Fermentation

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Fermentation throughout the ages

TABLE OF CONTENTS

a. advances in grape growing
b. advances in wine making
c. advances in preservation techniques

D. Wine making in the Middles Ages and Renaissance

E. Beer making from Antiquity to the 19th century

F. Microbiology revolution: Louis Pasteur
1. The scientific method
2. Pasteur's work in microbiology
3. after Pasteur
- biochemistry and fermentation
- yeast morphology and classification

G. Phylloxera, powdery mildew and modern grape growing

H. Exercises.

CHAPTER 3. THE GRAPES

A. Classification
1. Classification by botanical characteristics
a. Linneaus, evolution
b. types of grapes in wine making
- cepage
- varietal wines
c. pure bred versus hybrid grapes

2. Classification by flavor characteristics

B. Growing grapes
1. Climate
a. Degree - days
b. Mean temperature
- reading a graph
c. LTI
- calculating LTI
- relationship of LTI to wine quality
2. Rainfall
a. relationship with LTI
b. British and metric systems of measurement
- units in both systems
-dimensional analysis and unit conversion
3. Frost and grape growing

4. Soil conditions
5. Example of grape growing conditions: the Finger Lakes regions of New York State.
    a. conditions
        -topography
        -climate
        -selection of grape varieties
    b. growth cycle

C, Exercises.

CHAPTER 4. THE STRUCTURE OF PLANTS.

A. The plant body

B. The cell and its components.
1. Eukariots and Prokariots
2. More on the metric system
3. The components of the cell
    a. biological membranes
    b. cytoplasm
    c. nucleus
    d. chloroplast
    e. mitochondrion
    f. vacuole

C. Plant tissues
1. Meristematic tissues
    a. shoots
    b. roots
2. Permanent tissues
    a. xylem
    b. phloem

D. Anatomical regions
1. Vascular tissue
2. Cortex
3. Epidermis
    a. stomatas
    b. cuticle

E. Morphological structures
1. Root
    a. tap root system
    b. fibrous root system

  - c. vegetative reproduction
2. Shoot
3. Leaf
4. Flower
   - a. sepal
   - b. petal
   - c. stamen
     - filament
     - anthers
     - pollen
   - d. pistil
     - stigma
     - style
     - ovary
5. Fruit
6. Seed
   - a. seed coat
     - -function
     - -dormancy
   - b. endosperm
   - c. embryo

F. Exercises.

CHAPTER 5: YEAST

A. Classification

B. Biology of fungi

1. Role in nature
2. Morphological comparison to bacteria

C. Reproduction

1. DNA
2. Mitosis: asexual reproduction
3. Meiosis: sexual reproduction
4. Inheritance of genes
5. Evolution and cell division
   - a. mutations.
   - b. cross-overs, duplications and deletions.

D. Fermentation

1. Animals use sugar in the presence of oxygen
2. Alcohol is one result of anaerobic use of sugars
3. Measurement of sugar

4. Carbon dioxide
    a. production in fermentation
    b. poisons
        - definition in ecological systems
        - criteria for defining poisons
5. Yeast and Fermentation
    a. Beer making
        - diversity
        - selection criteria
    b. Wine making
        - still wine and champagne
        - selection criteria
6. Contamination

E. Exercises.

CHAPTER 6. WINE MAKING.

A. Introduction.

1. Definition of wine
    a. strict definition of grape and other fruit fermentations
    b. adulteration
2. Types of wine.
    a. red wines
    b. white wines
    c. rose wines
    d. naturally sweet wines.
    e. fortified wines.
        - Sherry
        - Port

B. Initial steps in wine making.

1. Introduction.
2. Grapes.
    a. sugar levels
    b. acid levels
        - elements and electronic configuration
        - bonds between atoms: compounds
        - covalents compounds
        - ionic compounds
        - acids and bases
        - concentration: the concept of mole and molarity
        - hydrogen ion concentration
        - neutralization reactions
        - titrations

- measure of acidity in grape juice

c. adulteration

3. Choice of grapes.
4. Pressing.
   a. effect on juice color
   b. free run
   c. carbonic maceration
   d. organic material contnet of juice
5. Sterility and the use of sulfur.
6. Fermentation.
   a. preparing a yeast starter
   b. starting wine fermentation
   c. primary fermentation
   d. secondary fermentation
   e. differences between red wine and white wine
   f. making red wine.
   g. fermenting white wine.
   h. secondary fermentation: malolactic fermentation
   i. racking
   j. fining.
   k. filtration.
   l. tasting and blending
   m. bottling
7. Equipment for making wine at home
8. Additional fermentations: sparkling wines.
   a. introduction.
   b. history of champagnization.
   c. making champagne
      - methode champenoise
      - transfer process
   d. other methods
      - bulk method.
      - $C0_2$ pumping method

E. Exercises.

CHAPTER 7. BEER MAKING.

A. Introduction

1. Definitions
   a. ale
   b. lager
2. Variety of beers in the world
3. History of modern beer making in the United States

B. The beer making process

1. Malting
    a. grain selection
    b. two versus six-row barley
    c. breeding new varieties
    d. malting process
        - objectives
        - enzymes
        - process
        - kilning
2. Mashing
    a. objectives
    b. adjuncts
    c. steps in mashing
    d. sparging
3. Post mashing
    a. hops
    b. process
    c. filtration
4. Fermentation
    a. yeast selection
        - ale
        - lager.
    b. criteria for yeast selection
        - high yeast count at racking
        - pH value
        - balanced production of flavor compounds
        - attenuation power
        - flocculation properties
        - head formation
        - haze level
        - taste test

5. Fermenting systems
    a. traditional ale fermenting system
    b. lager system
    c. continuous fermentation system
6. Maturation and conditioning
    a. lager
    b. ales
    c. ten-day-beer
7. Pasteurization

C. Diversity in beer making methods

D. Exercises.

CHAPTER 8. LIQUORS, SPIRITS AND DISTILLATION.

