzymes was a setback for vitalism, which responded by making a distinction between "organized ferment" which was living, and the isolable enzymes that catalyzed simple reactions like hydrolysis. Diastase and pepsin are water-soluble hydrolytic enzymes, in contrast to the organized ferments responsible for more complicated processes, like alcoholic fermentation (*5*).

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Coming on the heels of the enzyme breakthroughs was a discovery put forward independently by three scientists in 1837, Charles Cagniard de la Tour (1777-1859), a physicist/engineer; Theodor Schwann, discoverer of pepsin; and Friedrich Traugott Kützing (1807-1893), a pharmacist who did research on algae. Using improved microscopes that became available at this time (*6*, *7*), they showed that yeast is a living organism not, as some had thought. a precipitated byproduct of the fermentation process. In addition, they provided strong evidence that the live yeast organisms were responsible for the fermentation process (*8*). One reason that this discovery was so important is that it linked the alcoholic fermentation reaction, a complex process whose reactants and products were well known, with a living organism, yeast. This made alcoholic fermentation the fulcrum of biochemistry for the next century. Yeast was readily available for study as a by-product of beverage and industrial alcohol production. The high industrial and strategic importance of alcoholic fermentation helped attract interest and funding.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

Opposition from Chemists

The next episode in the story reflects poorly on three chemists whose influence extends to our own time, Jöns Jakob Berzelius (1779–1848), Friedrich Wöhler (1800–1882), and Justus von Liebig (1803-1873). Berzelius is credited with the modern system of chemical formulas. Wöhler, who had been a student of Berzelius (*9*), was the first person to synthesize a biological compound from inorganic starting materials. Liebig, together with Wöhler, originated the concept now known as the functional group that still serves as the organizing principle in organic chemistry. Liebig's five-bulb *Kaliapparat* (Figure 1) is a symbol on the logotype of the American Chemical Society. Berzelius, Wöhler, and Liebig did not just disagree with the concept of fermentation being carried out by a living organism. They *hated* it. They used every means at their disposal, which regrettably did not include much evidence, to attack the work of Cagniard de la Tour, Schwann, Kützing, and that of the many scientists who provided confirmation. Berzelius attributed fermentation to his concept of a catalytic force (which turned out to be correct) but rejected the participation of living organisms. In 1839 Liebig published a complicated theory that, to modern eyes, seems to look back to the doctrines of Georg Ernst Stahl (1659?-1734), who originated the phlogiston theory (*10*). Liebig held that fermentation was due to putrefaction of plant substances and the transfer of their instability to the substance being fermented. The controversy took a turn in the direction of a smear campaign with the publication of an anonymous skit, obviously by Liebig and Wöhler, in which they make a satirical report of ridiculous observations of microscopic animals eating sugar, defecating alcohol, and urinating carbon dioxide (*6*). The strident opposition by the most respected chemists to the idea that fermentation was a life process, delivered with the full weight of their authority, was a big setback for biochemistry.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

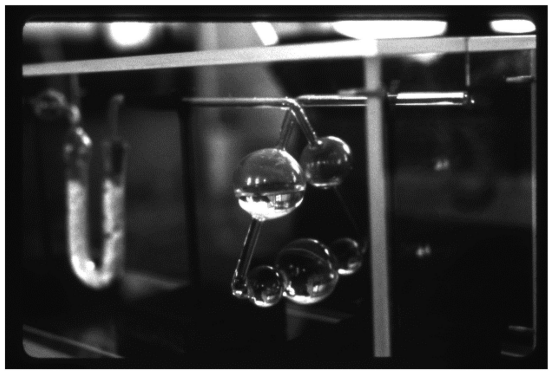

Figure 1. Kaliapparat, Robert K. Wismer Collection of Chemical Museum Slides. Chemical Heritage Foundation Archives.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

Pasteur

In 1847 Louis Pasteur (1822-1895), then a 24 year old graduate student at the École Normale Supérieure in Paris, submitted theses in chemistry and physics. It would hardly have been expected that these disciplines would be the educational foundation for a seminal figure in microbiology. Pasteur's physics thesis and his subsequent work at the École Normale were on crystallography and optical rotation. He worked on crystals of compounds whose solutions rotated plane polarized light. One such compound was sodium ammonium tartrate. A compound that was thought to be an isomer, sodium ammonium paratartrate, did not rotate light. Close examination of the paratartrate crystals revealed two forms that were mirror images. Pasteur separated these with tweezers, finding that one form rotated light to the right, and the other to the left. This was the first resolution of a racemic mixture. In addition to launching Pasteur into the Parisian scientific elite, it also focused his attention on optically active compounds, which are generally associated with biological systems (*11*). The optically active (rotates light) form of tartaric acid is naturally produced in grapes and other fruit. Tartaric acid made industrially from maleic acid is *paratartaric* acid, the mixture of left and right-handed forms (enantiomers) (*12*). In general the production of one of the enantiomers of an asymmetric molecule is a normal occurrence in molecules of biological origin, and can only otherwise occur by the participation of asymmetric molecules or fields. This is the major, and not conclusively explained, difference between compounds of biological origin and others (*13*). It is said that the presence of optically active "amyl alcohol" in fermentation products led Pasteur to the conviction that alcoholic fermentation correlates with life and is not a degradation or putrefaction process (*14*). Amyl alcohol presumably refers to active amyl alcohol, now designated (S)-2-methylbutan-1-ol (Figure 2), which has an asymmetric carbon at position 2. Active amyl alcohol occurs at

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

concentrations in the range of hundreds of parts per million in grape wine (*15*). This minor fermentation product seems to be the link between Pasteur's early work on crystallography and optical activity, and his turn to fermentation and from there to become one of the founders of modern microbiology.

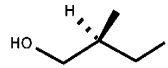

Figure 2. Active amyl alcohol: (S)-2-methylbutan-1-ol.

Pasteur is a towering figure in fermentation of wine and beer to the extent that his contributions are often misstated. Pasteur did not discover yeast; yeast has been known since prehistory. Pasteur did not discover the living nature of yeast, but he did bury the last of the opposition to the concept. Nonetheless, Pasteur's actual contributions were central to the development both of microbiology and biochemistry. Pasteur established that different organisms give different fermentation products (*16*). Modern concepts of sanitation in alcoholic fermentation, and indeed the preoccupation with microbiology in alcohol production facilities derive from this discovery. Pasteur showed that yeast does not need protein as a source of nitrogen; it can use nitrogen from inorganic ammonium compounds (*17*). This was an early hint of the complexity of the processes in living organisms. On the negative side, Pasteur's insistence that there could be no fermentation without life may have delayed the discovery of cell-free fermentation until three years after his death in 1895.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

Sugars

Molecular asymmetry, the phenomenon that drew Pasteur to study fermentation, was first explained in 1874 independently by Dutch chemist Jacobus van't Hoff (1852-1911), winner of the first Nobel Prize in Chemistry, and French chemist Joseph Le Bel (1847-1930). Their explanation, based on the tetrahedral arrangement of bonds about a carbon atom, is the standard model used today. The success of this work made the next step in the advance of biochemistry possible, which was the determination of the structures of sugars by Emil Fischer.

Hermann Emil Fischer (1852-1919) determined the structures of the complete set of six-carbon aldehyde sugars, including glucose. He did this with derivatization reactions, some of which he devised himself. The only physical techniques he had available were melting point determination and optical rotation. Today's organic chemists, weaned on routine access to infrared spectroscopy, nuclear magnetic resonance, mass spectrometry, X-ray diffraction, and many other techniques, look on Fischer's contributions with awe. Fermentation played a direct role in Fischer's work. Fischer's father, a successful businessman, had a financial interest in a brewery in Dortmund. He had his son Emil work at the brewery to help deal with yeast issues. Fischer remembered deriving great benefit from this experience in his later work on sugars. Fischer used micro-scale

fermenters to test the sugars that he characterized including those fully synthesized in his laboratory. He noticed large differences between the fermentability of sugars whose only difference was the stereochemical configuration about carbon atoms (*18*). He was able to use these differences to separate enantiomers, which are mirror images of one another and which have identical physical properties. The observed selectivity of the fermentation reactions led him, in 1894, to propose the lock and key model that, with modern modifications, forms the basis for our understanding of enzyme action. In 1899 Fischer turned his attention to proteins. By 1906 he had discovered three new amino acids, and even more significantly, he was able to condense amino acids into dipeptides, making a peptide bond (*19*). In 1902 Emil Fischer became the second recipient of the Nobel Prize in Chemistry.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

Yeast Juice

While Fischer was making foundational discoveries based on carefully planned, goal-oriented work, Eduard Buchner (1860-1917), working with his brother on a completely different problem, stumbled on a way to conduct fermentation in the absence of cells. The story begins with Eduard's older brother Hans, a professor at the Institute of Hygiene in Munich. The objective was to find a way to extract proteins from bacterial cells. Yeast was used to perfect the technique. Sugar was introduced to suppress bacterial growth (*20*). The breakthrough to fermentation without any cells or parts of cells was a lucky accident. Yet, had Eduard Buchner failed to notice bubbling or failed to realize its significance, progress in biochemistry could have been delayed for years.

Buchner rinsed brewing yeast (available in abundance in Munich), pressed it dry, added abrasives, and ground it with a huge mortar and pestle. He added water, wrapped it in filter cloth, pressed it in a hydraulic press at 400-500 atm, and filtered the resulting liquid. When any of several sugars was added, fermentation began in 15-60 minutes and continued for days (*3*). Buchner called the fermentation enzyme zymase. Today we ascribe the alcoholic fermentation process to twelve enzymes, plus several cofactors. The discovery of cell-free alcoholic fermentation was of profound significance in the rise of biochemistry, not so much for its own content, but for the studies that it enabled. Prior to Buchner, fermentation could only be observed in intact cells. A cell is a complex, ever-changing apparatus that makes an unpromising target for chemical research. A cell-free extract, by contrast, offers a myriad of possibilities for experimental exploration with a reasonable chance of being able to interpret the results. Buchner himself got the ball rolling by demonstrating that the fermentation was unaffected by various substances that would have killed live yeast. Soon there were several preparations of cell-free yeast extract available to researchers. Buchner was awarded the seventh Nobel Prize in Chemistry in 1907 for the discovery of cell-free alcoholic fermentation.

Muscle

As early as 1907 W. M. Fletcher (1873-1933) and F. Gowland Hopkins (1861-1947) discovered that when frog muscle is exercised without oxygen, lactic acid (Figure 15) accumulates. When oxygen is present or is provided later, the

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

lactic acid disappears (*21*). Hopkins was awarded the Nobel Prize in Physiology or Medicine in 1929 for the discovery of vitamins. Lactic acid indirectly links alcoholic fermentation with anaerobic (no-oxygen) muscle action, which is a type of fermentation. Ethanol (Figure 17), the end product of alcoholic fermentation can, in theory, be made by removing carbon dioxide from lactic acid. It turns out that both ethanol and lactic acid are made from a common intermediate, pyruvic acid (Figure 14). The discovery of some of the same phosphate esters in muscle as in yeast-juice fermentation tightened the link. In 1912 Gustav Embden (1874-1933), encouraged by the successes with cell-free yeast juice, began making cell-free juice from animal muscle (*22*).

By 1925 Otto Fritz Meyerhof (1884-1951) and others realized that alcoholic fermentation in yeast juice was mostly the same as lactic fermentation in muscle. Meyerhof was even able to stimulate lactic acid production by adding yeast juice to muscle juice whose fermentation capacity had been exhausted. He was awarded the Nobel Prize in Physiology or Medicine in 1922. The merging of yeast and muscle work not only enlarged the field of observations bearing on the fermentation problem, but also increased the centrality of the work. A pathway that is applicable both to yeast, a non-motile single-cell eukaryote, and to animal muscle cells must be central to the life processes of all eukaryotic life. We now know that glycolysis in some form is used by every living cell including prokaryotes (bacteria). Muscle brought another issue into focus, energy. A muscle is a device that converts chemical energy to mechanical work. Meyerhof was especially interested in the thermodynamics of metabolism, that is, the function of the fermentation reactions in providing available chemical energy (*23*). Progress picked up.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

Glycolysis

The glycolysis reactions were not discovered in order, nor were all the products and reactants understood at the time a reaction was discovered. We will use standard chemical nomenclature rather than the specialized jargon of biochemistry. To avoid the complication of varying degrees of protonation, we will depict acidic compounds in the protonated form, ignoring possible ionization. Our objective is to explain some of the key pieces of evidence for the glycolysis pathway and its continuation to ethanol or lactic acid.

Step 1: Glucose Phosphorylation

The first step of glycolysis is the transfer of a phosphoryl group from ATP to glucose (Figure 3). In 1914, Arthur Harden (1865-1940) and Robert Robison (1883-1941), at the Lister Institute in London, discovered a hexose monophosphate during the fermentation of glucose or fructose by yeast-juice in the presence of sodium hydrogen phosphate (Na_2HPO_4). The compound was isolated by precipitation with barium ion. Elemental analysis was consistent with $C_6H_{11}O_6PO_3Ba$ (*24*). It was not until 1931 that Robison purified the hexose monophosphate and determined it to be glucose-6-phosphate (G6P) (*25*).

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Meyerhof noticed glucose phosphorylation in muscle in 1927 and was able to isolate the enzyme for it, now called hexokinase. Adenosine triphosphate (ATP, properly termed adenosine-5′-tripolyphosphate) and some inkling of its role in phosphate transfer were discovered in 1929 in muscle by Cyrus Hartwell Fiske (1890-1978) and Yellagaprada SubbaRow (1896-1948) at Harvard University (*26*) and by Karl Lohmann (1898-1978) at the Kaiser Wilhelm Institute for Biology in Berlin in 1929 (*27*). Meyerhof determined that ATP was the second reactant for this step in 1935 (*28*). Other details were eventually added, including the involvement of magnesium ions and, most importantly, the role of ATP in the transfer of energy. In 1941 S. P. Colowick and H. M. Kalckar, at Washington University in Saint Louis, published mechanistic details of this reaction catalyzed by hexokinase extracted from yeast (*29*).

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

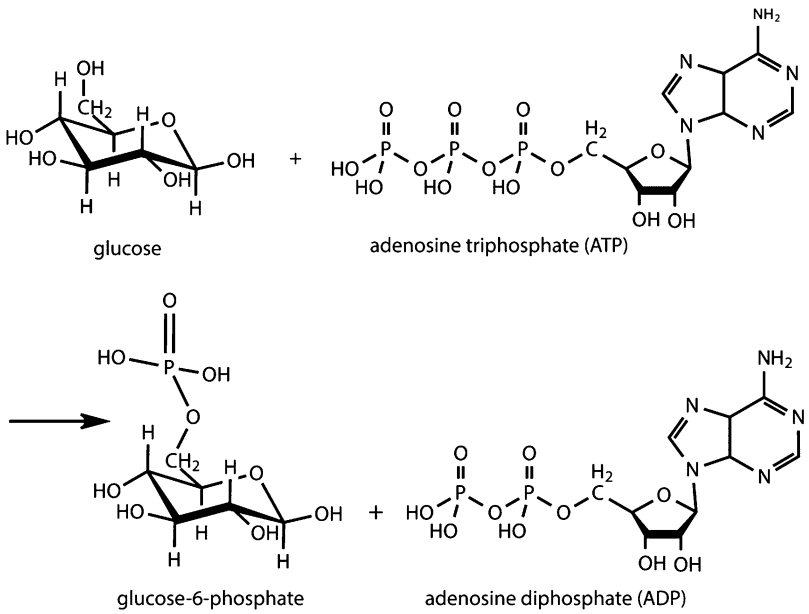

Figure 3. Glycolysis step 1.

Step 2: G6P Isomerization

In step 2 G6P is isomerized to fructose-6-phosphate (F6P), as shown in Figure 4. F6P was first prepared artificially from fructose-1,6-diphosphate, a fermentation product, by Karl Neuberg (1877-1956) (*28*). Robison isolated it from yeast-juice fermentation in 1932 (*30*). The enzyme for this reaction, glucose-6-phosphate isomerase, was found in muscle in 1933 and in yeast in 1947 (*28*).

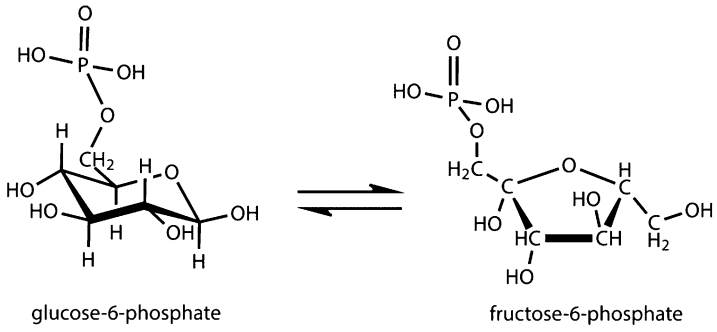

Figure 4. Glycolysis step 2.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

Step 3: F6P Phosphorylation

Step 3 is a second phosphorylation giving fructose-1,6-diphosphate (FDP), also called fructose bisphosphate. Figure 5 shows step 3 using familiar biochemistry notation for reactions involving a cofactor.

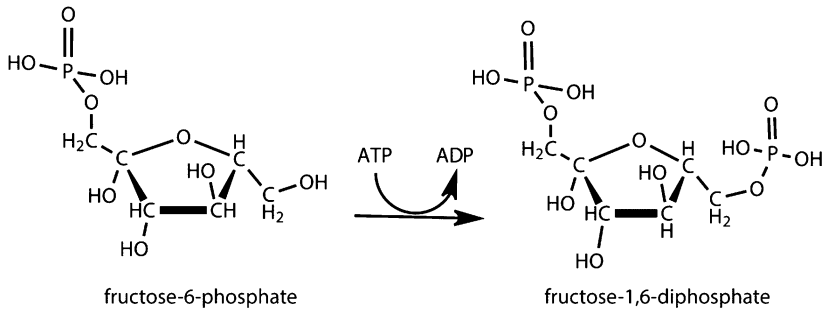

Figure 5. Glycolysis step 3.

Buchner's press juice produced alcoholic fermentation, but it was a poor substitute for living yeast. Fermentation started slow and got slower, dying off completely well before the sugar supply was exhausted. Researchers at the time suspected that proteolytic (protein breaking) enzymes in the press juice were destroying the fermentation enzymes. Harden, working with William Young (1878-1942), tried adding various forms of deactivated yeast juice to an active sample, in the hope that the protein fragments from the proteolysis would bind to the proteolytic enzyme, decreasing its ability to destroy the fermentation enzyme. This worked quite well, even for addition of boiled yeast juice. But the antiproteolytic effect and the enhancement of fermentation did not vary in

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the same way. Harden and Young inferred that there was something else in the inactivated additions that was necessary for fermentation. In a series of papers (*31–33*) summarized in Harden's book (*34*), Harden and Young reported the following instructive series of discoveries regarding cell-free alcoholic fermentation.

- If no phosphate is added, the fermentation slows down to a slow steady-state rate.
- When phosphate is added, the fermentation rate increases temporarily to a much higher rate.
- The additional production of carbon dioxide and ethanol is equal to the moles of phosphate added.
- After the period of enhanced fermentation activity, the phosphate exists in a bound organic form that does not form a precipitate with magnesium citrate or with uranium citrate. It does form a precipitate with lead acetate.
- The phosphate is released from the organic part after fermentation stops.
- The lead precipitate has the formula $C_6H_{10}O_4(PbPO_4)_2$.
- The lead can be removed by treatment with hydrogen sulfide giving $C_6H_{10}O_4(H_2PO_4)_2$.
- The organic phosphate can be hydrolyzed to give phosphoric acid and a mixture of sugars, mostly fructose.
- The yeast-juice contains an enzyme that hydrolyzes the (presumed) phosphate ester.

Harden and Young introduced the practice of following the progress of fermentation continuously by measuring carbon dioxide generation with the apparatus shown in Figure 6. This enhanced accuracy and productivity; more could be learned from each fermentation run.

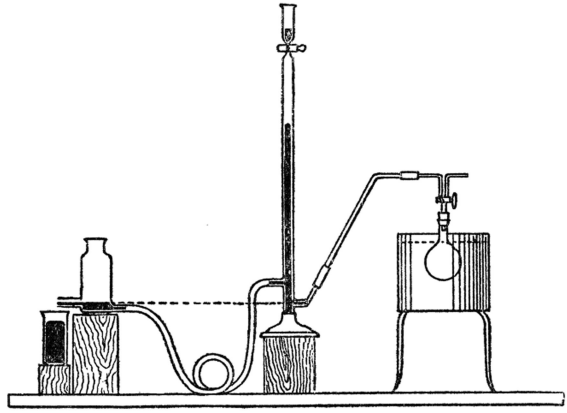

Figure 6. Carbon dioxide measuring apparatus, Harden, 1923 (34). Othmer Library of Chemical History. Chemical Heritage Foundation.

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Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

Fructose-1,6-diphosphate (FDP) was the first of the sugar-phosphate esters to be discovered in alcoholic fermentation because it accumulates when step 6 is blocked by removal the cofactor NAD, as explained in step 6. Step 3 is irreversible, but steps 4 and 5 are reversible; when their products accumulate, their reactants do as well, backing up the pathway up to the step 3 product, which allowed Harden and Young to recover and analyze it in 1908 (*32*). Even though the exact structure of the hexose phosphate ester was not known, its discovery is attributed to Harden and Young. For a long time it was called the Harden-Young ester. It was not until 1928 that the Harden-Young ester was identified as fructose-1,6-diphosphate (*35*). The discovery of this phosphate ester was an essential step in understanding the glycolysis process. It foreshadowed the central role of the phosphate group in the handling of energy and in biological signaling, such as G-coupled protein systems. Eventually a series (Table 1) of six-carbon sugar (hexose) phosphate esters was discovered in association with alcoholic fermentation (*24*, *30*).

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

Table 1. Hexose Phosphate Esters

ester	*year*
fructose-1,6-diphosphate	1908
glucose-6-phosphate	1914
fructose-6-phosphate	1932

The enzyme for step 3, phosphofructokinase, was discovered in muscle in 1936 and in yeast in 1947 (*28*).

Step 4: Splitting of FDP

In step 4, FDP is split into two monophosphorylated trioses (three-carbon sugars). Figure 7 shows fructose-1,6-diphosphate in the open chain form to make it easier to understand the decomposition reaction. The products are the phosphates of simple sugars, dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GA3P). The enzyme is called aldolase.

This step was difficult for fermentation chemists to elucidate. It was known that ethanol, the final product of yeast fermentation, and lactic acid, the final product of muscle fermentation derive from pyruvic acid, a three-carbon compound (see step 9). So some sort of decomposition of hexose to three-carbon intermediates was expected. In 1911 Alexander Nikolaevich Lebedev (1861-1938), the developer of another type of yeast juice, found that FDP is formed during the fermentation of the triose phosphates, DHAP and GA3P (*28*). This suggests that this part of the fermentation process is reversible; it can go from six-carbon sugars to three-carbon sugars and back. Step 4 products are

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

present in low concentration, because if the fermentation reaction is going well, they are rapidly consumed. If the process is blocked at one of the subsequent steps, the equilibrium favors the reactant, FDP. Another problem was that FDP itself ferments very slowly under ordinary circumstances. This led many to conclude that the phosphate esters were by-products rather than intermediates. In 1930 Ragnar Nilsson provided the first clue to the central role of the phosphate esters. He used fluoride ion to inhibit the formation of pyruvic acid, one of the late steps in fermentation, and he supplied acetaldehyde (Figure 16), produced in an even later step. FDP was consumed, the acetaldehyde was hydrogenated to ethanol, and there was a large accumulation of 3-phosphoglyceric acid (Figure 11) (*28*). The correct solution was provided by Embden in 1932. He reasoned that phosphoglyceric acid could be an oxidation product of GA3P, which, along with DHAP, would be logical to expect from the splitting of FDP. He looked for these products and found them. This allowed Embden to propose an essentially correct, but still incomplete scheme for glycolysis (*36*). Had Embden not died the year after this contribution, he might have won a Nobel Prize. Meyerhof confirmed that DHAP and GA3P are produced in equal amounts by stopping the next step with hydrazine.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

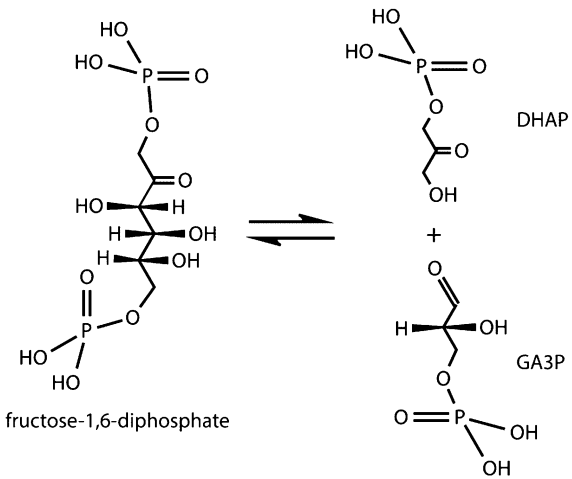

Figure 7. Glycolysis step 4.

Step 5: DHAP Isomerization

Step 5 is the isomerization of DHAP to GA3P (Figure 8), catalyzed by triose phosphate isomerase.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

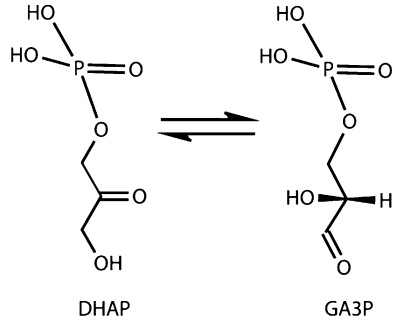

Figure 8. Glycolysis step 5.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

Meyerhof observed rapid equilibration of these two triose esters. So far, one molecule of glucose and two molecules of ATP have been consumed. The rest of the pathway goes from the two molecules of GA3P that have been produced. If step 12 is blocked by sulfite ion, the hydrogen atoms that would have been used to convert acetaldehyde to ethanol can end up reducing DHAP to glycerol phosphate, leading to glycerol (Figure 9). Glycerol for nitroglycerine was produced by this process during World War I (*28*).

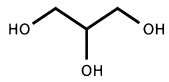

Figure 9. Glycerol.

Step 6: Oxidation/Phosphorylation of GA3P

In this step, shown in Figure 10, glyceraldehyde-3-phosphate, an aldehyde, is oxidized to an acid and simultaneously converted to the anhydride with phosphoric acid, 3-phosphoglyceroyl phosphate (3PGP), also called 1,3-diphosphoglycerate. The enzyme is called glyceraldehyde phosphate dehydrogenase. The key clue to this step came in 1937 from muscle studies by Dorothy Needham (1896-1987), who noticed that when step 10 was poisoned with fluoride, inorganic phosphate continued to react to give an organic phosphate. She discovered that the amount of phosphocreatine (a storage form of reactive phosphate in muscle) was more than the amount of phosphorus transferred in step 11. She proposed that GA3P was undergoing a second phosphorylation (*22*). 3PGP was discovered by Erwin Negelein (1897-1979) and Heinz Brömel (1914-1941) in the laboratory of Otto Warburg (1883-1970) in 1939 (*28*).

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch003

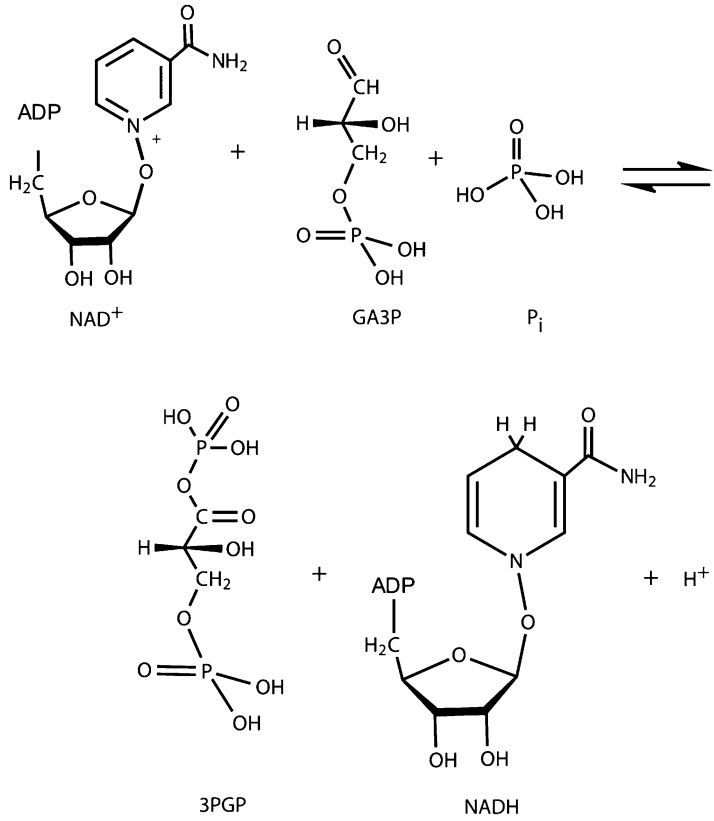

Figure 10. Glycolysis step 6.

Nearly 35 years earlier in 1905, Harden and Young made another key discovery, the coenzyme that we now call nicotinamide adenine dinucleotide (NAD, formerly known as DPN). When yeast juice was subjected to dialysis using gelatin as a semipermeable membrane, the juice separated into two fractions, neither of which could ferment sugar. A mixture of the fractions caused vigorous fermentation. This showed that in addition to the yeast enzyme(s), and phosphate, fermentation requires another small (compared to an enzyme) molecule. The coenzyme (using today's jargon) was not destroyed by boiling, unlike the enzyme fraction (*31*, *37*). The discovery of NAD, like that of fructose diphosphate, had fundamental significance. Since the time of Lavoisier, chemists had focused their attention on oxygen, especially in reactions involving significant transfer of energy. The release of energy from food was, and still is, understood as a combustion process. NAD and related coenzymes serve as carriers of oxidizing capacity, not by the transfer of oxygen, but by the transfer of hydrogen, as shown in Figure 10. Oxygen enters the respiration process only as the final acceptor of electrons. It took years before this could be understood, but the first step was the discovery of NAD.

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Step 7: First Phosphorylation of ADP

In step 7 Adenosine diphosphate (ADP, properly termed adenosine-5′-pyrophosphate) is phosphorylated by 3PGP, as shown in Figure 11. This is the first step that makes ATP. At the completion of this step, consumption and production of ATP are even. Two molecules of ATP have been consumed to make FDP (steps 1 and 3) and two are produced in this step (taking into account that each step after the splitting of FDP in step 4 occurs twice.

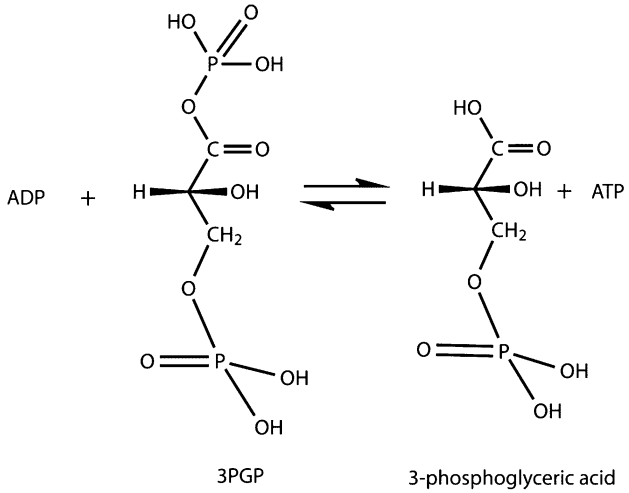

Figure 11. Glycolysis step 7.

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In 1932 Embden determined that 3-phosphoglyceric acid was an intermediate in the formation of pyruvic acid. Fifteen years later in 1947, working in Warburg's laboratory, Theodor Bücher (1914-1997) found the enzyme for this reaction, phosphoglycerate kinase, in yeast juice (*28*).

Step 8: Phosphate Shift

In step 8, the phosphate group shifts from carbon 3 to carbon 2, as shown in Figure 12. In 1933 Meyerhof found an enzyme, called phosphoglycerate mutase that catalyzed this reaction (*28*). It is interesting that steps 7, 8, and 9 are all equilibrium reactions, indicating that the Gibbs energy of the products is close to that of the reactants (*28*).

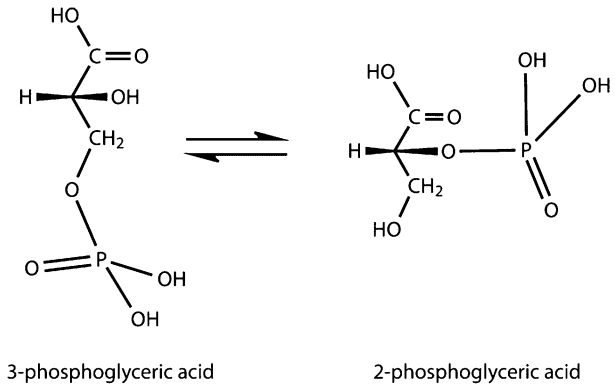

Figure 12. Glycolysis step 8.

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Step 9: Phosphoenolpyruvic Acid Synthesis

In step 9 the 2-phosphoglyceric acid is dehydrated to phosphoenol pyruvic acid (PEP), as shown in Figure 13. The recognition of PEP came from a series of discoveries in the period from 1910 to 1920 by Carl Alexander Neuberg (1877-1956), that pointed to pyruvic acid (2-oxopropanoic acid, Figure 14), a dephosphorylated version of PEP, as a key intermediate in alcoholic fermentation. Neuberg showed that pyruvic acid is decarboxylated to acetaldehyde, and acetaldehyde is hydrogenated to ethanol. In addition, if the hydrogenation of acetaldehyde is inhibited by trapping it with sulfite, fermentation of a hexose yields glycerol, carbon dioxide, and the sulfite-trapped acetaldehyde, but no ethanol.

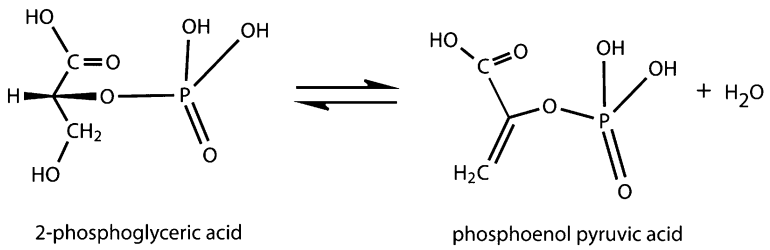

Figure 13. Glycolysis step 9.

In 1933 Embden showed that 2-phosphoglyceric acid releases water and phosphate to give pyruvic acid. In 1934 Meyerhof showed that this process involves three reactions (steps 8, 9, and 10). When yeast juice is subjected to dialysis, ADP is removed, blocking step 10. This allows phosphoenolpyruvic acid (PEP) to accumulate, otherwise it is a fleeting product of the second reaction

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(step 9). The enzyme for this reaction, phosphopyruvate hydratase, also called enolase, was crystallized in 1941 by Otto Warburg, who won the Nobel Prize for Physiology or Medicine in 1931 for discoveries on cellular respiration (*28*).

Step 10: Second Phosphorylation of ADP.

Step 10 is the phosphorylation of ADP by PEP, shown in Figure 14. This step yields the second pair of ATP molecules made in glycolysis. One ATP is consumed in step 1, and one in step 3. Two ATP molecules are made in step 7 and two in this step. The net production of ATP is two molecules of ATP for each molecule of glucose. The other change in cofactors is the reduction of one molecule of NAD^+ to NADH + H^+. Glycolysis itself ends at this point with pyruvic acid, ATP, and NADH entering other pathways. In the absence of oxygen, there are additional steps to oxidize the NADH. In the case of muscle fermentation there is one more step. For alcoholic fermentation, there are two.

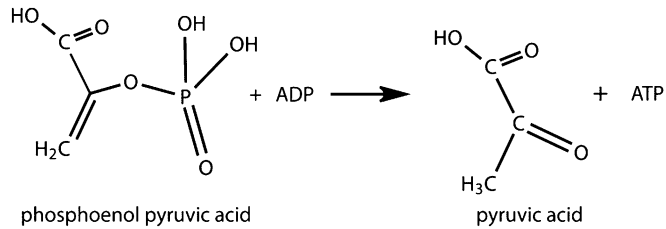

Figure 14. Glycolysis step 10.

In 1934 Jakob Parnas (1884-1949) and coworkers reported the formation of ATP from glycolysis. They agreed with Embden that it came from phosphoglyceric acid. Hermann Lehmann (1910-1985), who was working for Meyerhof because the Nazis wouldn't let him take the medical exam in 1933, showed that the PEP was the source of the phosphate transferred to ADP. The enzyme for this reaction, pyruvate kinase, was discovered by Meyerhof and Karl Lohmann (not Lehmann) in 1934 (*28*).

Lactic Acid Synthesis

The reduction of pyruvic acid to lactic acid, shown in Figure 15, was one of the reactions discovered by Neuburg in 1910 (see step 9). The pattern of discovery shows that it was easier to work out the first steps, those close to known reactants, and the last steps, which are close to known products, than to decipher the intermediate steps. For a long time it was thought that synthesis of lactic acid was integral to the action of muscles. We now know that the key product of this reaction is NAD^+, without which step 6 cannot proceed.

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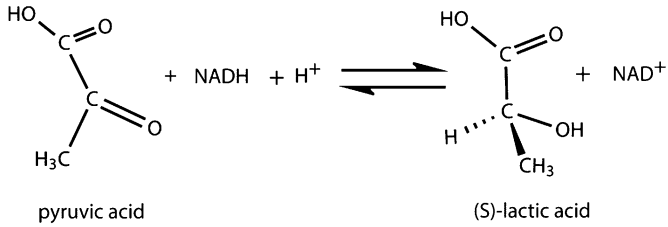

Figure 15. Muscle: lactic acid synthesis.

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Pyruvate Decarboxylation

Once pyruvic acid is known to be involved, the reaction shown in Figure 16 can be expected, especially given that carbon dioxide has been known as a product of alcoholic fermentation since the days of Antoine Lavoisier (1743-1794). Neuberg discovered this reaction in the fermentation of pyruvic acid by yeast in 1911. The enzyme, pyruvate decarboxylase, was isolated from yeast in 1941 by Fritz Kubowitz and Wilhelm Lüttgens, assistants of Warburg (*28*).

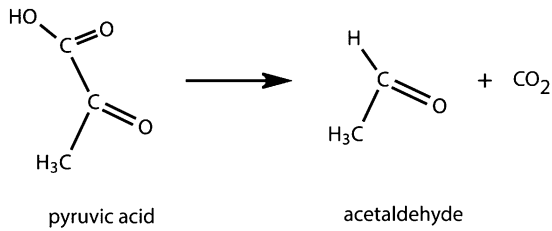

Figure 16. Acetaldehyde synthesis.

Ethanol Synthesis

The hydrogenation of acetaldehyde to ethanol was known to classical organic chemistry. The reaction shown in Figure 17 was discovered by Neuberg in 1910. It took longer for the function of NADH to be understood. An enzyme for this reaction, alcohol dehydrogenase, was isolated from a liver preparation in 1909. Ethanol dehydrogenase was purified from yeast in 1937. This reaction regenerates NAD^+ during alcoholic fermentation, allowing step 6 to continue (*28*).

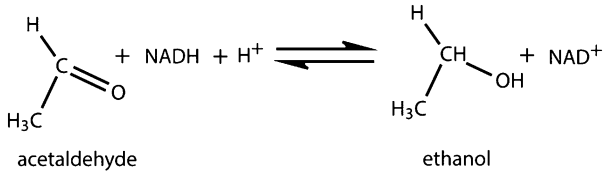

Figure 17. Ethanol synthesis.

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Energy

The biological purpose of the glycolysis pathway was only understood after its reactions had been completely revealed. Muscle studies led to the key discovery of adenosine triphosphate (ATP, properly termed adenosine-5′- tripolyphosphate) in 1929 by Fiske and SubbaRow (*26*). This discovery was made possible by their spectrophotometric method for phosphate analysis. The process of discovering the transfer of a phosphoryl group from ATP to glucose to give the first of the phosphate esters took thirty years.

- 1905 Phosphate needed for fermentation by yeast-juice, Harden and Young.
- 1908 Fructose diphosphate (FDP) discovered, Harden and Young.
- 1914 Discovery of glucose-6-phosphate (G6P) in yeast-juice fermentation, Harden and Robison.
- 1927 Hexose kinase: phosphorylates glucose, fructose, and mannose, Meyerhof.
- 1929 ATP, Fiske and SubbaRow.
- 1931 Structure of G6P determined, Robison.
- 1935 ATP is the source of the phosphate transferred by hexose kinase, Hans von Euler-Chelpin (1873-1964).

A complete account of the storage and release of energy by the ADP/ATP system was provided by Fritz Lipmann (1899-1986) (*38*). He devised the term "energy-rich bond," which has misled generations of students into thinking that such bonds are like loaded springs that release energy when broken. Lipmann shared the 1953 Nobel Prize in Medicine or Physiology with Hans Krebs. This completed the picture. Glycolysis converts one molecule of sugar to two of pyruvic acid and uses the energy released to phosphorylate two molecules of ADP to ATP. The ATP carries energy to other systems. During the process, one molecule of NAD^+ is reduced. In the absence of oxygen, NADH is regenerated by the hydrogenation of pyruvic acid to lactic acid in muscle or of acetaldehyde to ethanol in yeast. If oxygen is present, NAD^+ can be regenerated by subsequent respiratory processes. Some form of glycolysis occurs in every living cell.

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Conclusion

Modern biochemistry started with alcoholic fermentation. The first biochemical pathway was investigated with the alcoholic fermentation reactions effected by cell-free yeast juice. The success of this approach led to similar methods being applied to muscle. The combination of these two strands led, over a period of 35 years, to the discovery of all the reactions of the glycolysis pathway. The methods and insights of this multinational project continue to drive biochemistry forward to this day. Nonetheless, glycolysis is not a completely worked out problem. Control and feedback in the enzymatic reactions are still under active investigation. Yeast will continue to play a central role. Yeast is available in abundant supply and in many pure strains. The generation time is a few minutes. Research review committees do not protect the rights of yeast. The first eukaryotic organism to have its complete genome sequenced was the yeast *Saccharomyces cerevisiae* in 1997 (*39*). We can confidently expect that the many advantages of yeast as an experimental organism will continue to keep alcoholic fermentation at the forefront of research on metabolism.

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Chapter 4

Beer and Brewing: Credible and Cultured Capstone Chemistry

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The instruction of prospective brewers in the matter of beer and brewing is far more than a means for teaching chemistry in the context of what is for many a desirable and pleasurable product. A good appreciation of the essential principles of chemistry and biochemistry is certainly an a priori requirement to fully understand the processes of converting crops into the world's favorite adult beverage. However it is equally important for a student to have a decent grasp of plant physiology, chemical engineering, microbiology, physics, sensory science and more besides. They need to be energized with a passion for the process and the product. The most valuable brewing courses in academia are taught by (or at the very least coordinated by) individuals with a genuine understanding of the realities of life within the industry.

Principles, Appreciationn and Understanding - Not Memorization

Sir Hans Krebs is perhaps the most eminent biochemist of all time. Amongst other things, he of course delivered us the Tricarboxylic Acid or Citric Acid Cycle, more frequently referred to using the great man's name.

Krebs was lecturing one day about this very topic.

"And we must use as our starting point the end-product of the glycolysis pathway, pyruvic acid."

At which point he moved to chalk up the formula on the board, hesitated, turned, picked up his notes, and copied the structure therefrom.

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Hence I say to my students that if Krebs could not remember CH3COCOOH then they don't need to either. Not that, and certainly not that of iso-α-acids, or polyphenols, or the 300+ components of the essential oil of hops.

Which is not to say in any way that chemistry is not important. It is critical to have a fundamental understanding of the principles underpinning chemistry if one is to understand brewing. Equally it is important to have a grasp of microbiology, physics, chemical engineering, sensory science, botany, plant physiology…the list goes on.

A Brewer Does Not Thrive by Molecules Alone

I fervently believe that brewing is not a handy-dandy way to teach chemistry, as seems to be the approach taken in the burgeoning number of universities and colleges that seek to educate the brewers of tomorrow. The education of brewers is far more than this. They don't need to memorize formulae, but they need to be able to apply chemical principles in a brewing context, no more and no less than they need to be able to understand how a pump works, how to kill a bacterium or how to nucleate a bubble and thus draw on relevant elements of engineering, microbiology and physics respectively.

I believe that the teaching of brewing is an ideal capstone to a university education that embraces a breadth of fundamental science and engineering courses. At UC Davis we find that this is conveniently packaged within a Food Science major. I have steadfastly avoided establishing a major in Brewing per se: it is not a good idea in my opinion to saddle oneself with too narrow a name on a certificate if, at the end of a university career, you have decided not to be a brewer or if no suitable openings arise. Fermentation Science would be acceptable, but nowadays we choose to take the even more encompassing Food Science route, the expectations of which can be inspected at http://foodscience.ucdavis.edu/undergraduate/bsmajor/foodsciencebrewingoption.html.

However many of the students in my brewing classes are majoring in other disciplines, especially Biotechnology, Chemical Engineering and Viticulture & Enology (in this latter case you may see what I mean about "narrowness"). Any student taking the upper division brewing classes (FST102A and FST102B, which are described in Sidebars 1 and 2) must have passed the necessary prerequisites, notably in terms of fundamental chemistry and biochemistry.

Perusal of this material will, I hope, convince the reader that a successful student must be able to draw upon a diversity of sciences, not chemistry alone.

Practical Realities

And yet still I find myself saying to students that no matter how much I teach them in the classroom (drawing on 36 years' experience in the brewing industry – I fervently believe that whoever teaches brewing should have industrial credibility) they will learn far more by working within a brewery. Even in a pilot scale brewery (and we are very proud of ours at UC Davis, which comprises one 1.5 barrel brew length system and four 5 gallon sculptures) one cannot teach the practical realities

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of day to day life in a commercial operation. Things happen that you will not read about even in the best brewing textbooks. A major plus is internship experience, especially one where a brewery will allow a student to gain genuine hands-on involvement in operational activities.

In this context, then, there is much to be said for a brewer's education to take what I might call the opposite route: learn at the sharp end first by working in a brewery and then splice in the academics afterwards through shorter courses. At UC Davis we do this through Extension programs (https://extension.ucdavis.edu/areas-study/brewing). The difference between this route and the on-campus approach described earlier is that, for some of the courses, a student may not necessarily have quite the fundamental science background that is necessary. For the one week class on practical brewing, for example, I send out some pre-reading to cover the basic chemistry and biochemistry (*1*, *5–9*). Perusal of these articles will give the reader an idea of just how much fundamental chemistry and biochemistry I personally believe is necessary to prepare a student for a basic appreciation of brewing science.

One of my books, currently in its Third Edition, is entitled *Beer: Tap Into the Art and Science of Brewing* (Oxford University Press). The word Art for me encompasses the ethos of beer and brewing: the culture, the traditions, the diversity, the passion. To my mind it is essential that a student embraces these dimensions – and in my lower division General Education class at Davis we address these things (Sidebar 3). Teachers of tomorrow's brewers have a responsibility far beyond chemical formulae.

Sidebar 1. Upper division Malting and Brewing Science Lecture Class (FST102A) at UC Davis

(a) Content (2 x 2h classes per week for 10 weeks ; there are also two field trips, one to a microbrewery and the other to a regional brewery)

- Outline of malting and brewing
- Styles of beer
- Overview of quality
- Barley and malting
- Barley biochemistry
- Sweet wort production
- Water
- Hops
- Wort boiling, clarification and cooling. Sugars
- Yeast and Fermentation
- Microbiology
- Beer Flavor
- Haze stability
- Processing of beer
- Flavor stability
- Light stability
- Foam and gushing

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- Hygiene
- Packaging
- Environmental

(b) Learning outcomes: At the end of this course students will be able to:

- explain the essential compositional features of barley, water, hops, yeast and other ingredients as they pertain to brewing process performance and product quality
- explain the chemistry, biochemistry and physics underpinning the processing treatments that are used to render barley, water, hops, yeast and other components into forms suitable for brewing
- illustrate and describe the unit processes leading from barley to packaged beer
- name and give examples of the key microbial threats to brewing and beer and outline how they are detected and dealt with
- summarize the basic principles of plant cleaning and sanitation
- name the key contributors to beer flavor, outline the pathways by which they arise and discuss the factors that influence the levels at which they are found in different beers
- explain the chemistry, biochemistry and physics of quality attributes, notably foam, gushing, color, haze and physical stability

(c) Class text: Bamforth, C.W. (*2*)

Sidebar 2. Upper division Malting and Brewing Science Laboratory Class (FST 102B) at UC Davis

(a) Content (weekly for ten weeks: one 2h open discussion session; one half day laboratory session)

Students alternately address analytical issues (methodology primarily from the standard methods of the American Society of Brewing Chemists) and perform practical brewing either on the 1.5 barrel brewery or one of the 5 gallon breweries. There is an "Iron Brew" competition in which students (as small groups) design, brew, analyze and present their beers for expert judging. The winning beer is brewed commercially at a local brewery.

(b) Learning outcomes: At the end of this course students will be able to:

- differentiate the principles of Quality Assurance from those of Quality Control and outline the essential components of a quality system in the malting and brewing industries

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Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch004

- explain the basic statistics relevant to making, reporting and interpreting analytical measurements in a brewing context
- describe the concept of Standard Methods of Analysis, explain how the methods emerge and evolve and summarize how they are employed
- summarize and practice key analytical tests on barley, malt, hops, yeast, wort and beer
- interpret the key analytical parameters applied to water for brewing
- interpret data sheets for barley, malt, adjuncts, water, hops, yeast, wort and beer
- plan and calculate all relevant process parameters in the production of beers in the experimental breweries
- formulate brewing recipes and produce beers in the experimental breweries

(c) Class text: Bamforth, C.W. (*3*)

Sidebar 3. Lower division Beer and Brewing Class at UC Davis

(a) Content (2 x 1.5h classes per week for 10 weeks)

Note: Guest speakers vary from class to class. Others include Vinnie Cilurzo (Russian River), Brewmasters from Anheuser-Busch-Inbev, Mont Stuart (maltster) from Miller-Coors, Fritz Maytag (Anchor).

The basics of beer and brewing
Malt and hops: the soul and spice of beer
Ryan Fry and Trenton Yackzan: *Sudwerk*
Yeast and water: Godesgood and how beer is water with added value
A walk through the brewery: from milling to cardboard boxes
The sociology and business of brewing
History of beer and brewing – world and US
Some great Brewing companies
Beer styles and types
Paul Ghiglieri: *Rice*
The quality of beer: heads, hues and haze etc
Ken Grossman: *Sierra Nevada*
Beer as part of the diet: the pro's and the cons
Responsibility: everything in moderation
Dan Gordon: *Gordon Biersch*
Film: *Brewdogs*
Review session

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(b) Learning outcomes: At the end of this course students will be able to:

- list the successive unit stages in malting and brewing
- state what the purpose of each of those unit stages is and briefly describe what occurs in them, how long they take and recall what the temperature ranges are for each of the unit process stages
- list the major ingredients used in the production of beer and identify the key properties demanded of them
- identify the major beer markets in the world
- identify the major brewing companies in the world
- describe the evolution of the brewing industry in America (English and German influence, prohibition, consolidation, microbrewing revolution)
- outline the major styles of beer and state how they differ in their raw materials and production protocols
- describe how the strength of beer is quantified (alcohol and Plato) and indicate what the approximate strength of different styles of beer is
- summarize what the major substances are that contribute to beer taste and aroma, foam, clarity, gushing and color and identify their origin
- explain where the major contributors to flavor are detected (nose, mouth, trigeminal sense)
- indicate the relationship between beer composition and bodily health, in terms of beer composition (alcohol, calories, vitamins, minerals, antioxidants, fiber, antimicrobials, pH) and the basic rationale for the U-shaped or J-shaped curve
- describe the concept of a "unit of alcohol"

(c) Class text: Bamforth, C.W. (*4*)

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Chapter 5

Introduction to Brewing Science Courses

Comparison of General Studies and Full Program Options

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Brewing Science courses have been implemented in many institutions across the country. These courses have included many curriculum options and serve a wide variety of purposes within the departments in which they are housed. The present discussion centers on comparison of courses within a general studies curriculum and within a program in Brewing Laboratory Science. While the differences seem significant at the surface, there is a great deal of overlap between course objectives, student learning outcomes, and interest in these topics. Herein we outline both curriculum and courses within two programs that lie at the extreme ends of the spectrum. Their comparison highlights the students' greater appreciation for the interdisciplinary nature of Brewing Science and for the application of chemistry to 'real-world' problems.

Introduction

Craft brewing has experienced tremendous growth in the last decade, with no signs that this growth is slowing. For example, 175 breweries were in operation in Colorado in 2013, 151 were operating in 2012, and 126 in 2011 (*1*). This is a nationwide trend that is reflected in the growth in craft beer marketshare increases (17.2% increase in 2013), the economic impact within the country, and the huge demand for employees in this industry.

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To address student demand for training in this field, many new programs focusing on brewing science have been put in place by both 2- and 4-year higher education institutions across the country (*2*). The myriad of courses that have been developed span the curriculum from special topics courses within a major, to general studies courses for non-majors, to introductory courses within developing brewing science programs. The level of each course has reflected student and curricular demand based on institutional requirements.

Traditionally, school curricula have been largely based on the concept that instruction should be separated into distinct subjects for ease of understanding and then reassembled when complex applications are required (*3*). In recent years, interdisciplinary instruction, in which a common theme is studied in more than one content area (*4*, *5*), has gained popularity. Further, it has been shown that interdisciplinary instruction can enhance knowledge transfer by sustaining enthusiasm on the part of the students (*6*). Introductory brewing science courses, by their very nature, fall nicely into this interdisciplinary instruction motif.

At the University of Northern Colorado (UNCo), an interdisciplinary course has been created that serves as the introductory course for an undergraduate Minor and Certificate program in Brewing Laboratory Science. The course requires that students have met minimum pre-requisite coursework in science. The topics in the course are geared for the student interested in this area, with the idea of preparing the student for post-graduation work in a brewery.

At the University of Nebraska at Kearney (UNK), a course has been created that employs the same interdisciplinary science discussion centered on the theme of classical beer brewing. The class is intended for the student who dislikes (or 'hates') science. It is hoped that the central theme will keep students motivated and engaged. The science discussed in the class is presented at the general studies (liberal arts) level, and offered as a senior capstone class in the university's General Studies program.

These courses operate at the extremes of the spectrum of brewing science instruction. While the immediate impression is that they are vastly different in almost every area, the similiarities between the courses indicate that they both serve to introduce the overarching topic in a way that improves student understanding, provides an outlet for de-compartmentalizing multi-disciplinary topics, and advances a strong appreciation for beer, brewing, and the science behind this interesting topic.

Brewing Science Program at UNCo

The mission and vision of the University of Northern Colorado is to provide transformative learning experiences that improve students' competencies in critical thinking, communication, and problem solving (*7*). These experiences are designed to enhance a student's experience in leadership and the application of technology to address needs for the state of Colorado. University funding for the internal development of new programs that address critical needs in employment within the state was recently made available. Given the huge increase in demand

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Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch005

for graduates capable of working within the state's breweries, a new program housed within the Department of Chemistry and Biochemistry was created focusing on Brewing Laboratory Science.

Students in the program work to complete three courses within brewing science (Figure 1); an introduction to Brewing Science (CHEM 370), a laboratory-based practical experience in Brewing Science (CHEM 470), and a capstone experience within a new campus brewery (CHEM 479) that unites the material from the introduction and the laboratory to real-world practice of brewing. The goal of the program is to develop qualified candidates for employment in the brewery laboratory, with specific skill sets in the successful operation of American Society of Brewing Chemists (ASBC) Methods of Analysis (*8*) and experience in development and implementation of quality assurance protocols.

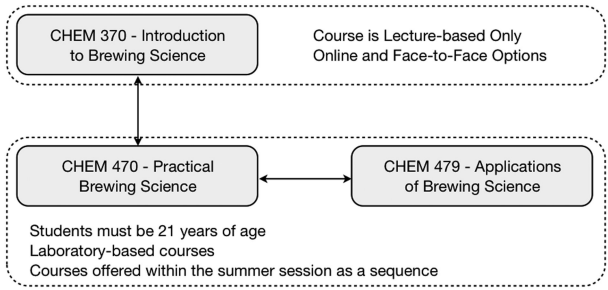

Figure 1. Brewing laboratory science program.

Students enrolling in the program will be juniors or seniors who have probably completed the majority of their liberal arts curriculum. Thus, students will have at least two semesters of science, one semester of mathematics, and various specialized courses in areas such as the arts, history, culture, and social categories. Given the nature of the courses, students must be of legal drinking age by the time they enroll in the second course within the sequence.

Brewing Science Program Details (UNCo)

The syllabus for the introductory course (CHEM 370) within the Brewing Laboratory Science program, illustrates three main features. As any introductory course in brewing science would be, the course is very multi-disciplinary in nature. It involves discussion of topics in history, politics, energy, chemistry, biology, and many other subjects. Second, the course is a science course. Students use information that they have learned in their previous studies in biology, chemistry, mathematics, and physics to evaluate and solve problems in brewing science. Finally, the detail of the course is such that students become prepared to learn, practice, and evaluate methods of beer analysis in the subsequent laboratory-based courses.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch005

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

The course is organized into topics that are aligned with each of these major disciplines, as shown in Table 1. In the first five weeks of the course, students are exposed to the role that beer has played in society. Topic are focused on the history of beer and its impact on society at the economic, political, and social levels. Topics also include the development of styles as a function of that history. In the second portion of the course, students learn an overview of the modern production of beer at the industrial level. The level of discussion includes the design of brewing vessels, pumps, valves, and the movement of liquid through piping. The final portion of the course focuses on the chemistry and biology of the production of beer.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch005

Table 1. Introduction to Brewing Science Syllabus at UNCo

Week	*Topic*
1	Responsibility, Units, Definition
2	History of Beer and Brewing (the ancient world)
3	History of Beer and Brewing (the modern world)
4	Beer Styles – Lagers and Meads
5	Beer Styles – Ales
6	Brewing Process – Malting, Milling, Mashing
7	Brewing Process – Fermentation and Conditioning
8	Brewing Process – Maturation and Packaging
9	Brewing Process – Fluid Movement (Pumps, Valves, Piping)
10	Chemistry of Brewing - Water
11	Biology of Brewing – Barley and Malt
12	Chemistry of Brewing – Hop Oil and Isomerization
13	Biology of Brewing – Yeast and Other microbes
14	Biochemistry of Brewing – Yeast Metabolism
15	Quality Control and Assurance

This introductory course assumes no prior knowledge or understanding of beer or brewing. During the first week, students learn the basic definitions of beer and brewing, understand the responsibilities associated with drinking (and the penalties for failure to be responsible), and learn the units associated with beer measurements (barrel, firkin, °Plato, the SI system, etc.) The course then covers a thorough discussion of the history of beer and brewing from pre-historic times with a focus on the societal and political impacts of beer.

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This is followed by an overview of beer styles based on the Beer Judge Certification Program (BJCP) style guidelines (*9*). Students learn not only the style names and properties, but also some of the history associated with each of the general classifications. Because this introductory course does not have an age requirement, 'hands-on' discovery of each styles' taste is not conducted. However, those students who are 21 or older are encouraged to evaluate those styles on their own. The BJCP style guidelines were chosen for this course, because of the association of the styles into classifications that illustrate inter-relationships. Other style guidelines do exist and could be used instead (*10*).

The brewing process portion of the course outlines each of the steps within the production of beer. While students are exposed to a brief overview in the first week, this portion of the course covers each step in great detail. Discussion on the engineering principles and potential options at each step are highlighted. For example, students are exposed to Burton Unions, Yorkshire Squares, and the Cylindroconical Vessel (CCV) during discussion of fermentation.

The final portion of the course outlines the science behind each of the steps in the process. Students learn the malting process at the molecular level, discover the chemistry of water and its influence on beer style, and explore the compounds found in hop oils and what they impart to the final product. This section also includes discussion of the utility of the quality control and assurance in the brewing process.

The topics of the course are, in some cases, highly integrated. While the bulleted topics list above suggests that the topics are compartmentalized, this is not the case in practice during classtime. For example, during discussions on the process of cooling wort as it moves from the whirlpool to the fermenter, the counterflow chiller is introduced, dissected, and the theory behind its operation explored. This is excellent timing to also introduce the thermodynamic changes within the process and present multiple problems involving heat exchange.

Weekly quizzes punctuate the key topics of the syllabus. A midterm and final examination, essay style, require that students make and evaluate connections between multiple topics and then use that information to solve problems in brewing science. By the end of the semester, the students are sufficiently well versed in the process and key topics of brewing. Their level of preparation by the course provides them with the ability to input details on laboratory analyses into their understanding.

The second course in the brewing laboratory science sequence at UNCo, CHEM 470, is laboratory-focused and project-driven. Students learn the use of ASBC laboratory methods of analysis in great detail. Students perform basic brewing techniques at the one-gallon scale at the start of the semester, and use these project beers to practice many of the analyses for malt, wort, and beer. The methods are supplemented with additional laboratory activities outlining the use of refractometers, densitometers (hydrometers), gas chromatography instruments, and UV-vis spectrophotometers. A focus on the comparison of methods and on drawing conclusions from the available data prepares the students to handle the final course in the sequence.

The final course in the program is the application of brewing laboratory methods in the campus seven-barrel brewery. Students prepare and act upon a

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch005

quality assurance plan that dictates process management during the production of beer for sale by the campus Dining Services. Along the way, students learn key sampling points and perform the appropriate laboratory analyses. By the time a student has completed the three course sequence, he or she is well prepared to properly collect samples, identify errors in the brewing process, and work in a brewery laboratory. Separate from the three courses, students are also presented with the option to conduct mentored undergraduate research projects or participate in external internships with local microbreweries. Students exercising these options have an increased appreciation for the science behind brewing and can make valuable contributions to the understanding of this field.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch005

Brewing Science at UNK

The mission of the General Studies (GS) program at the University of Nebraska at Kearney is to help students acquire knowledge and abilities to understand the world, make connections across disciplines, and contribute to the solution of contemporary problems (*11*). Since the primary purpose of education is intellectual development, the GS program is designed to provide broad intellectual knowledge of the diverse academic disciplines. The liberally educated person, free to explore knowledge and wisdom from a broad perspective of human culture and experience, is able to think independently, to question, to analyze, to interpret, and to judge. The structure of the program centers on a diverse set of "distribution" classes in categories of Natural Science, Social Science, Humanities, Aesthetics, Analytical Thought, and Wellness. The distribution classes are taken after Portal classes and before Capstone classes.

Portal classes, taken by first-semester freshmen, are designed to help students develop critical thinking skills, interpret an argument through engaged discourse, and construct a cogent argument pertaining to the course topic. Critical thinking has generally been defined as "challenging assumptions" that may be inherent within the problem (*12*). The central assumption of this perspective is that there is no one correct answer to each question or not one right solution to each problem. Further, this philosophy tends to reduce a student's inherently biased approach to reasoning (*13*, *14*).

Capstone classes, on the other hand, are generally taken at the end of the student's GS program. With a diverse set of background content knowledge and fully developed critical thinking skills, the Capstone classes are designed to develop and demonstrate the following abilities:

a. Evaluate information from more than one academic discipline.
b. Formulate logical connections between disciplines as they relate to the topic.
c. Employ the approach of more than one academic discipline in completing a Capstone project.
d. Synthesize knowledge related to the topic in completing a Capstone project.
e. Communicate effectively in the medium chosen for the Capstone project.

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Details of the Brewing Science Capstone at UNK

The Brewing Science course at the University of Nebraska at Kearney employs the approaches of chemistry, biology, and physics to the development of student understanding of the course topic. Each capstone class, as outlined above, requires a project to be developed by the student. In this class, the students design a recipe for a given beer style, brew the beer, and analyze their beer after it has conditioned.

The curriculum for this one-semester course is organized in thirds. The first third is devoted to the history, and most importantly, the process of brewing. Students learn the essential elements to brewing, such as how to calculate needed grains and hops for a given beer style. The assumptions and empirical formulae used at this stage are those popularly used by home brewers. The major difference between this course and that designed exclusively for home brewers is that the students ultimately measure the vital parameters of the beer they produce, such as percent alcohol by volume (%ABV), bittering (IBU), and standard reference method (SRM) color. Students use these analyses to critically analyze the validity of their design parameters. During the laboratory portion of the course in the first third of the semester, students brew two different beers to practice both the process and calculations. This engages the students early in the semester and establishes interest, setting the stage for the last two thirds of the semester.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch005

Table 2. Laboratory Curriculum for Brewing Science at UNK

Week	*Topic*
1	Measurement and cleanup, Microbial swab, Lab book etiquette
2	Brew day: Amber
3	Brew day: Stout
4	Prime/Bottle Amber and Taste spectrum of Malt
5	Prime/Bottle Stout and Taste spectrum of Bitter (Hops)
6	Mashing: enzyme activity and temperature
7	Heat: Specific heat, latent heat
8	Brew day: Project brew
9	Sugar content: buoyancy and index of refraction
10	Prime/Bottle Project beer
11	Yeast metabolism
12	Color: Lambert-Beer law, SRM
13	Quantitative Chemistry: IBU and ABV on project beers

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The last two-thirds of the semester are devoted to the science behind what the students have accomplished during their "brew days". The topics covered include the biology of malting, roasting and Maillard reactions, mashing biochemistry, thermodynamics, buoyancy, index of refraction, soils and the biology of the hop plant, yeast metabolism, hop chemistry in the boil, color theory, physiology of taste, and "off flavors". This class is unique within the General Studies capstone courses at UNK since there is also a required laboratory to accompany the class. Typical laboratory topics covered in the semester are listed in Table 2.

As an example to illustrate the academic level of the class, the laboratory for Week 9 is discussed (from Table 2). By the end of the lab experiment, the students will have been exposed to the principles and limitations of the equipment often used to measure sugar content, and they will understand the assumptions made in those measurement. In this lab, the students use several known concentrations of sugar solution and measure:

a. Specific Gravity with Hydrometer
b. Specific Gravity and °Brix with a commercial refractometer
c. Index of Refraction with an open trough.

The last measurement is the most challenging since it is primitive and requires significantly more computation. Students must measure the entrance (angle α in Figure 2) and exit angles (β) of light traversing a tray of the solution. Then, using Snell's law and geometry, students compute the index of refraction (*15*).

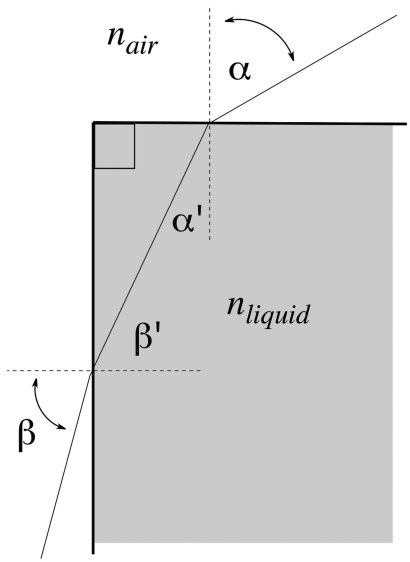

Figure 2. Water tray geometry used to measure the index of refraction of an unknown liquid.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch005

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Students are then asked to measure the "percent sugar concentration" in three "unknowns"; a 10% sugar solution, a 10% salt solution, and finally a 50% ethanol solution – all made from food grade supplies. The students are invited to taste the unknowns and discuss discrepancies and assumptions. Obviously, the salt solution gives a large specific gravity measurement due to the increased dissolved solids, but the index of refraction is roughly that of pure water. The alcohol solution will indicate a solution less dense than water based on the hydrometer measurement. In contrast, however, it will suggest a very large sugar concentration based on measurements using optical methods because of the relatively large index of refraction of ethanol.

The capstone project is an essential part of the curriculum of this course. Students design and brew a beer consistent with one of the styles listed in the BJCP styles guidelines (*9*). Since this class is a science class, we also require certain elements of scientific communication as part of the project. After the students have brewed two test batches and had sufficient instruction on recipe design, the students are required to give an oral presentation. They must present some history of the selected beer style, and fully justify the recipe parameters before brewing of their project can begin. Students are allowed to select any beer style from the BJCP, as long as it is consistent with the *Reinheitsgebot*, (*16*) (contains only water, malt, hops, and yeast) and that the design %ABV is less than 8%. The first restriction is designed to steer students away from fruit-bearing recipes, which can sometimes be problematic within the process of brewing. Since the sugar and color contributions from non-grain ingredients becomes increasingly complex to predict, use of those adjuncts in a student's project beer will make testing of the predicted values of %ABV and SRM difficult. The second restriction is due to the limited time in the semester needed to properly age a higher %ABV beer.

During the remaining two-thirds of the semester, students write mini-papers. The topic of each paper correlates to the science instruction that is occurring in class at the time, but as applied to their own project beer. These papers, with the two analytical lab reports, are combined at the end of the semester into a final project paper. In the comprehensive final paper, students are expected to review the same material presented in the oral proposal, explain the science used in producing their beer, and use the final analysis of their beer to critically analyze the assumptions in design. In addition to writing the paper, the students are expected to orally present a condensed version at a "tasting" event where the public (of legal age) is invited. This event is well received and increases awareness of the class.

Challenges

There are many challenges unique to the implementation of these types of classes. Although some challenges are specific to the school introducing the courses, there are some commonalities in the challenges. First, administrative buy-in to the implementation of the course must be obtained. For the two programs outlined here, this approval saw both ends of the spectrum. At UNCo, the approval was easily obtained and support was given at every stage of the approval process. At UNK, it was difficult to overcome the administration's view

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch005

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

that offering a course in brewing would promulgate a long-past perception of a "party school". The necessary approval arrived only after careful explanation of the benefits of rigorous, interdisciplinary science instruction applied to a common and interesting problem. Further support related to the recent increase in craft brewing in the U.S. provided credibility that the endeavor was worthy of study for graduating students. As part of the analysis, students are required to taste their beer, and compare it to other well defined, commercially brewed, standards. This is an essential element in the critical analysis of their project, as well as the discovery of, and solution to, off-flavors. To make this element of the class more palatable to administration, there were several layers of control placed on the class. Both the UNK and UNCo courses require that registration for a course where alcohol is tasted is strictly limited to students of legal drinking age. This generally is not an issue for enrollment since students often take the course as seniors. To ensure compliance with tasting methods, the departments offering the course at both UNK and UNCo maintain a breathalyzer. Students must demonstrate an alcohol blood level of less than one-half the legal limit for driving before being allowed to leave class on days where beer is tasted. For those students that do not pass this test, they must remain in class until they can pass the breathalyzer test. In practice, this has never been an issue at either institution since the students are tasting small volumes and following proper tasting procedures.

The other major challenge to the implementation of this type of class stems from the diverse population of students enrolling in the class. Since the class is open to all majors, both science and non-science majors, it is difficult to strike a balance of academic rigor that thoroughly engages the science majors but, yet, not so difficult that the subject is incomprehensible to the non-science majors. For the course at UNK, the instruction is offered at the General Studies level where the science majors (about 34% of the enrollment) tend to out-perform the other students when considering grade distributions, as illustrated in Figure 3. At UNCo, the distribution is more uni-modal due to the more uniform distribution of students with science majors and primarily within Chemistry.

A related challenge in the offering of the course at both UNK and UNCo is based on somewhat unrealistic expectations on the part of some students. In more than a few cases, it appears that the students register for the class thinking the class will be easy to complete, or that the class is simply a course in consuming beer. For example, in the first few semesters of offering this class at UNK, approximately 10% of enrolled students did not take their role in the course seriously or did not enter the class with the assumption it would be rigorous, and withdrew from the course by the first exam. This issue is quickly being resolved as word-of-mouth communication has conveyed the seriousness of the class. Even so, the relative percentage of science majors and non-majors has not changed significantly from 34% and 66%, respectively.

A final challenge experienced by instructors at both UNK and UNCo is the availability of textbooks that suitably present the material at the level needed for the specific courses outlined here. There are many well-written brewing science texts available (*17–19*), but the science tends to be presented at a level inappropriate for the students of the course. The typical textbook is often written either at the level of the novice homebrewer, or at the level of the graduate student

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch005

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

in food science. A textbook that begins with novice level introductory chapters, but includes the rigor and depth of topic needed for a scientific exploration of brewing has proven very difficult to find. The lack of a suitable textbook that was available when these courses were created greatly increased the preparation time for the construction of the syllabi and materials for the courses at both institutions. As a result, the UNK course uses Daniels (*20*) for the initial weeks covering recipe design, then a series of instructor notes and selected readings from other general studies science texts. The UNCo course uses a series of books (*21–23*) and instructor-prepared handouts to cover the material at the level appropriate for the course.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch005

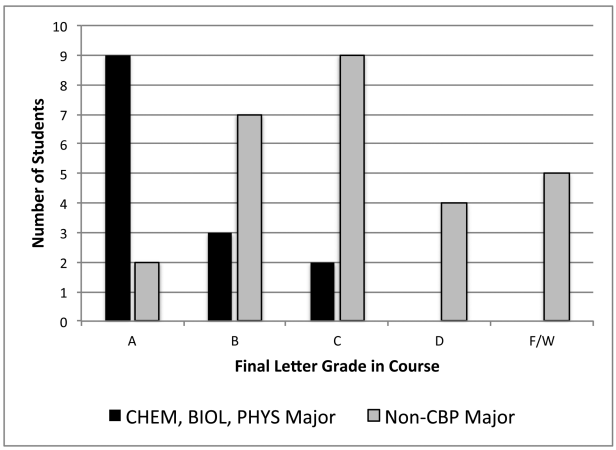

Figure 3. Cumulative grade distribution over four offerings of Brewing Science at UNK organized by student major.

Conclusions

While the student population and desired outcomes of the courses at UNK and UNCo contrast sharply, there are some striking similarities between the two courses. First, the courses begin with the assumption that the students have little or no prior knowledge of beer or brewing. This assumption, thus far, has essentially proven to be correct. Second, these courses are serious explorations of the science behind beer and the brewing process, and students that are either ill-prepared or misadvised about the rigorous nature or level of science within the courses tend not to succeed in the programs. Third, by the end of the semester, the students completing the course have developed a strong background in the science of brewing and an enhanced appreciation for the subject. Fourth, students report that after completion of the course, their experience with beer and brewing has changed to be more responsible than prior to the course. Finally, students from both courses recognize and appreciate the vast multi- and inter-disciplinary

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

nature of Brewing Science. Students are able to see the need to approach a problem in Brewing Science using theory, understanding, and practice from a variety of disciplines.

Overall, the time invested in preparing syllabi, writing lectures, setting up experiments, obtaining approval, and implementing a course in Brewing Science is well worth the effort for the instructor, or team of instructors in the case of a team-taught course. The benefits associated with student learning, appreciation for science, and understanding of the application of science and other subjects to solving problems in the real world far outweighs any challenges to the design and implementation of these courses.

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Chapter 6

Engaging Non-Majors in Chemistry through Brewing and POGIL

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Interest in fermentation and fermented beverages makes courses that focus on the science associated with fermentation a ripe area for engaging students who might not otherwise consider taking chemistry courses. This chapter describes a course about the chemistry of brewing taught at Linfield College for upper division non-majors using POGIL and hands-on activities to engage students in learning chemistry.

Research has recently shown that courses with significant student-centered active learning components lead to fewer students withdrawing from or failing introductory STEM courses (*1*, *2*). Process oriented guided inquiry learning (POGIL) actively engages students, and has been shown to be successful in a variety of introductory and upper-division STEM courses (*3–5*). Instructors in classes that have implemented POGIL (*6*) act as facilitators of learning, helping students work through activities, and asking questions of the students to check understanding. Students at Linfield College are required to take an upper-division course that is not in their major area of study. This chapter describes a course that is designed to introduce chemical concepts in the context of brewing using POGIL and hands-on activities.

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About the Course

The Art and Science of Brewing (CHEM 300) has been taught at Linfield either on campus or abroad during each of our campus' January terms since 2010. A topical course on the chemistry of brewing ensures student interest, and a wide variety of different majors have enrolled each year. The diversity of student background knowledge in the course means care must be taken in designing the learning space, POGIL activities, and other course components. Students who take the course at Linfield must be of legal drinking age because they will be brewing and tasting beer during the term. The course meets two hours a day, five days a week for four weeks. Typical enrollment has been between 20 and 28 students.

Nearly every class meeting involves a POGIL activity designed around one or two course-related learning goals. Table I lists the POGIL activities for the course. Low-stakes quizzes are given at the beginning of each day to provide formative assessment of student. On the second day of class, students work in groups of three to four to brew malt-extract beer using recipes developed by students in the previous year's class. Most students in the class have never brewed, and this experience engages them early in the course. During the following two to three weeks the students can observe the changes in their beer as it ferments which the class activities are designed to build on.

Students are placed into groups of three to four on the first day of the term, with well-defined roles (for example: manager, spokesperson, recorder, and technician). Group roles rotate each class, and group membership changes each week. POGIL activities in the class frequently begin with a question designed to have students discuss their prior knowledge, or build on knowledge developed during a previous activity.

POGIL Activities

POGIL has been described in detail elsewhere (*3–6*); a brief description is given here. POGIL is based on the central idea that each student constructs his or her own knowledge of a subject, and that this knowledge is influenced by the student's prior knowledge. Along with developing content knowledge, POGIL specifically develops process skills, such as critical thinking, problem solving and communication. The instructor in a POGIL classroom facilitates learning rather than lecturing. Students work in collaborative groups with well-defined roles that help them manage their work using activities that have been developed specifically to accomplish both the acquisition of content knowledge and development of process skills.

Content knowledge is taught through a learning cycle approach, in which students first explore a model, invent a concept or term, and then reinforce their understanding through application of the concept (*7*). Students have to draw conclusions together by analyzing models and data and discussing ideas – being actively engaged in the material when they are in class.

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The 18 POGIL activities listed in Table I form the majority of the in-class work. Each activity is designed around one or two learning goals, which are related to chemical concepts or skills that are being developed in the course. Figure 1 is an example of one of the course activities, "*How are Sugars Produced During the Mash?"*. In this activity students follow a learning cycle to first understand the composition of starches, followed by an exploration of how alpha and beta amylase convert starches into fermentable sugars during the mash. The final model in the activity has students learn that not all starches can be broken down into fermentable sugars by these enzymes.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch006

Table I. POGIL Activities for The Art and Science of Brewing

1. How is Beer Made?
2. What Constitutes a Style?
3. What is Malt?
4. What Atoms and Ions are Important in Beer?
5. Is Beer Chemical-Free?
6. What Compounds are Responsible for Off Flavors and Aromas?
7. What Makes Good Brewing Water?
8. What is Mashing?
9. How are Sugars Produced During the Mash?
10. What Happens During the Boil?
11. What Gives Beer its Color?
12. What Compounds do Hops Provide in Beer?
13. How is Ethanol Produced During Fermentation?
14. How Do You Carbonate Beer?
15. How Do You Plan a Recipe?
16. What Flavors and Aromas Characterize American and British Ales?
17. What Flavors and Aromas Characterize Lagers?
18. What Flavors and Aromas Characterize Belgian Ales?

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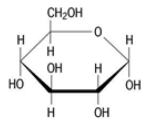
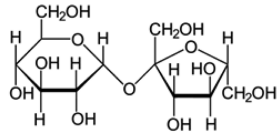
How Are Sugars Produced During The Mash?

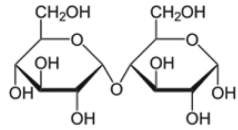
Model 1 – Some Sugars



Glucose
$C_6H_{12}O_6$



Sucrose
$C_{12}H_{22}O_{11}$



Maltose
$C_{12}H_{22}O_{11}$

Sugars are a type of **carbohydrate**. Carbohydrates consist of only C, H and O. Glucose is an example of a **monosaccharide** – it is a single sugar unit.

Critical Thinking Questions

1. How many sugar units are there in sucrose and maltose? What do you think these types of sugars are called?

2. Maltose is a disaccharide that is made up of two glucose units. Circle on the glucose molecule where the bonds are formed to make maltose.

3. Sucrose (table sugar) is a disaccharide made up of glucose and fructose. Circle the fructose.

4. What is the ratio of H atoms to O atoms in these sugars?

5. Do sugars contain any double bonds? Double bonds are indicated by two lines (=), single bonds are indicated by single (-) lines.