A. Introduction

B. Distillation
1. The states of matter.
   a. molecular attractions
   b. temperature
2. Vapor pressure and evaporation.
3. Distillation
4. Types of distillates
   a. Brandy and Cognac
   b. grain distillates
   c. other distillates
   d. liqueurs and cordials
5. Production and distillation of vinegar
   a. the chemistry of vinegar
   b. how vinegar is made

C. Exercises

CHAPTER 9. WINE AS FOOD AND MEDICINE.

A Introduction

B. Chemistry of wine
1. Organic chemistry
2. Content of wine.
   a. alcohols
   b. aldehydes
   c. acids
   d. carbon dioxide
   e. nitrogen compounds
   f. coloring
   g. extract and minerals
   h. esters
   i. vitamins

C. Physiological effects of wine
1. Alcohol
   a. ethanol
   b. other alcohols
2. Acids
3. Carbon dioxide

4. Tannins
5. Minerals.

D. Wine as food

E. Wine as medicine
1. Digestive system
2. Cardiovascular and respiratory systems.
    a. respiratory system
    b. cardiovascular system
3. Neuromuscular system
4. Miscellaneous

F. Alcoholism
1. Genetic alcoholism
2. American Indians and alcohol

G. Exercises

CHAPTER 10. MAKING CHEESE AND FERMENTED MILK

A. Introduction

B. A brief history of cheese making

C. Types of fermentation in milk
1. lactic acid formation
2. propionic acid fermentation
3. alcoholic fermentation
4. citric acid fermentation
5. butyric acid fermentation
6. vinegar and carbon dioxide fermentation

D. The composition of milk
1. evolution of mammals
2. characteristics of mammals
3. composition of milk
    a. proteins
    b. fats

E. Fermented milk products: yogurt

F. Cheese
1. the curdling process
2. removing the whey

3. molding and shaping
4. salting and shaping
5. ripening

G. Nutrition

1. proteins
2. fats
3.vitamins and minerals

H. Exercises

CHAPTER 11. OTHER USES OF FERMENTATION. (work in progress)

A. Fermentation and the making of

1. pickles
2. sauerkraut, fermented vegetables and Kimchi
3. salamis and other sausages
4. the production of:
   a. coffee
   b. chocolate
   c. vanilla

B. Fermentation and biotechnology

C. Fermentation and oil spill cleanup

D. Microbe therapy, biomining and waste treatment

Lesson 1

INTRODUCTION TO FERMENTATION AND FERMENTED FOODS

2 Figures
1 Table

A. INTRODUCTION

When we talk about fermentation, most of us assume that we are referring to the process used to make wine or beer, i.e. production of alcohol by microorganisms; however, fermentation plays a much greater role in our lives. In fact, the fermentation process accounts for a large part of the foods we eat. Sour cream, pickles, vinegar, sour dough, yogurt, sausage, cheese, bread, and sauerkraut have one thing in common: they are all fermented foods or they mimic fermented foods i.e. where humans introduce in the food the chemical products normally produced by microorganisms.

The definition of fermentation is actually very broad and encompasses various phenomena but in each case, has one thing in common - microorganisms introduced in food, voluntarily or not. These microorganisms can be bacteria, yeast or molds but all of them use the sugars in these foods to grow: bacteria, yeast and mold all break down the sugar to extract the energy, but they do it in different ways, with different end products. This means that there are different types of fermentation. (Fig. 1.1)

- You or I will break down sugar to CO_2 + H_2O if we have access to oxygen. If we are running out of oxygen (When does that happen?), we will produce lactic acid which crystallizes in the muscle cells with painful results.
- Yeast will break sugar down to CO_2 + H_2O in the presence of oxygen, but to alcohol + CO_2 in the absence of oxygen.
- Some bacteria will break sugar down to alcohol, acetic acid (vinegar) and lactic acid (Leuconostoc).
- Other bacteria will break sugar down to acetic acid only (Acetobacter).
- Some will produce lactic acid (Pediococcus or Lactobacillus) to help curdle milk in the cheese making process.
- Other bacteria will break sugar down to CO_2 and compounds that give the food an off-flavor or toxic properties that make it unfit for consumption (Pseudomonas, Klebsiella, Clostridium).

Therefore, we will define fermentation as a process that involves one of the following:

- Alcohol and alcoholic beverages
- Spoilage of foods by microorganism. Fermenting bacteria are not always good and the products they form may be considered undesirable in some foods but desirable in others. Vinegar is desirable in pickle fermentation but not in milk fermentation.

Toxins are also produced by some bacteria that cause illness or death.

- Metabolic process that release energy from a sugar without the use of oxygen. In such cases the microorganism does not need O_2 to grow.

For example, yeast will convert sugar to ethanol in the absence of oxygen. Other microorganisms however convert sugars into a variety of compounds that are also of use to humans, such as lactic acid, acetic acid (vinegar), acetone, methanol and glycerol. Table 1.1 and figure 1.1 show some of the compounds produced by these microorganisms.