6. Are all the hydrogens shown in maltose? If not, add them based on the glucose and sucrose structures.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch006

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Information
Glycans are compounds made by linking glucose units. Maltose is actually bent – it is not a linear unit as depicted in the model. Cellobiose is a linear disaccharide made up of two glucose units.

Model 2 – Polysaccharides made of maltose and cellobiose.
Amylose is a starch formed from repeating maltose units. Amylose is a straight chain polysaccharide that forms in a helical pattern.
Cellulose is a straight chain polysaccharide formed by repeating cellobiose units. It is an important component of most plants, like the husks in barley.

Critical Thinking Questions
7. The model shows two segments of two polysaccharides. Indicate which is likely to be amylose and which is likely to be cellulose.

8. Which is the more important polysaccharide in brewing? Amylose or cellulose? Explain your choice.

Model 3 – An Amylose Chain, α Amylase and β Amylase.
This model shows imaginary snapshots of how the two enzymes act on an amylose chain, and ultimately what happens to the chain during the mash.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch006

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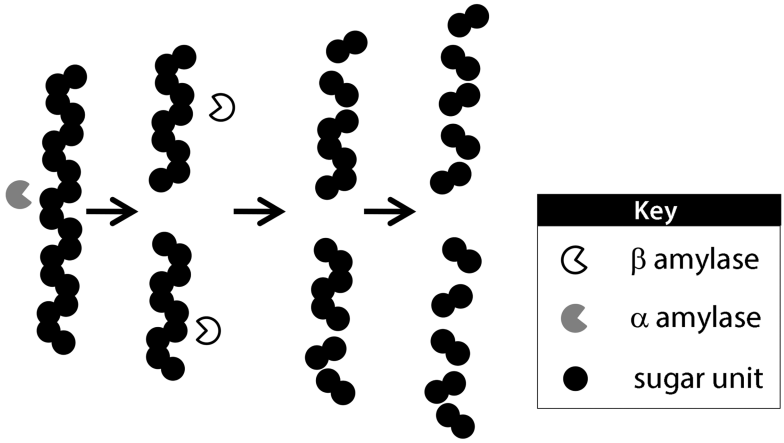

Critical Thinking Questions.

9. Do α and β amylase act on different parts of an amylose chain? Describe the difference as specifically as you can.

10. What types of compounds are formed during the mash?

Information

Amylopectin is approximately 75% of the malt starch. It is a highly branched polysaccharide made of repeating glucose units:

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch006

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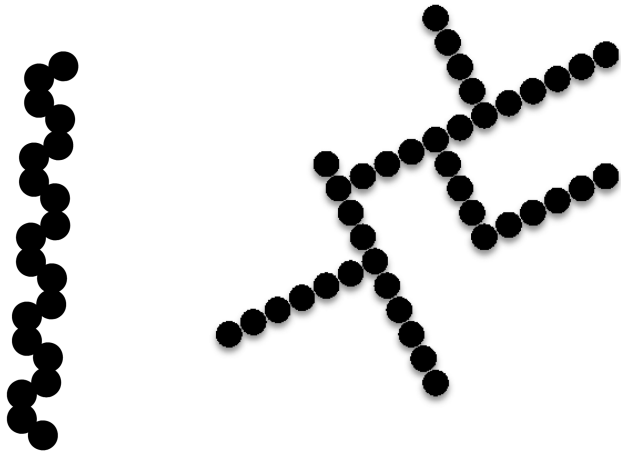

Glucose units at the branching points are joined to form cellobiose linkages. Alpha- and beta-amylase can't cleave these linkages, resulting in the formation of dextrins – unfermentable fractions of amylopectin that contain four – thirty glucose units.

Critical Thinking Questions

13. Indicate where in the amylopectin alpha- and beta-amylase will not be able to cleave glucose linkages.

Figure 1. Excerpt from a POGIL activity on the enzymatic conversion of starches to fermentable sugars during the mash.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch006

Hands-on Activities

In addition to POGIL activities, students engage in several hands-on activities that give them practical experience in brewing and assessing beer: brewing and bottling a batch of beer, sensory analysis for off flavors and aromas, tasting classic beer styles, and visiting local breweries and brewpubs. The goal of these activities is to reinforce knowledge gained during classroom activities.

Students brew a five gallon batch of malt extract beer on the second day of class. Each batch is different, and is based on a recipe developed by students the previous year. Recipes utilize some specialty malts for color and character and are crafted so the beer will ferment out and condition within the four week

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term. Many of the POGIL activities are written so that students can connect the conceptual aspects of brewing to observable changes in their beer as it ferments. Beers are bottled near the beginning of the third week of class. These beers then serve as the basis for a final exam on the last day of the class during which students are judged on their ability to discuss the characteristics of the style of beer they brewed, flavors and aromas that characterize it, and any flaws in their beer (the quality of the beer itself is not part of the final exam grade). Judges at the tasting include faculty, staff, students and professional brewers.

The first tasting session focuses on off flavors. In this session, a baseline beer (a mass produced light beer) is spiked with common off flavors, like diacetyl (green apples), dimethyl sulfide (cooked vegetables), and isoamyl acetate (banana), using readily available standards from the Siebel Institute (*8*). The off-flavor spikes are made to be several times more concentrated than the accepted threshold for each, so the aromas and flavors are fairly obvious. Models and critical thinking questions for the accompanying POGIL activity have students identify the typical sources during brewing and fermentation that can lead to off flavors, and learn how to use the American Society of Brewing Chemists flavor wheel for later tastings (*9*).

The next three tasting sessions help students develop their palates, understand differences between and within styles, and learn how to identify the common off flavors found in beer. The first tastings focus on beers that students may be more familiar with, American and British style ales. Two other tastings focus on lagers (primarily Czech and German styles) and Belgian beers. These tastings often introduce students to flavors and aromas whit which they are unfamiliar. Tasting sessions follow a POGIL model, and students use the beers with notes form the Beer Judge Certification Program (*10*) as models. Critical thinking questions ask the students to try to identify flavors, aromas, and colors in the beers.

Three tours of different breweries in the area provide a chance for students to see how beer is made in a variety of different scales. The class visits two breweries in McMinnville: a brewpub that produces a variety of ales and lagers, and another that is a small brewery dedicated to brewing lagers. A third visit is usually to a brewery in Portland, either to Widmer Brothers (the largest brewery in the Pacific Northwest), or to one of the other breweries in the city. During tours, students see how the brewing process is essentially the same for homebrewers as it is for professionals, but the scale changes. Tours also provide opportunities for students to interact with professional brewers who can answer many practical questions students have, such as the cost of starting a brewery, the cost of producing a pint of beer, or the hours brewers typically work.

Practical Considerations

There are several practical considerations that are perhaps specific to this course, especially since students brew and bottle a batch of beer, taste beer and visit breweries. Students who enroll in the course must be of legal drinking age. Our college worked with the Oregon Liquor Control Commission (OLCC) to ensure that the course would be legal and that any state regulations regarding the production and consumption of alcohol related to the course were being met. In

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Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch006

this instance the OLCC regards this course as a homebrew club and there are no state licensing fees necessary.

Our department used existing funds to purchase a total of seven basic homebrew kits. A per student course fee covers replacement of equipment, purchase of ingredients for brewing, purchase of beers for tasting, purchase of the off-flavor kit from the Siebel Institute, and college van rentals for out of town tours. Students are responsible for providing their own clean and empty bottles for bottling (which provides a useful unit conversion exercise for the students).

The equipment for the brewing set ups was based on experience and recommendations in common homebrewing books. Seven kits were purchased and students brew in groups of 2-4 depending on class size. The equipment in each brewing setup is listed in Table II.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch006

Table II. Brewing Kits

Five-gallon stainless steel pot (for 2.5 gal. concentrated wort boils).
Propane burner (Camp-Chef).
Propane tank.
Six-gallon glass carboy for fermentation.
Large stainless steel spoon.
Six-gallon food grade plastic bucket with siphon valve for bottling.
Bottling wand and plastic tubing.
Racking cane and plastic tubing.
Hydrometer and hydrometer jar.
Bottle capper.
Fermentation lock and carboy cap.

Students share bottle caps, priming sugar, cleanser (PBW), and sanitizer (Star-San). Brewing is done outside in a courtyard in front of the chemistry building, and the beer is fermented in an office, and bottled indoors.

Outcomes

There are no standardized ACS exams for a brewing course, so other methods are needed to assess student outcomes. Two are described here, daily knowledge surveys (KS) conducted before and after each POGIL activity and the Students Assessment of Learning Gains (SALG) survey administered at the beginning and end of the term (*11*, *12*).

The daily KS are based on specific learning objectives for each POGIL activity and ask students to rate their ability to meet the learning goals before and after completing each activity. An example survey item is given below.

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I can explain how alpha and beta amylase break down starches and how the actions of these two enzymes differ from one another.

1. I can't do this now.
2. I can do some of this now, but do not have full understanding.
3. I feel I can do this now with full understanding.

Students respond to the KS items via clickers, and the class' averaged responses are collected. Figure 2 shows the averaged responses for the class before (dashed) and after (solid) completing each POGIL activity in January, 2012 (N = 24 students). The gap between the two traces reflects the gains in content knowledge the students report after completing the activities.

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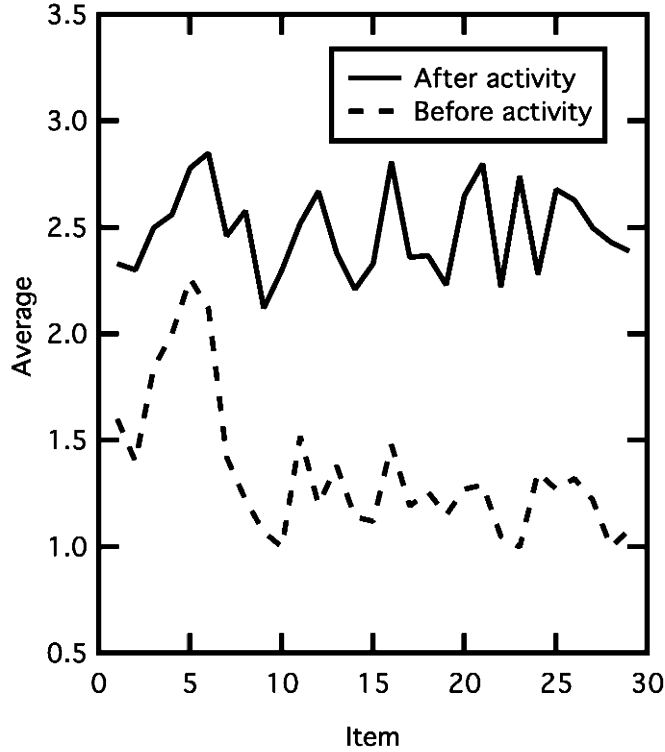

Figure 2. Average scores on Knowledge Surveys before and after classroom activities.

The pre- and post-activity averages were 1.4 ± 0.3 and 2.5 ± 0.2, respectively. Note that in the earlier activities, students rate their starting average confidence relatively high. As the term progresses, this drops, while their confidence after each activity remains relatively constant. This likely is a reflection of how the relative complexity of course concepts progresses in the POGIL activities. Earlier in the term, course concepts are fairly straightforward, and many students may

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already have some prior knowledge about how beer is made. Later in the term, students are exposed to more detailed chemistry concepts, such as enzyme action, which most non-majors have not learned about in previous classes.

Table III. SALG Survey Results from January 2011

I understand...	*Baseline (N = 23)*	*Final (N = 17)*
The brewing process	3.3 ± 0.9	4.8 ± 0.4
Germination	2.6 ± 0.7	3.8 ± 0.7
Malting & Kilning	2.4 ± 0.7	4.3 ± 0.7
Mashing	2.6 ± 0.7	4.3 ± 0.6
Brewing (boil)	3.0 ± 1.0	4.8 ± 0.6
Fermentation	3.4 ± 0.8	4.8 ± 0.4
Color in beer (chemicals)	2.3 ± 0.8	4.1 ±0.9
Bitterness in beer (chemicals)	2.7 ± 0.9	4.4 ± 0.8
Aroma in beer (chemicals)	2.7 ± 1.0	4.7 ± 0.6
Mineral content in brewing water	2.4 ± 0.7	4.4 ± 0.8
I can...		
Understand brewers during tours	3.0 ± 1.3	4.7 ± 0.5
Understand flavors & aromas and their source during brewing	2.6 ± 0.8	4.6 ± 0.5
How to brew beer at home	2.0 ± 0.7	4.6 ± 0.6
Propose a recipe for a beer	2.1 ± 0.6	4.1 ± 0.7
Analyze the style of a beer	2.5 ± 0.8	4.6 ± 0.5

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The SALG survey focuses on broader learning goals than the KS. Students were asked to rate their understanding ("Presently I understand…") of general course concepts as well as their skills ("Presently I can…") in a baseline survey (first day of class) and final survey (last week of class). Students rate their understanding and skills on a 1-5 scale (1 is low and 5 is high). The results of these surveys are given in Table III from January 2011. Students reported an average of 2.7 ± 0.4 for items asking about their understanding of the brewing process at the beginning of the class. This increased to an average of 4.4 ± 0.3 by the end of the class. Similarly, students reported an average of 2.4 ± 0.4 confidence in the skills portion of the class at the beginning, which increased to 4.5 ± 0.2 by the end of the term.

In terms of understanding course concepts, students reported their largest gains in being able to understand which chemicals influence the aroma of beer and how they are formed, as well as how mineral content in brewing water

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influences the characteristics of beer. The smallest gains were for understanding the germination process, an item that is not a major part of any single activity in the class. Students reported their greatest gain in skills to be their ability to brew beer at home.

Engagement in a class is somewhat difficult to measure, but student comments on the SALG survey provide some sense of how engaged students were in the course:

> "I was very intimidated by chemistry before beginning this class. I like how we approached everything in a way where everyone could understand and I think I am not so intimidated my it now."
> "This was not only a very engaging class, but I also have taken so much from it. Beer nerds are awesome."
> "I really enjoyed doing the group beer activities that we did in class because it tied in with the texts and it gave us a better understanding."
> "I did not know much about chemistry before this course so it was nice to see sort of how things come together and make a product."
> "At the start I had zero understanding of chemistry, but after taking this class I have gained so much knowledge and understanding of chemistry by relating it to beer. "
> "I strongly believe that this course should be taken prior to biology because it's an inspiring and real life application in what is going on around us. "

Conclusion

A course that teaches chemistry in the context of brewing is a good opportunity to engage upper division non-chemistry majors in chemistry. Students generally are motivated and interested to learn the chemistry so they can know more about how beer is produced. This course uses a combination of classroom POGIL activities, hands on brewing activities, tours, and student projects to help students learn basic chemical principles in the context of brewing. Student's self-assessment of their learning indicates that the course is successful.

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Chapter 7

Beer as an Introduction to Chemistry

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The characteristics of a themed introductory chemistry course, specifically with a beer theme are discussed. Issues of suitable mathematical level and chemistry notation are addressed. Resources for launching a beer-themed introductory chemistry course are provided.

Introduction

Beer is virtually unique in its ability to reach students and arouse their interest. Alcohol has been an integral part of academic culture for a long time. Many milestones, good and bad, in a student's life involve the consumption of beer and other fermented beverages. The willingness of many students to run the risks and suffer the consequences of indulgence in beer suggests that that their interest is a real commitment. On the other side of the coin many important aspects of chemistry are involved in the production, handling, and packaging of beer. These include periodic table, water chemistry, organic chemistry, biochemistry, thermochemistry, colloid and surface chemistry, redox chemistry, materials chemistry, tests and measurements, and many others. In terms of engaging students and teaching the key elements of chemistry, beer makes a splendid theme. Beer can be an ideal introduction to chemistry(*1*).

Characteristics of an Introductory Course

An introductory chemistry course is aimed at a non-technical audience. Students in the sciences or health professions will normally be assigned to a sequence beginning with General Chemistry or to a specialized course or sequence. Usually the introductory course will be one of several science courses

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that satisfy a general education or distributive requirement. Programs vary, but it would be unusual for an introductory chemistry course to be an absolute requirement. The students can be expected to come from a wide variety of backgrounds, including business, social sciences, humanities, and arts. Some characteristics for the typical introductory course are:

- No prerequisite. For most students it will be their first and only college chemistry course.
- The course is aimed at (but not necessarily restricted to) non-science majors.
- The course meets a general education requirement.
- Many of the students will have a very different skill set than what we expect of our usual clientele.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

Student Expectations

An introductory course usually fits in as science distributive requirement. It competes with other courses, some of which have reputations for being fun and easy. If the students do not elect your class, you can't teach them any chemistry at all. One factor that may need to be overcome is a negative experience with chemistry in secondary school. A student whose high school chemistry class spent several weeks on some boring bookkeeping issue like significant figures is likely to shy away from additional exposure to the discipline. Some factors needed to appeal to students are not at all like the priorities of traditional chemistry courses:

- Entertainment
- Moderate level of effort
- High grade distribution
- Minimal math
- Deals with existing student interests.

Themed Course

A themed course is focused on some object or idea from the larger culture taking the point of view of an academic discipline. Recently, themed courses in all disciplines have become popular. The theme can be some general area like the environment ("Humans and the Environment", Penn), or some specific aspect of student experience like food or drink ("Food Fire and Physics", West Chester University), an aspect of popular culture ("Breaking Down Breaking Bad", SUNY-Buffalo), or even something totally imaginary ("Zombies", San Diego State). Here we will be drawing from my experience at West Chester University teaching "The Chemistry of Beer" every semester since 2009.

A theme presents a number of challenges and opportunities for the instructor. In following the theme, one is drawn across disciplinary boundaries. I am a physical chemist, but my course has components of organic chemistry,

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biochemistry, and biology. Learning about the theme itself as well as about the related aspects of the other disciplines took significant effort. The theme needs popular appeal, which makes it likely that there will be a good deal of information, much of it unattested at best, in popular sources. Some misinformation may even find its way into standard reference works.

In my experience, the opportunities exceed the challenges. Since the course started, I made presentations to chemists, home brewers, clubs, and even a reunion of retired faculty. I wrote papers, a book, and chapters to other books. I met brewers, brewing scientists, journalists, science historians, archaeologists, and many others. In addition I purchased hundreds of dollars' worth of books for myself, and I had the University library acquire a strong collection on beer and brewing. Some of these books are mentioned under Beer Chemistry Resources. The intellectual rewards of devising a themed course exceeded all expectations.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

Learning Considerations

Chemistry Learning Goals

It is essential to articulate realistic goals that make sense to non-technical students. Virtually any aspect of chemistry that would be expected to be covered in an introductory course finds a natural place in a beer-themed course. Here are some of the highlights of the chemistry goals in The Chemistry of Beer.

Periodic Table

Associate the position of a main group element on the periodic table with its usual charge and valency. Locate metals, non-metals, and semi-metals on the periodic table.

Water Chemistry

Identify the ions responsible for pH, hardness, and alkalinity. Describe how these ions affect beer character and style. Give formulas for forms of carbonate, indicate how they vary with pH. Describe various methods of water treatment. State Le Châtelier's principle and give an example of its application. Students are not expected to do equilibrium problems.

Organic Chemistry

Know one's way around a molecule. This includes knowledge of the conventions of chemical structure diagrams. Recognize functional groups like alcohols and identify features like *a carbonyl carbon atom that is connected to a second oxygen atom*. Pick from a group of structures ones that have been

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

mentioned in connection with the course, for example, select the drawing of dimethyl sulfide when asked which molecule is responsible for a cooked vegetable off-flavor. Students are not expected to become familiar with organic nomenclature.

Biochemistry

Identify terms like enzyme, protein, active site, denature, channel, pump, and receptor. Identify carbohydrates, sugars, starches, and dextrins. Identify features like the R group from a drawing of an amino acid, the anomeric carbon(s) on a drawing of a carbohydrate, peptide bonds, glycosidic bonds, ATP, and asymmetric carbon atoms. Describe the processes of gelatinization, liquification, and saccharification. Distinguish between the action of alpha and beta amylase and explain how these affect extract and fermentability.

Thermochemistry

Identify steps in the brewing process that consume large amounts of energy. Be able to identify ways in which energy could be saved or recovered. Understand the role of fermentation in terms of providing energy to yeast cells. Be familiar with issues of heat release during fermentation and fermenter temperature control.

Colloid/Surface Chemistry

Describe the processes leading to foam and haze (cloudiness), both of which are colloids. Distinguish between particle growth by coalescence and disproportionation. Identify terms like surface tension, surfactant, foam, haze, nucleation, and head retention. State Henry's law for dissolved gases and understand its application to beer foam and its relationship to Le Châtelier's principle. Identify the role and mechanism of action of finings, materials added to clarify beer.

Redox Chemistry

Determine oxidation numbers from Lewis structures. Identify oxidation and reduction. Identify oxidation products responsible for stale flavors in beer.

Materials Chemistry

Describe the characteristics of metals. Describe the steps in the extraction of aluminum and the fabrication of beverage cans. Identify the raw materials for aluminum and glass.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

Tests and Measurements

Learn the principles and advantages and disadvantages of various types of thermometers used for measurement or control. Learn about methods for estimating carbohydrate content of beer wort and alcohol content of finished beer. Understand the concepts of density and specific gravity. Distinguish between systems of color measurement. Do simple calculations involving flavor units and flavor threshold. Distinguish among primary, secondary and background flavors.

Upper Level Goals

Although the course described is at the introductory level, the beer theme can be used to introduce or reinforce many upper level concepts. Among these could be:

Biochemistry: Glycolysis, Enzymatic kinetics and mechanisms, Genetics and mutations, Bioenergetics and ATP, Carbohydrates, Proteins.
Analytical Chemistry: Water analysis, Gas and liquid chromatography, Colorimetry, Titration.
Inorganic Chemistry: pH, Complex formation, Alkaline earth ions, Oxidation/Reduction.
Organic Chemistry: Bonding, Delocalization, Functional Groups, Mechanisms, Free radicals.
Physical Chemistry: Chemical Equilibrium, Phase Equilibrium, Chemical kinetics, Thermodynamics, Colloids and surfaces, Spectroscopy.

Beer Learning Goals

Learn the steps of the brewing process, why each step is done, and the equipment used for the process. For example, students learn the principles of two methods of wort separation: lauter process and mash filtration. They learn some advantages and disadvantages of each, and the types of equipment used for each. Students learn about various beer styles and the relevant reactions and flavor molecules involved. They learn about style characteristics, like initial gravity, and how the ingredients and processing can affect these characteristics. In the final part of the course, brewing at home is covered in detail.

General Education Learning Goals

The Chemistry of Beer course at West Chester University is designed to meet two of the goals of the General Education Program.

- Employ quantitative concepts and mathematical methods (Q).
- Think critically and analytically (C).

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.
Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

Table 1 shows how the coverage in the course addresses these goals.

Table 1. Course Content and Goals with Examples of Application

Topic	*Goals: C = Critical Thinking; Q = Quantitative Concepts*
Introduction and History of Beer.	C: Objective of prohibition compared to results, modern-day prohibition ("war on drugs"), evidence-based beer history vs. legends. Role of beer here and now compared to that in earlier times or in different parts of the world.
What is Beer?	C: Defining beer in a way that makes scientific sense. Conflict with the marketing definitions. Changes in the concept of beer in time and place. Q: Beer composition.
Chemistry Basics	Q: Organizational principles of chemistry: hierarchy of stuff: elements, compounds, mixtures. Bonds and geometry, geometry and molecular behavior. Representing compounds: formulas. Representing chemical reactions: chemical equations. Mixtures: types and composition. C: inferences from molecular structure.
Chemistry of Water	Q: Strength/weakness of acids and bases. Compositon of solutions. pH, isoelectric point, water: hardness and alkalinity. C: loaded language: pollutant vs. component.
Organic Chemistry	Q: Graphic representations of molecules (structural formulas). Classification of compounds.
Carbohydrates	Q: geometry and symmetry
Mashing	Q: Levels of structure/geometry. C: Inferences about molecular interactions.
Boiling	C: Evaluating different methods of wort separation. Evaluating hop products. Q: Energy for heating and cooling.
Fermentation	Q: Energy conservation. Comparison of energy from fermentation and respiration. C: Benefit of ethanol to yeast. Correcting misconceptions about bond energy and energy storage in ATP.
Tests and Measurements	Q: Concepts in measurement. C: advantages and disadvantages of various measuring devices.
Chemistry of Flavor	Q: Quantifying flavor. C: Distortions in sensory evaluation.
Chemistry of Beer Styles	Q: What goes into a beer formulation? C: Off-flavors vs. style characteristics. Defining beer styles in a way that makes chemical sense. Classicism vs realism in brewing.
Foam and Haze	Q: Particle size and surface. Surface energy and surface tension. Structure of surfactants. Henry's law.
Beer Packaging	C: Packaging and perception. Bottles vs cans: the role of coolness. Bisphenol A. Economics vs. esthetics. Q. Crystal structure of metals. Toughness/brittleness.

Continued on next page.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

Table 1. (Continued). Course Content and Goals with Examples of Application

Topic	*Goals: C = Critical Thinking; Q = Quantitative Concepts*
Beer Flavor Stability	Q: Allocating electrons. C: Acceptability of additives.
Brewing at home	Q. Costs. C: Advantages and disadvantages of brewing methods.

Mathematics

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

Chemistry has a well-deserved reputation for being mathematically oriented, especially in the first course. To some extent the emphasis on stoichiometry and calculations derives from the history of chemistry. The analytical balance carried chemistry from its alchemical origins to a legitimate science. None of this resonates with students in an introductory course. For the course to reach a wide audience, the bookkeeping aspects of the discipline need to be minimized. Nonetheless, even if we strip most of the calculation out of the chemistry, the beer theme comes with an emphasis on measurement and requires some mathematical thinking. So what sort of mathematics is appropriate and tolerable to the students? I will discuss this with an example.

Molecules vary in their ability to elicit a flavor response in humans. This is measured by the *flavor threshold*, which is the smallest concentration that the average taster can discern. The thresholds of the flavor compounds that are potentially important in beer vary over nine orders of magnitude. To allow comparisons of the flavor contributions of compounds whose actual concentrations vary over such a wide range, the concept of a *flavor unit* was developed (*2*). The concentration of a compound expressed in flavor units is the actual concentration divided by the threshold concentration. The flavor units can be thought of as the concentration expressed in multiples of the threshold. As part of a quiz on flavor chemistry, students were assigned versions of the following problems (for security, all problems are issued in two versions).

The course directly addressed the definition of flavor units: it is the concentration of a substance divided by its flavor threshold. One of the questions put the problem in this format. *The flavor threshold for isohumulone is 4 ppm. How many flavor units does isohumulone contribute at 48 ppm?* Student performance on this problem (combined with a version with ethanol) was 93% correct. Another question, issued on the same quiz, turned the problem around. *The flavor threshold for 4-vinylguaiacol is 300 ppb. How much 4-vinylguaiacol would be needed to provide 3 flavor units?* The outcome for this question, combined with a version involving isohumulone was 66% correct. The difference in outcomes of 42 students for the two problems was significant with a p-value of 0.002. This result indicates that students may be better at numerical problems than one might have thought. More students can plug into a definition than can work out a mathematically equivalent problem, but the majority can do both.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

Chemical Structures

Chemical structure drawings are used to represent various aspects of molecules in a simplified, conventional way. Chemists pass easily from one convention to another without much thought. Not so introductory chemistry students. Many sources, even those intended for a general readership, present highly conventionalized structures with no explanation at all. Some use notation that makes it difficult for the uninitiated to understand the geometry of the molecules and the distinctions among them. A few sources go into a good deal of detail about various compounds and their role in beer without showing any structures or formulas at all. Most chemists would be uncomfortable with this latter approach. Some of the common structure conventions and their limitations are listed here.

Lewis Structure

The Lewis structure of ethanol is shown in Figure 1. The Lewis structure can be easy to understand because nothing is omitted. Molecules with many atoms and bonds can become overcrowded and messy. The conventions behind this structure are:

- The symbol of an element represents an atom of that element.
- A line represents a covalent bond, which is a shared pair of electrons.
- Unshared valence electrons may be shown as dots.
- The drawing shows the connections among atoms, but is not an accurate three-dimensional picture. The structure may make some effort to represent the bond angles accurately, sometimes using wedge-shaped bonds to represent a three-dimensional effect.

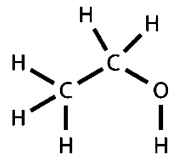

Figure 1. Lewis structure of ethanol.

Condensed Structure

The condensed structure is convenient to represent a molecule in a single line of type. This convention is used only for very simple molecules. It works best for molecules in which all the polyvalent atoms are in a single chain. Branches and rings are clumsy to represent. The condensed structure for ethanol would be

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

CH_3CH_2OH. Sometimes dashes are used to show the bonds between the polyvalent atoms: $CH_3{-}CH_2{-}OH$, in which case care must be taken to avoid unwanted line breaks. The condensed structure makes no effort to represent the arrangement of the atoms in space.

- All atoms are shown with elemental symbols.
- Hydrogen atoms (and other monovalent atoms) form only one bond.
- Each monovalent atom is bound to the atom that it follows in the formula. Subscripts may be used for multiple monovalent atoms of the same element bound to the same polyvalent atom.
- The polyvalent atoms are bound to one another in order.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

Semicondensed Structure

The semicondensed structure is akin to the Lewis structure, but bonds to hydrogen and sometimes to other monovalent atoms are not drawn. A semicondensed structure of ethanol is shown in Figure 2. The omission of the bonds to hydrogen allows for the structure of a more complicated molecule than can comfortably be represented as a full Lewis structure. Conventions for the semicondensed structure are as follows:

- All atoms are shown with element symbols.
- Hydrogen atoms are shown near the atoms to which they are bound, with a subscript if there is more than one hydrogen atom.
- Covalent bonds between polyvalent atoms are shown as lines.

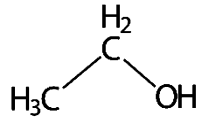

Figure 2. Semicondensed structure of ethanol.

Skeleton Structure

The skeleton structure is highly conventionalized, as shown for ethanol in Figure 3. Neither carbon atoms nor hydrogen atoms bound to them are shown. Atoms other than carbon and hydrogen are always shown, as are any hydrogen atoms bound to them. Advantages of the skeleton structure are that the hetero atoms, which are usually in the important functional groups, are highlighted. Also removal of most of the hydrogen atoms can make the underlying shape of the molecule easier to see. An effort is usually made to represent the geometry of the

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

molecule accurately. The main disadvantage is that it takes experience to be able to mentally add the implied atoms and bonds. Students usually need practice in going from Lewis structures to skeleton structures and the reverse. The conventions for skeleton structures are:

- A line represents a bond, which is shared pair of electrons.
- Ends of line segments represent carbon atoms, unless marked.
- Carbon must have four bonds. Any not shown are to hydrogen.
- Atoms other than carbon and hydrogen are shown with their element symbol.
- Hydrogen atoms not bound to carbon are shown, although not always their bonds.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

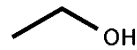

Figure 3. Skeleton structure of ethanol.

It is not unusual for different parts of the same structure to be represented with different conventions, giving a myriad of hybrid structures. To highlight the result of different conventions with a more complicated molecule, the Lewis (Figure 4), hybrid skeleton/semicondensed (Figure 5), and skeleton (Figure 6) structures of isohumulone, a bitter compound from hops, are shown. The Lewis structure of such a complicated molecule is crowded and confusing.

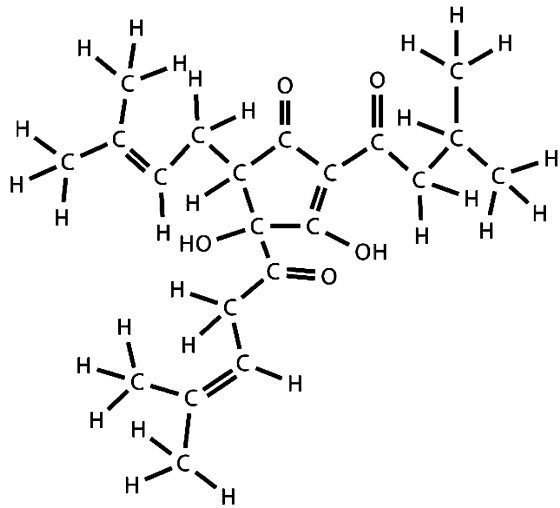

Figure 4. Lewis structure isohumulone.

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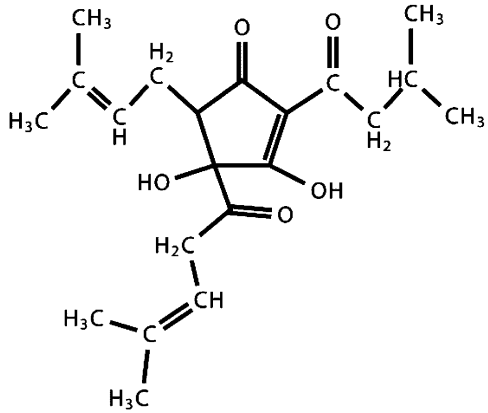

Figure 5. Hybrid structure isohumulone.

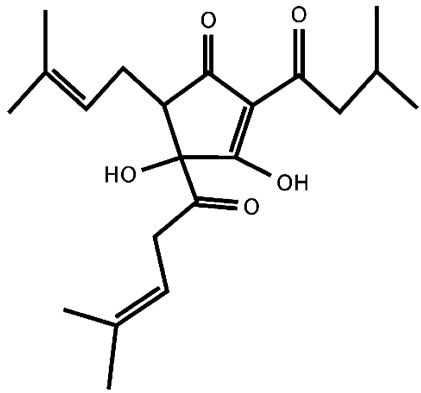

Figure 6. Skeleton structure isohumulone.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

Sugar Structures

Sugars relevant to brewing have multiple asymmetric carbon atoms. The only difference between many of the sugars is the configuration about an asymmetric carbon. To help identify individual sugars and to help elucidate their structure, Emil Fischer devised a scheme called the Fischer projection. The conventions are as follows:

- The sugar is shown in the open-chain form with the carbon chain arranged vertically
- Hydrogen atoms and hydroxyl groups extend to the right and left.
- The carbon atoms are usually not marked.
- Vertical lines represent bonds that extend away from the viewer; horizontal lines extend toward the viewer.

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The Fischer projection for D-glucose is shown in Figure 7.

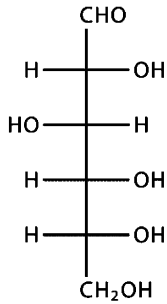

Figure 7. Fischer projection D-glucose.

A limitation of the Fischer projection is that it ignores the hemiacetal ring configuration that is common in pentose and hexose molecules. Another limitation is that if the structure is rotated 90 degrees to the right or left about an axis perpendicular to the paper, the configuration about every asymmetric carbon atoms becomes reversed, so the enantiomer is represented. A 180 degree rotation is permissible. Rotation by 180 degrees about an axis in the plane of the paper also reverses the configurations.

To allow representation of sugars in their ring forms, the Haworth projection is often used.

- The conventions of a skeleton structure apply.
- The ring is shown flat and viewed at an angle of about 60 degrees above the plane. Often the ring bonds closer to the viewer are drawn more heavily.
- Groups drawn on the right in the corresponding Fischer projection are shown going down, below the plane of the ring.

The Haworth projection for α-D-glucose is shown in Figure 8.

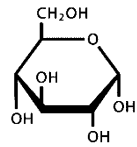

Figure 8. Haworth projection α-D-glucose.

The major limitation of the Haworth projection is that it must be shown in the standard orientation to be understood. This leads to serious distortions when representing polysaccharides. Figure 9 shows a regrettably common representation of maltose.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

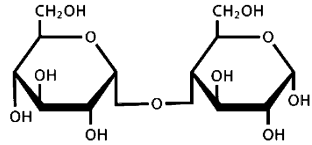

Figure 9. Distorted representation of maltose.

The right angles to the left and right of the bridging oxygen are not intended to represent carbon atoms. They are only to keep the rings in the standard orientation for the Haworth projection. Such drawings should not be used in any context, least of all for introductory students. Figure 10 shows maltose represented in a way that highlights the bent structure of the molecule. The shape of maltose results from the axial (α) configuration of the glycosidic bond. When glucose molecules are linked in the axial configuration the polysaccharide forms a helix characteristic of starch. If the configuration is equatorial, the disaccharide is cellobiose, a straight molecule, and the polysaccharide is cellulose, which forms fibers.

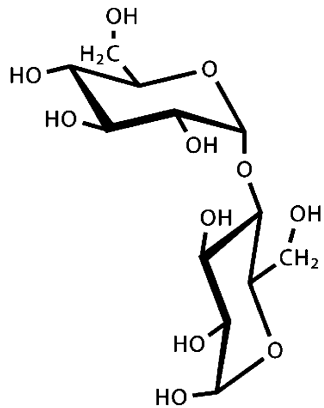

Figure 10. Skeleton structure maltose.

Conclusion

Teaching a beer-themed chemistry course is rewarding to you, your students, your department, and your discipline. The course is fun to teach. One meets a diverse variety of interesting students who otherwise would never be seen in a chemistry classroom. Some actually stay late to discuss beer issues. Beer is a great way to introduce students to chemistry. Everything that would normally be a part of an introductory course finds a natural place. The course can greatly benefit your department's credit production and other economic indicators, especially if you can teach it as a moderately large lecture with no lab. The course attracts to chemistry students who otherwise would be out of reach. This enhances public awareness and appreciation of the discipline.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

Beer Chemistry Resources

Textbook

Barth, R. *The Chemistry of Beer: The Science in the Suds.* Wiley 2013. ISBN 978-1-118-67497-0.

Professional Societies

American Society of Brewing Chemists.
Master Brewers Association.

Literature

Journal of the American Society of Brewing Chemists. ISSN 0361-0470.
Journal of Agricultural and Food Chemistry. ISSN 0021-8561.
Cerevisia. ISSN 1373-7163.
Technical Quarterly - Master Brewers Association of the Americas.

Web Sites

How to Brew: www.howtobrew.com
Home Brew Talk: www.homebrewtalk.com

Reference Books

Briggs, Dennis E; Boulton, Chris A.; Brookes, Peter A; Stevens, Roger *Brewing Science and Practice*, CRC Press, 2004. ISBN 978-1-8557-3490-6.

Hornsey, Ian S. *Alcohol and its Role in the Evolution of Human Society*, Royal Society of Chemistry, 2012. ISBN 978-1-84973-161-4.

Hough, J. S. *The Biotechnology of Brewing and Malting*, Cambridge University Press, 1985. ISBN 978-0-521-39553-3.

Lewis, Michael J.; Young, Tom W. *Brewing*, Springer, 2001. ISBN 978-1-4615-0729-1.

Priest, Fergus G., Stewart, Graham G., Eds.; *Handbook of Brewing*, 2nd ed.; CRC Press, 2006. ISBN 978-0-8247-2657-7.

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Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch007

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Chapter 8

Brewpub and Brewery Operations

Mixing Science and Business

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The 400-level course Brewpub and Brewery Operations was created in 2006 and offered between 2006-2013 by the Department of Hospitality Management at Indiana University of Pennsylvania (IUP). The class was open to any IUP student, faculty, or staff, provided that the student was 21 years old. Course components included lectures on brewing processes and responsible alcohol service issues, a one-day brewing laboratory session, field trips to regional brewing facilities, and a brewery design feasibility project. Students were assessed by their performance on multiple journal entries, quizzes, a group project report, a beer-food pairing, and a final exam. Course components, instructor reflections, and future directions are described.

Introduction

In recent years, several universities have offered a range of beer- and/or brewing-related courses (*1–9*), short courses (*10–13*), and degrees or certifications (*12*, *14–20*). College classes vary from freshman introductory chemistry

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

courses that fulfill a General Education or Liberal Studies science component, to upper-level advanced brewing courses that involve beer brewing and tasting. Courses have been delivered in several different departments, including Chemistry (*1–3*, *5*, *6*, *9*, *10*, *21*), Food and Nutrition or Food Science (*4*, *7*, *8*), Hospitality Management (*13*, *20*), and Biology (*2*).

Specialized degrees or certification programs have been offered for years at UC Davis (*12*) and The Siebel Institute (*17*). The growth of the craft beer industry in the U.S. has spurred interest and demand for such programs, and potential students for these programs may wait over a year before being admitted.

Indiana University of Pennsylvania (IUP) is the second largest University in the Pennsylvania State System of Higher Education (PaSSHE). Located in the town of Indiana, the seat of Indiana County, IUP serves almost 15,000 graduate and undergraduate students in 136 undergraduate, 57 masters, and 11 doctoral programs.