Why do we ferment food? These products of fermentation will change the character of foods and either:

- Increase preservation aspects: acetic acid (vinegar preserves cucumbers for long periods of time as pickles.
- Increase flavor or aroma: these microorganisms often leaves in the fermented foods flavors that appeal to our taste buds; thus we say that fermentation enhances organoleptic properties of foods and drink.
- Increase nutrition: eating fermented foods means eating the microorganisms present in those foods with the vitamins that they have produced. Yogurt is more nutritive than milk in that respect.
- Food can be cleaned up by fermentation (chocolate, coffee). The chocolate bean is covered with pulp that is digested away by bacteria and yeasts (the process takes about a week). The end product is a bean that has been loosened from its liquefied pulp. The fermentation process also changes the flavor, aroma and color of the cocoa bean.

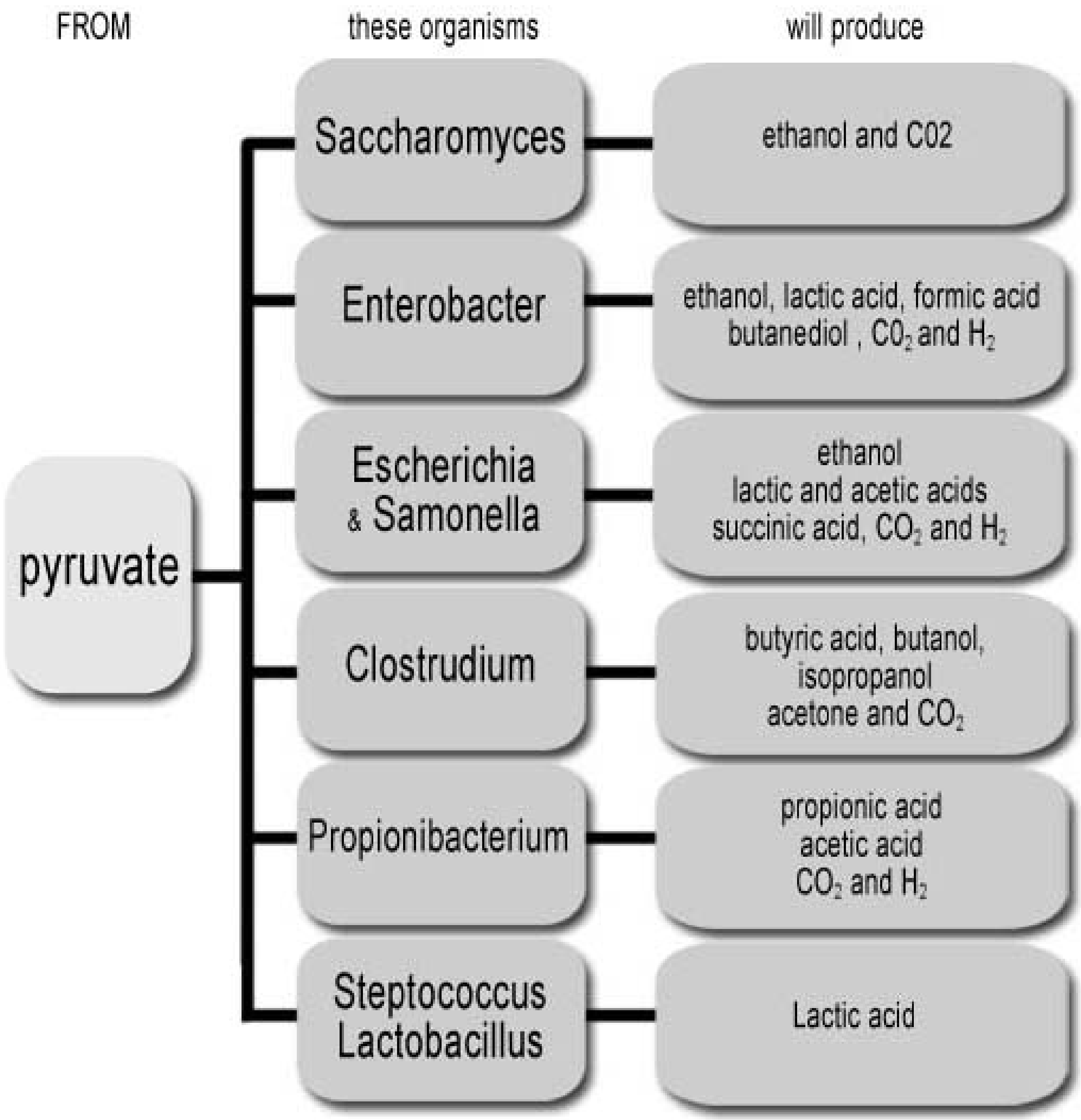


Figure 1.1 The products of fermentation for various organisms when the starting material is pyruvate. Notice that the end product will vary.

This Organism	uses	to produce	in
Saccharmoyces Cerevisiae (FUNGUS)	grain extracts	ethanol	beer
Saccharmyces ellipsoideus (FUNGUS)	fruit juices	ethanol	wine
Saccharomuces Streptococcus (BACTERIA)	milk	lactic acid	cheese
Lactobacillus Bulgarious (BACTERIUM)	grain, sugar	lactic acid	rye bread
Pediococcus (BACTERIUM)	meat, cabbage	lactic acid	sausage and sauerkraut
Acetobacter (BACTERIUM)	alcohol	acetic acid	vinegar
Propionibacterium Freudenrichii (BACTERIUM)	milk	propionic acid	swiss cheese
Aspergillus (FUNGUS)	molasses, soy, milk tofu	citric acid	flavorings soy sauce and tempeh
Clostridium Acetobutylicum (BACTERIUM)	molasses	acetone, butanol	industrial products
Clostridium	agricultural wastes	methanol	fuel
Saccharomyces cerevisiae	molasses	glycerol	industrial products

TABLE 1.1 Fermentation Products

B. ORIGINS OF FERMENTATION:

Go back to when humans were barely such, ignorant of their worlds, ascribing natural phenomena to the works of the Gods. However, human emerging intellect enabled them to remember experiences and learn and profit from them. Even if they did not understand these phenomena they could still take advantage of them.

Using their experience, humans began the transition from “gatherer” to “food producer” through agriculture and animal husbandry. These people had to realize quickly that food could be produced only periodically during the year and the abundance at production time (harvest) had to be stretched over periods of non-production. Storage and preservation as well as the methods to attain such goals were a matter of luck, random occurrence and the keen sense of observation by individuals of the group.

EXAMPLE: 10-15,000 years ago, certain animals could be milked and the milk stored in crude bags or pouches made of animal skins. When stored this way, two possible types of change could occur in the milk:

- The milk became sour, thicker, or turned to a solid curd. The curd had pleasant acidic odor and was soft and smooth. It could be eaten without health problems and preserved for long periods of time: Lactobacillus bacteria were responsible for these changes and the end product was cheese.
- The milk became roiled and gassy, acquiring a non-appetizing flavor. It induced sickness if eaten. Salmonella bacteria were responsible for these changes.

Humans learned to differentiate between these 2 types of fermentation through careful observation. They also learned the circumstances that led to either outcome and selected the proper process for cheese making (through the use of starters i.e. the introduction of some cheese in a fresh batch of milk) versus spoiling. These methods produced foods that were edible, stable upon storage (acids and alcohol act as preservatives) and, in some cases, possessed certain exhilarating properties (alcohol). Note again that while these observations were the result of random or accidental occurrences, over the next few thousand years, humans were able to build on these experiences to develop a reliable and reproducible process.

1. Bread

Humans, as farmers, soon discovered that grasses provided a source of foods that were nutritious and could be stored easily: Sugar cane, bamboo, wheat, corn, rice, oats, barley, millet are such grasses. What is millet? Millet is commonly known as sorghum; it is a grass similar to wheat and barley that is fed mostly (with its grain) to animals.

In the storage areas of early Indian cities in Nevada, we have recently found 1000 year old corn kernels. These corn seeds were so well preserved that some of them germinated. In the Bible Joseph recommended that the Egyptians stockpile grain for the seven lean years to come. In proper conditions of humidity and temperature grass seeds can be stored for years after drying without spoiling. This helped humans survive through droughts and other disasters – until their next harvest. And, we still do the exact same thing today. The world continues to maintain reserves of wheat, corn rye etc. in case of drought or famine.

Around 7,000 BC humans (probably Egyptians) somehow learned to grind grains in water and heat the mix on hot stoves to make unleavened bread. Later (around 3,500 BC) the Egyptians found that the bread became airy and lighter if the dough was left unattended several hours prior to baking. The dough expanded and the resulting bread was light and spongy. While the Egyptians attributed this phenomenon to the action of benevolent Gods we now know that the leavening of bread is due to the production of carbon dioxide by yeast that deposited on the dough when left unattended for some time before baking. Leavening (or fermentation) was used from then on in bread making. This process soon spread to other cultures as the Egyptians conquered more lands. While the Jews most likely learned from the Egyptians they continued to use unleavened bread in religious ceremonies.

Grinding the grains between stones made for crude bread. That meant that the bread made back then had very little resemblance to what we have today. The leavening process used did not make the bread as “fluffy" as what we eat today because the shards left by the crude grinding methods burst the bubbles of carbon dioxide and reduced the rising. In fact, I tried a crude grinding method a few years ago and the result was a very heavy, dense bread. Grinding methods improved over the millennia and as the Roman writer Pliny stated - a quality bread depends on good wheat and fine flour (well ground).

The baker had to maintain a yeast starter for his bread since he could not buy his yeast in packets at the supermarket. And since aseptic techniques were not known back then the dough was actually populated by a mixture of yeasts and bacteria. Thus the breads of Antiquity were more like coarse sourdough breads made by a mixture of bacteria and yeast that produced CO_2 , alcohol and acids. Through trial and error they obtained a mix of bacteria and yeast in their starter that would sour the dough (pleasantly) and thus prevent spoilage. Did you ever eat sour dough bread? What did you think?

This means that they had to observe, over centuries, that carrying over a small amount of dough from a previous batch of bread soured the new bread, preserved it and made leavening a uniform process (leaving the dough unattended for hours may not have always introduced yeasts). Romans also used yeasts collected from wines to make bread.

Today we leaven bread with yeast, bacteria, or a mix. The latter 2 are called sour dough such as rye bread or pumpernickel bread. Most sourdoughs are obtained with a mixture of yeast (Saccharomyces cerevisiae) and bacteria (Lactobacillus brevis and Lactobacillus plantarum).

2. Milk

Humans soon realized that milk was very nutritious; a source of fat, vitamins and proteins and they became faced the challenge of preserving it after production (after the young were born) because they could not make these animals lactate year round as we do today. Milk was revered (as well as the cow and goat) and used extensively as a remedy, food, cosmetic, and in religion.