The purpose of the Brewpub and Brewery Management course was to provide a general elective for upperclassmen that would harness the interest and enthusiasm of students for beer to learn topics related to beer production, service, and ancillary areas. Though the course was offered exclusively in the Department of Hospitality Management (HRIM) (*22*), students from any major (and faculty/staff) were encouraged to enroll. There were no course prerequisites that required previous HRIM or other majors' courses, though each course attendee was required to be 21 years of age by the first day of class.

Included in the course was a one-day practical experience in the beer brewing process, science and business lectures related to the brewing process and brewing industry, beer styles lectures with related tasting sessions, a food-beer pairing exercise, and a group project. The lectures were geared towards a diverse student audience in order to provide a broad spectrum of topics related to the beer industry.

The group project was the design of a new brewery or brewpub based on the information attained during the course, including brewing equipment needs and cost, restaurant needs and cost, licensure, marketing, real estate location, demographic studies, and personnel issues. Groups were assigned to maximize the diversity of student majors on each project team, with the expectation that a more robust feasibility study and creative approach would be seen from their skill sets and knowledge bases than had a single major been represented in a group.

History of the Course Creation

This course originated as "guest lectures" by Dr. William Dietrich (Biology), an experienced home brewer, for the course HRIM 402, Beverage Management, that was taught by Dr. Thomas Van Dyke. Because of the time constraints imposed by a regular semester schedule, the scope of these guest lectures was limited and confined to three sessions: 1) the brewing process, 2) bottling and beer styles, and 3) a final tasting activity.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

In HRIM 402, three to five student volunteers were recruited to help with the making of a single batch of beer using malt extract and grain adjuncts on the afternoon before the class met for the first guest lecture on the brewing process. During that lecture, Dr. Dietrich and his student volunteers described what they had done during the brewing process on the previous day, including the brewing steps and relevant biochemical processes that were occurring during brewing and fermentation. Later in the semester, all of the students helped to bottle the beer (wine was also produced and was bottled at this time). Lecture time in this session was given over to the types and styles of beer from around the world and relating it to the brewing process. The final class meeting was dedicated to a review of the course material, and a tasting of the beer produced in class and of others provided by the instructors. Students and instructors prepared a food item to bring to the tasting that would complement the beer produced.

Drs. Van Dyke and Dietrich noted that expanding what was done in the Beverage Management course could make an interesting elective. As a result, a syllabus for a general university elective was written, submitted and approved as a three-credit "481 course", a designation that indicates it was "experimental". The design of the expanded course included brewing, visits to brewpubs and breweries, responsible service training, lectures by a Prevention Specialist on alcohol abuse, and training by Pennsylvania Liquor Control Enforcement (PLCE) officers. In order to provide all of the requisite experience for the course topics, two instructors – a scientist and a hospitality management professor – were required to teach the course.

The capstone of the course was a project by groups of three to four students that would result in presentations describing a new brewpub that might attract potential investors. This project included potential locations, theme and design, types of beers, cost estimates of brewing and kitchen equipment, building, and food, and legal considerations. The students would use that information which was learned in this intensive course and, simultaneously, learn to share work and knowledge from their respective majors.

The course was advertised campus-wide as a general elective through flyers, student newspaper ads, and cafeteria table tents. Students were required to be 21 years of age by the first day of class, but there were no other prerequisites with regards to major area of study or other HRIM or science courses. The course was presented during the three-week May intersession between the spring semester and the summer sessions in an effort to recruit a wider demographic of the student population. During the second week, students were taken on field trips to local brewpubs, breweries, and related businesses. To avoid legal issues, students were transported in IUP vans, each holding 12 passengers. With two faculty drivers, this limited the course participation to a maximum of 22 students.

The course was first presented by Drs. Van Dyke and Dietrich in 2006 with an enrollment of 15 students and again in 2007 with 16 students enrolled. The course was offered by Drs. Van Dyke and McElroy in 2009 with 15 students enrolled. The course was repeatedly offered during the May intersession until 2013. Low enrollments, however, prevented the course from being approved at the College level during those times.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Course Components

Beer Brewing Lab

On the first day of the course, students and faculty participated in a beer brewing session in the kitchen of Ackerman Hall on the IUP campus. Ackerman Hall houses the Hospitality Management (HRIM) and Food & Nutrition (FDNT) departments. In addition to faculty offices and instructional classrooms, the building contains a professional kitchen and dining room where students can experience food service and related activities in their coursework.

Prior to the first day of class, all students were contacted by email to inform them of the proper dress code for working in the kitchen area. Course participants were required to wear long pants, hard-soled shoes, and a hat.

Faculty prepared for the brewing demonstration by purchasing raw ingredients at a local homebrew shop for making the beer and by supplying their personal home brewing equipment for use in the kitchen during the demonstration. A typical equipment list for brewing 5-gallon batches of beer can be found in most homebrewing books such as Papazian (*23*), Palmer (*24*), Goldammer (*25*), and Noonan (*26*).

The students were divided into two working groups. Half of the students worked with one instructor to brew a 5-gallon batch of an ale; the other group worked with a second instructor to brew a 5-gallon batch of a 'lager'. The lager was not a true lager in the sense that it was not matured at colder temperatures. However, the wort was fermented with lager yeast so that students could appreciate the differences in fermentation and final product.

The procedures for making a 5-gallon batch of homebrew with malt extract and specialty grains can be found in most homebrewing books, such as those listed above. Each instructor guided their respective group through the brewing process while explaining the reasons behind each step, and the same steps were taken by each group.

Chemistry and Biochemistry Lectures

During the first week of the course, several lectures were delivered in a traditional classroom setting that covered a host of brewing topics, ranging from a brief history of beer and brewing to the brewing process to the chemistry and biochemistry of key brewing components and processes.

Lecture 1: The History of Beer and Brewing

A short lecture describing the milestones of beer history was presented. Certainly, beer is woven into the fabric of modern society from its ancient roots. Beer, after all, is nothing more than a water solution of sugars, derived from grains that is spiced and then fermented by microorganisms, not too dissimilar from wine or mead. Modern archeological techniques have come to bear on its origins somewhere in Africa, China or Mesopotamia about 6,000-7,000 years

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

ago. The importance of beer in early societies was as food, payment, and as a religious prop (*27*).

After the advent of agriculture, milestones such as the introduction of hops in Europe in the late Middle Ages, the advent of lagers, Pasteur's discovery of yeast, and the German Purity Law were introduced. A discussion on the variation of beer styles in different parts of Europe illustrated how different ingredients can be derived from very similar processes yet produce drastically different final products because of *terroir* and water chemistry profiles. Finally, "Beer in America" was covered from the ales of the Revolution and the lagers of German immigrant culture, to industrial breweries and finally the craft brewing revolution.

Lectures 2−5: The Brewing Process and Beer Ingredients

These presentations were designed to describe each key brewing step and to reinforce and further explain the steps that the students took during the first day of class. Topics included:

- Malting and the various grains used in brewing
- Water and brewing water chemistry
- Hops
- Yeast
- Milling of grains
- Wort production, including mashing and sparging
- Wort boiling and hopping
- Wort chilling and clarification
- Fermentation, both primary and secondary
- Bottling and kegging, storage
- Serving

The bulk of the science in this class was presented over the next two and a half days in lectures about beer, beer ingredients, the and brewing processes used by both homebrewers and commercial brewery operations.

First, the four main brewing ingredients were discussed, including key chemical and biological information. The process of malting barley was described, requiring treatment of the chemical structure of sugars and the polymer starch. The action and specificity of enzymes was introduced in the breakdown of starch to sugars and limit dextrins by amylases. The sensitivity of these reactions to their environment allowed the instructors to bring the importance of water into the discussion; pH, mineral composition and temperature were related to enzyme activity in the mashing and lautering process. Isomerization, extraction and other chemical changes were brought into the discussion when hops were introduced. Finally, the primary metabolism of yeast was introduced as a way of capitalizing on those considerations and phenomena already discussed. Consequently, when the linear process of beer brewing was described, the reactions were "reviewed" in the context that was the theme of the course. Different styles of beer could be attained by alterations in conditions and ingredients.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

Beer descriptors such as ABV (alcohol by volume), degrees Lovibond (°L) and Standard Reference Method (SRM) for color, and IBU (international bittering units) were described while reviewing composition and process in a section called "beer by the numbers". These parameters are relevant to what the students sense when they taste beer. Finally, anyone who has paid attention to modern beer in the U.S. knows that many beers contain other ingredients such as sugars, spices, and fruit. These are added at appropriate times to augment or add flavors already in the beer.

HRIM Lectures

The topic of beer styles was introduced as a discussion of the types of beer students like. Most students were familiar with and had preferences for at least two or three styles of beer, such as pale lagers, stouts, and light beer. Several other styles of beer were introduced through in-class tastings, such as porters, German wheat beers, India pale ales, and Belgian-style ales. All of the styles discussed were related to history of beer, ingredients and processes of the specific regions where the styles originated.

The "Beeriodic Table" (*28*) and the flavor wheel (*29*) were introduced during this topic as students considered what was sensed during beer tasting. The class also discussed the question of whether or not there was a true American beer style.

A video presentation on "beer clean" glasses was shown, highlighting the importance of and methods for providing clean glassware in which to serve beer.

Dr. Van Dyke provided lectures in class regarding several issues related to operating an establishment that serves alcohol. Topics included business liability and other legal issues of running a brewpub in Pennsylvania, proper alcohol service, care of the product such as proper storage, cleanliness of beer lines and glassware. Methods for increasing alcohol sales, and employee and customer issues that arise when having alcohol present at the workplace were addressed.

Pennsylvania offers training for retail operators that serve alcoholic beverages. The Responsible Alcohol Management Program (RAMP) (*30*) is conducted by a certified trainer (Dr. Van Dyke), who is approved by the Pennsylvania Liquor Control Board (PLCB). The students received a minimum of three hours of training, including a discussion of the chemical components of alcohol and how they effect the brain, absorption rate factors, signs of the levels of intoxication, measures of intoxication (visible and blood alcohol content), drink equivalences, refusing service, minors and the law, carding persons, acceptable identifications, fake identifications, proof of carding, liabilities (administrative, criminal, civil), and incident documentation forms. A state-approved examination was given at the end of the training. Students who scored 80% or better on the exam received a certification of training card that is valid for two years.

Field Trips

During the second week of the course, four days were dedicated to field trips to local breweries and related establishments. For each trip, two university vans were used, each capable of carrying 11 passengers plus a faculty driver. Two months

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

prior to the course start date, local breweries were contacted via mail or phone. The brewers were informed about the nature of the course and how they could assist the students by hosting a field trip tour and brief Q&A session. The majority of brewers and/or establishments who were contacted enthusiastically accomodated our visits.

Day 1: Split Field Trip

Due to the tour capacity and distance from campus of two of the breweries involved, the class was split into two groups in order to visit two different sets of breweries. In previous years, different establishments were visited – the most recent are described below as an example of the diversity of the students' experiences.

Group 1 traveled first to Marzoni's Brick Oven & Brewery in Duncansville, PA, followed by Otto's Pub & Brewery in State College, PA. At Marzoni's, the group met head brewer Bill Kroft at 10am for a brewing demonstration on a 7-barrel (bbl) system. Students were given a tour of the brewhouse, the fermentation room, and shown the kegging system. Mr. Kroft explained his brewing process, including sanitation and the serving system at the bar area of the brewpub. He allowed students to rake spent grain from the lauter tun and explained how local farmers collected the spent grain as silage for their cattle.

Group 1 then traveled to State College, PA for a lunch at Otto's Pub & Brewery, followed by a tour of the brewery and bottling line. In addition to beer, Otto's also makes root beer for in-house service.

Group 2 traveled first to Red Star Brewing Company in Greensburg, PA. There, they were allowed to watch beer brewing by head brewer Jeff Guidos in their 7-bbl brewhouse. Following this tour, the group then traveled to Rock Bottom Brewing in Pittsburgh, PA for lunch and a brewery tour.

Day 2: Pittsburgh

On the second day, all students traveled to Pittsburgh to visit a large bottle shop, a Belgian-themed pub, and a microbrewery. The choice of locales was intended to show students some difference methods by which craft beer was produced and/or distributed. D's Six Pax & Dogz is a large craft beer store that specializes in craft and rarer import beers that are available for sale in single bottles, which in the mid-2000's was rarer in Pennsylvania than at present because of the alcohol sales laws at the time. Most of the students had only experienced package stores that were limited to sales of cases of beer or six-packs.

The second stop was for lunch at the Sharp Edge Brasserie, a gastropub focused on Belgian cuisine and beer. After lunch, the group was given a tour by beverage manager Hart Johnson, who showed students their comprehensive 40-tap, anti-foaming serving setup. He explained the precautions taken to provide the freshest quality product.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

The third and final stop for this trip was a visit to East End Brewing Company, a microbrewery established in 2005 by Scott Smith. At the brewery, Mr. Smith provided a tour of the brewery, had students taste and smell brewing ingredients while explaining his brewing processes, and provided samples of his current beers for sale in growlers.

In addition to the field trip, students were invited but not required to attend the monthly meeting of the Indiana Pennsylvania Alemiths (IPAs) homebrew club that evening at a local restaurant in Indiana.

Day 3: Pittsburgh

On the third day of field trips, the students traveled again to Pittsburgh to visit two different brewpubs.

The class first visited the Hofbräuhaus, whose Pittsburgh location was established in spring 2009. Student enjoyed lunch inside this German beer hall, followed by a brewery tour and presentation by head brewer Eckhard Kurbjuhn.

The second trip was to the Church Brew Works. Established in 1996, this brewpub is housed in the former St. John the Baptist Catholic church, with the brewhouse situated on the former altar. Brewmaster Brant Dubovick provided a tour of the brewery, maturation and storage areas, and their yeast lab.

Day 4: Monroeville

On the final day of field trips, the class traveled to Monroeville, an eastern suburb of Pittsburgh, to visit Rivertowne Pour House, a brewpub established in 2009. Brewmaster Andrew Maxwell and assistant brewer Barrett Goddard provided a tour of the facility, then Mr. Maxwell gave a one-hour presentation during lunch which described all of his trials in starting the brewery. He provided his experiences and advice for establishing recipes, corresponding food menus, expenses and equipment, and personnel issues.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

Guest Speakers

During the course, two guest speakers were brought into the classroom to address specific areas of responsible alcohol service.

The first presentation was given by representatives of the Pennsylvania Liquor Control Enforcement (PLCE). These officers described the laws regarding alcoholic beverage service, including topics such as: checking identification, refusal of service, and liabilities of establishment owners with regard to the law.

The second presenter was a Certified Preventative Specialist with the Armstrong-Indiana County Intermediate Unit (ARIN IU). The speaker dealt with topics related to alcohol abuse and its effects on families. Thus, the social responsibilities of alcohol service, and hence the brewing industry, were emphasized.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Feasibility Study

Groups of 3-4 students were assigned so as to maximize the diversity of majors, including hospitality management, chemistry, culinary arts, geoscience, and business. Each group was assigned the tasks of researching and creating a fictional brewpub or brewery in Pennsylvania and giving an oral presenation of this creation. This project was intended to involve students in critical thinking. Groups were evaluated on both the professionalism and rigor of the written and oral reports and the creativity of the brewery/brewpub. Areas of research and creation for the project included:

- Suitable location – groups chose a specific town in Pennsylvania and searched local real estate options for lease or sale; in addition to a building or development site, groups also researched basic demographic data such as population, median age and income of population, and competing businesses.
- Cost analysis – groups were required to create a realistic budget and search for brewing equipment, related supplies, ingredients, and utilities.
- Licensure – groups were responsible for searching the availability of alcohol licenses in the county for which they planned to build their business. Students were directed to the online Pennsylvania Liquor Control Board Search System (*31*).
- Employees – groups were asked to budget for a core staff for their business and justify their choices. For example, those choosing to build a brewpub looked at a brewer, a chef, and a manager.
- Theme – groups were asked to create a unique theme for their brewery or brewpub.
- Beers – groups were responsible for choosing 4-5 beer styles, including a flagship product, that they felt would sell well for their target demographic and compete with local products. Students were also asked to come up with creative names for their beers that would fit in with the theme of their business.
- Menu – for those groups creating a brewpub, students were required to create a simple menu of signature dishes that would pair well with their chosen beer styles and fit in with their chosen brewpub theme.

In addition to working on the project outside of class, one class period was dedicated as a group working session in a computer lab so that students could be shown relevant websites for license (*31*) and real estate searches, demographic data (*32*), and distributors of brewing equipment and ingredients (*33*).

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Student Evaluation

The students were evaluated on the basis of the following:

- Attendance – attendance was required for all lectures and field trips.
- Journals – all students were required to keep a journal during the field trips in which they described each of the businesses they visited, notes on the brewers, and reflections on their experiences.
- Final exam – a comprehensive final exam was given on the last day of class, covering the instructor lectures, beer brewing lab, guest lectures, and materials provided during the course.
- Group project – an oral (PowerPoint) report was presented by each group on their fictional brewery or brewpub.
- Food-beer pairing – each student was required to provide a food dish on the final day of class to be paired with either the ale or the lager brewed on the first day of class. Each student provided a recipe and a brief justification for their choice.

Instructor Reflections

Student Demographics

The majority of students who registered for the course (whether it ran or not) were majors from the Department of Hospitality Management or IUP's Culinary Institute. However, other majors included Geoscience, Chemistry, Biology, Business, and English. Although the course content would be most applicable to students in the service industry, inclusion of other majors provided a more rich learning experience because of the different perspectives and experiences with beer and brewing of the participants.

The majority of students were undergraduate upperclassmen. A few graduate students and faculty also participated in the class. There were a handful of students who were already brewing at home, though the majority had no brewing experience prior to the class.

Examples of Student Projects

One of the more rewarding aspects of teaching this course was to see the creative concepts of the student groups for their feasibility study project. The diversity of the student groups in terms of age, experience, and major provided a rich learning and collaborative environment. The depth of background research and creativity for designing a fictional business was impressive. A few of these examples included:

- WaterWorks Pub and Brew House – an authentic English pub experience set in an upper-middle class retail area in suburban Pittsburgh. Their goal was to focus on retail patrons, local families, and commuters by providing

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

traditional English pub fare and brewed-on-premise ales with a tie-in to local place names. The group provided their start-up costs for a 10-bbl system and key personnel, provided a menu and beer list, and required per-patron charges needed to maintain their operations and overhead.

- The Beer Loft – a neighborhood brewpub in Pittsburgh with an artistic theme. The group created a sample menu and beer list that incorporated famous and local artist names (e.g. IPAcasso, Raphe-Ale, Warhol Wings). In addition to showcasing works of local artists, they included the idea of a wall inside the pub for local artists to use as a canvas that would change every quarter. They also included plans for the donation of unused food and a percentage of profits to local food banks and homeless shelters.
- Cinema 5 Pub & Brewery – a brewpub set in an existing movie theater in State College, PA. The group envisioned a stadium-style seating arrangement, expanded to include tables, from which patrons could watch one of three different movie presentations during dinner. The menu and beer selections reflected the Hollywood theme (e.g. Brave Hart's Scotch Ale, Red Hot Stuffed Chili Peppers, One Flew Over the Chicken Breast), as did the lobby and dining area décor.
- Wilchakas Brew Factory – a microbrewery located in York, PA. This group focused their attention on creating a brewery rather than a pub, and provided detailed information on their proposed building, brewing and packaging equipment, and local demographics. Their beer names reflected places and personalities from the York area, and they provided a comprehensive marketing strategy for their brewery (e.g. billboards and print media, growler sales, and tastings).

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

Advantages and Disadvantages to the Course Format and Delivery

Students from a variety of the Colleges in the university took this course. While the number of science majors, specifically chemistry and biochemistry, was virtually non-existent when compared with hospitality and business majors, their interaction in designing a brewpub/brewery was good to see. The presentations and written papers showed that they were indeed learning from one another.

Teaching the course in the Intersession had both advantages and disadvantages. The time of year was good for visiting breweries and for obtaining transportation. Students, however, seemed weary at the end of the academic year and needed prodding to do the work.

The topic of the course seemed to be of concern to the parents, who were leery of a course about beer, while the students had a strong interest in the topic.

Fortunately, some graduate students and professors took the course, and their maturity allowed them to lead undergraduates during the project phase.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Future Directions

Undergraduate Course Opportunities

At present, no faculty member in Hospitality Management has offered HRIM 481 since the time of Dr. Van Dyke's retirement. Drs. Van Dyke and McElroy created an alternate regular course, HRIM 404, which was an expanded, six-credit Brewery Operations course with additional focus on the marketing and service components of a brewpub or brewery. This course was approved by the University Senate in 2012 and first offered in summer 2013, but was cancelled due to low enrollment. We expect that HRIM 404 or a variant of HRIM 481 will again be offered in the near future by one of the newer HRIM faculty members.

There is no doubt that using brewing to interest non-science majors in science can lead to a popular undergraduate general education science course (*2*, *21*, *34*, *35*). Such a course is a common requirement (100 level) of the General Education or Liberal Studies Curriculum and is usually fulfilled by General Biology or Physical Science (a mixture of Physics and Chemistry). In recent years, several universities have offered similar courses with varying degrees of alcohol-related activities depending upon the laws of a particular state. Nonetheless, allowing students to see science in a context of a business or application is a topic near and dear to the undergraduate heart that would draw them in, and sparking an interest in science often occurs in the context of an application.

In summer 2015, Dr. McElroy will offer CHEM 281 – The Chemistry of Beer as a three-credit, non-laboratory science elective. The course will focus on advanced general and basic organic chemistry, and basic analytical methods focused on beer and the brewing process. There will not be a brewing or tasting component to this course, thus there will be no age restrictions. In order to facilitate more meaningful chemistry content delivery, students must have completed a freshman two-semester chemistry course as a prerequisite.

Graduate Course and Internship Opportunities

At IUP, graduate (M.S. level) curricula in biology and chemistry may benefit from either an elective course in brewing science or possible available internships at local breweries. Previously, the Biology Department had a course in Industrial Microbiology in which beer was brewed, and currently the Chemistry Department has a Professional Science Masters in Industrial and Applied Chemistry. One requirement of the PSM degree is an industrial internship, an opportunity that local breweries may be able to provide.

List of Course Resources

The following resources were used in the creation and delivery of course content. Resources marked with an asterisk (*) were from the personal libraries of the instructors and were placed on reserve at IUP's Stapleton Library for the students' use.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

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Smith, G. *Beer in America: The Early Years 1587-1840*; Brewers Publications: Boulder, CO, 1995; ISBN: 978-0937381656

Websites

American Homebrewers Association (www.homebrewersassociation.org)
Beer Judge Certification Program (www.bjcp.org)
Brewers Association (www.brewersassociation.org)
Pennsylvania Liquor Control Board (www.lcb.state.pa.us)
RateBeer (www.ratebeer.com)

Journals and Periodicals

Journal of American Society of Brewing Chemists
Brew Your Own
Zymurgy

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch008

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Chapter 9

"Beer Is Good for You" as a Message in Academia

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There is a tightrope to be walked in the teaching of alcoholic beverages on a university campus, especially when it comes to discussing the relative merits and de-merits of drinking. It is critical, however, that students are exposed to a clear and unprejudiced hearing of the harmful consequences of consuming alcohol balanced against the growing appreciation that there are medical advantages of moderate alcohol consumption. At UC Davis the topic of beer and health is presented as a metaphor for a "middle way" between neo-prohibitionism and abstention at one extreme and abuse on the other.

In one of the most popular classes on the UC Davis campus the students are taught that beer is empty calories. I suspect the instructor is using this gambit to caution students about heavy drinking. For myself, I prefer to tell it the way it is: beer is not empty calories, in fact far from it. However I too caution against heavy drinking. And I believe that to hear the brewing professor advise moderation is all the more powerful.

The challenge that I have, whether talking to students on campus (and I reach them not only in the classroom but also by invitation for "fireside chats" in their halls of residence) or to the greater world is that my business card says "Distinguished Professor of Malting and Brewing Sciences". Straightway this positions me for the naysayers about alcohol in the "prejudiced and not to be trusted" camp. A lifetime spent in and around brewing is, for them, all the evidence they need to distrust anything I have to say on the topic. Even more it

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disturbs some that there are academic institutions that have programs dedicated to the study of alcoholic beverages.

As I articulated in my book *Beer is Proof God Loves Us* (*1*), to me beer is a metaphor for the Buddhist concept of the middle way. Strict abstention and abusive drinking of alcohol are polar extremes. Whilst there may be religious foundations for the former, there is burgeoning evidence that the healthiest of lifestyles can be enjoyed when alcoholic beverages including beer are consumed moderately and indeed regularly. Yet the very act of pointing this out with strict cautionary words about excessive consumption is something that rather sticks in the craw of those who would contend alcohol to be simply a "no no".

One of the more ridiculous things that anyone has said to me is "oh, yes, my wife and I do drink beer, but never in front of the kids". To me that statement encapsulates the somewhat deceitful approach to the consumption of alcohol in the United States. In many European societies beer or wine is just another normal and unexceptional part of a day to day experience. Children grow up seeing their parents and grandparent enjoying moderately their preferred beverage. It is not hidden away behind cloaks of mystery and challenge. Small wonder that children grow up with an unhealthy attitude, culminating in ridiculous drinking rituals when they reach the legal drinking age. It is an issue that I confront directly in one of my classes, spelling out an actual example of a UC Davis student dying after such an alcohol (and probably drug) fueled celebration. Yes, there are episodes of drunken stupidity in countries such as my native England – and to be present in many a city center on a weekend evening is to witness unpleasant episodes of ribaldry fueled by booze - but somehow there do not seem to be the same fatal excesses.

Thus, without fear and sometimes with favor, I talk to the students both formally and informally about healthy approaches to beer. I am the loudest voice deploring stupidity like Beer Pong. I talk of the negative impacts of abusing alcohol – notably accidents, offensive behavior and so on. But I also give what I feel to be a balanced view on the positive and negative impacts of alcohol on the body.

In this context I also draw attention to the respective perceptions of wine and beer, something I addressed in some detail elsewhere (*2*). There is a widespread belief that wine is the more wholesome beverage, whereas the reality is that beer has substantially more nutritive value and is at least the equal of wine in the key beneficial impacts that are to be offered. Yet still there are those who would view the title of another of my books as an oxymoron (*3*). It isn't.

Reported Benefits of Beer

There is now ample evidence that the active component in wine that is responsible for the so-called French Paradox (lower than expected rates of atherosclerosis in a society that consumes large amounts of cholesterol-inducing foodstuffs) is not resveratrol, but rather ethanol (*4*, *5*). As such, any alcoholic beverage in the same equivalent quantities in terms of alcohol presents the same advantage. I emphasize to the students that this is likely to be a more significant benefit in older populations. However I do not shy away from the observation

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that it is daily moderate consumption that is of most benefit in this context (*6*), but that the weekly dose cannot be taken in a single sitting, which constitutes the very harmful binge drinking.

Considering that coronary heart disease is the number one cause of death in the U.S. (*7*) and that moderate alcohol consumption offers some protection against it, perhaps it is surprising that there seem to be few reasonable and informed individuals in politics and medicine who are addressing the message. Rather they focus on the perceived negative elements – a clear case of not taking the middle way.

From head to toe there are many studies drawing attention to the benefits of moderate alcohol (including beer) consumption, to add to the defense against coronary heart disease:

(a) Improved cognitive function (*8*)
(b) Reduced risk of Parkinson's Disease (*9*)
(c) Reduced incidences of dental caries (*10*)
(d) Promotion of lactation (*11*)
(e) Reduced risk of late onset diabetes (*12*)
(f) Reduced instances of gall stones (*13*)
(g) Reduced instances of kidney stones (*14*)
(h) Reduced instances of osteoporosis (*15*)
(i) Reduced risk of rheumatoid arthritis (*16*)
(j) Increased fertility (*17*)

In terms of chemistry, beer has been demonstrated to be a significant source of

(a) Some B vitamins, especially folic acid (*18*)
(b) Silicate (*19*)
(c) Antioxidants (*20*)
(d) Fiber (*21*)
(e) Anti-mutagens (*22*)

Reported Adverse Impacts of Consuming Beer

The customary response to claims that beer is a healthier choice than wine is to draw attention to the "beer belly" and that there is no "wine belly". Wannamethee (*23*) stressed that the beer belly is a myth, and that it is simply a case of balancing calories in (eating and drinking) with calories out (activity). The main source of calories in any alcoholic drink is in the form of alcohol per se. The problem is much more likely to be that a beer drinker may well have a more sedentary lifestyle involving a less healthful diet as compared to a wine drinker (*24*).

Excess beer consumption has been linked to gout, but so too has wine (*25*).

People suffering from celiac disease have long since been advised to avoid beer. However many commercial beers contain extremely low levels of problematic proteins and their degradation products (*26*) and it is certainly

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possible to make beers from malted barley that are devoid of the challenging peptides (*27*).

In many ways the most sensitive area for discussion about impacts of alcoholic beverages concerns cancer. There are many reports linking alcohol consumption to the risk of various cancers (*28*). However there are studies registering the opposite (*29*). This highlights the challenges in drawing correlations generally between lifestyle and diet and impacts on the body. Man or woman does not live by alcoholic beverages alone. There are major challenges in excluding the risk of secondary correlations. For example: there is an accepted link between smoking and cancer. However there is also a correlation between smoking and drinking beer. As such, it is entirely possible to draw a correlation between beer drinking and cancer, whereas there is equally probably no causal relationship whatsoever.

Which brings me back to the concept of the middle way. There is no challenging the fact that excessive consumption of alcohol can have severe adverse impacts – for example cirrhosis, whilst noting that this condition demands abuse with hard liquor (*30*). Equally there is an understandable caution to expectant mothers to avoid drinking, yet it is likely that serious problems such as fetal alcohol syndrome (*31*) are really linked to abusive levels of intake and that the occasional beer or wine is not going to present a major problem. A woman who is used to drinking moderately should not despair if she realizes that she has taken an occasional drink before realizing she is pregnant (*32*).

I believe it would be doing students a disservice to do other than present them with all the facts as we know them and to give them advice that is perhaps best summed up in two phrases found etched into the temple of Delphi: *Know thyself* and *Nothing in excess.*

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Chapter 10

Using a Simulated Commercial Wine Laboratory To Teach Quantitative Analysis Laboratory

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At Adrian College, quantitative chemical analysis is a two semester course with the lecture component in the Fall Term followed by the laboratory component in the Spring Term. The lecture focus is conceptual, addressing measurements, data management and statistics, as well as fundamental analytical concepts and methodology.

During the laboratory course, a simulation of a commercial wine analytical laboratory is employed as an application of a variety of analytical methods and techniques. During the first half of the term, students are required to conduct analyses found in wines and grape juice, e.g., alcohols, carbohydrates, sulfites, organic acids, and total, fixed and volatile acidity.

The assignment for the term is to achieve all of the functional prerequisites for designation as a Certified Wine Chemist by the Alcohol and Tobacco Tax and Trade Bureau (TTB) of the U.S. Department of the Treasury and additional analytes defined by the course Instructor. Time in the lab is self-paced and self-scheduled. Students can come in and work in the lab at their discretion, provided a faculty member is in the area and available at all times.

The benefit of this approach is a high level of student engagement because of the contextual nature of the unknown and the breadth of analytes and methodology..

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Introduction

Quantitative chemical analysis at Adrian College has historically been conducted in two courses as follows:

1. Analytical Chemistry (CHEM 303 – 3 Credit Hours), (formerly entitled Quantitative Analysis) is offered annually in the Fall semester. The focus of the lecture component is conceptual, addressing measurements, data management and statistics, as well as fundamental analytical concepts and methodology. The textbook used is Harris, "Exploring Chemical Analysis, 5th Edition (*1*)"
2. Analytical Chemistry Laboratory (CHEM 304 – 2 Credit Hours), (formally entitled Quantitative Analysis Laboratory) is offered annually in the Spring Semester. It is nominally a 6 hour per week lab.

During the laboratory course, a simulation of a commercial wine laboratory is employed as an application of a variety of analytical methodologies and techniques. During the first half of the term, students are required to conduct analyses on a standard wine to develop their techniques for analyzing for a variety of analytes found in wines and grape juice, e.g., alcohols, carbohydrates, sulfites, organic acids, and total, fixed and volatile acidity.

Their assignment is to achieve all of the experimental requirements for designation as a Certified Wine Chemist by the Alcohol Tax & Trade Bureau (TTB) of the U.S. Department of the Treasury, plus additional determinations specified for this class. While their work is self-scheduled and self-paced, there is a hard deadline for submittal of their final report. The "final exam" is an oral debriefing held with the Instructor of Record.

CHEM 303 and 304 are required for the Bachelor of Science (B.S.) Degree in Chemistry, the B.S. degree in Biochemistry, the Bachelor of Arts (B.A.) in Chemistry and for a minor in Chemistry. Many Biology majors elect to pursue a minor in Chemistry, especially those who intend to enter medical or dental school.

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Mission

To achieve all of the functional qualifications consistent with those specified by the Alcohol and Tobacco Tax and Trade Bureau (TTB) of the United States Department of the Treasury for designation as a Certified Wine Chemist and, additionally, to demonstrate proficiency in the determination of additional analytes as defined by the Instructor of Record.

Accordingly, subsequent to demonstration of methodological proficiency, each student is provided with one 750 mL bottle of wine. The assignment is for each student to conduct an analysis of the unknown sample of wine for the analytes specified in the template shown below and to submit a comprehensive report of results to the Instructor of Record.

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TTB

The Alcohol and Tobacco Tax and Trade Bureau, statutorily named the Tax and Trade Bureau and frequently shortened to TTB, is a bureau of the United States Department of the Treasury. The TTB offers a Chemist Certification Program for the export of beverage alcohol to foreign markets. Many countries accept a report of analysis from a TTB-certified chemist accompanying the export shipment as a condition of entry. To facilitate the export of domestic wine, the TTB offers a certification program twice per year as a service to the alcohol beverage industry (*2*). The TTB requirements for certification are as follows:

a. The applicant must be employed by or otherwise affiliated with a qualified laboratory. TTB does not certify laboratories, but rather applicants who perform the analyses.
b. A "qualified laboratory" is defined as a laboratory equipped to perform all necessary analyses for certification of wine or distilled spirits and employs an individual certified by TTB to perform or supervise the analyses.
c. In conjunction with the application for certification, an appropriate official of the laboratory must consent to the inspection of the laboratory premises or other appropriate TTB validation of the claims made in the application.
d. Certification is dependent upon continued employment with the qualified laboratory.
e. A certified individual may retain his or her certification in the event that he or she takes a position with another qualified laboratory.

To qualify for Wine Analysis certification by the TTB, the applicant must:

(1) Have at least:

    (i) A Bachelor's degree in chemistry; or
    (ii) A Bachelor's degree in any physical, chemical, or biological science and at least 30 credits of chemistry; or
    (iii) A Bachelor's degree in enology.

(2) Have access to laboratory equipment and facilities as are necessary to perform the required wine analyses.
(3) Receive two 750 mL bottles of wine for each cycle of testing. Upon completion of the analyses, each participant must submit a report of analysis by the specified deadline that includes all analyses specified in Table 1 (*2*) below for each wine analyzed.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Table 1. Required Analyses for TTB Wine Chemist Certification

Analysis	*Reported to the nearest:*
Alcohol by volume	0.1% by volume
Total extract	0.01 g/100 mL
Total Acidity as Tartaric Acid	0.01 g/100 mL
Volatile Acidity	0.001 g/100 mL
Citric Acid	0.1 g/L
Total Sulfur Dioxide	1 mg/L
Residual Sugar (expressed as glucose + fructose)	0.1 g/100 mL
Sorbic Acid	1 mg/L
Methanol	0.01% by volume

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

The Adrian College analog to the TTB requirement for analysis specifies 20 analyses be performed for one wine sample.

Learning Objectives

The learning objectives for CHEM 304 are to achieve proficiency in the fundamental techniques of quantitative chemical analysis. In particular, the following are among the critical competencies to be realized:

1. The interpretation and application of data generated by wet chemical methods, such as titrimetry and gravimetry, and instrumental methods, e.g., UV-VIS spectrophotometry, atomic absorption (AA) spectrophotometry, gas chromatography (GC), and high performance liquid chromatography (HPLC).
2. Effective functioning in a simulated commercial laboratory environment.
3. Methods for the effective documentation of procedures and results, i.e., the laboratory notebook.
4. The efficient and effective use of limited laboratory time and resources.
5. Setting priorities and managing time to complete an extended, complex project.
6. Learning to work independently.
7. Statistical handling of data using Microsoft Excel and the reporting of accuracy and precision.
8. Concise, accurate written communication of results and conclusions.
9. Good laboratory practices, especially continuous attention to safe laboratory practices, the importance of good housekeeping and the proper and appropriate disposal of waste materials.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Sequential Stages

I. ***Qualifying Exam***: Students must demonstrate knowledge and comprehension of the critical areas of quantitative analysis prior to beginning their experimental work. Accordingly, a qualifying exam must be taken and passed by all students with the exception of those students who scored higher than 70% on the CHEM 303 Final Exam. In order to proceed to Stage II, students must achieve a score of at least 70% on the Qualifying Exam. If a score of at least 70% is not achieved, the exam must be retaken until the qualifying score is achieved. The qualifying score increases by 5% for each successive attempt at qualification.

II. ***Methodology Development and Certification***: During Stage II, students must develop, demonstrate and document their competency with all appropriate methodology utilizing standard wine samples provided by the Instructor. Each student receives a sample of either a red or white wine which they are to use to develop their skills for performing each of the analyses required for the analyses to be carried out for their unknowns. The samples are drawn from a box wine so all of the students in a peer group receive the same sample. A master round-robin chart is put on a white-board in the lab. Accordingly, students can hone their laboratory skills and gain confidence in their ability to generate creditable results. It is not until a student has demonstrated competency for conducting each of the protocols that they receive their unknown sample, - a 750 mL bottle of wine.

III. ***Analysis of Unknown/Submission of Final Report***: Each student receives a 750 mL sample of wine on which they must carry out analyses for each of the defined analytes. The samples are drawn from commercial 3 liter box wines, typically a red or white. However, samples also may also be a fortified wine, an ice wine or other specialty wines. Many of the samples are quantitatively adulterated with one or more of the components, e.g., ethanol, glucose, fructose, potassium metabisulfite, copper, acetic acid, malic acid, lactic acid, citric acid or tartaric acid.

The Final Report must be a comprehensive report written in accordance with standard scientific report format. The Final Report must be submitted no later than a time and date defined at the beginning of the term. Late reports will only be accepted with a *bona fide* documented reason, but at a substantial point penalty. Failure to submit the Final Report will result in a grade of F for the course.

This report consists of a cover letter and a tabular summary of results for all analytical determinations required for certification. Copies of all appropriate pages from the laboratory notebook are submitted along with the Final Report. If the Instructor of Record is unable to determine how the results were determined, including raw data and calculations, the student receives a zero for that analyte, even if the result is correct.

IV. ***Oral Debriefing and Exit Interview***: Subsequent to submission of the summary report of results, a one-hour oral debriefing is scheduled and conducted with each student. At this meeting, students are asked questions to ensure they have an in-depth comprehension of the analytical method as well as an understanding of why it is important to a winemaker to have this information. In other words, what does each of these analytical results tell the winemaker about the wine and what remedial actions are indicated.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Ground Rules

While this laboratory is self-scheduled and self-paced, students are required to submit a weekly time-sheet by 5:00 pm each Friday during the term to document the number of hours spent in the lab. Each following Monday morning, a cumulative bar chart is posted in the lab indicating the total hours worked in the lab for each student to date. The nominal total of hours to be achieved for the term is 72 hours, i.e., 12 week at 6 hours per week.

The Golden Rules of the Lab

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

The following rules were developed to ensure safe functionality, conscientious and professional behavior and good housekeeping in the lab.

1. If you open it, - close it. (balances, drawers, doors)
2. If you turn it on, - turn it off. (hot plates, condenser water, lights)
3. If you dirty it, - clean it. (glassware)
4. If you empty it or use it all, - refill it or replace it. (squirt bottles, DI water carboys, solvents)
5. If you break it, fix it. If you can't fix it, - find someone who can or dispose of it properly and safely. (glassware)
6. If it belongs to someone else, - get permission to use it. (equipment, tools)
7. If you borrow it, - return it. (equipment, tools)
8. If you make a mess, - clean it up. (spills, paper towels)
9. If you get it out or move it, - put it back. (stools, equipment, glassware, - the sinks are not "back")
10. If you don't know how to operate it, - leave it alone or ask someone who does. (instruments)

The Bottom Line: Leave it like you found it.