Egyptians used butter and cheese around 3000 B.C. Butter and Cheese were made from cow's milk. The Greeks used goats and the Romans used sheep so for the most part used cheese as preservative rather than sour milk.

a. Fermented milk

Fermented milk was made in various parts of the world and varied in texture and in taste depending on the type of microorganism used and the conditions of fermentation. Around 3000 B.C. Sumerians fermented milk by pouring fresh milk in small-mouth containers that permanently harbored the cultures of bacteria. Jewish tribes (Abraham was said to have done this) fermented milk into leben as did the Egyptians, Syrians and Palestinians. Different names are given to fermented milk in different parts of the world:

- Yogurt is made by Turkish and Caneasus people.
- Taettemjolk is made in Scandinavia.
- Skyr is made in Iceland.
- Mazun is made in Armenia.
- Cieddu is made in Italy.
- Dahi is made in India.
- Kefir is made in the Balkans (an acid-alcoholic milk drink similar to yogurt in texture but containing up to 3% alcohol).
- Kumiss is made in Mongolia

b. Cheese

Cheese is the curd obtained when bacteria produce acids that coagulate the milk leaving behind a clear liquid, the whey. It is different from fermented milk in that it is precipitated into a solid mass. Fermented milks do not coagulate and remain more liquid.

You can make cheese without using bacteria by putting in the milk, the acid produced by bacteria or a similar compound that will coagulate milk: vinegar and/or rennet, a compound produced by lining of the stomach. This sour milk curdles into a cottage cheese-type - a relatively flavorless product. These curds can then be aged with other bacteria or molds to produce flavored cheeses. For examples of the variety of effects produced by the aging processes look at brie (aged with bacteria) versus camembert, Blue cheese, cheddar or Roquefort (aged with molds). Incidentally while we tend to dislike molds for the green or black fuzz they cover foods with when left in the refrigerator a little too long they actually are cousins of yeasts and belong to the same Kingdom, the Kingdom of the Fungi.

The various processes, bacteria used, aging methods etc. have given rise to about 1000 different types of cheeses. A famous ruler in 13th Century France (Mazarin) once said that it was impossible to unite and rule a country (France) that produced 325 different types of cheese. At that time England produced only one cheese - the cheddar.

3. Fermented Vegetables

This practice started in Antiquity from the observation that vegetables packed in closed containers (no air) with salt or brine, changed flavor but remained good tasting and could be preserved. Remember that in Antiquity preservation was indispensable since refrigerators had not yet been invented.

The pickling process may have started in Southeast Asia. Understand that today, we pickle by adding vinegar (acetic acid) to vegetables. In Antiquity, they could only hope that the organism making the acid (lactic acid or acetic acid) would be the first and only one to grow. Salts helped in that they prevented the growth of other, salt-sensitive organisms which would have spoiled the vegetables.

Documents show that the Great Wall of China was built by Ch'in Shih Huang Ti around 300 B.C. He provided fermented vegetables as part of his workers' ration (all kinds of fermented vegetables were used). This diet kept his workers healthy and the great undertaking could therefore be completed.

Romans liked pickled cucumbers but also grew cabbage and used it to make fermented foods such as sauerkraut. They also pickled olives in brine and added them to wine to mask off-flavors. Both the Greeks and Romans ate pickles and sauerkraut as food and used them as medicine. The Germans who were nomadic, must have learned the art of making kraut from the Romans.

Today 75% of Americans eat fermented vegetables at least once a week, mostly cucumbers but also onions, cauliflower, celery, beets. We now know that any vegetable can be fermented. To do that one needs sugar (from the vegetable), salt to inhibit the growth of harmful microorganisms and no air (to inhibit aerobic bacteria).

As shown in Figure 1.2, in a first step, Leuconostoc mesenteroides starts the fermentation and destroys compounds in the vegetable that could slow bacterial growth. Then, Lactobacillus brevis, Pediococcus cerevisiae and Lactobacillus plantarum will grow in sequence, producing lactic acid in anaerobic (with no oxygen present) conditions. The environmental conditions, number and types of organisms present, salt concentration and temperature will influence the course of fermentation as well as which organism proliferates first followed by which, and, of course, the distinctive taste of the final product, its color, texture etc.

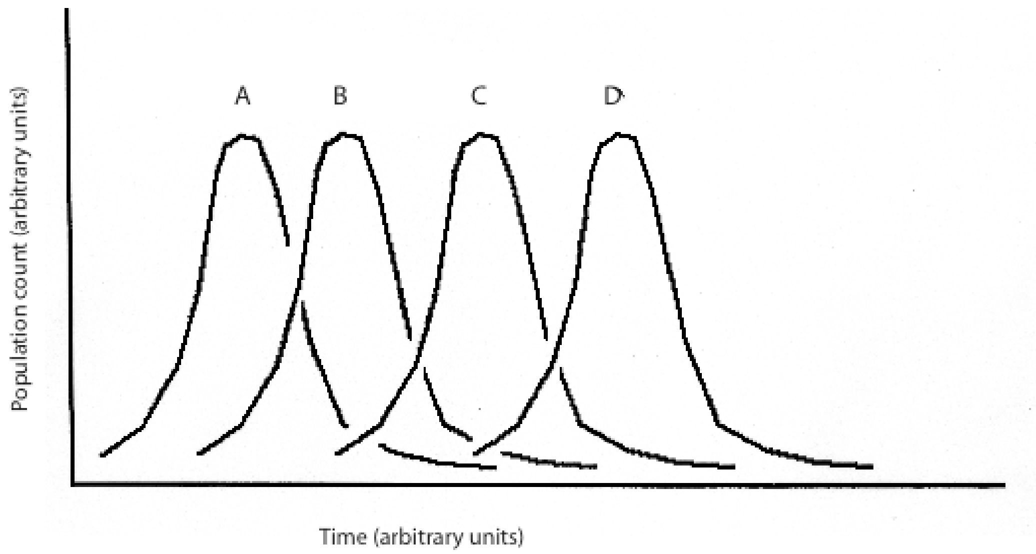


Figure 1.2. Life cycle of four organisms involved in the fermentaton of sauerkraut. Leuconostoc mesenteroides (A) develops first. As it dies out Lactobacillus brevis (B) takes over followed by Pediococcus cerevisiae (C) and finally Lactobacillus plantarum (D).

In fact, meats (pepperoni, salamis etc...) are fermented the same way as sauerkraut, using the same sequence of organisms and similar fermentation conditions. The Romans were famous for their fermented meats and provided them to their solders during campaigns.

4. Beer and Wine

The production of alcohol is probably the best known of the fermentation processes. However, again, for fermentation to occur, sugar is needed. For wine, the fruit whose juice is fermented supplies the sugar. In the beginning wild yeasts that grew naturally on fruits supplied the fermentation drive. Wine making may have started as early as 3500 B.C. in Assyria, but again, that was a process of chance and fermentation was started by whatever organism grew on the fruit. Later, probably the Greeks and surely the Romans used starters to inoculate fresh fruit juice. The starters were taken from previous batches of their best tasting wines which took over the fermentation of the juice instead of the indigenous species of yeast. The latter would have to go from a dry state to a wet one before they started fermenting and that took time. The starter yeast, in contrast, was already in solution and ready to go.

For beer, the process is a bit more complicated because beer is made from grains such as barely. There, the sources of energy are concentrated in starch, i.e. long chains of sugars that the yeast can use only after it has been broken into sugars again – a process that yeast does very poorly, if at all. It was necessary to find a way to break starches into sugars before adding yeast. This is what is called the malting process (more on this later). The malting process simply involves letting the grains germinate for a few hours. The Babylonians originally made beer without malting. They would grind barley in water and let it ferment but this resulted in very low alcohol levels and therefore poor preservation potential. In fact bread and beer making were closely associated and used the same type of yeast. As we shall see later the first steps of both processes were the same.

DID YOU KNOW:
The world oldest known recipe is for beer.

5. Sausage

Since meats could not be saved for long periods of time, people learned early to chop it finely, mix it with salt and spices and let it ferment and dry in rolls that were the ancestors of sausage. Babylonians ate fermented sausage around 1500 B.C. as did the Greeks and Romans later. Even American Indians prepared fermented sausage.

The term salami comes from the Latin word "Salumen" which describes a mix of salted meats. Caesar's legions used sausage as a secret weapon that enabled them to keep their strength, even during long marches and battles far away from Rome. Roman butchers prepared sausage by cutting beef and pork finely, mixing salts and herbs, stuffing the meat into skins and letting them dry and age in special rooms. These rooms and their shops were infested with the right lactic acid bacteria and micrococci which gave the sausage its tang and prevented growth of other, spoiling organisms.

Today's sausage can be fresh, cooked, smoked, dry and semi-dry. The last 2 are fermented; salami and pepperoni. The strains of bacteria used for this process are the same as in sauerkraut fermentation: Lactobacillus, Pediococcus and Leuconostoc. As in sauerkraut fermentation the process as well as the interactions between these microorganisms is very complex. Fermentation does however alter the flavor, smell and taste of the final product, thus conferring onto it characteristic attributes. These sausages are cured in warm, humid conditions in order to encourage growth of the bacteria involved in the fermentation process. Sugar is added as a food source for the bacteria during the curing process and lactic acid is produced by the bacteria as a waste product, lowering the pH and coagulating and drying the meat. The acid produced by the bacteria prevents the growth of other, dangerous bacteria and imparts its tangy flavor to the sausages.

Note that you can also pickle beef heart, tongue, salmon (lox) etc. Have you ever tried any of these?

6. Other fermentations used in food preparation.

Chocolate is made from cocoa beans. Discovered by the Aztecs, it was mostly made into a drink that became very popular in Spain and Britain after the discovery of the New World. Cocoa is made from the seeds of the cocoa trees. The seeds are trapped in strong pods. Workers split the pods in half to reveal the beans embedded in white pulp that is the inner layer of the fruit shelf. At this point, fermentation is used to free the beans from the pulp and enrich the flavor, aroma and color of the bean. Fermentation (about 9 days) allows for the complete digestion of pulp. The fermentation produces a water loss which results in an apparent increase in fat content.

There are two stages of fermentation: yeasts first, then bacteria (15 species of the first, several of the second) produce vinegar and other acids that will digest the pulp and add flavor to the beans.

The vanilla bean is also a native of South America and comes from only one of 20,000 varieties of orchid. Vanilla beans are also contained in pods and the typical flavor is absent from the unfermented bean. The pods must be harvested unripe (ripe pods are useless) and fermented for two to six months. During this time, several types of bacteria grow and produce profound changes in color, taste and aroma. The most important of these changes is the transformation of a compound in the bean into vanillin which is responsible for vanilla flavor.

Other compounds are formed that contribute to the fragrance of vanilla. Some of these changes are due to the reactions in the bean itself, some to the bacteria and others to a combination of both. The bacteria provide the right conditions (acid) to favor certain reactions inside the bean which would not normally take place.