In addition, each student was assigned accountability for an assigned unit operation to ensure that the units were properly maintained and that there were sufficient inventories of all consumables to ensure continuity of operations.

Analytes: Occurrence, Significance, and Analytical Methodology

The following analytes are required for the term project. It is important to note that this class is not a class in methods development. The procedures employed were reported in the two fine books by Iland *et al* (*3*) and by Zoecklein *et al* (*4*). In addition, the Official Methods of Analysis of AOAC (*5*) is used as a reference source.

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Ethanol

Ethanol in wine is derived from the naturally occurring fermentable carbohydrates, especially glucose and fructose. During the fermentation process, approximately 51% of the mass of these carbohydrates is converted to ethanol. The remainder of the carbohydrates is converted predominantly to carbon dioxide.

The primary basis for wine taxation is the alcohol content. Table wines must contain between 14 and 24% ethanol. In the dessert wine category, sherry must have a minimum ethanol content of 17%, whereas other dessert wine types have an 18% minimum. A measure of the "alcoholic strength" of wine is required to meet legal requirements and for labelling purposes.

The physical and sensory properties of wines are dependent to a degree on ethanol content. In particular, ethanol imparts a slight "sweetness" to the wine.

Alcoholic strength may be measured by a variety of methods. Because of the significance of the determination of ethanol in wines, three methods are used in CHEM 304.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

1. Ebulliometry

The ebulliometric determination of ethanol utilizes the reduction of the boiling point of an aqueous solution of ethanol. A mercury-in-glass precision thermometer is necessary because, the boiling point needs to be determined to within 0.02 °C. Since the boiling point is dependent on the prevailing atmospheric pressure, it is necessary to determine the boiling point of pure water. The "Ebulliometric Degrees" for the sample is defined as the boiling point of pure water minus the boiling point of the unknown sample. The relationship between Ebulliometric Degrees as a function of ethanol content is nonlinear. However, in the region of ethanol contents up to 16%, a calibration curve can be constructed graphically or by fitting a third degree polynomial to the data points generating a third degree polynomial using Microsoft Excel. Since the ebulliometric method utilizes a colligative property of the solution, false results can be generated caused by a significant content of other solutes, viz., carbohydrates. Accordingly, this technique cannot be reliably utilized for the determination of ethanol content is sweet wines.

2. Gas Chromatography

Gas chromatography (GC) is ideally suited for the separation and quantification of ethanol in aqueous solutions. To improve quantitation, n-butanol was employed as an internal standard. An internal standard is a known amount of a compound, different from the analyte, that is added to the unknown. The peak area of the analyte is compared with the peak area from the internal standard to determine the quantity of analyte present in the unknown sample. In our case, the peak area ratio of ethanol to n-butanol was compared with the area ratio obtained from the injection of a standard ethanol/n-butanol mixture as described by Zoecklein *et al* (*2*).

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3. High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is also well suited to the quantification of ethanol. Standard solutions of ethanol in water were prepared at 4, 8 12 and 16% (v/v) and were injected into the mobile phase, which was 0.01N H_2SO_4. A refractive index detector was employed to quantitate the peak areas. The resulting calibration curve was determined to be linear over the range of interest.

Organic Acids

Grape juice, and therefore wine, contains a complex mixture of organic acids. The prevalent acids in grape juice are generated during the maturation of the grape berry, primarily tartaric, malic, citric and succinic acids.

Hydrogen ion concentration plays a significant role in winemaking. These acids manifest their influence from physical-chemical and biological concerns to the organoleptic attributes and potentially, faults. The pH of white wines is typically 3.4 or less. Higher values are usually observed for red wines. This is primarily because of the juice and the skins prior to and during the fermentation process. The acid content of wine is of importance, in that it influences the taste and mouth feel. Moreover, it affects pH, color, stability and shelf life of the wine. Titratable acidity in wines is typically between 5.0 and 16.0 g/L

1. pH

pH was determined using a Hanna HL222 pH meter.

2. Titratable Acidity (TA)

The determination of titratable acidity measures the concentration of all available sources of hydrogen ions present in a sample of wine. It provides a measure of hydrogen ions that are both free in solution (as H^+) and those that are bound to undissociated acids and to anions. The concentration of the available hydrogen ions may be determined by titration of a known volume of wine with a standard solution of strong base, e.g., 0.1 M NaOH. Upon the incremental addition of the base, the free protons are consumed and the undissociated acid releases hydrogen ions in an attempt to reestablish an equilibrium condition consistent with the equilibrium constant of the weak acid. Eventually, all of the protons are neutralized.

The equivalence point for the reaction of a weak acid and a strong base occurs at a value greater that pH 7. The predominant acid in wines, tartaric acid, has pH of 8.2 at the equivalence point. If using an indicator for the detection of the equivalence point, the indicator should be chosen to change colors at pH 8.2, e.g., phenolphthalein. If determination of the equivalence point is to be detected by a pH meter, a pH of 8.2 should be employed as the end point.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

3. Volatile Acidity (VA)

The volatile acids found in wine include primarily acetic acid and, to a lesser extent, other acids such as butyric, formic and propionic acids. They are termed volatile because they can be detected organoleptically and can be steam distilled. The determination of acetic acid is common. The concentration of acetic acid during fermentation is usually less than 500 mg/L, primarily determined by the strain of yeast employed. Higher amounts of acetic acid may be produced by bacteria or fermentation yeast activity during and after fermentation. Ethyl acetate is produced concurrently with acetic acid, in the ratio of 1 part ethyl acetate to between 5 and 10 parts of acetic acid.

High volatile acidity is objectionable and is an indication of spoilage or improper storage of the wine. Accordingly, monitoring changes in VA is important to detect the onset of spoilage. In the method used, the volatile acids are steam distilled, thereby separating them from the fixed acids, primarily tartaric and malic acids. The steam distillation was carried out using a modified Cash still, shown in Figure 1. The distillate is collected and subsequently titrated to an end-point of 8.2 with 0.01M standard NaOH.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

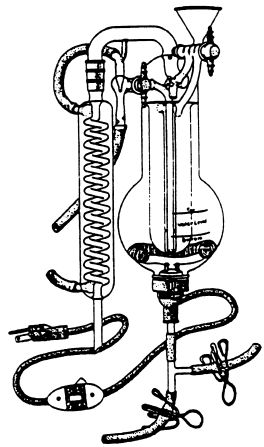

Figure 1. Modified Cash Still.

The threshold for detection of acetic acid in wine is generally accepted to be 700 mg/L (ppm). Values in excess of 1,200 – 1,300 mg/L are regarded to be sufficient to result in an unpleasant odor.

4. Tartaric Acid

Tartaric acid and its salts, potassium tartrate and calcium tartrate, are normal constituents of juice and wines and important to stability. Crystalline deposits can occur as wine ages and are unwelcomed by consumers. Accordingly, winemakers strive to reduce the occurrence of precipitation in the bottle.

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The tartaric acid content in wine typically ranges from 1.5 to 4.0 g/L and varies in accordance with region, varietal, maturity, soil and viticultural practices.

Tartaric acid is determined spectrophotometrically in accordance with the Rebelein method. The wine sample is mixed with a 0.1 M solution of silver nitrate in 30% (v/v) acetic acid solution, decolorizing carbon and ammonium vanadate. The tartrate ions in the sample react with the ammonium vanadate, forming an orange-yellow complex. The absorbance of the complex is determined by using a UV-VIS spectrophotometer at 525 nm and quantified using a previously constructed calibration curve.

5. Malolactic Fermentation

The process of malolactic fermentation (MLF) occurs in wines when bacteria convert malic acid ("apple acid") to lactic acid ("milk acid"). Typically, this process is initiated by the winemaker in order to replace the tart malic acid with the softer lactic acid. The conversion can be subjectively monitored by utilizing paper chromatography. Paper chromatography does not provide quantitative results, but rather a qualitative indication of the extent to which the conversion has been accomplished.

Bromocresol green is incorporated in the solvent mixture. This indicator reacts with the acid components and generates a yellow spot, subsequent to removal and drying of the chromatogram. Each of the acids present in the wine will evidence a spot on the chromatogram upon air drying. The spots resulting from the wine samples are identified by comparison of the elution distances with the standard samples

6. Malic, Lactic, and Citric Acids

These three weak organic acids all occur naturally in the grape berries and together are the primary source of acidity in wines. In concert, these acids species are responsible for determining the pH and TA of the wine.

Enzymatic analysis is an option when it is important for the winemaker to know the concentrations of each. The enzymatic analysis protocol generally relies on the increase or decrease in absorbance at 340 nm associated with the formation or depletion of NADH as a result of an enzymatic reaction. The amount of analyte present in the sample is calculated from the change in absorbance recorded prior to and following the addition of the enzyme. The calculation relates this change in absorbance to the extinction coefficient of NADH, precluding the necessity of constructing a standard curve.

Commercial enzymatic kits have been developed and are available commercially from r-Biopharm.

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Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

Reducing Sugars (Glucose and Fructose)

A number of carbohydrates occur naturally in grape juice. Glucose and fructose are the primary sugars, but others occur in relatively small amounts. The designations "reducing sugars" and fermentable sugars are used as descriptors for the different properties of sugars. Fermentable sugars are those which can be used by yeast during the alcoholic fermentation. The reducing sugars present in the must are not fully converted to ethanol and carbon dioxide during the fermentation process. The actual amount will vary. Accordingly, the residual sugar remaining when a wine has been fermented to "dryness" is approximately 0.2 g/100 mL.

If the fermentation is deliberately concluded prior to completion in order to produce a sweeter wine, an appropriate amount of residual glucose and fructose will be present. The amount of sugar remaining at the completion of fermentation is referred to as residual reducing sugar (RRS). This can be quantified by a number of analytical methods, most commonly HPLC.

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Sulfur Dioxide

Sulfur dioxide (SO_2) is added to must, juice, or wine to aid in the inhibition of oxidation and spoilage. When SO_2 is added to any of the above, it exists in either a free or bound form. The free form consists of molecular SO_2, the bisulfite anion and the sulfite anion. These forms exist in a pH-dependent equilibrium. Accordingly, the percentage of the free SO_2 that exists as the active molecular form is dependent upon the pH of the wine. The bound form occurs when the SO_2 binds to components present in the wine, e.g., aldehydes, pyruvic acid, and sugars. Total SO_2 is reported as the sum of the free and bound species.

Because the amount of free and bound SO_2 in a wine is dependent upon pH, it is difficult to predict the relative amounts of the two species. Accordingly, it is necessary to determine the actual concentration of the free and bound forms present.

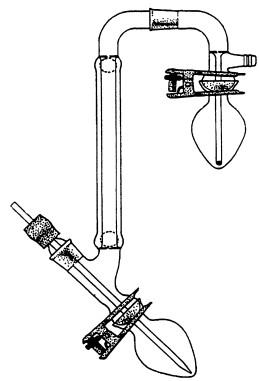

Figure 2. Aeration/ Oxidation Apparatus.

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The most commonly used method is referred to as the aeration/oxidation method shown in Figure 2. Free SO_2 is removed from the wine by passing air through an acidified sample. The released SO_2 is passed through a hydrogen peroxide solution containing an indicator that is a mixture of methyl red and methylene blue indicators. This mixed indicator is green in basic solutions and turns purple in acid solutions. During the aspiration process, the SO_2 in the stream is oxidized to H_2SO_4 by the peroxide accompanied by a color change from purple to green. This solution is titrated with 0.01 M NaOH back to the original green color.

Subsequent to the determination of the free SO_2, the receiver flask is returned to the A/O apparatus and the process is repeated, this time heating the analyte solution to boiling with an alcohol burner. This heating drives off the SO_2 that had been bound, restoring the original purple color. After an appropriate period of aspiration, the receiver solution is once again titrated to the original green color and the bound SO_2 content is calculated. The free and bound totals are added to define the total SO_2 content of the original sample.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

Sorbic Acid

Sorbic acid (2,4-hexadienoic acid) is a short-chained unsaturated fatty acid commonly used in the wine industry as a chemical preservative. Due to the low solubility of sorbic acid in water, it is generally added by the winemaker as potassium sorbate. The primary function of sorbic acid is as a fungistat. It also adds a slight, non-sugary, sweetness to the wine. Sorbic acid appears to operate in a synergistic manner with sulfur dioxide. Ough and Ingraham (*6*) reported inhibition of yeast growth in wine with additions of sorbic acid at 80 mg/L coupled with free sulfur dioxide levels of 30 mg/L.

The flavor threshold for detection of sorbic acid in wines has been reported by Ough and Ingraham to be as low as 50 mg/L.

The method for quantification of sorbic acid in wine was reported by Ziemelis and Somers (*7*). In this protocol, the sorbic acid is quantitatively extracted from the wine sample with 2,2,4 trimethyl pentane (iso-octane). Subsequent to extraction, the sorbic acid content is measured using a UV/VIS spectrophotometer at 255 nm relative to an iso-octane blank. Calibration standards were prepared to cover the anticipated sorbic acid concentration and a calibration curve was constructed. The procedure was performed on the unknown sample of wine and the concentration determined from the calibration curve.

Copper

The presence of copper in wines may be attributed to three sources: (1) vineyard sprays; (2) winery equipment; and (3) the addition of copper salts to the wine for the remediation of wine faults attributed to hydrogen sulfide or mercaptans. In the laboratory, copper was determined utilizing atomic absorption spectrophotometry. A calibration curve was contructed to cover the anticipated copper quantities.

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Ash

"Ash" is the amount of inorganic materials present in wine.

1. Total Ash

This is determined by heating the wine to dryness in a laboratory oven, followed by calcining the residue in a muffle furnace at 600 °C for 3 hours.

2. Alkalinity of Ash

Subsequent to ashing the wine, the ash was slurried in deionized water to extract all soluble components. The extract was then titrated with 0.01 M NaOH. The alkalinity of the ash was reported as mL of .01 N H_2SO_4/100 g of ash.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

Instrumentation

The following instruments were used for instrumental analyses:

- Atomic Absorption Spectrophotometer (AA): Shimadzu AA-6300
- High Performance Liquid Chromatograph (HPLC): Shimadzu Prominence LC-20AD; RID-10A Refractive Index Detector; Rezex ROA-Organic Acid H+ (8%) column, 300 x 7.80 mm
- Gas Chromatograph (GC): Shimadzu GC-2010; flame ionization detector; RESTEK Stabilwax column, 30 m x 0.32 mm i.d., Catalog Number 10624.
- UV Spectophotometer: Shimadzu UV-1800
- Residual Sugar Spectrophotometer: Hanna HI 83746
- Tartaric Acid Photometer: Hanna HI 83748

A Standard Operating Procedure (SOP) was provided for each of the instruments listed above to ensure compliance with instrumental procedures.

Final Report

Students are required to submit a Final Summary Report as shown in Table 2 below prior to a specified date and time. Late submissions are not accepted.

Table 2. CHEM 304 Wine Chemist Certification Results Template

Submitted by: _____ Date: _____

No.	Analysis	Units	Method	Method Source	No. of Detns.	Result ± Std. Dev.
1	Alcohol by volume	% (v/v)	Ebulliometric	Iland		±
2			GC (Int. Std. Method)	Zoecklein		±
3			HPLC	JPR		±
4	pH	unitless	Meter	Iland		±
5	Total acidity (Titratable Acidity)	g/L (as tartaric acid)	Titrimetric	Iland		±
6	Volatile acidity	g/L (as acetic acid)	Titrimetric (Steam distillation)	Iland		±
7	Tartaric acid	g/L	Spectrophotometric (Hanna H_2T Meter)	Unit Instructions		±
8	Malic acid	g/L	Spectrophotometric (enzymatic)	Kit Instructions		±
9	Lactic acid	g/L	Spectrophotometric (enzymatic)	Kit Instructions		±
10	Malolactic fermentation	Yes/Partial/No	Paper chromatography	Iland		±
11	Citric acid	g/L	Spectrophotometric (enzymatic)	Kit Instructions		±
12	Sorbic acid	g/L	Spectrophotometric	Technical article		±
13	Sulfur dioxide - free	mg/L	Titration (Aeration/Oxidation)	Iland		±
14	Sulfur dioxide - total	mg/L	Titration (Aeration/Oxidation)	Iland		±
15	Reducing sugar	mg/100mL (as G+F)	Spectrophotometric (Hanna RS Meter)	Unit Instructions		±
16	Glucose	mg/100mL	HPLC	Zoecklein		±
17	Fructose	mg/100mL	HPLC	Zoecklein		±
18	Copper	mg/L (ppm)	Atomic Absorption Spectroscopy (AA)	Zoecklein		±
19	Ash	g ash/100 mL	Gravimetric			±
20	Alkalinity of ash	mL 0.05M H_2SO_4/100 mL	Titrimetric			±

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

Assessment

Grading for CHEM 304 is defined by the following criteria:

1. Qualifying Exam (100 pts)
2. Methodology and Technique Development (100 pts)
3. Final Report of Unknown (400 pts)
    a. Accuracy
    b. Statistical treatment of data
    c. Replicates
    d. Significant figures
    e. Efficiency Factor, i.e., number of results divided by total hours spent in lab
4. Laboratory Notebook (50 pts)
    a. Table of Contents
    b. Content
    c. Legibility
5. Compliance with safety rules and waste handling procedures (100 pts)
6. Lab practices and technique, including housekeeping and compliance with the "Golden Rules of the Laboratory" (100 pts)
7. Performance of Duties – Lab Functional Assignment (100 pts)
8. Oral Debriefing (150 pts)

Total Available Points: 1,100

Student Feedback

At the conclusion of each course, students are requested to submit a Course Evaluation report to the Department of Academic Affairs. These evaluations are closely reviewed by the Instructor of Record, the Department Chair and the Vice President of Academic Affairs. In addition to stock questions in a Rubric format, students are invited to concurrently submit anonymous comments related to their experience in the class. Typical examples of comments submitted for CHEM 304 follow:

- "Personally, I loved this class. We were given the freedom to work at our own pace. This course taught me a ton on critical thinking and lab techniques (*8*)."
- "This has been my most favorite chemistry class to date. It was a lot of fun working with wine as a teaching tool. This course has helped me get very comfortable using the HPLC and GC instruments. Overall, a very interesting and challenging course (*9*)."

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- "I really enjoyed being in this class. I learned a lot from it. I learned to be independent in lab and how to use leading chemistry equipment. It helped me to develop strategies for working on long projects that are due at a specific deadline. This class helped me to determine that I want to go to graduate school and working in the lab is what I want to do (*10*)."
- "I felt as though this was one of the best labs I have had at Adrian College. This is the only lab that has really prepared me for life outside of college (*10*)."
- "I learned an incredible amount of information in this class. It has improved my lab skills, improved my precision, and taught me how to use chemical instrumentation. Also, I'm pretty sure I can hold me own in any conversation about the quality of wine.
  Overall, this is one of the best courses I have ever taken, and I'm sure the skills I learned will carry through to my career (*10*)."
- I was scared to death to take Quant Lab. I had no confidence in my laboratory abilities, and I was sure I was going to ruin an instrument, if not fail. By the end of the semester I felt not only confident in my abilities, but confidence in being able to teach others.
  This class was the best I have ever had at Adrian in terms of the Chemistry Department, even in terms of all my classes here.
  I have 7 days left before my last final at Adrian College, and I can tell you that if I had not chosen this college, I would have never had the opportunities to take classes like Quant and get hands-on experience with instruments like HPLC, GC, AA and UV. As intimidating as this class was, this was the closest to a real workplace experience as it gets. Also, I feel like I can now have very classy conversations with sophisticated people on wine and its components and production (*10*)."
- "I really enjoyed this course. I think having the lab for six hours in a different semester really helped. We had plenty of time to understand the methodology and the reasons for each of the tests. Having our own wine sample to test really helped push me to take the time with all of my tests and make sure my numbers made sense. This course also showed me how it would be to work with others in a lab (*10*)."
- "This lab was by far my favorite lab I've ever taken. It was difficult to get all of the tests done because of the limited number of instruments; however, it may be beneficial to have the tests run in cycles rather than as a free-for-all to provide more organization to the lab. It may also be helpful to not have as many people take the lab at the same time (*11*)."
- "This course was my favorite laboratory course. I only wish it was worth more credits, and that there were not as many people in here at once because there is not enough room for everyone or enough instruments (*11*)."

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

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Conclusions

This contextual approach to the teaching of quantitative analysis has been shown to be very effective in developing time management skills, intermediate goal setting in a complex project consistent with the project goals, as well as establishing specific technical capabilities and confidence with the complex analytical instrumentation employed.

Prior to the Spring, 2014 offering, the maximum number of students in enrolled in CHEM 304 was 10. In the Spring, 2014 offering, the enrollment was 20 stuedents. Even with the scheduling flexibility, this caused frequent log-jams for instrument time and for personal student/instructor interactions. It is highly recommended that, when using the approach described in this chapter, the enrollment be limited to a maximum of 12 students.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch010

Epilogue: Capstone Project

As a follow up to the positive experience with the approach detailed in this paper, our Capstone course has been modeled after it. The capstone specifies a product category and each student participant receives a specific product in that category. In the past two years, the categories have been coffee and energy drinks. Each student receives a specific brand sample and must conduct analyses for analytes specified by the Instructor of Record.

However, unlike the Quantitative Analysis Lab project, they are not provided with methodology. They must do a literature search to learn about the product and its chemistry. Further, each must decide how to adapt what has been done and chronicled in the literature to the specific product received. The scope of the Capstone has been expanded to incorporate as many of the sub-disciplines as possible, e.g., organic chemistry, inorganic chemistry, biochemistry, thermodynamics, kinetics, technical writing, and oral presentation to ensure a broad scope for the project.

Students must submit a written technical report and conduct an oral presentation to all members of the Department of Chemistry and Biochemistry. The faculty then meets as a group to reach consensus on the grade earned by the student. In many cases, they are instructed to conduct remedial work to achieve the scope and quality as possible.

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Chapter 11

Teaching Analytical Chemistry with Grapes and Wines

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Grapes and wine are an excellent medium for teaching the principles of analytical chemistry to undergraduate students. Analytes in grapes and wines range in concentration from percent levels to μg/L (ppb) and lower. As a result, a variety of analytical methods may be used depending on the analyte of interest and the time during winemaking when the analyte is measured. In this chapter we describe basic goals and content of a course taught at UC Davis using applications in grapes and wines to demonstrate basic analytical chemistry principles. The methods can be readily applied to any undergraduate analytical chemistry course using real-world samples that students can understand and readily relate to.

The Department of Viticulture and Enology at the University of California was established as a research and teaching institution in 1880 by the California legislature. The Department's goal was and continues to be to place the study of viticulture (grape growing) and enology (winemaking) within a scientific foundation in order to ensure the production of sound, defect-free wines in an economically sustainable manner. The research and teaching programs focus on grape and wine chemistry; microbiology; chemical, biochemical, and process engineering; plant biology and physiology; genetics; and sensory science in order to cover winemaking from the field, through processing and storage, and to the consumer.

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Undergraduate students complete preparatory courses in Chemistry, Organic Chemistry, Biology, Microbiology, Physics, Calculus, and Statistics to obtain the foundation for subsequent classes and laboratories. These courses encompass all aspects of viticulture and enology including Wine Production, Grape and Wine Analysis, Viticulture Practices, Sensory Evaluation of Wines, Wine Processing and Stability, Wine Microbiology, and Wine Technology and Winery Systems. Additional information about the Department and its courses and programs can be found online (*1*).

The Grape and Wine Analysis course, taught at the Junior/Senior level, provides an overview of the fundamental principles of analytical chemistry as they relate to specific methods used in winemaking. The course includes both a lecture and a laboratory component, typically taken concurrently. While students in the course are typically completing a Bachelor of Science (B. S.) degree in Viticulture and Enology, students in other majors, including chemistry, food science, environmental chemistry, etc. also take the course.

This chapter will provide a brief overview of the Grape and Wine Analysis course topics and student laboratory exercises and will include some example experiments and student-generated data to demonstrate important concepts. The information provided here could be used to develop a quantitative analysis course in a traditional chemistry major; by linking the analyses to winemaking processes, students are introduced to concepts of 'Fitness for Purpose' in ways that are not typically possible in traditional quantitative analysis courses. This can be an advantage over more traditional course structures, providing students opportunities to evaluate the parameters needed to obtain quality data in a 'real-world' situation. Alternatively, the analytical methods can be used in place of existing analyses and demonstrations in a general chemistry or quantitative analysis course. The methods described here provide an excellent and unique opportunity to apply and demonstrate basic chemical principles and analytical chemistry techniques using a food (grapes) and beverage (wine) that students are familiar with and that are readily available throughout the country and the world.

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Course Outline

While the course could be taught in a variety of ways (*e.g.*, sequentially covering methods as they would be encountered during the winemaking process or discussing all potential methods for a single analyte together), we have generally introduced topics so that analytical chemistry principles and related techniques are covered together (Table 1). For example, methods using acid-base titrations are covered together followed by discussions of oxidation-reduction titrations; relevant laboratory exercises using these methods are simultaneously discussed and performed, including the determination of titratable acidity and sulfur dioxide concentration in wines. In general each analysis is completed in one laboratory section (the laboratory course meets for ~3 hours each week).

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Table 1. Analytical Chemistry Principles and Applications to Grape and Wine Analyses

Principle	*Analysis*	*Reference*[a]
Sampling	Discussed throughout course Demonstration based on Clement (*2*)	(*3*, *4*)
Statistical Analysis and Quality Control	Descriptive statistics, mean, standard deviation, relative standard deviation Student's t-tests Quality Control Charts	
Weighing, Pipetting, Dilutions, and Density Measurements	Calibrating pipettes Making dilutions Sugar/Density/Specific gravity measurements	(*5*); OAC 920.56 (*6*)
Potentiometry	pH measurement Selective ion electrodes (*e.g.*, NH_3)	Manufacturer's manuals; AOAC 960.19 (*6*); (*7*)
Titrations: Acid-Base	Standardizing titrants Titratable Acidity[b] Volatile Acidity[c]	AOAC 962.12 (*6*) AOAC 964.08 (*6*); (*8*)
Titration: Oxidation-Reduction	Standardizing titrants SO_2 analysis (Iodimetric titration; Free and Bound SO_2)[d]	
Spectroscopy: Colorimetric Analyses; UV-Visible Spectral Analyses	Wine color and phenolics α-Amino nitrogen Enzyme linked analyses (*e.g.*, glucose and fructose)	(*9*–*11*) (*12*) AOAC 985.09 (*6*)
Spectroscopy: Atomic Absorption and Atomic Emission	Copper Potassium	AOAC 970.18 (*6*), (*13*) AOAC 963.13 (*6*)
Spectroscopy: Infrared/FTIR and Indirect Spectroscopic Measurements	Ethanol	
Chromatography	GC-Ethanol HPLC-phenolics and/or organic acids	AOAC 983.13 (*6*) (*14*, *15*); AOAC 2013.12 (*6*)
Capillary Electrophoresis	Organic acids—malic and lactic acids	(*16*)

Continued on next page.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch011

Table 1. (Continued). Analytical Chemistry Principles and Applications to Grape and Wine Analyses

[a] With a few exceptions, reference methods for all analytes can be found in (*5*, *17*, *18*) Additional reference methods and modifications are cited here. [b] Titratable Acidity for winemaking purposes is defined as the total concentration of available protons (H^+ and HA) and is reported in the U.S. as g tartaric acid equivalents/L. Titratable acidity in the U.S. is determined by titration with standardized NaOH to the pH8.2 (or phenolphthalein) endpoint. [c] Volatile Acidity for winemaking purposes is defined as the volatile acids that can be steam distilled from wine. The main volatile acid in wine is acetic acid, but other short chain acids are also included (*e.g.*, formic, butyric, and propionic). Volatile acidity should not include lactic, succinic, sorbic, carbonic, or sulfurous acids. Volatile acidity is reported as g acetic acid equivalents/L. [d] SO_2 in wine typically occurs bound to aldehydes and ketones such as acetaldehyde, pyruvate, glucose, etc. This bound form can be released by strong acid, strong base, and/or heat. Free SO_2 is measured before hydrolysis, total SO_2 is measured after hydrolysis. [Free SO_2] + [Bound SO_2] = [Total SO_2]. In addition to the iodimetric titration, SO_2 can also be measured by Aeration-Oxidation (*5*, *19*) which is a modification of the AOAC approved Monier-Williams procedure Method 940.20 (*6*).

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Our goal at UC Davis is to teach students the principles of the methods, giving them fundamental information on advantages, disadvantages and sources of error and interferences. Students perform the analyses in the laboratory, and there is an emphasis on learning how to validate methods, monitor results using quality control and quality assurance principles, and trouble-shoot when problems arise. There are several good reference books on grape and wine analysis (*5*, *17*, *18*) and we encourage students to have at least one of these books for their future professional careers. However, currently none of these books have the depth of information on analytical chemistry principles that is needed to provide the background information that many students require to successfully achieve the course goals we have set for them. Therefore, we encourage students to have an analytical chemistry textbook for reference and study (*e.g.*, (*20*, *21*)) and we make copies available for purchase in the campus bookstore and on loan in the campus library. A comprehensive general reference on the principles of winemaking is also available (*22*).

In addition to these textbooks, we provide extensive information to students via on-line review and reference materials, relevant journal articles, and practice problem sets including problems linked to the Analytical Chemistry textbook. I have authored a laboratory manual containing background readings on the analytical methods, step-by-step procedures for the analyses performed in class, and questions and templates to guide data collection, analysis and reporting of results.

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Teaching Quality Control Principles

When analyzing grapes and wines in an industrial and production setting there are rarely 'right' answers for the analytes of interest. Therefore students need tools to know when the methods they are using meet the expected standards of performance. As a result, an important component of the course is giving students the information needed to evaluate the quality of the data they are producing.

As a first step in this process, we emphasize to students that analytical methods should be 'Fit for Purpose' and we encourage them to establish good analytical practices of defining the analytical problem, determining how the results will be used, identifying the appropriate methods to meet the stated objectives, and establishing the restrictions on cost, timing, accuracy, and precision that are required for each analysis. Throughout the course we emphasize that different analytical methods may be used depending on the purpose of the analysis and the time during winemaking when the results are used (*e.g.*, sugars/density in grapes and must at the beginning of fermentation and residual sugar levels remaining in wine at the end of fermentation and prior to bottling as discussed further below). In addition, results from different methods are compared so that students gain an understanding of how methods may differ in their applications, limits of detection, accuracy, and precision.

Second, we define and discuss principles of quality assurance, quality control, and method validation. Students are introduced to the use of quality control charts and basic statistical analyses are discussed and used to aid in data interpretation (mean, standard deviation, relative standard deviation, and t-tests). Finally, in laboratory exercises we provide and emphasize the use of reference standards to monitor accuracy and precision of analytical methods. Students use class data collected with these standards to discuss sources of error in their analyses and they use quality control charts to begin to identify when methods are 'out of control' and may need re-evaluation. Reference or Quality Control standards that we typically use for in-class analyses are provided in Table 2. In addition to these standards, which are analyzed along with wine samples of unknown analyte concentrations, students learn to calibrate pipettes by weighing known volumes of water and correcting for density at room temperatures. This provides students the opportunity to check and validate their own pipetting techniques as they learn to use various types of pipettes.

Proper recording of laboratory observations and data in the lab notebook is also emphasized. Students submit their laboratory observations and raw data for instructor review and comment on a weekly basis.

Many wine industry laboratories participate in an inter-laboratory proficiency testing program (*23*). Reports from recent testing rounds are available and summaries of results have periodically been reported in the literature (*24–26*). These reports and articles provide excellent information that can be highlighted during class discussions and in student-written laboratory reports, providing documented examples of method performance, matrix effects, and equipment variables that can influence reported results.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch011

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Table 2. Selected Reference Standards for Calibrating and/or Monitoring Analytical Method Performance

Analyte	*Reference Standard*	*Preparation or Source*[a]
pH	a. pH 4.00 ± 0.01 (25°C) NIST buffer b. Saturated Potassium Hydrogen Tartrate Solution, pH 3.557 at 25°C	a. Purchased commercially b. $KC_4H_5O_6$ added to deionized water to saturation (*21*)
Brix (Soluble Solids)[b]	20.0 Brix Standard	20.0 g sucrose added to 80.0 g deionized water (total solution weight 100g)
Ammonia	1,000 mg/L Nitrogen	3.786 g NH_4Cl/L deionized water (*7*)
Ethanol	12.0% (v/v) ethanol solution	Absolute (200 proof) ethanol diluted to volume with deionized water[c]
α-Amino Nitrogen	5.0 mM Isoleucine standard	0.656 g isoleucine/L deionized water (*12*)
D-Glucose	0.5 g/L	0.50 g D-glucose/L deionized water or purchased with enzyme kit (R-Biopharm, Washington, MO)
Copper	0.100 g/L	0.2512 g anhydrous $CuSO_4$/L deionized water
Potassium	0.010 g/L	0.0191 g KCl/L deionized water or purchase 1.0 M reference standard and dilute with deionized water

[a] All chemicals purchased from Sigma-Aldrich unless otherwise noted. [b] Soluble solids in grape juice include all components that are dissolved in solution (*e.g.*, sugars, organic acids, amino acids, pectins, etc.); in practice >90% of soluble solids in grape juice are glucose and fructose. Brix is a measurement of soluble solids and can be determined by density (or more correctly as specific gravity) or by refractive index. For grape juice, Brix is defined as g sucrose/100g of total solution. [c] NIST also sells ethanol-in-water reference standards (6%, 25%). Due to cost and volume needs for teaching purposes, we typically prepare our own solutions, recognizing that some errors in preparation may be possible.

Whenever possible we analyze samples in class that are obtained from a Winemaking Practices course that is taught simultaneously. This helps to reinforce to students that the information can and should be used to guide winemaking decisions. However, any of the analyses can be performed on commercial wines or juices that can be readily purchased.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch011

Example Applications

To demonstrate the applications of these analytical principles several selected examples of laboratory exercises and student-generated data will be discussed. In this section we have combined analytical methods according to the analytes measured, in order to emphasize comparisons among the methods. Here, we will focus on laboratory exercises for analysis of sugars and alcohol/ethanol with brief mention of approaches for acids, phenols, nitrogen containing yeast nutrients, SO_2, and trace elements.

Sugars

Sugars are among the most important analytes measured in grapes; levels are monitored at all stages of the winemaking process. In the vineyard, soluble solids (measured as Brix; g sucrose/100 g solution) are monitored to determine optimal harvest levels; wine grapes are typically harvested at 22-25 Brix depending on the variety and the wine style intended. During winemaking, decreases in soluble solids throughout fermentation (as yeast converts sugar to ethanol and carbon dioxide) are monitored to ensure that fermentations are proceeding to completion. At the end of fermentation and prior to bottling residual sugar concentrations are measured to ensure the wines will be stable from further yeast metabolism and to adjust/blend to desired sweetness levels. Wines are considered 'dry' or lacking in sensory sweetness at residual sugar levels of less than 0.2% (2 g/L), however some sugars may remain unfermented, *e.g.*, pentoses not metabolized by yeast.

The predominant sugars in grapes are glucose and fructose, making up more than 90% of the total soluble solids. In the vineyard, sugar/soluble solids levels are typically monitored using a hand-held refractometer. Proper grape sampling is critical for accurate estimations of sugar levels in a vineyard and sampling protocols are discussed during class (*3*, *4*). Following crushing of the grapes and for further monitoring fermentation progress, the specific gravity of the solution is monitored using a Brix hydrometer (units of Brix are used in the U.S.; units of measurement may be different in other countries). Because ethanol has a very high refractive index, refractometers cannot be used to measure soluble solids levels during fermentation. Speed and simplicity are often the main factors driving the choice of analytical method during the first stages of winemaking, making the refractometer and hydrometer measurements ideal.

In class, students measure the soluble solids level of a sample juice using both a refractometer and a hydrometer; a reference standard (Table 2) is analyzed simultaneously. Students record their own data in their laboratory notebook and data from all groups are entered into an on-line database. The entire class data is then downloaded into a spreadsheet and provided to the students for further data analysis and interpretation.

The reference standard allows students to assess the accuracy of the method itself. Actual student-generated data (Table 3) shows that for a reference Brix standard, both methods provide accurate and nearly identical mean values, but the precision of the hydrometer measurements is poorer, possibly due to greater propensity for errors in reading the hydrometer scale compared to the

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

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refractometer. For the juice sample, the mean hydrometer value is slightly, but significantly, higher (t-test, $p < 0.01$) than the mean value of the refractometer measurement (Table 3). One sample may be an outlier in the refractometer measurements (Group #7). Student data is always discussed with the entire class each week and the individual groups are encouraged to describe sources of error they encountered during their analyses; students can then support each other in trouble-shooting and analysis of the data. For the juice sample, the mean hydrometer value is higher than the mean refractometer value, although the difference is not significantly different by Student's t-test. The comparison of the measured value between the two methods for calibrants and samples provides a valuable opportunity to discuss the potential impact of matrix components on measurement errors and sensitivity.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch011

Table 3. Student Generated Data for Soluble Solids Measurements of a Brix Standard and a Chardonnay Grape Juice

Student Group #	*20.0 Brix Standard*		*Chardonnay Juice*			
	Refracto-meter[a]	*Hydro-meter*[b]	*Refracto-meter*[a]	*Hydro-meter*[b]	*R-meter Number*	*H-meter Number*
1	19.8	19.9	22.2	23.0	C	794887
2	19.8	19.9	21.9	23.2	C	794887
3	20.0	19.8	22.0	23.1	C	794887
4	21.0	21.1	21.8	21.9	C	794887
5	20.0	21.4	23.0	23.5	D	30023
6	19.9	20.0	23.9	23.5	D	30023
7	20.0	20.0	17.5	20.7	C	30023
8	20.0	19.1	22.9	23.5	D	794887
9	19.5	19.9	22.4	23.1	C	794887
10	20.1	21.2	21.6	23.2	C	794887
11	19.7	20.0	22.9	23.5	D	30023
12	19.9	19.8	23.0	23.5	D	30023
13	20.0	20.0	22.9	23.4	D	30023
Mean	20.0	20.1	22.2	23.0		
SD	0.35	0.66	1.54	0.82		
RSD (%)	1.73	3.28	6.94	3.58		

[a] Hand-held refractometer has automatic temperature compensation. [b] For hydrometer measurements students also record the sample/standard temperature and apply the appropriate temperature correction based on standard tables that are provided.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Comparing the precision of the measurements for the reference standards and the juice samples also can provide information about potential matrix interferences in sample measurement. For data presented here (Table 3), operator error appears to be a greater source of error than matrix.

Finally at least two hydrometers and two refractometers were available to the students during this laboratory (Table 3). Each group recorded the instrument number, making statistical comparisons possible. For this data, no statistical differences in instruments for the two methods were observed.

Hand-held or table-top density meters are also increasingly used in the industry for monitoring juice soluble solids. These have a piezoelectric element that oscillates in a frequency inversely proportional to the density of the sample in the sample tube (*e.g.,* (*27*)*)*. Though they are not used in the Wine Analysis laboratory experiments, the operating principles of these meters are discussed and they are widely used in other undergraduate classes.

At the end of fermentation and prior to bottling, sugar levels may be low, less than 2%, and more accurate and specific methods are needed. Yeast preferentially use glucose and then fructose for metabolism so at the end of the fermentation concentrations of these sugars are low or below levels of detection, however, other non-yeast fermentable sugars may still remain. A number of methods for determining concentrations of residual sugars are available, however, the most common methods are enzymatic methods for glucose and fructose or chromatographic (HPLC) procedures (*15*). In class, glucose and fructose are measured spectrophotometrically in a finished wine using an enzymatic procedure (AOAC 985.09, (*6*)). While reagents for the enzymatic assay can be purchased individually, kits with all reagents provided at the appropriate concentrations are typically purchased. The enzymatic analysis is based on the enzymatic conversion of D-glucose and D-fructose to D-gluconate-6-phosphate. In the process, the co-factor NADP is stoichiometrically reduced to NADPH and the NADPH that is formed is measured spectrophotometrially at 340 nm (*28*).

The method typically works best when the total amount of glucose plus fructose in the cuvette is between 10 - 50 µg. Therefore an estimated sugar level of the sample is provided to the students based on prior instructor measurements and students then calculate the sample dilutions they will need in order to meet the method criteria. A standard reference sample that does not require any dilution is also provided to the students; the reference is simply pipetted into a cuvette using a volume specified by the instructors (if kits are purchased the information is specified in the instructions provided with the kit). Students add appropriate enzymes for measuring glucose alone and glucose plus fructose. A reagent blank is prepared simultaneously and used to zero the instrument.