Coffee beans can also be prepared by fermentation. Coffee is a berry's seeds (2 seeds per berry) that are fermented to separate the seed or bean from the mucilage covering the beans. Fermentation is natural (no inoculation) and it is hard to tell which of the bacteria found in there actually hydrolyzes the pulp. The fermentation process which lasts 16 to 36 hours is also thought to accentuate the body and flavor of the coffee beans.

Soy sauce is also a product of fermentation. The starting ingredient is soy beans which are fermented first by a mold (Aspergillus oryzae or Aspergillus sojae) for 5 weeks and then by yeasts (Pediococcus halophius, Saccharomyces rouxii, and Torulopsis versatilis) for 6 to 12 months. The soy beans are then pressed to yield soy sauce. Soy sauce comes in many different varieties and there are many differences between soy sauces but not all soy sauces available on the market are brewed. The aroma, color, consistency and flavor of the sauce show whether it has been fermented or whether it is a non-brewed chemical synthetic. In the synthetic recipe soybeans and wheat are boiled in hydrochloric acid for 16 hours, neutralized with sodium hydroxide and pressed into soy sauce. This product is commercially available in western supermarkets but not in Asia. An interesting variation on fermented soy beans is miso, a fermented soybean paste used as seasoning. Japan has been producing miso for 1000 years, from soybeans mixed or not with rice and barley. The process involves an Aspergillus oryzae fermentation of the soybeans followed by grinding into a paste.

C. FERMENTED FOODS IN THE WORLD.

It is hard to imagine yet it's true. A great majority of the foods we eat are fermented, from bread and cheese to soy sauce, yogurt, beer and wine. The variety of recipes using these products is also immense. In Yugoslavia cabbage is fermented whole with the same organisms used for sauerkraut preparation. The leaves are then stuffed with ground meat and baked into sarma. Fermented cabbage is also served the cold as a salad. The brine left after the fermentation of red cabbage is consumed as a pink appetizer.

At a Yugoslav cafe, you might have a lunch of bread, white cheese and sour goat's milk. Pick the unfermented food here. You might think that only Europeans eat these products. In the US people go to a Deli and order salami and cheese on whole wheat with relish and mustard, pickle, a beer and pretzels. Follow that with coffee and vanilla or chocolate ice cream (or milk shake). Pick the unfermented food here. Fermentation is involved in the production of many foods, including bread, beer, wine, sauerkraut, coffee, black tea, cheese, yogurt, buttermilk, pickles, chocolate, vanilla, ginger, catsup, mustard, soy sauce to name only a few.

It is true however that we have replaced the process of fermentation with the addition of chemical compounds (similar to those produced by the fermenting organisms) in the preparation of pickles, cheese, yogurt, soy sauce and sausage among others. While these synthetic products have flavors similar to those of the fermented equivalents modern science has not yet been able to duplicate the flavor compounds that make fermented products unique in taste, flavor and aroma.

Even though all people of the earth use fermentation some parts of the world use specific types of fermentation. While bacteria and yeast fermentation are common everywhere in the world only Asia makes extensive use of mold-fermentation (Aspergillus). Mold-fermentation is used in the production of soy sauce, Tempeh and miso. Similarly the use of molds in the ripening of cheese (Roquefort and Camembert) seems to be limited to Europe.

In the past 25 years a new area of pharmaceutical research has started using fermentation to produce medicines. Some drugs are produced naturally by microorganisms (penicillin is a product of the mold Penicillium). Others are produced by genetically engineered microorganisms. In these cases the gene coding for a particular drug is placed in the genome of a microorganism that starts producing it as if it were its own. Insulin produced in this manner is a lot cheaper to extract from fermentation vats than from a cow’s pancreas.

D. CONCLUSION

Until relatively recently have we started to study the microbiology and chemistry involved in fermentation. We understand very little of the complex interactions of these bacteria with each other and the medium that they are fermenting. We still do not know what makes a great wine, otherwise every year would be a vintage year and it is obviously not. The more we learn about these reactions, the more amazing it is that humans could have developed methods of fermentation without even knowing about microorganisms and even today, those using fermentations are still using "rules of thumb" methods developed over centuries. Fermentation is still considered more of an art than a science even though we learn more every day about the Microbiology and Biochemistry of fermentation.

EXERCISES

1. Define fermentation.
2. What types of organisms use fermentation?
3. Name four majors products of fermentation.
4. What is sour dough?
5. Explain the leavening process in bread making.
6. In Antiquity how did humans introduce yeasts in their bread dough?
7. Explain the difference between fermented milk and cheese.
8. Describe the role of fermentation in the preparation of chocolate.
9. Describe the role of fermentation in the preparation of vanilla.
10. What do molds and yeasts have in common?
11. Are microorganisms useful in medicine? Explain.
12. Do we eat molds in fermented products? Explain.
13. Draw a line connecting the organism (middle column) with its product (left) and where it can be found (right).