Actual student-generated data shows significant variability in the calculated glucose concentration for the reference standard (Table 4). Inspection of the raw absorbance data indicates much closer agreement in measured raw data before conversion to concentration. When the raw data are used by the instructor to recalculate the standard concentration, the results indicate that the method is accurate (± 0.003 g/L) with a relative precision of 4.47% (Table 4). Clearly calculation mistakes are a significant source of error for this analysis and this is readily demonstrated to the students from the data.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch011

Analysis of wine samples reveals additional potential sources of error (Table 5). While all groups indicated they diluted the sample in the same way, at least two groups (#2 and #3) either had variable dilutions or pipetting errors that resulted in nearly two times the amount of sugar being added to the cuvette than expected. One group (#5) appears to have only made the error on the glucose + fructose analysis, pointing to a pipetting error for this sample.

In summary, the enzymatic analysis of glucose/fructose in wine provides an ideal opportunity to discuss important analytical chemistry concepts such as method linearity and linear range, analytical detection limits, sample preparation and dilution, pipetting errors, and Beer's Law. Students can also be introduced to concepts of analysis costs and sample throughput; the reagents can be expensive and frequently have limited storage lifetimes, however, the method is relatively rapid and numerous samples can be prepared and analyzed at a time.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch011

Table 4. Student Generated Data for Enzymatic Analysis of a Glucose Standard

	Glucose Standard (0.5 g/L)		
Student Group #	*Measured Absorbance @ 340 nm*	*Student Calculated Glucose Concentration (g/L)*	*Instructor Calculated Glucose Concentration (g/L)*
1	0.566	0.486	0.486
2	0.598	5.980	0.513
3	0.578	9.420	0.496
4	0.616	0.528	0.528
5	0.534	0.458	0.458
6	0.552	0.474	0.474
7	0.581	0.499	0.498
8	0.587	0.504	0.504
9	0.613	0.526	0.526
10	0.567	0.486	0.486
	Mean	1.936	0.497
	SD	3.144	0.022
	RSD (%)	162	4.47

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Table 5. Student Generated Data for Enzymatic Analysis of Glucose and Fructose in a Red Wine Blend

		Glucose	*Glucose + Fructose*	*Glucose*	*Glucose + Fructose*
Student Group #	*Wine Dilution*	*Measured Abs. @ 340 nm*	*Measured Abs. @ 340 nm*	*Calculated Concentration (g/L)*	*Calculated Concentration g/L*
1	1/250	0.289	0.584	9.90	20.0
2	1/250	0.625	1.189	21.4	40.8
3	1/250	0.538	0.557	18.5	19.1
4	1/250	0.277	0.568	9.50	19.6
5	1/250	0.306	0.926	10.5	31.8
6	1/250	0.289	0.581	9.90	19.9
7	1/250	0.302	0.603	10.4	20.7
8	1/250	0.336	0.666	11.5	22.0
9	1/250	0.321	0.680	11.0	23.3
10	1/250	0.309	0.590	11.6	20.2
			Mean	12.3	23.8
			SD	4.12	7.04
			RSD (%)	33.44	29.53

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch011

Ethanol

In the U.S., wineries are required to have a means to measure alcohol content of wines on the premises of the winery (see (*29*)) and all wines sold must declare the alcohol level (as % v/v) on the bottle. The alcohol level in the wine determines the taxation level and also impacts flavor and mouthfeel.

Alcohol is measured by numerous methods. In order to meet the legal mandate of having an analysis procedure on the premises, some wineries use Ebulliometry, which is based on the principle of boiling point depression of ethanol/water mixtures (*5*). Although relatively simple and inexpensive, the Ebulliometer procedure is not readily automated and requires frequent calibration to existing atmospheric pressures. Distillation with pyncnometry and refractometry are standard AOAC methods (AOAC 920.57, 920.58, 920.59; (*6*)). Although refractometry and specific gravity measurements for alcohol analysis are not performed in class, students are able to apply these concepts to problems and questions about measuring alcohol concentration, since the theory has been introduced via soluble solids measurements.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

In the wine analysis course, students measure ethanol by GC and they are introduced to infrared and indirect spectrophotometric measurements using ethanol as a model analyte. The standard AOAC GC method is used (AOAC 983.13; (*6*)) and students prepare a calibration curve with absolute ethanol standards. Student preparation of the calibration standards and the standard curves reinforces concepts of dilution and calibration.

Students use the calibration curve to calculate ethanol concentrations in an unknown wine sample (Table 6). The GC experiment is performed toward the end of the quarter by which time students are becoming adept at dilutions, pipetting, and use of quantitative glassware so that these errors are less common.

The same wine sample is used for ethanol analysis by other methods, including IR spectrophotometry. UV-Visible and IR spectroscopy are increasingly combined with multivariate data analysis to predict and quantify the concentrations of numerous analytes in food and beverages, including grapes and wines. As a result there is a growing need to include discussions and applications of chemometric approaches for analyzing complex data sets in laboratory courses (*30*). In these spectroscopic analyses, the absorption spectrum of a sample is obtained and statistical pattern matching techniques (e.g., Principal Components Analysis (PCA); Partial Least Squares (PLS) regression, etc.) are used to develop mathematical models that can then be used to predict concentration of analytes in unknown samples. When developing the models, information on analyte concentration must first be determined using a standard, or 'primary', analytical method. Because the mathematical models are based on the analyte concentrations determined from the primary method and the absorption spectrum of the sample, they are considered to be indirect or 'secondary' methods. Therefore, the accuracy of the developed spectroscopic method, at best, will only be as good as that of the 'primary' method but often matrix effects increase the error. The matrix effects may not be directly proportional to the analyte concentration and hence the contribution to the error in prediction due to matrix effects can be variable.

The advantages of the indirect spectroscopic approaches are that they are generally rapid (an absorption spectrum can be collected in a few seconds or minutes) and in some cases, multiple analytes can be determined simultaneously. The disadvantages are that the statistical models that are developed must include samples with a wide range of analyte concentrations and matrix variables. Typically, hundreds of samples are required to build robust mathematical models that are capable of predicting analyte concentrations in unknown samples. Even then, if an unknown sample happens to have a matrix or analyte concentration that falls outside the range of samples used to build the model, the ability of the model to accurately predict the analyte concentration may be limited. The spectroscopic methods also require careful monitoring of the instrument with standards and quality control procedures to ensure that it is performing appropriately.

Indirect spectroscopic approaches such as this have been used to quantify a number of analytes in grapes and wines including polyphenols, anthocyanins, tannins, polysaccharides, sugars, alcohol, organic acids, pH, and SO_2 (*31–41*). An excellent review of IR spectroscopy applications for grape and wine analysis has been published by Bauer et al. (*42*).

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch011

Table 6. Student Generated Data for Ethanol Concentrations in a Red Wine Blend Using Gas Chromatography and near Infrared Spectroscopy (NIR)

	% Ethanol (v/v)	
Student Group #	*GC*	*NIR*
1	13.37	14.05
2	13.43	14.10
3[a]	13.15	
4	13.72	14.19
5	13.90	14.03
6	13.54	14.08
7	13.38	14.02
Mean	13.50	14.06
SD	0.24	0.07
RSD (%)	1.82	0.48

[a] Student-generated NIR data from Group 3 was inadvertently not reported.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch011

Instruments measuring in the UV-Visible (~230-900 nm), near infrared (NIR, ~800-2500 nm), mid-infrared (MIR, ~2500-50,000 nm), and far infrared (~50,000-1,000,000 nm) are most often used depending on the analytes and applications, although the far infrared is not often used for the analytes of interest in grapes and wines. Fourier Transform (FT) instruments are also common.

During the course, students use an FTIR instrument to collect spectral data on a set of wines, and they discuss the approaches used for developing analyte prediction models (Figure 1). A commercial NIR instrument to measure ethanol levels in wines is then used. This instrument is already programmed to use internal statistical models to convert the absorbance measurements to an ethanol concentration. Finally, the NIR results are compared to ethanol concentrations of the same wines determined by GC (Table 6).

Student-generated data from one class indicated unexpectedly and significantly different ($p < 0.05$) results between the ethanol levels in a red wine blend determined by GC and by NIR (Table 6). After consultation with teaching assistants it was determined that the calibration standard for the NIR instrument had been incorrectly prepared and following correct calibration no differences in the results between the two instruments were observed. This inadvertent error dramatically demonstrated to the students the importance of instrument calibration and highlighted that although the NIR measurement is simple and fast (and highly precise, *e.g.*, compare standard deviations of the two methods given in Table 6), operator error can still readily occur.

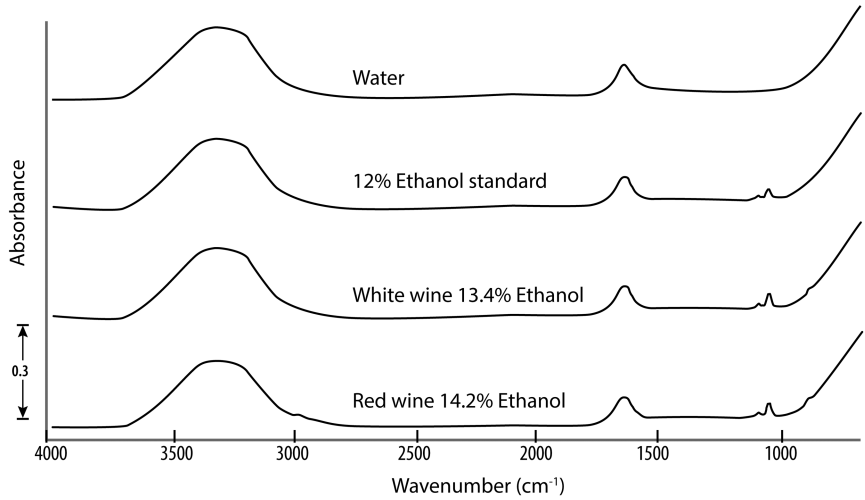

Figure 1. FTIR scans of water, 12% ethanol, and a white and red wine. Prominent peaks include strong O-H stretch of water/ethanol at 3400-3200cm^{-1}; weak C-H stretch at 2900-3000 cm^{-1} for ethanol in wine samples; weak C-O stretch from ethanol at ~1044 cm^{-1}.

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Acids

In grapes and wine, organic acids contribute to sensory properties (titratable acidity in particular influences perception of sourness) and they influence buffer capacity, chemical reactions and wine color. Grapes and wines have a pH range of approximately 3.0-4.0 and the main organic acids are tartaric, malic, acetic, citric, and lactic (lactic acid concentrations will depend on whether the wine has undergone a secondary bacterial fermentation with lactic acid bacteria). The important relationships between pH and acidity in grapes and wines have been reviewed (*43*, *44*).

Numerous methods are used for measuring acids. In the Wine Analysis course pH, titratable acidity, volatile acidity and individual acid concentrations using HPLC and/or capillary electrophoresis are determined (Tables 1 and 2).

Phenolic Compounds

Phenolic compounds, including proanthocyanidins, tannins, and anthocyanins, influence bitterness, astringency, color and antioxidant activity of grapes and wines. The Folin-Ciocalteau method is commonly used for determining total phenolics in grapes and wines and is based on the reduction of a phospho-molybdo tungsten reagent (Folin-Ciocalteau reagent) to produce a colored pigment that absorbs at 765 nm (*9*, *11*). Gallic acid is used as a standard and total phenolic concentration is reported in gallic acid equivalents (g/L). Individual phenolics are also measured by HPLC (*14*); in class, students compare

the information obtained by both HPLC and the Folin-Ciocalteau methods. Contrasting a method that measures total phenolics based on oxidation-reduction properties of the analytes as well as the HPLC method where individual phenolics are measured based on their UV-Visible absorbance properties reinforces these aspects of the chemistry of these interesting and highly varied wine components.

As an added component to the HPLC laboratory exercises, the principles of chromatographic separations are demonstrated using solid phase extraction cartridges to separate wines into three distinct fractions (*45*). As with the HPLC method, phenols are separated based on their polarity and the characteristic UV-Visible absorption spectra of each class can be compared and contrasted for tentative identification (*e.g.*, phenolic acids such as gallic acid, caffeic acid, λ_{max} 280 and 320 nm; catechins and anthocyanins including catechin, epicatechin, and malvidin-3-glucoside, λ_{max} 280 and 520 nm; and flavonols such as quercetin, λ_{max} 280 and 365 nm).

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch011

Nitrogen Containing Yeast Nutrients

Yeast requires a readily available nitrogen source for active growth and reproduction during fermentation. In class, an ammonia electrode is used to measure the ammonia content of a grape juice sample (Tables 1 and 2). Although ammonia is a readily assimilable form of nitrogen for yeast metabolism, α-amino acids are an equally important nitrogen source. There is little relationship between ammonia content of grape juices and the α-amino acid content (*46*), therefore measurements of both ammonia and α-amino nitrogen levels are often performed. If total assimilable nitrogen levels of a juice are low, winemakers may supplement to a concentration of about 140 mg N/L (*46*). The legal limit in the U.S. for supplementation with ammonium phosphate is 960 mg ammonium phosphate/L (203 mg N/L) (*47*).

α-Amino nitrogen is determined spectrophotometrically via reaction of α-amino acids with N-acetylcysteine and o-phthalaldehyde to form an isoindole chromagen that can be measured at 335 nm (*12*). Isoleucine is used as a calibration standard and total α-amino acid content is reported in mg isoleucine/L (Table 2). While proline is present in relatively high levels in grapes, it is not readily utilized by yeast as a nitrogen source and it does not react with N-acetylcysteine and o-phthalaldehyde (*12*).

SO_2

Sulfur dioxide is commonly added to juice, must (i.e., crushed grapes with juice, skins and seeds), and wine as an antioxidant and microbial inhibitor. Total levels of SO_2 are strictly regulated with 350 mg/L being the maximum allowable level in the U.S. (*48*). If levels of total SO_2 greater than 10 mg/L exist, the presence of SO_2 must be stated on the label (*49*). Wines may contain more than 10 mg/L total SO_2 even without addition of SO_2 due to yeast and bacterial metabolism (*22*).

SO_2 exists as both free and bound forms; the bisulfite species (HSO_3^-), which predominates at wine pH, readily binds to aldehydes and anthocyanins forming a hydroxysulfonate adduct. The bound form is readily hydrolyzed by strong base or

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heating under acidic conditions. Free SO_2 is responsible for antimicrobial activity and winemakers typically monitor free SO_2 concentrations during storage. Typical levels for free SO_2 in newly bottled wines are generally 15-40 mg/L depending on the wine style and pH.

Two methods are commonly used for SO_2 determinations, one of these, the Aeration-Oxidation procedure involves distilling an acidified sample into a trap containing a neutral hydrogen peroxide solution where the SO_2 is oxidized to sulfuric acid (H_2SO_4). H_2SO_4 can then be determined titrimetrically with NaOH. This procedure is a modification of the AOAC standard Monier-Williams procedure for total SO_2 (AOAC 940.20, (*6*); (*19*). The second method, commonly called 'Ripper' method is based on iodimetric titration of SO_2 with a starch indicator (alternatively a platinum electrode or redox electrode may also be used to detect the titration endpoint) (*5*). This redox titration is relatively fast and easy to do, however, other reducing species (*e.g.*, anthocyanins, sugars) can interfere and for sweet wines in particular other methods are recommended.

Accuracy of both methods for total SO_2 is similar, providing the analyses are performed carefully and SO_2 is not allowed to volatilize significantly following pH adjustments during the analysis. However, both the Aeration-Oxidation and Ripper methods can give only approximate measures of 'free' SO_2 concentrations since the free and bound forms are in equilibrium and both analytical techniques disrupt this equilibrium during analysis. In class, students analyze the same wine using both procedures and advantages and disadvantages can be readily observed and discussed.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch011

Elemental Composition

Knowledge of trace metals composition in grapes and wines is important for a number of reasons (*50*). While there are legal limits imposed in some cases (*e.g.*, the International Organization of Vine and Wine has established limits for As, B, Br, Cd, Pb, and Zn) (*51*), many elements are also important for vine nutrition and may be added as fertilizers or as agrochemicals to prevent pests and diseases (*e.g.*, copper sprays to prevent mold and mildew infections in the vineyard). Trace metals, particularly Cu and Fe, also impact wine stability by their influence on oxidation reactions and haze formation.

In addition to use in vineyard sprays as noted above, copper can arise from exposure of wines to brass fittings and copper salts may be added to remove objectionable H_2S and other thiol-related off-odors (*52*). U.S. regulations permit additions of up to 6 mg/L, as copper, in wines; however, residual levels in the wine after bottling cannot exceed 0.5 mg/L (as copper) (*47*). Therefore careful testing is required to ensure copper levels are below the legal limit.

Potassium is important for vine nutrition and levels in grape tissues may frequently be monitored (*22*). In addition, potassium influences wine acidity (*43*, *44*) and wine stability since precipitation of potassium tartrate crystals can occur depending on pH, tartrate, and ethanol concentration as well as storage temperature).

Atomic absorption (for Cu) and atomic emission (for K) are routinely used for monitoring levels of these elements in grapes and wines (Tables 1, 2). In

class students prepare calibration curves and analyze selected wines. Matrix effects, *e.g.*, inferences by ethanol in the determination of copper in wine, can be an important source of errors and the importance of matrix-matched calibration standards is emphasized. Dilutions are frequently necessary to ensure that samples are in the linear range of the instrument; student preparation and analysis of the diluted samples helps to re-inforce accurate dilution and calculation procedures.

Automation and Process Monitoring

By volume of wine produced, the wine industry in the U.S. is dominated by a small number of very large wineries. When considering the total number of wineries however, small and medium sized wineries predominate. These small and medium wineries typically do limited analyses in-house and most analyses are not highly automated. Methods taught in this course provide a balance between those that can be performed by wineries of all sizes, while recognizing that many laboratories, including contract testing laboratories, increasingly perform automated instrumental techniques. As a result, students are required to understand the principles of these instrumental methods and have an understanding of how to interpret results that are reported by these methods.

In addition, on-line or *in situ* methods are increasingly used for monitoring wines during fermentation and storage. These analyses include sensors and biosensors for measuring sugars and alcohol (*53*, *54*), color and total phenolics (*55*), SO_2 (*56*), and aroma volatiles (*57*). At UC Davis we have research programs focused on developing new types of on-line sensors to monitor wine fermentations. While these methods are not a specific focus of the wine analysis course, students in the Viticulture and Enology program have access to automated and precisely controlled research-scale fermenters; temperature, mixing, and sugar levels are continuously monitored and data is automatically transmitted to a computer server for on-line access from anywhere in the world (*58*, *59*). As a result, students are exposed to these state-of-the-art process monitoring systems that hold much promise for revolutionizing the future of wine analyses. Nevertheless, nearly all of these sensor systems require validation with standard, primary wet chemistry and chromatographic methods, so there will always be a need for students to have a strong foundational knowledge of analytical chemistry principles and techniques.

Conclusions

Grape and wine analysis involves numerous analytes and analytical methods that can provide a comprehensive approach to teaching the principles of analytical chemistry. New experiments and laboratory exercises continue to be developed as new analytical instrumentation and approaches become available, however, all rely on a strong understanding of the fundamentals of chemistry and analytical chemistry. The laboratory exercises described here provide examples of these fundamentals in an engaging way, demonstrating the application of chemistry to important real-life situations.

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Chapter 12

Chemical Profile of Texas Vodka

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Vodka is defined by the United States Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF) as "neutral spirits so distilled, or so treated after distillation with charcoal or other materials, as to be without distinctive character, aroma, taste, or color" (*United States Code of Federal Regulations Title 27, Section 5.22(a)(1)*). If this definition is to be strictly held, then why do people have strong opinions about any vodka on the market? What makes each vodka different, if there even is a difference? In this chapter, the authors study chemical differences between various Texas vodkas to help answer these questions by investigating the volatile compound content, conductivity, and metal-ions present in 19 Texas vodkas. A taste study was also performed to see how these data relate to consumer preference.

Vodka History and Production

Texas is known for its beers, most notable are Shiner and Lone Star, as well as being the home of one of the oldest soft drinks in America, Dr Pepper. Texas also boasts almost 300 wineries and vineyards, as well as a booming liquor and spirit industry. To date, there are over 21 vodka distillers, 12 whiskey and bourbon

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distillers producing over 25 different products, 2 gin producers, 7 rum producers making over 12 products, and one producer of a blue agave spirit in the same style as tequila.

Vodka, a homogenous solution of ethanol and water, is the focus of this chapter. The word vodka comes from the Russian word *voda*, meaning water. Modern vodka can trace its origins to either Poland ca. 8th century, or Russia ca. 9th century, and was traditionally distilled from fermented fruits and/or starches. Until the mid-19th century, there was no standard for vodka production. Dmitri Mendeleev is often misattributed as setting the standard for vodka production as part of his doctoral dissertation. While Mendeleev did study solutions of ethanol and water as part of his dissertation, these were generally at higher concentrations than found in vodka (*1*). Mendeleev did, however, support his research by running his own successful vodka distillery, still in existence to this day.

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Figure 1. Stages of vodka separation. Bottles (l-r): wheat grains; yeast fermentation with gravity separation; filtration; first distillation giving vodka. (Photo credit: Diana Mason.)

Vodka is made of the alcohol distillate from fermented fruits or starches. Vodka is traditionally assumed to be made from potatoes, but modern Russian vodkas are also made from wheat and other grains. American vodkas have also

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been made from corn. Texas vodkas are generally made from wheat, corn, or a mixture of grains. However, vodka can be made from any starchy or sugary plant material (e.g., black-eyed peas and sugarcane), and there are also Texas vodkas made with cactus, raisins, and rice. In the production of vodka, the plant material is chosen by the manufacturer and cooked into a mash to release the starches and sugars from the cells. This mash is then allowed to ferment by the addition of yeast. Once fermentation is complete, the resulting alcohol solution is filtered and distilled until it reaches 95% alcohol by mass. The alcohol solution is then diluted to 40% alcohol by mass with water and sold as vodka. Examples of the solutions at different stages in the production of vodka are shown in Figure 1.

One of the key aspects of vodka is its purity. Distillation is a method used to purify solutions of miscible compounds by boiling off each compound at a time. One of the problems with distilling ethanol from water is that these two compounds form an azeotropic mixture. An azeotropic mixture is a mixture of two liquids whose proportions cannot be altered by simple distillation. For ethanol and water, this proportion is 95.5% by mass. Once this ratio is reached via distillation, the ethanol cannot get any more pure without the addition of a drying agent to chemically remove the water. Subsequent distillations at this point only serve to extract other dissolved compounds. In addition to 4.5% water present in the ethanol solution, there is also a small amount of methanol. This methanol is a byproduct of the fermentation process, in which yeast ferments the sugars and starches from the plant material in an anaerobic metabolic pathway. When diluted to 40%, the total methanol concentration is generally around 0.4%, or 10 grams of methanol per liter of ethanol (*2*).

In Texas, the production of large quantities of ethanol is highly regulated. As a result, many Texas distillers purchase their alcohol from outside sources and perform subsequent dilutions in a Texas-based facility before diluting to 40% alcohol or they distill in small batches (varies from 40-200 gallons). Most distillers dilute their alcohol with Texas water, although there are a few who do not. For the purpose of this study, the authors have excluded any vodka that has not been diluted with Texas water. Table 1 gives a list of the plant materials used in each vodka for this study. It has been requested of the authors that brand names not be divulged; all subsequent data will utilize the labels given in Table 1.

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Gas Chromatography and Volatility

Flavor is a general concept that is primarily described by how certain chemicals smell and/or taste. From a biochemical standpoint, the way a chemical smells is characterized by its interaction with the nerves present in the nasal cavity, while the way a chemical tastes is determined by its activation of taste receptors on the tongue. There has been extensive research on the strong correlation between how food smells and its influence on the taste (*3*). This is illustrated when your sinuses are congested and food tastes differently because the smell is being physically blocked from interacting with the nerve receptors in the nose.

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Table 1. Conductivity and ICP-MS Output for Vodkas and Standard Solutions

Sample	*Alcohol Source*	*Conductivity ($\mu S\ cm^{-1}$)*	*Sodium-23 (counts s^{-1})*
A	Wheat	5.1 ± 0.8	0
B	Wheat	3.3 ± 0.4	354,400
C	Corn	18.7 ± 0.2	182,022
D	Corn	23.0 ± 2.6	112,933
E	Corn	72.1 ± 0.6	286,642
F	Corn	53.3 ± 0.3	159,875
G	Corn and wheat	18.3 ± 0.4	92,161
H	Wheat and raisins	42.8 ± 0.4	132,191
I	Corn and wheat	52.6 ± 0.3	254,064
J	Cactus	75.0 ± 0.4	100,153
K	Grains[a]	9.0 ± 0.4	165,378
L	Wheat and rye	7.4 ± 0.4	144,742
M	Grains[a]	7.7 ± 0.3	359,249
N	Grains[a]	3.2 ± 0.1	156,091
O	Grains[a]	3.5 ± 0.2	194,513
P	Grains[a]	5.6 ± 0.4	18,581
Q	Corn	37.1 ± 0.3	150,492
R	Wheat and rice	6.3 ± 0.3	23,530
S	Wheat	7.5 ± 0.4	40,264
Comparison	Russian wheat	15.7 ± 0.2	
Comparison	Austrian potato	5.4 ± 0.1	
Standard	95% grain alcohol	1.3 ± 0.0	282,925
Standard	40% grain alcohol	1.5 ± 0.2	0
Control	95% denatured ethanol	0.33 ± 0.01	5,469
Control	40% denatured ethanol	0.81 ± 0.09	0
Control	Deionized water	2.3 ± 1.1	152,103

[a] Specificity beyond "grains" is considered proprietary.

Smells can enter the nasal cavity either through the nostrils or through the back of the throat. In order for a chemical to interact with the nerves in the nasal cavity, it must first be in the gaseous state. The vapor pressure of a compound plays an important role in whether that compound will be smelled. The relationship between vapor pressure and temperature is described using

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the Clausius-Clapeyron equation, given as equation 1. In equation 1, ΔH_{vap} is the enthalpy of vaporization in units J mol^{-1}, R is the universal gas constant in units $J \cdot mol^{-1} \cdot K^{-1}$, T is the temperature in kelvin, and P is the vapor pressure of the liquid. Because vaporization is an endothermic process, this means that as temperature increases, the vapor pressure of a liquid will also increase. The reason foods or drinks smell differently at different temperatures is due to the differing concentrations of the different volatile compounds at those temperatures.

$$\ln\left(\frac{P_2}{P_1}\right) = -\frac{\Delta H_{vap}}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \quad (1)$$

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Gas chromatography (GC) is a technique that can be used to separate and identify the volatile compounds in a solution. In a typical GC experiment, a separation column is loaded with a stationary phase that is chosen based on the types of compounds that are to be separated, whether polar, non-polar, chiral, etc. The sample is then heated and vaporized upon injection to the column, where a carrier gas will push the sample through the separation column. The sample separates into the individual components based on their solubility in the stationary phase, with more soluble compounds staying on the column longer than less soluble compounds. A typical GC set-up is given in Figure 2. The oven can be controlled by the user to either run the separation isothermally or with a programmed temperature increase over time. The detector is also chosen by the user. Typical GC detectors that have wide applications include flame-ionization detectors (FID), mass spectrometer (MS), thermal conductivity detectors (TCD), and electron capture detectors (ECD) (*4*). FIDs are useful in the detection of hydrocarbons and other flammable compounds, mass spectrometers are useful for the detection of almost any compound that can be ionized, TCDs are the universal detector, and ECDs are useful for the detection of halogenated compounds (*4*).

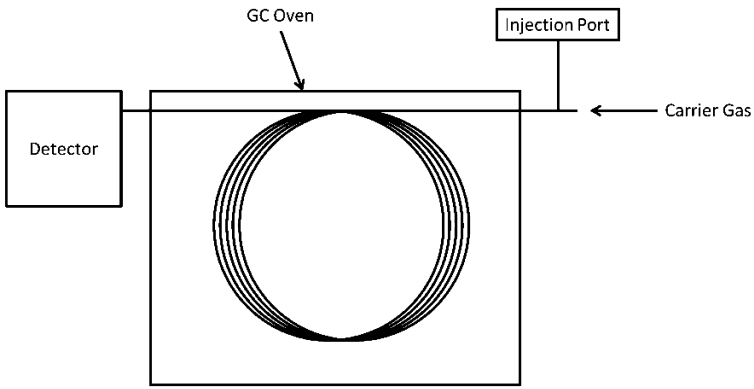

Figure 2. Basic gas chromatography setup.

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Mass spectrometers can also be used in the identification of compounds, depending upon the method of ionization. In a mass spectrometer, a compound is ionized and separated based on the mass to charge ratio (m/z). When coupled to a GC experiment, the mass spectrometer utilizes an electron impact method for ionization along with a quadropole separation technique. The electron impact ionization technique is considered a hard ionization method, and causes fragmentation of the parent molecule into smaller daughter fragments. Each parent molecule produces daughter fragments in a unique manner, and the identification of the daughter fragments and their abundance can be used to identify the parent molecule.

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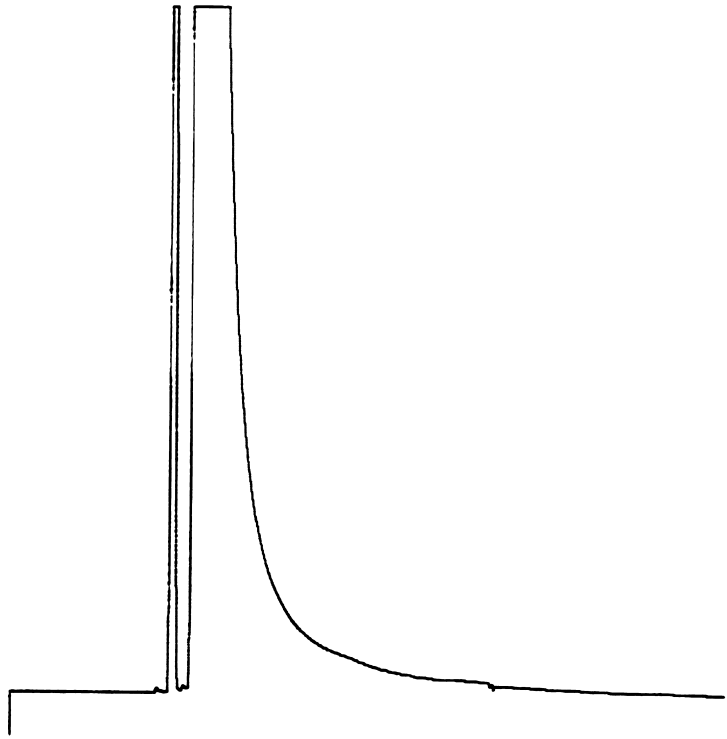

Figure 3. Gas chromatogram for 16 of the 19 vodkas studied, showing only the presence of ethanol and methanol in the vodka.

For this work, each vodka was injected directly onto a Carbowax 20M stationary phase and separated isothermally at 50 °C. A FID was chosen as the detector as a means to determine if other hydrocarbons were present in each vodka besides ethanol and methanol without identification of the molecules. The authors have been asked by the vodka distillers to not distribute qualitative or quantitative chemical information specific to the flavor of the identified vodkas.

Sixteen of the vodkas studied advertise at least six distillations resulting in the chromatogram shown as Figure 3. Only three vodkas (Figures 4-6) deviated from this chromatogram, which shows a very large peak corresponding to ethanol, and a second smaller peak corresponding to methanol. Vodka H (see Figure 4) was a surprising result because it had no discernible smell upon opening of the bottle, yet shows the presence of other organic compounds probably from the presence of raisins used in the fermentation process. As expected, vodka J shows evidence of other organic compounds (see Figure 5). By using cactus as the plant material for this vodka, vodka J is produced in small batches and advertises being distilled only once. This single distillation process leaves behind a lot of additional volatile organic compounds. Vodka Q (see Figure 6) was of note because it showed evidence of containing volatile organic compounds, whereas the other grain vodkas did not. Vodka Q is produced entirely in Texas from small-batch fermentation, and not obtained by the distiller as 95% alcohol. Vodka Q advertises three distillations before bottling. As a result, much of the organic content from the grain has not been distilled off from the alcohol content.

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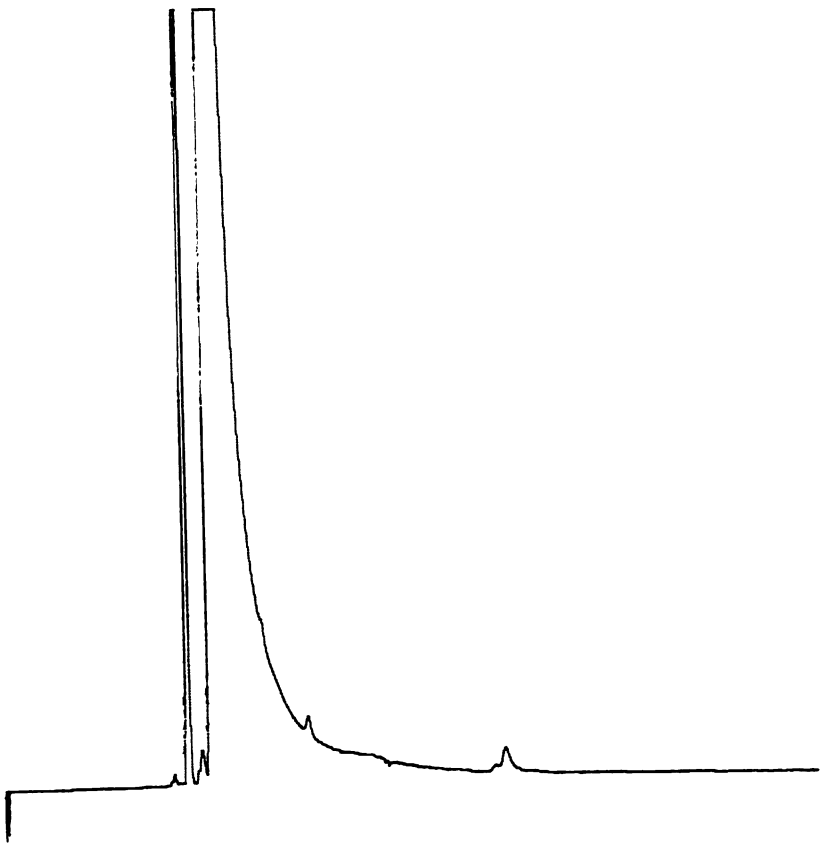

Figure 4. Chromatogram for vodka H.

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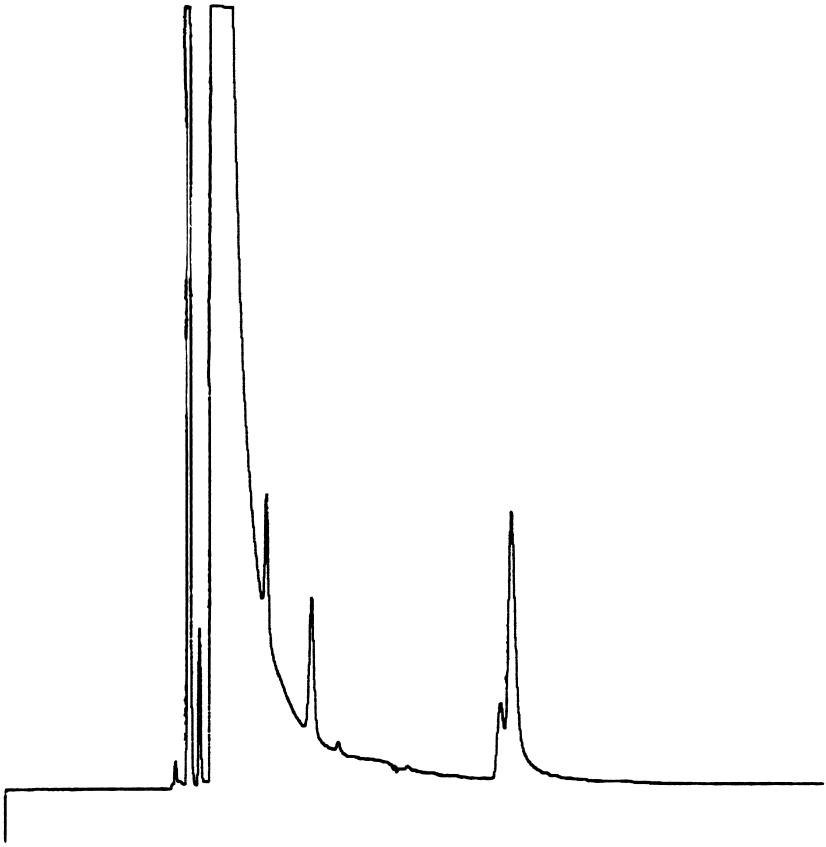

Figure 5. Chromatogram for vodka J.

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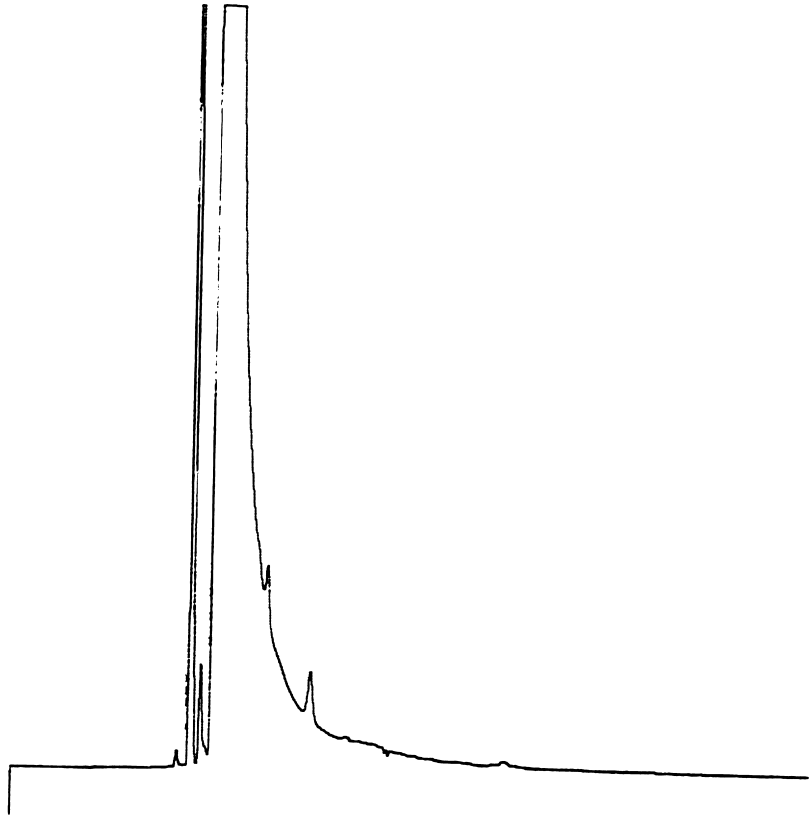

Figure 6. Chromatogram for vodka Q.

Conductivity and Taste

Earlier in this chapter, the importance of any volatile organic compounds and their role in the overall taste and flavor of vodka were discussed. As shown, the majority of vodkas tested only showed evidence of ethanol and methanol present in the solution. This begs the question as to what else can affect the overall taste of the vodka. Research performed by Lachenmeier et al. (*5*) indicated that the conductivities of vodkas are unique, and could be used as a quick and easy method to forensically identify different vodkas. Subsequent research performed by Breslin et al. (*6*, *7*) showed that sodium may be a factor in the suppression of bitter taste receptors on the tongue. The determination of metal-ion concentration will be discussed in a later section.

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The conductivity of a solution can be measured using an electrical conductivity meter to measure the resistance of the solution. The basic instrumentation is shown in Figure 7. The electrode shown in Figure 7 measures the resistance of the solution to the flow of current. The measured resistance correlates to the molar conductivity of the solution, Λ_m, with the relationships given in equations 2 and 3, where κ is the specific conductance, *R* is the resistance, *C* is the cell-constant, and *c* is the concentration of the electrolyte in solution.

$$\kappa = \frac{C}{R} \tag{2}$$

$$\Lambda_m = \frac{\kappa}{c} \tag{3}$$

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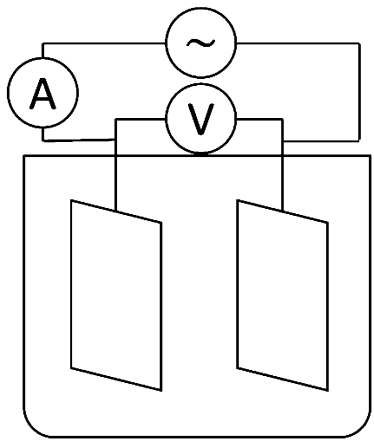

Figure 7. Basic conductivity apparatus.

The measured molar conductivity is dependent upon whether the electrolyte of interest is a strong electrolyte or a weak electrolyte. Strong electrolytes follow Kohlrausch's Law, shown in equation 4, where Λ_m^0 is the molar conductivity at infinite dilution and *K* is an empirical constant. The conductivities of weak electrolytes are dependent upon both the concentration of the electrolyte, and its equilibrium dissociation constant, K_{sp}, as shown in equation 5.

$$\Lambda_m = \Lambda_m^0 - K\sqrt{c} \tag{4}$$

$$\frac{1}{\Lambda_m} = \frac{1}{\Lambda_m^0} + \frac{\Lambda_m c}{K_{sp}\left(\Lambda_m^0\right)^2} \tag{5}$$

In general, conductivity is directly proportional to the amount of dissolved electrolytes. Since the identities of the electrolytes in the vodka solutions are unknown and varied, it is impossible to use conductivity exclusively as a viable quantitative method. Conductivity can be used to qualitatively determine the amount of dissolved electrolytes in each vodka. These data can be compared to data obtained from taste tests of the vodkas to evaluate any relationship between conductivity and taste.

The conductivity was measured at least three times for the 19 Texas vodkas, a Russian wheat vodka, and an Austrian potato vodka using the Thermo Orion 550A advanced pH/conductivity meter with two-point autocalibration from 12.9 mS cm^{-1} and 1,413 μS cm^{-1} sodium chloride solutions. These conductivities were compared to the following series of standard solutions: 95% grain alcohol, 40% grain alcohol prepared by diluting the 95% solution, 95% denatured ethanol, 40% denatured ethanol, and deionized water. Conductivities for each vodka and all standard solutions are given in Table 1.

As shown in Table 1, the majority of Texas vodkas have unique conductivities, supporting the claims by Lachenmeier et al. that all vodkas have unique conductivities (*6*). In all of these cases, the conductivities do not overlap within statistical error. Another interesting note about these data is the conductivity of the standard solutions. In the case of the four alcohol solutions, the conductivity of the diluted solutions either increased or stayed the same, but all were lower than the conductivity of the deionized water. This seems to indicate an increased resistance to electrical flow caused by the presence of the alcohol. The experiments were performed again using PASCO probeware that produced the same trends as shown in Table 1.

Lachenmeier et al. (*5*) tested the conductivity of a range of different grades of vodka, from bargain brands to premium brands. In all of the vodkas the Lachenmeier group tested, it was found that the conductivity did not show any statistical variance between different batches within the same brand. Furthermore, it was found that the more "premium" the brand of vodka, the lower the conductivity, with the so-called bargain brands having higher conductivities.

With the GC and conductivity data presented earlier, it became apparent that in order to understand how these data were important for vodka, the authors needed to connect these observations to the taste preference of average consumers. A pilot study performed by the authors in 2012 on six known Texas vodkas showed that there might be a strong connection between the conductivity and the preference of the vodka. By 2014, the number of Texas vodkas had more than tripled. The authors were able to obtain 19 Texas vodkas, and so the study was repeated to see

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if the relationship between taste preference and conductivity could be replicated. This study fell under a Protection of Human Subjects' exemption of the Federal Code, so Institution Review Board approval was not required (*8*). For the taste study, 30 people consisting of 14 males ranging in age from 33 to 71 and 16 females ranging in age from 21 to 65 were given samples of each vodka and asked to rank them based on aroma, flavor, aftertaste, and an overall ranking on a scale of one to three, with three being the most preferable. Participants were given 5-mL samples of each vodka and instructed in the "swish and spit" method preferred by wine tasters. They were asked to cleanse their palates after each sample with bottled water from Texas, eat oyster crackers, and/or sniff coffee beans. The vodkas were served neat at room temperature, labeled by lettered samples (A-S), and served grouped by broad geographical regions. The complete process included a mini-lecture on vodka and took about 1.5 hours. Vodkas B, G, L, N, O, and P were grouped together since they were distilled in the north-central Texas area. Vodkas C, D, E, F, I, and K were from the central Texas Hill Country region. Vodkas A, J, and M were from the south-central region. And vodkas H, Q, R, and S were form the southeastern area of the state of Texas.

The taste test was conducted by having participants rate within the small groups of 3-4 samples by general geographical region. No rule forbade tasters from rating each sample as 1, 2 or 3. If none of the samples were preferred each could be rated as 1, etc. These data were then complied and an overall average ranking calculated for each. Table 2 gives the final results of the taste study, as well as the measured conductivity of each vodka. Vodka J is the vodka that had the most volatile organic compounds dissolved, as shown in Figure 5, and it was ranked as the most preferred vodka. The most important factor is that it contains the most volatile organic compounds of all of the vodkas evaluated, and these compounds have a major influence on the overall taste. Vodka J is included in the analysis because it is marketed as a Texas vodka. However, it does not fit within the ATF regulation on vodka (*9*) because it has a distinct aroma and taste. Because of this peculiarity, the ratings were reevaluated with the exclusion of vodka J.

Data of interest in Table 2 are that in most cases the most liked sample had the highest conductivity similar to the trend observed in the pilot study. Also note that the plant source makes a difference in the conductivity measured. The vodkas distilled from wheat grains tend to have the lowest conductivities and then when mixed with corn or raisins, the conductivity increases. Corn has a large range of conductivity due to the wide-ranging varieties and whether or not is it fresh or dried as a grain. Corn as the single source of starting starch has relatively high conductivity. Table 3 gives the conductivity ranges for each plant material used to make the Texas vodkas analyzed. An extreme difference was observed for vodka J further supporting its elimination from the preferences seen in Table 4. These data illustrate that a large factor contributing to the conductivity of vodka is due to the presence of the volatile organic compounds that accompany the distillate. These analyses also give an appreciation of the ability of the human tongue to distinguish the subtleties of each vodka. Relative proportions of the various varieties and mixtures of plant material are trade secrets, so it is impossible to report any statistical correlations.

Table 2. Taste-Test Results by Location Given Conductivity and Plant Source

General Area of Texas	*Label*	*Conductivity ($\mu S\ cm^{-1}$)*	*Plant Material*	*Overall Taste Ranking*
North Central				
	N	3.2	Grains	19
	B	3.3	Wheat	13
	L	7.4	Wheat and rye	4[a]
	O	3.5	Grains	17 T
	P	5.6	Grains	16
	G	18.3	Corn and wheat	12[a]
Hill Country				
	K	9.0	Grains	11
	D	23.0	Corn	5
	F	53.3	Corn	6
	C	18.7	Corn	14
	I	52.6	Corn and wheat	10
	E	72.1	Corn	8 T[a]
South Central				
	A	5.1	Wheat	17 T
	M	7.7	Grains	2 T
	J	75.0	Cactus	1[a]
Southeast				
	Q	37.1	Corn	8 T
	S	7.5	Wheat	15
	R	6.3	Wheat and rice	7
	H	42.8	Wheat and raisins	2 T[a]

[a] Participants' favorite within geographical group matched with highest conductivity. T = tie. H, M tied for overall 2nd place; E, Q tied for 8th place; A, O tied for 17th place.

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Table 3. Plant Source and Conductivity Range

Source	*Conductivity Range ($\mu S\ cm^{-1}$)*	
	Low	*High*
Grains/wheat	3.2	9.0
Wheat and rice	6.3	6.3
Wheat and rye	7.4	7.4
Wheat and corn	18.3	52.6
Corn	18.7	72.1
Wheat and raisins	42.8	42.8
Cactus	75.0	75.0

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Table 4. Breakdown by Each Characteristic of the Taste Test

	Aroma	*Aroma (not J)*	*Flavor*	*Aftertaste*	*Aftertaste (not J)*	*Rank*	*Overall*	*Overall (not J)*
Women	J	L & I	M	J & H	H	H	H	H
Men	J	F	E	E	E	F	E	E
Overall	J	L & I	M	E	E	H	J	M & H

Table 4 shows a breakdown of each aspect of the taste test ranked in the study: aroma, flavor, aftertaste, and overall rank. Included in Table 4 are the results with the inclusion of vodka J and without. When looking at the results that exclude vodka J, the highest conductor at 75 μS cm^{-1}, it is interesting that vodka H was one of the overall favorites of the participants. Vodka H is one of the other vodkas that had dissolved volatile organic compounds present. This indicates that these compounds do play an important role in the taste of the vodka. Comparison to the results given in Table 2, conductivity does seem to play a role in preference. Vodka H (women's and overall favorite) did have a high conductivity of 42.8 μS cm^{-1}, but vodka M (tied as overall favorite) had a relatively low conductivity of 7.7 μS cm^{-1}. These two vodkas were the most preferred following the elimination of vodka J. Based on these results, no one geographical region of Texas produced the most preferred vodka by taste. The top five vodkas ranked were from the four identified geographical areas of Texas, but of interest is that the most preferred vodka in all but one of the six groups was the one with the highest conductivity.

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Determination of Dissolved Metals

Data presented earlier suggests that conductivity does play a role in the overall taste preference of vodka. There also must be other chemical components of the vodka that affect taste preference. Breslin et al. (*6*, *7*) stated that sodium plays a role in suppressing the bitter taste receptors on the tongue. In addition, the different vodkas were distilled using different distillation columns made from different metals such as copper or stainless steel. It is very likely that some of the metal from the distillation column dissolved into the vodka during the distillation process. Finally, the water for each vodka comes from a different source, many from natural springs and aquifers each with unique metal compositions. Any of these factors can affect the taste.

One method allows for fast quantification of dissolved metals in a solution, inductively-coupled plasma mass spectrometry (ICP-MS). ICP-MS can detect any atomic species from lithium to uranium with a limit of detection in the ppb region up to 100 mg L^{-1}. Typically, the plasma used for ICP-MS is generated from an argon source, which leads to spectral interference around 40 amu and 56 amu due to the formation of ArO^+ (*10*). Other sources of interference include HCO^+, COH^+, CO^+, H_2CO^+, and $HCOH^+$, all of which come from organic compounds and dissolved CO_2 (*10*).

An illustration of a typical ICP source, including sample introduction to the plasma, is given in Figure 8. In Figure 8, the liquid sample is made into an aerosol using a nebulizer before being injected into the plasma. The plasma is at a sufficiently high temperature, around 10,000 K, that the metals within the sample are ionized to their first ionization level, thus becoming singly charged. These ions can then be introduced to the mass spectrometer and separated by isotopic mass. The detector used will output the ion concentration in counts s^{-1}. This can be correlated to a series of standards of known concentrations to find the concentration of the analyte of interest.

For this experiment, each sample was diluted 1:100 using deionized water. Prior to any samples being run through the instrument, a blank was run so that any instrumental interference could be subtracted. These data can be used to give relative concentrations of sodium in each vodka. Table 1 gives the output concentrations for sodium-23 in counts s^{-1} for each of the Texas vodkas as well as the controls and standards. As shown in the last column of Table 4, vodkas H (women, overall), E (men), and M (overall) were the most preferred by the tasters. From Table 1, vodka M had the highest sodium concentration of all of the vodkas, followed by vodkas B and E. Since sodium interferes with the bitter-taste receptors on the tongue, this would mean that the vodkas with the higher sodium content should reduce the bitter taste of the alcohol. This argument is supported by vodka A, which was very low as a consumer preference (tied for 17th place of 19 samples) and had no discernable sodium-ion concentration. Vodka H was one of the few types of vodka that contained volatile organic compounds, indicating that these compounds factor into the overall taste.

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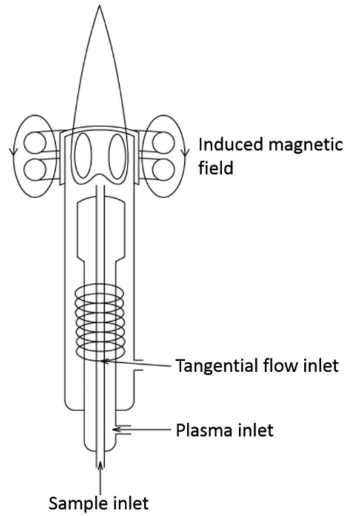

Figure 8. Schematic for ICP torch.

In addition to the sodium metal content, the authors also looked for the presence of lithium, beryllium, magnesium, potassium, calcium, scandium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, rubidium, strontium, cadmium, cesium, barium, mercury, and lead (see Table 5). For the iron content, the ICP-MS was set to detect the iron-57 isotope to eliminate most of the interference from the ArO^+ ion. For identification purposes, the metals that were detected at greater than 1,000 counts $s^{-1}$are labeled as being abundant, and the metals that were detected at less than 10 counts s^{-1} are labeled as being in trace concentrations.

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Table 5. List of Metals Present in Each Vodka

Vodka	*Abundant Metals*	*Intermediate Concentration*	*Trace Metals*
A	Mg, Ca, Sr, Ba	Sc, Mn, Fe, Zn, Rb	V, Cr, Ni, Cu
B	Na, Mg, K, Ca, Sr, Ba	Sc, Cr, Mn, Fe, Cu, Zn, Pb	Li, V, Ni, Cd
C	Na, Mg, K, Ca, Sr, Ba	Li, Sc, Cr, Mn, Fe, Cu, Zn, Rb, Cs, Pb	Co, Ni, As, Cd
D	Na, Mg, K, Ca, Sr, Ba	Sc, Cr, Mn, Cu, Zn	V, Fe, Ni, As, Cd
E	Na, Mg, K, Ca, Sr, Ba	Li, Sc, Cr, Fe, Cu, Zn, Rb	V, Mn, Ni, Cd
F	Na, Mg, K, Ca, Sr, Ba	Li, Sc, Cr, Mn, Fe, Cu, Zn, Rb	V, Ni, As, Cd, Hg
G	Na, Mg, K, Ca, Sr, Ba	Sc, Cr, Mn, Fe, Cu, Zn, Pb	Li, V, Co, As, Cd
H	Na, Mg, K, Ca, Sr, Ba	Sc, V, Cr, Fe, Cu, Zn, Pb	Ni, Rb
I	Na, Mg, K, Ca, Sr, Ba	Li, Sc, V, Cr, Fe, Zn	Mn, Cu, As, Cd
J	Na, Mg, K, Ca, Sr, Ba	Li, Sc, Cr, Mn, Fe, Cu, Zn, Pb	V, Rb, Cd
K	Na, Mg, K, Ca, Sr, Ba	Li, Sc, V, Cr, Fe, Cu, Zn, Rb, Pb	Ti, Cd
L	Na, Mg, K, Ca, Sr, Ba	Li, Cr, Fe, Co, Zn	Sc, V, Ni, As
M	Na, Mg, K, Ca, Sr, Ba	Li, Sc, Cr, Fe, Zn, Rb	V, Co, Ni, Cd
N	Na, Mg, K, Ca, Sr, Ba	Li, Sc, Cr, Mn, Fe, Zn	V, Co, As, Cd
O	Na, Mg, K, Ca, Sr, Ba	Li, Sc, Cr, Mn, Fe, Zn, Rb	V, Co, Ni, Cu, Hg
P	Na, Mg, K, Ca, Sr, Ba	Li, Sc, V, Cr, Fe, Zn	Cd, Hg
Q	Na, Mg, K, Ca, Sr, Ba	Li, Sc, V, Cr, Fe, Zn, Rb, Cs	Ni
R	Na, Mg, K, Ca, Sr, Ba	Li, Sc, V, Cr, Fe, Zn	Be, Mn, Co, Ni, Cu, As
S	Na, Mg, K, Ca, Sr, Ba	Li, Sc, Cr, Fe, Zn	Mn, Co, Cu

Vodkas B, C, G, H, J, and K showed measurable amounts of lead that were not classified as trace concentrations. Vodka C had a lead concentration of 54 counts s^{-1}, vodka G had a lead concentration of 16 counts s^{-1}, and vodka K had a lead concentration of 90 counts s^{-1}, all very low compared to the sodium concentrations listed in Table 1. Vodka J had a lead concentration of 1,536 counts s^{-1}. Vodka J advertises that it is distilled only once. Being made from cactus, it is very likely that there are lead compounds that are not being distilled off or discarded during production.

Vodkas C, E, F, and M all advertise the use of copper stills in the production of their vodka. Vodka H uses a copper-hybrid still, while vodkas D, G, and K all use stainless steel. The other vodkas do not advertise the metal of their still. For all of the vodkas, there was no discernible change in iron concentration, ranging from 6 - 38 counts s^{-1}. Vodka C showed the largest concentration of copper in the vodka with 898 counts s^{-1}. Vodkas E, F, and H showed low concentrations. Interestingly, vodka M, which was one of the overall favorites, showed no copper at all in the vodka, and vodka D showed the second highest concentration. This indicates that there is no dissolution of metals from the stills, and that these metals are coming from the plants sourced or the water used for dilution.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch012

Conclusions

On the surface, vodka may seem simple. It is a spirit with no "character, aroma, taste, or color" (*9*), and yet individual vodkas show different chemical compositions. Using Texas vodkas as an introduction to the chemical profile of vodka, the authors found vodkas that contained only ethanol and a small amount of methanol, as would be expected, but also found vodkas that contained other volatile organic compounds that impacted the taste of the vodka. Texas vodkas also offer more variety in the type of vodka to analyze, expanding from the more common potato, wheat, or other grains to include corn, cactus, rice, or raisins, as the plant material source for the alcohol. Using the research of Lachenmeier et al. (*5*), measured conductivities were recorded for each of the Texas vodkas and with the data from GC experiments, a taste study was performed to link these data with consumer preference. Further experiments with ICP-MS showed that the metal composition for each vodka is unique and based upon the water source used by the distiller in the production process.

This seemingly simple substance provides a wealth of opportunities to teach and/or practice a variety of laboratory techniques including those described in this chapter. Additionally, the hundreds of vodkas available across the globe allow students to practice designing experiments to evaluate the differences in physical properties and solution chemistry between products.

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Classroom Activities

• Obtain at least 10 vodkas from your state or region. If there are not 10 vodkas in your state or region, choose any of your favorite brands. Identify the starting plant materials of each and the location of the water source. Measure the conductivity of each vodka. What is the trend between the plant source and conductivity? Determine the most abundant and trace metals present in each vodka. What is the minimal lethal dose (MLD) of the abundant metals found in your vodkas? Refer to the IUPAC Gold Book. What is the lethal limit associated with methanol?

• Obtain five flavored vodkas that appear to have a variety of tints as detected by the human eye. Develop a fingerprint of each based on their colorimetry, conductivity, pH, sodium-ion content, and other metal ions present. Publish your fingerprints to the class and ask your peers to identify each of your vodkas based on taste preference. Was any one data point more helpful than another? If yes, why? Can distilled spirits have a reproducible common fingerprint? (Refer to Eric V. Anslyn's research on red wine.) Identify the sources of error that might have occurred in the collection of the above data?

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch012

Further Questions for Thought

• What is moonshine? How is it made? What is meant by proof (historically and today)? Why is the presence of any methanol permissible in commercial spirits?

• Discuss the mechanism of separation. Give the specific column chemistry, mobile phase composition and the detector.

• Describe three methods that are used to relate an instrument signal to an analyte concentration.

• Chemical analysis is affected by two types of noise, one of them being chemical noise. What are some sources of chemical noise?

Acknowledgments

The authors acknowledge our acquaintances, friends and family for their helpful contributions to gathering the taste-test data, especially the participants from the Texas Exes Denton County Chapter. Thanks are extended to Blain Mamiya for his assistance with the Texas Exes taste-test event, José Calderón for the use of his GC, Guido Verbeck, IV for the use of his ICP-MS, and Teresa Golden for the use of her conductivity meter. Thanks are also extended to Phillip Mach and Andrew Drumwright for obtaining the dissolved metal data. The authors declare no competing financial interest.

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Chapter 13

Analysis of Liquid Patent Medicines Archived at the Henry Ford Museum, via ^{1}H NMR Spectroscopy

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The Henry Ford, located in Dearborn, MI, has an extensive series of patent medicines and nostrums housed in its collection. These materials were produced in the late 19th century, before the formation of a United States Food and Drug Administration. Their ingredients were routinely kept as trade secrets by those who produced them, for fear that successful recipes would be stolen and used by competitors. Thus, while archivists are able to find the time during which such medicines were sold, and through period advertising know what claims were made for each material, nothing is known of the ingredients. Thus, for liquid samples, proton nuclear magnetic resonance (^{1}H NMR) was performed in an attempt to determine specific chemical components of each material.

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Introduction

The Henry Ford Museum in Dearborn, Michigan has a large collection of nineteenth century patent medicines, nostrums, and apothecary supplies which appears to have begun with Henry Ford's personal collecting interest, and has grown significantly with the passage of years as specimens were acquired through a variety of donations. While these containers are labeled, this set of ^{1}H NMR analyses was undertaken to expand on the rather scant existing information that the labels and promotional claims provided about the actual composition of the medicines.

The term patent medicine may be misleading in a modern context, as the term does not mean that the material was patented under US law. Rather, the term hearkens back to the idea of some form of official sanction or imprimatur being given to a process or material – in this case a sanction as to the usefulness of the medicine or nostrum. By the later 1800's, numerous patent medicines were being sold both through stores and by itinerant salesmen, and there were no restrictions on what claims could be made by the purveyors of these materials, or indeed what ingredients could be added to such (*1*, *2*). The inlcusion of alcohol as an ingredient, or of pain killers such as heroin or cocaine, had certainly been established by this time. At the same time, the temperance movement that would reach its zenith with the passage of the 18th Amendment to the US Constitution in 1920 (and which was repealed by the passage of the 21st Amendment in 1933) was recruiting members and gathering strength.

Throughout history, alcohol has had religious, medicinal, social, and nutritive uses, and thus has found applications in easing pain and supposedly in curing numerous diseases. As well, it has been a component of social gatherings in many cultures, including the United States, where for the first hundred years of the nation's existence, alcohol was an important part of the voting process (*3*). At the time when the patent medicines examined here were produced, in the 1880 – 1910 time frame, it was not uncommon for members of the population in the United States to utilize alcohol medicinally in a variety of applications. It was also not uncommon to use opiates as pain killers.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch013

Evolution from Household Medicines to Mass Produced Products

The mass produced medicinal products used today all must go through an exhaustive system of testing to be determined to be useful for some condition or disease, and not to have deleterious side effects that would significantly offset their health benefits. The patent medicines examined here did not have to undergo any such scrutiny. Yet that lack of examination and oversight does not automatically mean that these medicines did not have any beneficial effects. Indeed, often patent medicines were created from herbal recipes that had been handed down from one generation to the next (*4–6*). As well, the purveyors of such medicines knew that if their products were found to be harmful or poisonous, they would ruin their chances for long-term and repeat sales. However, egregious cases of mis-

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representation during this time frame, of what a medicine could do or what it could cure, led eventually to the establishment of the US Food and Drug Administration in June 1906 (*7*).

Choice of NMR as Primary Analytical Technique

The entire suite of patent medicines provided by the Henry Ford included several solids as well as a smaller number of liquids. Energy dispersive X-ray fluorescence spectrometry (EDXRF) was run on all the samples, to determine the elemental composition of each. This technique is useful for solids as well as for liquids, and results of these examinations have been reported (*8*). It was felt that NMR would be useful as a second analytical technique, in large part because it is so ubiquitously used for the determination of organic materials in samples, and also because one can easily imagine that most patent medicines, especially liquid samples, would be water-based and thus soluble in D_2O. While the technique may not be as complete or quantitative as GC-MS or LC-MS, it is well suited to determine quickly the presence of any organic compounds with a diagnostic peak or series of peaks. A single drop of liquid sample in 1 mL of D_2O or $CDCl_3$ was found to be sufficient to gather a usable spectrum in each case.

Interestingly, since a few medicines that were originally marketed as patent medicines have survived until the present time and are still sold – the most famous being Bayer aspirin – it was also felt that easy comparisons could be made between the samples provided in this study and similar medicines in their modern form.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch013

Experimental

A Jeol 300-MHz multi-nuclear FT NMR was utilized for these analyses, and was run for ^1H NMR, with a standard 16 counts gathered per sample. Single drop samples were solvated in $CDCl_3$ and in D_2O to ensure that both non-polar and polar materials in the liquids were observed. Samples were also analyzed via energy dispersive X-ray fluorescence spectrometry, the results of which have already been published (*8*).

Discussion

The samples that were examined are shown in Table 1. This is a summary list of the liquid samples examined plus the modern Lydia Pinkham Herbal Liquid Supplement which is still marketed today. It groups the medicines in terms of those which have alcohol present, and those with an opiate present (*9*).

Table 1. Liquid Sample Findings

Sample Name	*Claim*	*Alcohol*	*Opiate*
Mrs. Winslow's Soothing Syrup	"Likely to sooth any animal or human"	Yes	Yes
Honey Syrup of Tar, Tolu, and Wild Cherry	"For cough and grippe"	Yes	Yes
Ayer's Hair Vigor	"Composition for coloring and dressing human hair"	Yes	No
Lax-a-tesia	Laxative	No	No
Dr. Pierce's Compound Extract of Smart-Weed	"Cures every pain"	Indeterminate	No
Lydia Pinkham Herbal Liquid Supplement*	"Nutritional support to help you feel better during menstruation and menopause."	Yes	No

* Modern formulation.

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Alcohol

The ^{1}H NMR triplet of ethyl alcohol which appears at 1.2 ppm and the quartet at 3.7 ppm show through clearly in the spectra of "Mrs. Winslow's Soothing Syrup," of "Honey Syrup of Tar, Tolu, and Wild Cherry," and of "Ayer's Hair Vigor." These peaks did not occur in other patent medicines, and in only one case, "Dr. Pierce's Compound Extract of Smart-Weed," was the spectrum cluttered enough in the aliphatic region that a determination could not be made with certainty. One medicine that has existed since the time when patent medicines were common, and that still is marketed today, is "Lydia Pinkham Herbal Liquid Supplement." A modern sample was purchased, and since the ingredients list includes alcohol at 10%, a ^{1}H NMR spectrum of it was run, as a comparison to the three patent medicines in which alcohol was found. This product, originally marketed to the public in 1856, was met with commercial success, became a household name and commonly advertised medication, (and even inspired the folk song Lilly the Pink). Figure 1 is the ^{1}H NMR spectrum of "Mrs. Winslow's Soothing Syrup" and Figure 2 is the spectrum of "Lydia Pinkham Herbal Liquid Supplement."

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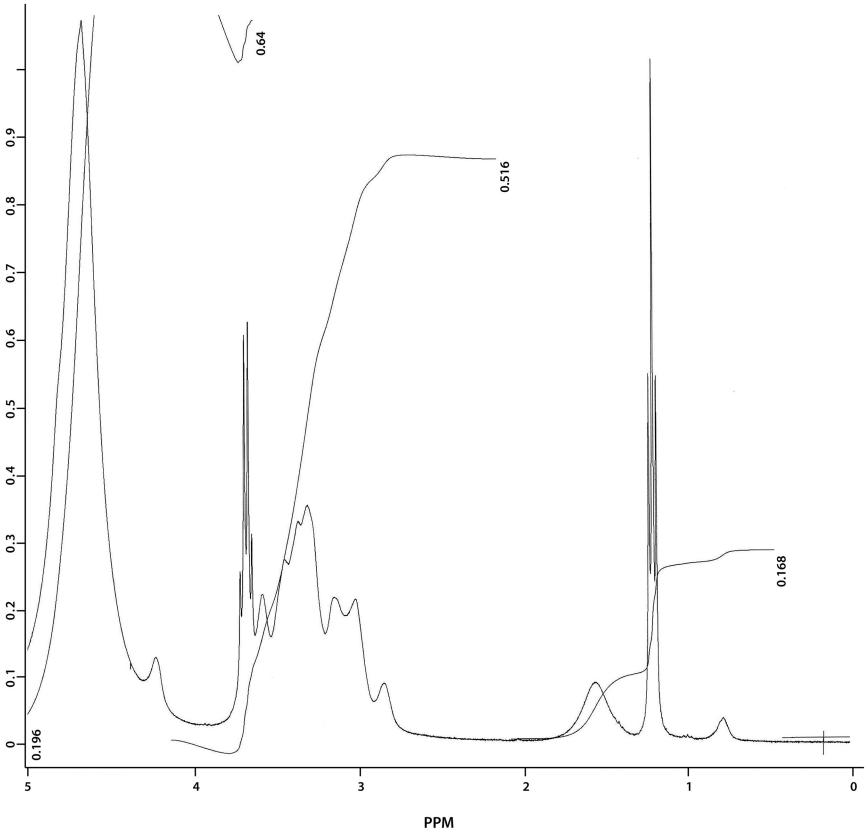

Figure 1. ^{1}H NMR of "Mrs.Winslow's Soothing Syrup".

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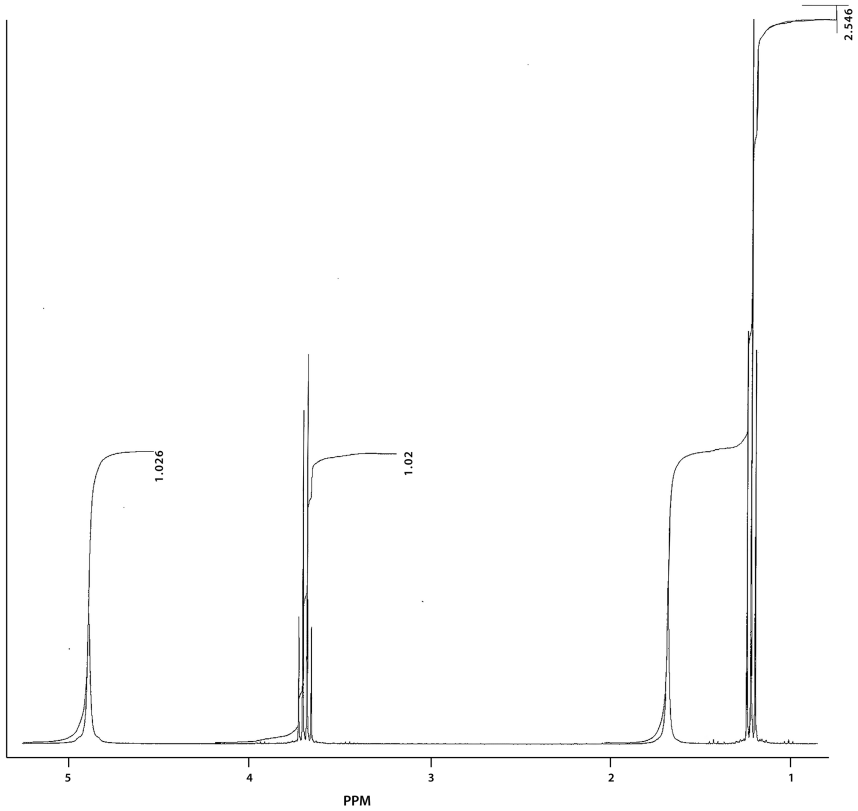

Figure 2. [1]H NMR spectrum of "Lydia Pinkham Herbal Liquid Supplement".

Opiates

Another aspect of patent medicines of this time is that various opiates could possibly have been ingredients. From Bayer heroin, which was sold legally for some years, to cocaine toothache drops, which were also sold legally, one can surmise that the commercial success of these and similar products may have been because of their established pain killing effects. Using ACDLabs Chem Sketch programming, the spectra of heroin, codeine, and morphine were generated from their structures to use as points of comparison with the patent medicine samples, as shown from top to bottom in Figure 3. Two of the liquid samples, "Mrs. Winslow's Soothing Syrup" and "Honey Syrup of Tar, Tolu, and Wild Cherry," do have morphine compounds listed in their recipes, with Figure 4 being the [1]H NMR spectrum of the former, shown from -2 ppm to 12 ppm.

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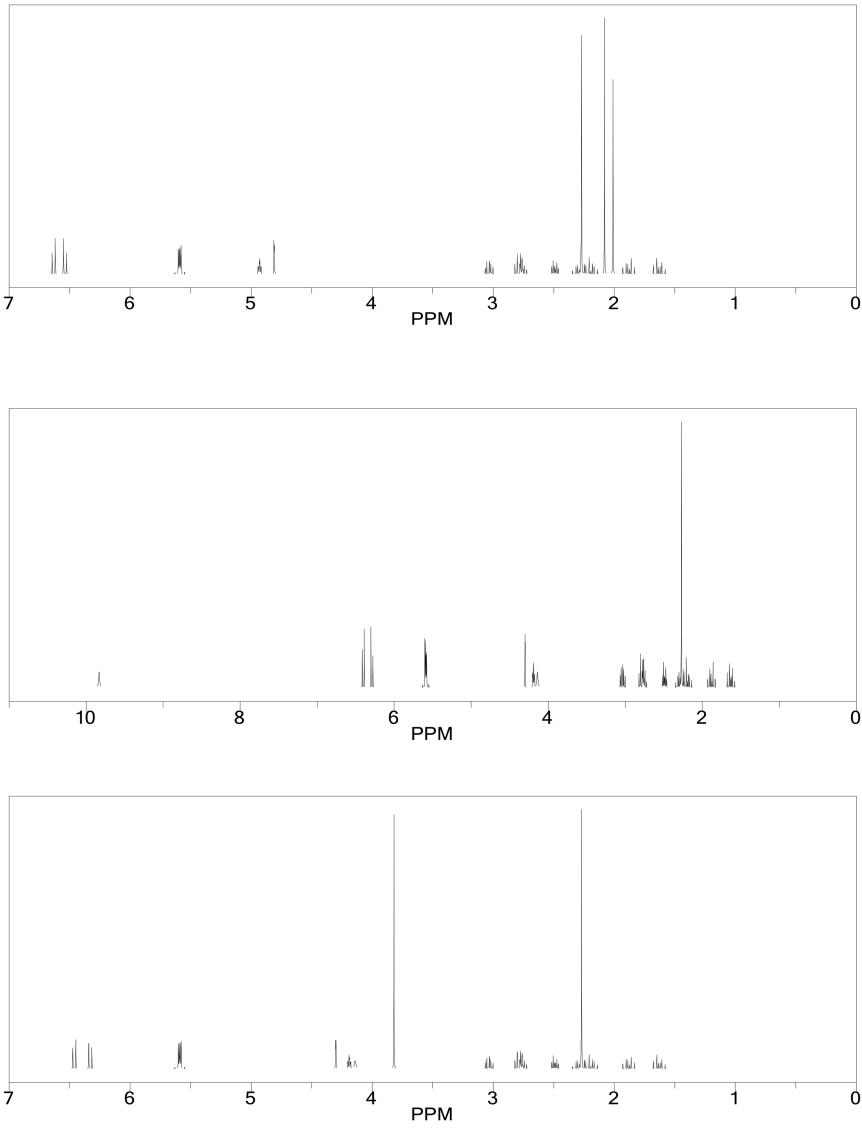

Figure 3. Simulated NMR Structures of Heroin, Morphine, and Codeine, from top to bottom.

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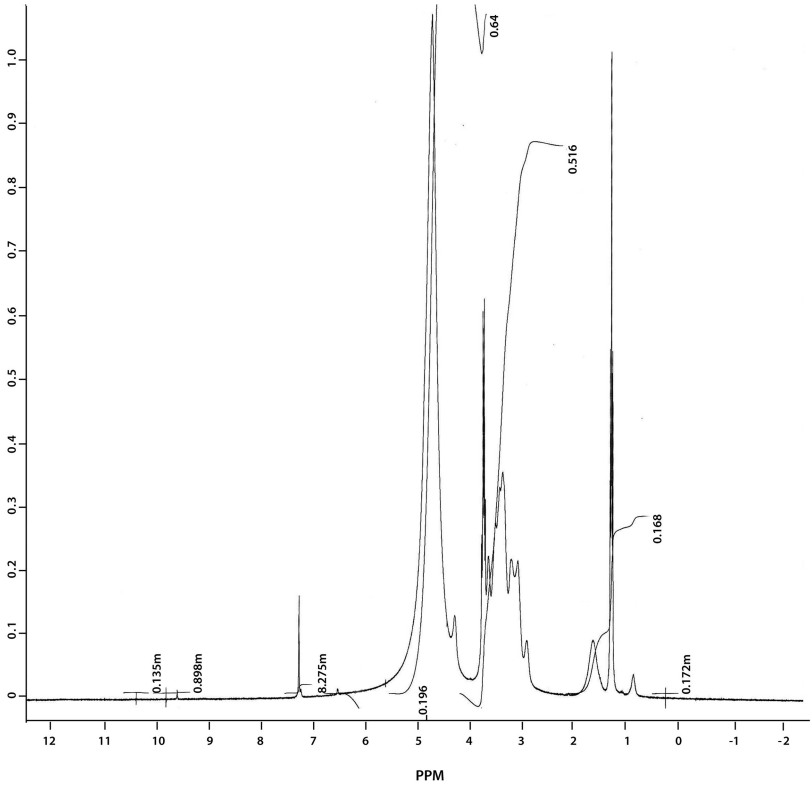

Figure 4. [1]H NMR of "Mrs. Winslow's Soothing Syrup," from -2 to 12 ppm.

Spectra for the patent medicines tend to be cluttered in the aliphatic region of the NMR, as evidenced by Figure 4, and can have peaks that are overwhelmed by the water of the solution. However, for the three opiates: heroin, codeine, and morphine, the region between 6.0 and 8.0 ppm displays a characteristic set of two doublets, which are the resonances of the two protons in the only aromatic ring. As well, the simulated spectrum of morphine shows a distinct signal at 9.8 ppm. In the patent medicine samples, these two doublet peaks, seen in Figure 4 at 6.5 and 7.2 ppm appear to be evidence for the presence of an opiate, and the singlet at 9.6 ppm further indicates that this is morphine.

While the two doublets in Figure 4 do not precisely align with those in the simulated spectra of the three opiates, it must be noted that the simulated spectra are based on estimates built into the program. Importantly, concerning the actual samples, it must also be noted that the stability of these opiates in any solution, aqueous or otherwise, over the course of 120 years has never been examined or reported. Essentially, the opiates in these patent medicine samples have been in

solution for longer than a century, and how heroin, cocaine or morphine in these conditions exists or degrades after such a time has never been determined, although it appears that at least one has not degraded past the point of identification.

Conclusions

Four of the liquid patent medicine samples examined in this study contain ethyl alcohol, which is proven unambiguously by their ^{1}H NMR spectra. As seen in the ^{1}H NMR of a sample of a current Lydia Pinkham compound, some such medicines survive even to modern times, contain ethyl alcohol, and continue to be marketed. Two of the liquid samples appear to contain an opiate, again as evidenced by their ^{1}H NMR spectra. Despite the cluttered aliphatic region of these ^{1}H NMR spectra, diagnostic peaks in the aromatic region could be compared to simulated spectra of heroin, morphine, and codeine, leading to the conclusion that morphine is the opiate that is present in these two samples. It should be noted that the actual liquid sample spectra, and the simulated opiate spectra do not match exactly, but that those peaks being used as diagnostics do match in shape and multiplicity.

It appears that these medicines may have had a therapeutic effect on the body, due to the significant alcohol content of many of them, and the opiate in two of them. They may also have served as a form of socially acceptable alcohol consumption at a time when the temperance movement was growing in strength and numbers, since the regulation of medicinal opiates and their identification was far less stringent at the time these medicines were produced than today.

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Chapter 14

The Role of Disulfiram in Alcohol Metabolism and the Treatment of Alcoholism

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Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the two major enzymes responsible for the metabolism of ethanol. ADH converts ethanol to acetaldehyde and ALDH converts acetaldehyde to acetate. Disulfiram is an irreversible inhibitor of ALDH. The increase in serum aldehyde concentrations when alcohol is consumed in the presence of disulfiram produces acute, unpleasant symptoms known as the disulfiram-alcohol reaction. The mechanism of inhibition is believed to involve a disulfiram metabolite-cysteine adduct in the active pocket of ALDH.

After caffeine, alcohol (ethanol) is the second most widely used psychoactive drug in the world. A psychoactive drug may be defined as a chemical substance that principally acts on the central nervous system (CNS) to alter mood, thought processes, or behavior. The National Institute on Alcoholism and Alcohol Abuse (NIAAA) estimates that 17 million people age 18 years and older in the United States suffer from either alcoholism or alcohol abuse (*1*). Alcoholism refers to an alcohol use disorder in which the person has a physical dependency on alcohol and suffers withdrawal symptoms when alcohol consumption is ceased. Alcoholics tend to develop tolerance and often need to drink more alcohol to experience the same effects. They spend more of their time seeking enough alcohol to drink and recovering from its effects than on their normal activities. This places an enormous burden on family, friends, employers, and society at large. Various

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treatments exist to treat alcohol addiction; Antabuse (disulfiram) is used during the maintenance phase of treatment. This chapter integrates general, organic, and biochemistry principles to illustrate how the enzymes in the liver that detoxify alcohol are irreversibly inhibited by disulfiram.

Pharmacology of Alcohol

Alcohol (Figure 1a) is considered amphiphilic by biochemists because its structure contains both polar and nonpolar regions. The polar alcohol functional group (–OH) gives ethanol hydrophilic properties, which means it readily dissolves in water and, therefore, the bloodstream. The hydrogen atom in the hydroxyl group carries a partial positive charge, while the oxygen atom carries a partial negative charge. These atoms form a hydrogen bond to the water molecules of the bloodstream. Hence, alcohol is readily transported throughout the body. Hydrogen bonds are electrostatic forces of attraction between partial positively charged hydrogen atoms and unshared electron pairs.

The nonpolar ethyl group (CH_3CH_2–) imparts hydrophobic properties to ethanol allowing it to dissolve readily in lipids and nonpolar solvents. Therefore, alcohol undergoes passive diffusion through the bilipid membrane layers of cells and is considered lipophilic. Lipid membranes are present in the stomach lining and the walls of the intestines.

If taken on an empty stomach, approximately 20 percent of the alcohol in a beverage is absorbed directly through the stomach lining; the remaining 80 percent is rapidly absorbed through the walls of the small intestine (*2*). Food delays the movement of alcohol into the small intestine and results in a decrease in the rate of absorption; carbonation, on the other hand, increases the rate. The amount of alcohol that accumulates in the bloodstream is reported in g/100 mL, known as blood alcohol concentration (BAC). All 50 states define a motorist as driving impaired (DUI) at a BAC level of 0.08 and above. Alcohol does not diffuse as rapidly into fatty tissue as body fluids; hence, gender and lean body mass play a role in actual BAC levels. In other words, women have higher amounts of fatty tissue and higher BAC levels than men of the same body weight.

The metabolism of alcohol is primarily accomplished in two steps by two major enzymes: alcohol dehydrogenase (ADH) and aldehyde dehydrogenase 2 (ALDH2). These enzymes are directly responsible for the disulfiram-ethanol reaction discussed later in this chapter. Knowledge of how these enzymes and alcohol interact and the chemical reactions involved provide a means to understand the effects of disulfiram on the body.

Alcohol dehydrogenase metabolizes nearly 95 percent of the alcohol ingested by a person. Liver alcohol dehydrogenase is responsible for 85 percent of this metabolized amount; gastric ADH accounts for the other 15 percent (*2*). In addition to these enzymes, some alcohol is converted into acetaldehyde (Figure 1b) by cytochrome P450 CYP2E1, 1A2, and 3A4 enzymes and catalase (*3*). About five percent of alcohol is excreted unchanged, mainly through the lungs, which accounts for the smell of alcohol on a person's breath and forms the basis for breathalyzer tests.

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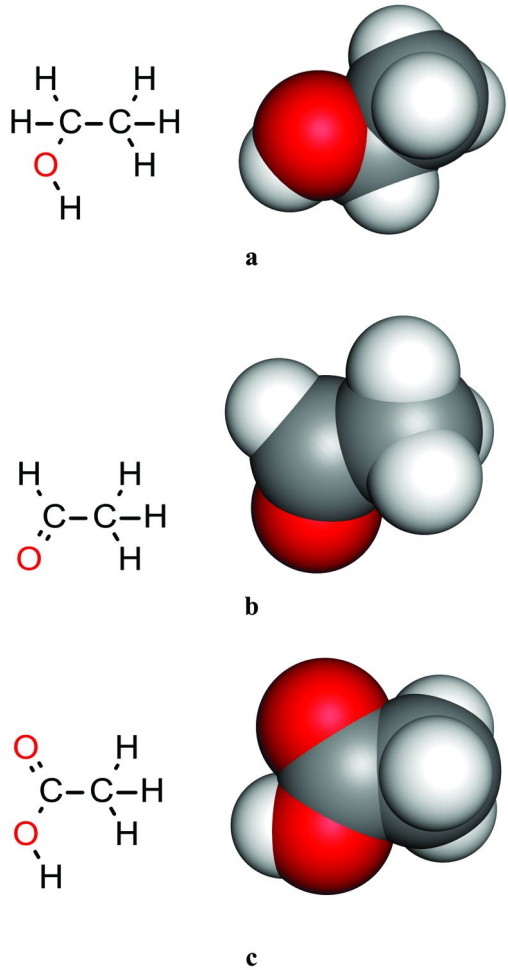

*Figure 1. Line formulas and spacefill structures of ethanol (**a**), acetaldehyde (**b**), and acetic acid (**c**).*

The dehydrogenase enzymes metabolize alcohol at the rate of about 6-8 grams per hour. This metabolic rate is independent of BAC and follows zero orde kinetics, which means that BAC is linear with time. If a standard drink contains about 14 grams of alcohol and the activity level of the enzymes is constant over time, then it takes the body about two hours to metabolize one drink. The number of drinks consumed in an hour has a direct effect, therefore, on an individual's BAC. If a 175-lb male consumes one 12-oz can of beer in one hour, he is considered to have consumed one standard drink or 14 grams of alcohol. If this

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amount of alcohol is distributed in 50 liters of body fluid, his initial BAC is about 0.028. If this individual, instead, consumes three 12-oz cans of beer in an hour, his initial BAC increases to 0.084. After one hour, 6 grams of alcohol are metabolized and the BAC decreases by 0.012 to 0.072. To estimate a BAC before driving, use the BAC calculator at this website: http://www.intox.com/drinkwheel.aspx.

Dehydrogenases are enzymes that catalyze oxidation-reduction (electron transfer) reactions in the body. The products of the two-step enzyme oxidation of alcohol are the same as those obtained in the organic chemistry laboratory but under milder conditions and greater control of the reaction. While metal ions are used as the oxidizing agents in the laboratory, enzyme cofactors act as the oxidizing agents in the body. Oxidizing agents are the chemical species that accept electrons and undergo reduction.

The conversion of ethanol to acetaldehyde is a two-electron oxidation that occurs with the removal of two hydrogens in the form of H^+ and H^-. Nicotinamide adenine dinucleotide (NAD^+, Figure 2) is the enzyme cofactor that serves as the oxidizing agent that accepts the two electrons as a hydride transfer and is subsequently reduced to NADH. The reaction can be broken into oxidation and reduction half-reactions. Oxidation half-reactions show the loss of electrons as products. Reduction half-reactions show the gain of electrons as reactants. Equations 1 and 2 show the simplified oxidation and reduction half-reactions, respectively, for alcohol. Equation 3 shows the net reaction equation for the enzyme-catalyzed reaction.

$$CH_3CH_2OH \rightarrow CH_3CHO + 2H^+ + 2\,e^- \quad (\mathbf{1})$$

$$2\,e^- + 2\,H^+ + NAD^+ \rightarrow NADH + H^+ \quad (\mathbf{2})$$

$$CH_3CH_2OH + NAD^+ \rightarrow CH_3CHO + NADH + H^+ \quad (\mathbf{3})$$

The similarity in the size and structure of the spacefill models of ethanol and acetaldehyde (Figure 1a and 1b) explains the ability of ADH to function in the reverse direction when necessary.

Acetaldehyde is toxic and is rapidly metabolized by ALDH2 to acetate (acetic acid, Figure 1c); however, it may also form adducts with other biochemical molecules, such as proteins and DNA, and cause harmful effects to organs. The NADH is oxidized back to NAD^+ in the mitochondria by the electron transport chain (*3*).

The conversion of acetaldehyde to acetate also involves a two-electron oxidation and uses NAD^+ as the oxidizing agent. In this oxidation, however, an oxygen atom is added to acetaldehyde via hydride transfer and hydrolysis of a thioester bond. The simplified oxidation and reduction half-reactions are shown in Equations 4 and 5, respectively, for acetaldehyde. Equation 6 shows the net reaction for the enzyme-catalyzed reaction. Acetate ion is shown as the product rather than acetic acid (CH_3COOH) because at cellular pH (~7.40) the acid-ionization equilibrium ($pK_a = 4.72$) favors acetate formation.

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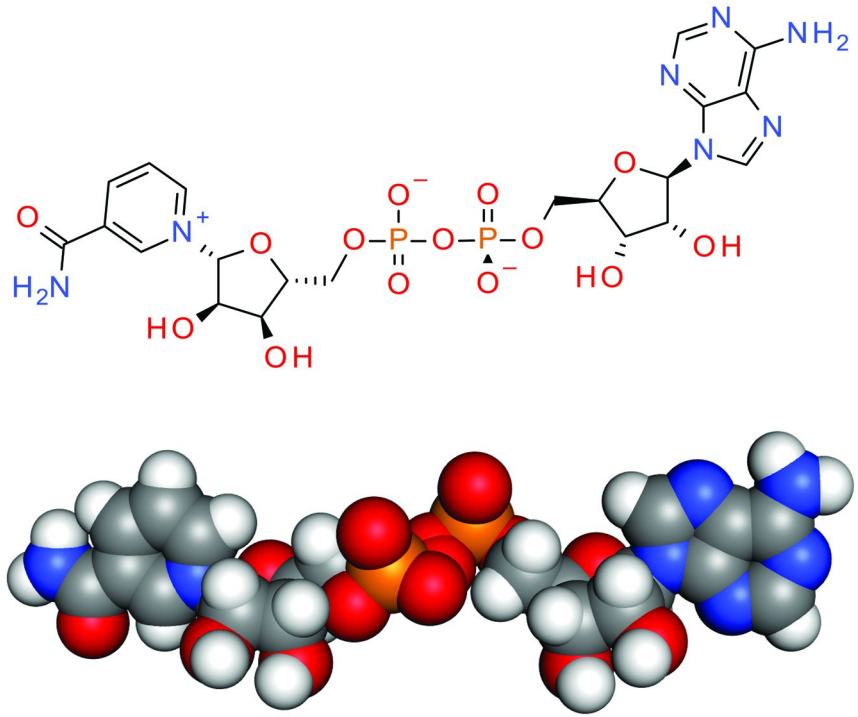

Figure 2. Line formula and spacefill structure of NAD^+.

$$H_2O + CH_3CHO \rightarrow CH_3COO^- + 3\ H^+ + 2\ e^- \quad (4)$$

$$2\ e^- + 2\ H^+ + NAD^+ \rightarrow NADH + H^+ \quad (5)$$

$$H_2O + CH_3CHO + NAD^+ \rightarrow CH_3COO^- + 2\ H^+ + NADH \quad (6)$$

Acetate enters the bloodstream and is further oxidized to CO_2 and water in the heart, brain, and skeletal muscle by the tricarboxylic acid cycle. Figure 3 shows the oxidation reactions responsible for the metabolism of ethanol.

Ethanol can be used as a fuel in the body. The eventual conversion (burning) of ethanol to CO_2 and water supplies energy. Equation 7 shows the overall reaction equation for the complete oxidation of ethanol.

$$CH_3CH_2OH + 7/2\ O_2 \rightarrow 2\ CO_2 + 3\ H_2O \quad (7)$$

The metabolism of ethanol into CO_2 and water releases 28.3 kJ/g of free energy. The calories obtained from ethanol are considered “empty” calories as the body does not use them to build important biomolecules, such as proteins or carbohydrates. In the electron transport chain, the oxidation of NADH to NAD^+ is coupled to ATP synthesis.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

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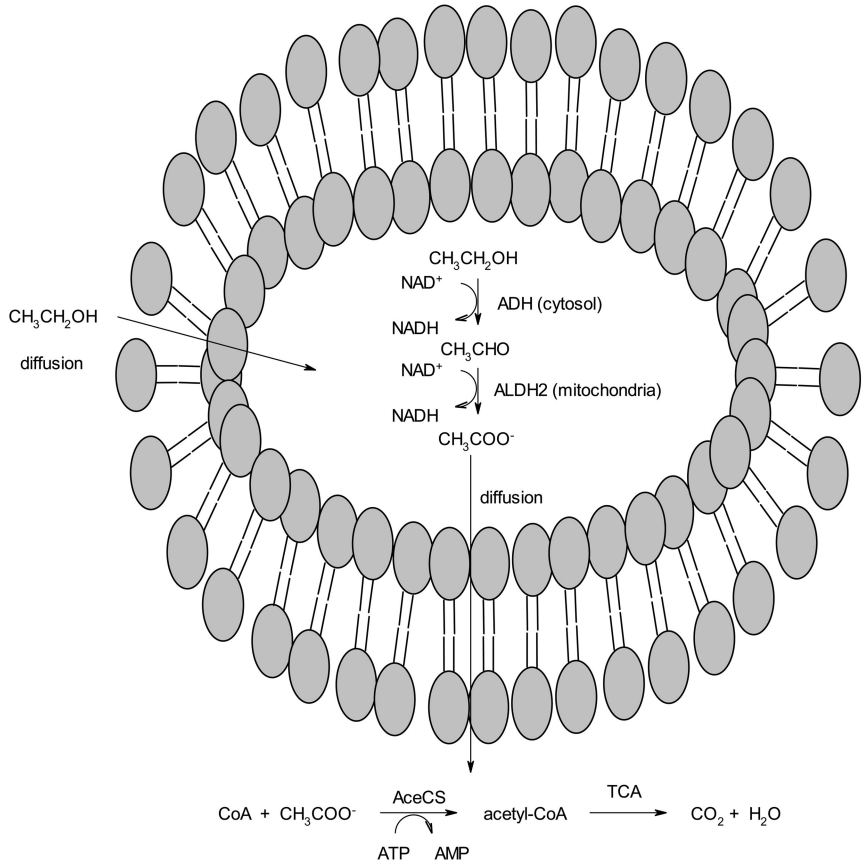

Figure 3. Metabolism of alcohol by liver enzymes.

Alcohol Dehydrogenase

Dehydrogenases are proteins that exhibit the secondary alpha helices and β-pleated sheet structures found in other proteins. Hydrogen bonding between NH and C=O along the polypeptide backbone is responsible for these secondary structures. The polypeptide backbone forms by removing a water molecule from the α-amino group on one amino acid and the carboxylic acid group on the next amino acid in the protein structure. The folding of the enzyme into its proper shape depends on tertiary interactions of the side chains. When nonpolar side chains are involved, hydrophobic interactions are important. The hydrophobic nature of these side chains cause folding towards the interior region of the enzyme to avoid contact with water. When uncharged polar side chains are involved, hydrogen bonding becomes important. When charged polar side chains

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

are involved, ionic salt bridges are important. Both hydrogen bonding and salt bridges are found on the surface of the enzyme and in the interior domains. Dehydrogenases exhibit all three types of tertiary interactions. The secondary and tertiary structures are crucial for binding the correct substrates and coenzymes. Loss of this functionality, which occurs in the case of the drug disulfiram, inactivates the enzyme. The structures of these two enzymes are discussed in detail to provide a basis for understanding disulfiram's role in alcohol metabolism and its use in the treatment of alcoholism. The proposed mechanism of oxidation by each enzyme is discussed for further clarification.

Amino acid residues are numbered from the N-terminus to the C-terminus end. Alcohol dehydrogenase is a homodimeric zinc metalloenzyme with 374 amino acids and two zinc atoms in each subunit (*4*). The coenzyme NAD^+ of each subunit occupies the binding domain formed by residues 176-318. The secondary structure of 45 percent of these residues is helical and 32 percent β-pleated sheets. The domain consists mainly of a central β-pleated sheet of six parallel strands surrounded by five α-helices. The folding of the polypeptide chain creates a crevice of mainly hydrophobic side chains. This unique set of nonpolar side chains is necessary to bind the adenine moiety of the NAD^+ coenzyme. The pocket lining includes the nonpolar amino acids phenylalanine, valine, isoleucine, proline, and glycine. In addition to the hydrophobic interactions, NAD^+ is held in place by hydrogen bonding and salt bridges to the charged amino acids aspartic acid, lysine, and arginine and the polar uncharged amino acids threonine and asparagine. The side chain interactions function to correctly position NAD^+ for ease of hydride transfer to the nicotinamide ring during alcohol oxidation.

The catalytic domain (*4*) has two sets of residues: 1-175 and 319-374. Thirty-five percent of the residues form three distinct and almost exclusively anti-parallel β-pleated sheet regions. Only 19 percent of the residues assume helical secondary structure. These secondary structures twist to form four separate hydrophobic cores within the domain. In addition, the two zinc atoms in the subunit act as Lewis acids (electron pair acceptors) and form coordinate covalent bonds to ligands in this domain. This is important to the ability of ADH to bind alcohol during its oxidation to acetaldehyde.

The coenzyme-binding domain and the catalytic domain are separated by a cleft that contains a broad and deep hydrophobic pocket (*4*). This pocket has been identified as the most probable site for substrate (alcohol) binding. Only four of the residues lining the pocket are polar. The remaining residues are the nonpolar amino acids leucine, valine, phenylalanine, proline, isoleucine, and methionine, which create an extremely hydrophobic environment for the active site. One of the zinc atoms, namely the catalytic zinc atom, is located at the bottom of this pocket. This atom is bound to the sulfurs in two cysteine residues and a nitrogen from a histidine residue. Tetrahedral coordination is completed by water or OH^-. Alcohol replaces this fourth coordination site during catalysis (Figure 4). Cysteine is an amino acid with a thiol group (–SH) at the end of the side chain. Thiol groups are known to undergo oxidation to remove the H atom. This often results in a disulfide bridge when two cysteine residues are involved. Disulfide bonds can be reduced back to thiols, a reaction which occurs during the metabolism of disulfiram in the body.

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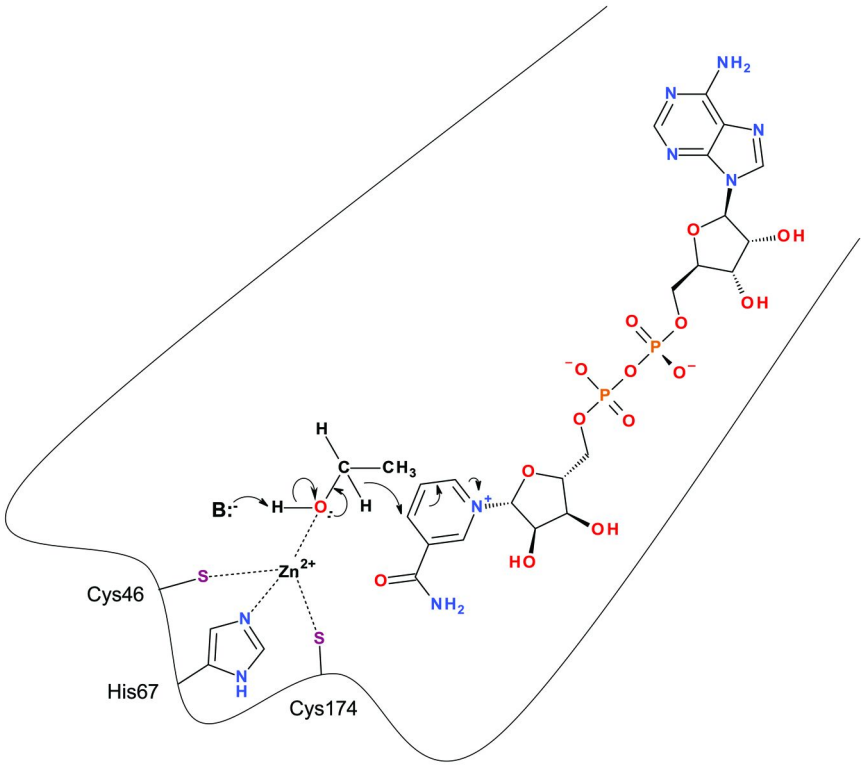

Figure 4. Mechanism of ethanol oxidation in the active site binding pocket of ADH.

The second Zn atom is found nearer the globular surface of the catalytic domain. Sulfur atoms from four cysteine residues are bound to this zinc atom in a distorted tetrahedral arrangement. The function of this zinc atom is unknown at the present time.

The two subunits combine to form the dimer mainly through their coenzyme-binding domains (*4*). Both hydrogen bonding and hydrophobic interactions are responsible for producing the strong attraction between the two subunits, with the hydrophobic interactions making the larger contribution. A central core develops as the domains approach each other. The overall shape has the appearance of a dumbbell with a catalytic domain at each end. Two active site pockets form at the junctions of the two catalytic domains and the core. The inner region of each active site contains the catalytic zinc atom, the nicotinamide group of NAD^+, and the reactive part of the substrate (alcohol). Each active site is buried inside its own subunit. Figure 5 shows the pocket with the products of the reaction between ethanol and NAD^+.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
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Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch014

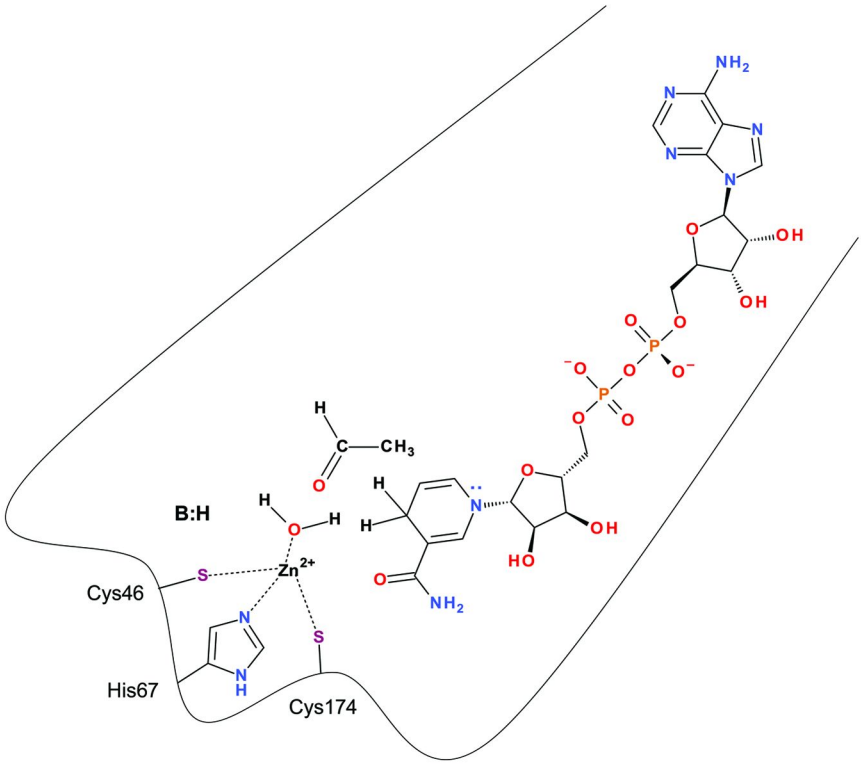

Figure 5. Acetaldehyde and NADH in one of the two active binding pockets of ADH.

Aldehyde Dehydrogenase

Aldehyde dehydrogenase is a homotetrameric enzyme with 500 amino acids in each subunit (*5*). Approximately 39 percent of the amino acids in the subunit form a set of fourteen α-helices and 25 percent form nineteen β-sheets. Each subunit has a coenzyme-binding domain, a catalytic domain, and an oligomerization domain. The coenzyme-binding domain for NAD^+ includes residues 8-135 and 159-270. The first set of residues has four β-strands and three α-helices. The second set has five β-strands and five α-helices. These residues interact with the adenine ring through hydrophobic interactions. The adenosine ribose unit uses its hydroxyl groups to bond to the protein. The phosphate linkage also hydrogen bonds to the protein. The nicotinamide ribose is held in place by two hydrogen bonds and a single hydrophobic interaction. The nicotinamide ring is held in position by hydrophobic interactions and a single hydrogen bond. As in ADH, the side chains play an important role in positioning NAD^+ for optimal hydride transfer to the nicotinamide ring. The catalytic domain is composed of residues 271-470. Seven β-strands and six α-helices are found within this domain.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch014

Residues 140-158 and 486-495 form a three-stranded antiparallel β-sheet structure known as the oligomerization domain (*5*). This domain provides the interactions necessary for the tetramer to assemble. Initially, two subunits form a dimer through contact between an α-helix and β-sheet in one subunit with an α-helix and β-sheet in another subunit. The two α-helices involved in dimer formation are in the coenzyme-binding domains. The β-sheet interactions bridge both the catalytic and oligomerization domains. The tetramer assembles when a β-strand in the oligomerization domain of one dimer forms an antiparallel interaction with a β-strand in the oligomerization domain of the second dimer.

The active site is located at the base of a hydrophobic tunnel lined mainly with aliphatic and aromatic side chains (*5*). The tunnel lays 12 Å below the surface of the enzyme. The base is separated into two halves by the nicotinamide ring. A cysteine residue in the catalytic domain region of the active site is believed to act, after deprotonation, as a nucleophile in the oxidation mechanism. A tetrahedral thiohemiacetal oxyanion transition state is proposed (Figure 6).

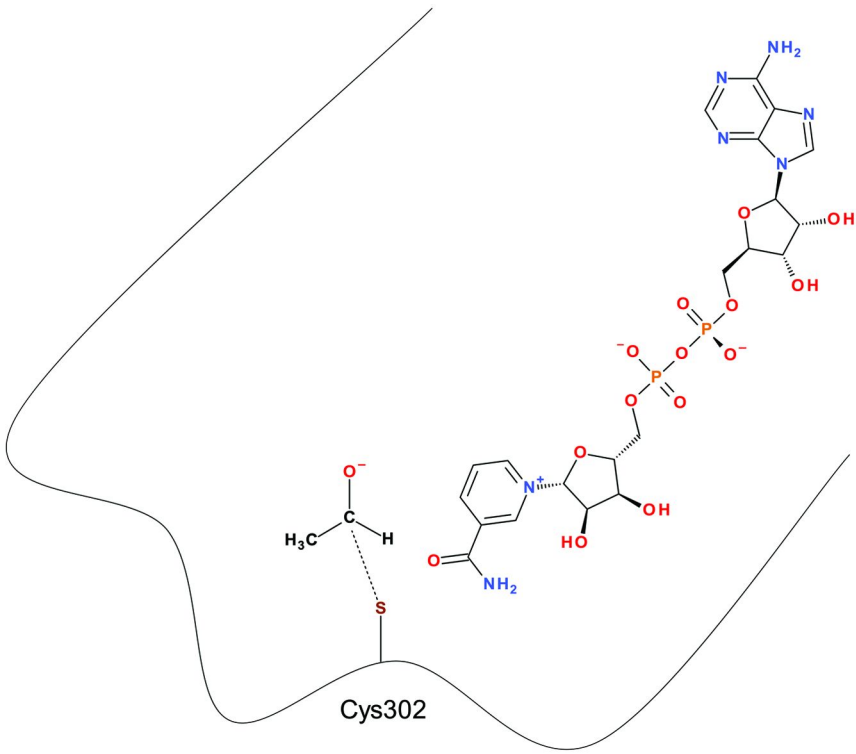

Figure 6. Tetrahedral intermediate for acetaldehyde in the active site of ALDH2.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

The active sites of ADH and ALDH are also capable of oxidizing methanol and ethylene glycol. The secondary and tertiary structures of these enzymes have a shape that also accommodates the shape of these alcohols and binds them in the active sites. Upon ingestion, methanol is converted to formaldehyde and ultimately formic acid. Formic acid is known to cause blindness in humans. Glycolic and oxalic acids are oxidation products of ethylene glycol that are toxic to the human body. Since ADH preferentially binds ethanol, ethanol is often used as an antidote in these types of poisoning cases to give the body time to flush the toxins from the bloodstream.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch014

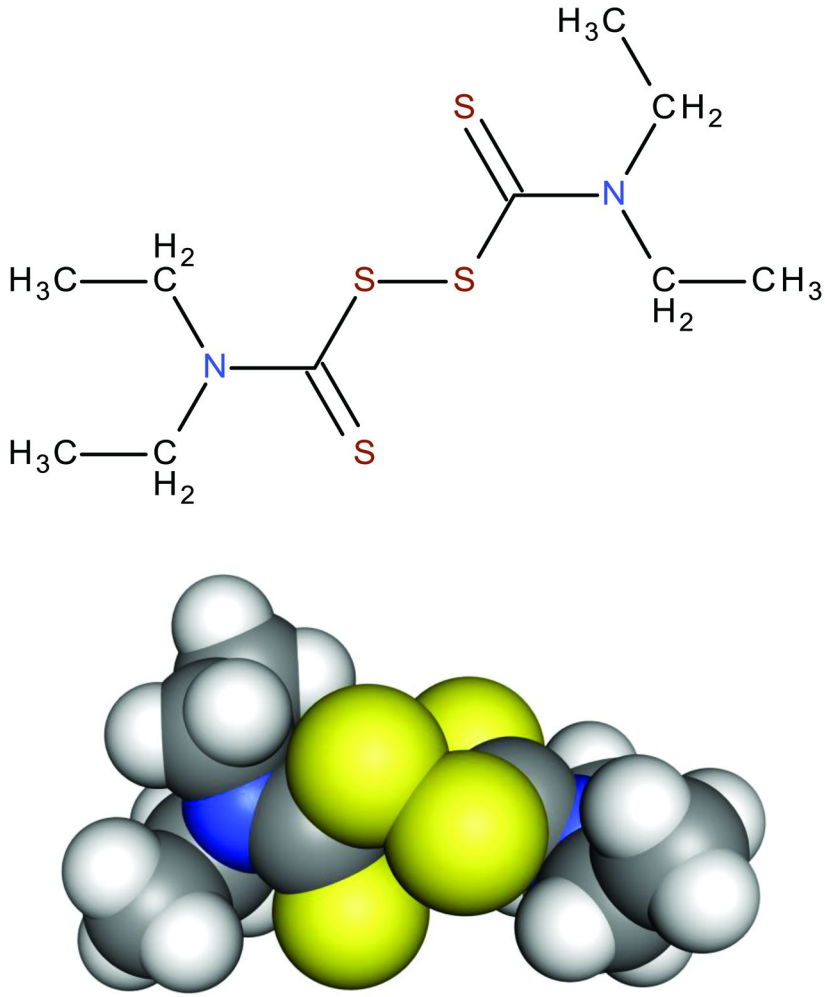

Figure 7. Line formula and spacefill structure of disulfiram.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch014

Disulfiram

Disulfiram, trade name Antabuse, is an FDA-approved alcohol-aversive agent, which is prescribed for chronic alcoholism as part of a psychosocial program to eliminate alcohol consumption in patients who express a desire to abstain from drinking. Treatment usually involves taking a daily 250-mg tablet until the patient exhibits long-term abstinence.

Chemically, disulfiram has the IUPAC name 1, 1′, 1″, 1‴-[disulfanediyl-bis(carbonothioylnitrilo)]tetraethane (Figure 7). The disulfide bond is important in its pharmacological action. Disulfiram is an irreversible inhibitor of ALDH, but not ADH, enzymes found in the cytosol and mitochondria of liver cells. Inhibition of ALDH slows the oxidation of acetaldehyde to acetate when alcohol is consumed. Acetaldehyde is a toxic substance that produces a set of unpleasant symptoms (Table 1) as the serum levels rise 5 to 10 times the concentration seen in normal alcohol consumption. This pharmacological condition is known as the disulfiram-ethanol reaction (*6*).

Table 1. Possible Effects of the Disulfiram-Alcohol Reaction

Body part affected	*Moderate*	*Severe*
Body skin	Sweating	None
Respiratory system	Hyperventilation Respiratory difficulty/ dyspnea	Respiratory depression
Head, neck, and throat	Acetaldehyde breath odor Blurred vision Head and neck throbbing Thirst	None
Stomach, digestive system	Nausea/vomiting	None
Chest, heart, circulatory system	Chest pain/palpitations Hypotension Tachycardia	Cardiovascular collapse Arrhythmia
Brain/nervous system	Vertigo Syncope Marked uneasiness Confusion	Seizures
Other	Weakness	Death

(Adapted from Ref (*6*). Copyright 2009. Substance Abuse and Mental Health Services Administration).

The disulfiram-ethanol reaction has an onset of action within 10 to 30 minutes after the ingestion of alcohol. BACs of 0.005-0.010 produce mild effects; symptoms are fully developed at BACs of 0.05-0.10. Unconsciousness may result

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

if the BAC reaches 0.125-0.150. The drug appears to have no effect, however, on the rate of alcohol elimination. A patient's awareness of the aversive responses given in Table 1 is believed to provide the motivation to abstain from drinking.

Some of the disulfiram-ethanol reaction effects are induced by the release of neurotransmitters and other signaling molecules when an organ detects an increase in aldehyde concentration. The release of epinephrine and norepinephrine in the peripheral nervous system leads to negative cardiovascular consequences, while the release of histamine and bradykinin produce vasodilation and facial flushing (*7*).

One of the mechanisms proposed for the inhibition of ALDH by disulfiram involves the formation of an internal disulfide bond by two cysteine residues (*8*). This leads to an irreversible inhibition of the enzyme. The body now needs to synthesize more ALDH to replace the inactive enzymes. Since this is a slow process, the disulfiram-ethanol reaction may persist for up to two weeks (*6*), even in the absence of disulfiram dosing.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch014

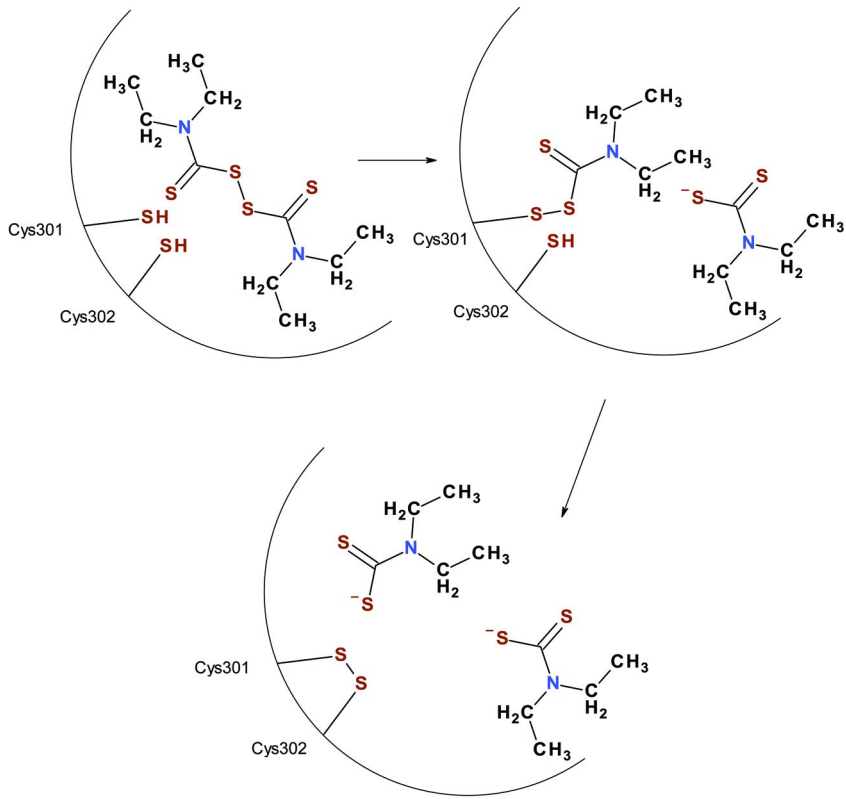

Figure 8. Mechanism of disulfiram inhibition of ALDH.

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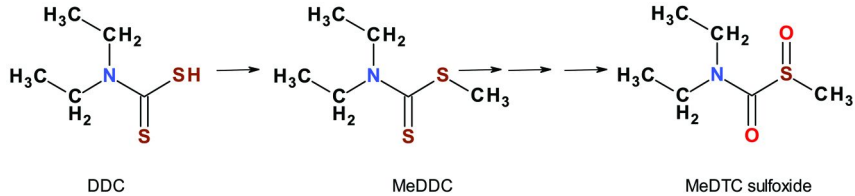
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

The proposed mechanism for the inactivation of ALDH by disulfiram involves the reactions (*8*) shown in Figure 8. A covalent bond is formed between diethyldithiocarbamate (DDC) and a cysteine residue in the active site pocket after the disulfide bond in disulfiram undergoes reduction. This prevents the thiol group from forming the oxyanion tetrahedral intermediate with acetaldehyde. DDC is released when the internal disulfide bond between two cysteine residues forms.

The body further metabolizes the DDC produced to its methyl analogue (MeDDC). This metabolite is further oxidized and ultimately converted into *S*-methyl-N,N-diethylthiocarbamate sulfoxide (Scheme 1), which is thought to be the actual active inhibitor of ADLH (*9*).



Scheme 1

(Adapted with permission from Ref. (*9*). Copyright 2001. The American Society for Pharmacology and Experimental Therapeutics.)

About 80-95 percent of an oral dose of disulfiram is absorbed from the gastrointestianl tract. It has a high lipid solubility and is rapidly distributed to tissues and organs with an elimination half-life of 7–8 hours. The elimination of disulfiram is believed to involve the metabolites above as well as diethylamine and carbon disulfide. Carbon disulfide is expelled during respiration. Diethylamine is eliminated in the urine. DDC metabolites are typically eliminated in the urine as glucuronide conjugates (*10*).

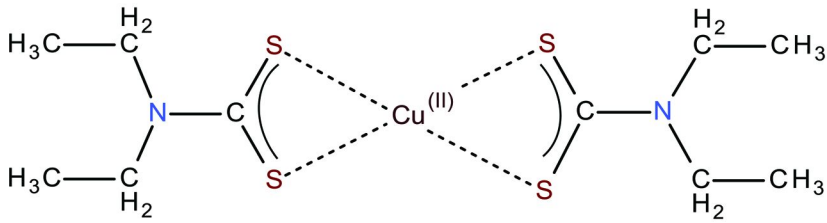


Figure 9. Chelation of Cu(II) with DDC.

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch014

Disulfiram and its metabolite DDC are also being studied as anticancer agents for the treatment of melanoma, breast, and ovarian cancers (*11–13*). Several mechanisms for this cytotoxicity have been proposed: covalent bonding to enzyme thiol groups, which blocks the active site; chelation of divalent copper and zinc ions (Figure 9), which inhibits copper- and zinc-dependent enzymes by depleting the intracellular availability of these ions as cofactors; redox cycling of the metal ion in the chelation complex, which induces cellular oxidative stress; and inhibition of proteasomal degradation of proteins, which interferes with cancer cell protein homeostasis.

In the central nervous system, dopamine and norepinephrine are neurotransmitters that provide positive feelings of reward and reinforce drug behavior. Disulfiram inhibits dopamine β-hydroxylase, an enzyme necessary for the conversion of dopamine to norepinephrine in noradrenergic neurons (*14*). Dopamine β-hydroxylase is a copper-dependent enzyme that can be inhibited by the DDC metabolite of disulfiram by the same mechanism found in cancer cells. This inhibition lowers the norepinephrine to dopamine ratio, which leads to changes in the responses of the brain's reward pathway and an increase in aversion effects, such as anxiety, headache, and fatigue. These unpleasant side effects are central to the use of disulfiram to promote cocaine abstinence and prevent relapse during and after treatment for addiction.

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch014

Summary

NAD^+ is the coenzyme in both ADH and ALDH2 that acts as the oxidizing agent in ethanol metabolism. ADH and ALDH have thiol groups at their active sites that participate in tetrahedral coordination with their substrates. Disulfiram bonds to thiol groups at the active site in ALDH and irreversibly inhibits the oxidation of aldehyde to acetate. This leads to high acetaldehyde serum levels and the disulfiram-ethanol reaction. This reaction serves as a deterrent to drinking in motivated individuals. In addition, disulfiram is being studied for its anticancer properties and as a possible treatment for cocaine addiction.

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Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ch014

Subject Index

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

C

D

E

F

G

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ix002

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ix002

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

V

Publication Date (Web): June 15, 2015 | doi: 10.1021/bk-2015-1189.ix002

In Ethanol and Education: Alcohol as a Theme for Teaching Chemistry; Barth, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.