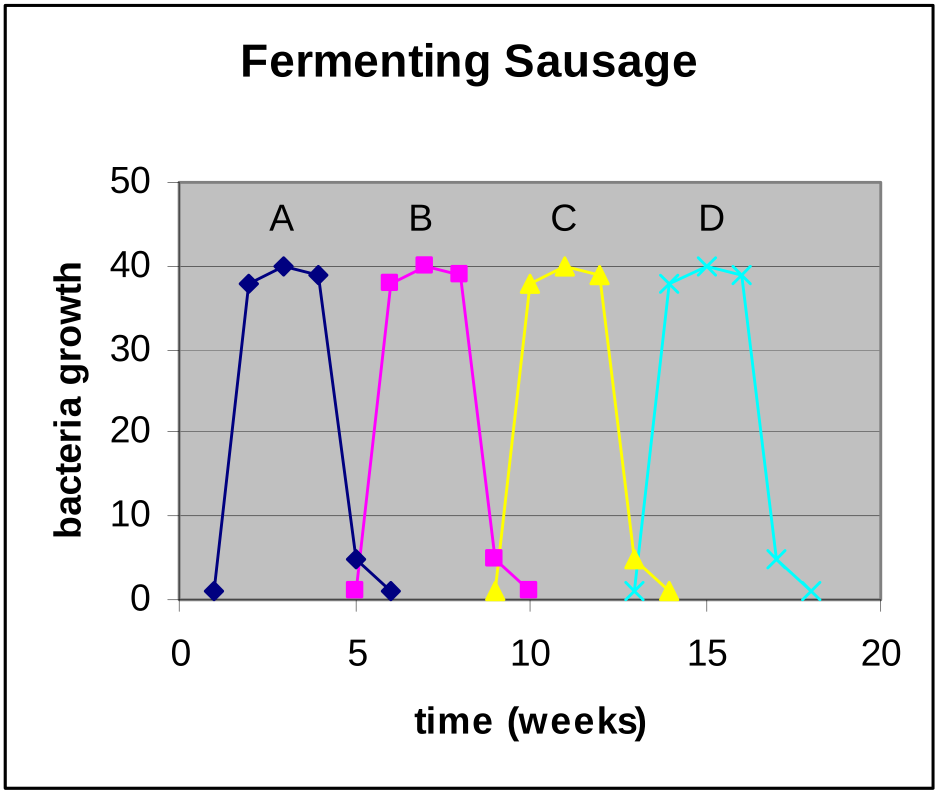
Product	**Organism**	**Where it can be found**
Citric acid	Saccharomyces Cerevisiae (fungus)	Fuel

Methanol	Saccharomyces Ellipsogdeus (fungus)	Wine
Lactic acid	Lactobacillus (bacteria)	Soy sauce
Acetic acid	Streptococcous (bacteria)	Cheese
Ethanol	Pediococcous (bacteria)	Sauerkraut
Lactic acid	Acetobacter (bacteria)	Propionic acid
Ethanol/glycerol	Aspergillus (fungus)	Vinegar
Lactic acid	Clostridium (bacteria)	Beer
Swiss cheese	Propionibacterium freudenrichi (bacteria)	Cheese

14. What advantages did fermentation have for the people of 6000 B.C. versus the people of 2005 A.D.?

15. What are the possible reasons for England making only 1 type of cheese and France making 350 types of cheeses in 1650 A.D.?

16. From Fig 1.2, Why does organism C grow at 10 weeks and organism B die at 10 weeks? What is the difference between organism C and B at 10 weeks?



17. Explain why the following organisms are important in fermentation:

a. Saccaromyces cerevisae
b. Acetobacter
c. Clostridium
d. Aspergillus
e. Lactobacillus

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Lesson 2. HISTORICAL ASPECTS OF WINE AND BEER MAKING

1 figure
no table
no illustration
1 appendix

A. INTRODUCTION

It is very possible that the first fermentation occurred by accident, when grain or fruit was left unattended; yeasts that are normally ubiquitous in the air, especially on the skin of most fruits fermented that juice or grain. The product did not spoil but rather gave a "crude" alcoholic beverage with a more or less pleasant taste. It is estimated that this happened along the river Tigris in the empire of Mesopotamia (now Iraq) 6-7000 years ago. As the early rulers of Mesopotamia, the Sumerians, were succeeded by the Babylonians around the second millennium BC, the beer making process became more sophisticated and the variety of beers available grew. While other cultures used different grains to make beer (millet, wheat, rye, rice and corn) the inhabitants of Mesopotamia adopted barley and emmer (an ancient wheat) in their beer making practices.

B. BEER IN ANTIQUITY

PLEASE READ "Big Brewhaha of 1800 BC by Judith Stone, Discover, January 1991.

The brewing of beer probably paralleled the baking process since they are very similar and of course, both use yeast. Today, different strains of yeasts are used for the two processes. These yeasts have been selected or "engineered" to optimize the processes but it is likely that the same organism were originally used for both beer and bread making. Since beer and bread making came before writing, little documentation exists to describe the first 1000 years of bread and beer making; however, there are indications that beer making may have pre-dated the making of bread. Beer making probably started in Sumer 5-7000 BC. The oldest document known to man is an ancient clay tablet depicting the preparation of beer for sacrificial purposes, inscribed in Babylon in 6000 BC. They called it **bousa** or boozah and, by variations in barley sources and flavoring agents, produced 20 different varieties. Several authors mention the production of eight barley beers, eight emmer beers and three mixed beers. While it is indicated that several types of grain were used to make beer it is clear whether barley ever established itself as the grain of choice for beer making in Sumer. Spices, aromatic plants, extra honey, cereals and malt were added to increase its alcohol content and help in its preservation.

Barley

Barley, the major ingredient of beer is one of humanity’s oldest cultivated plants. Again, modern barley carries six rows of grains. It has evolved from the older two row barley through Plant Breeding techniques to "improve" its performance, facilitate mechanical harvest and increase nutritive aspects; however ancestors of modern barley have been found in the Middle East at Jarmo which date to 6750 ± 250 years B.C. The spike carrying the grain had 2 rows while modern barely can have two or six rows. The **two row barley** actually contains more starch and more flavor than its 6 row counterpart and is to this day, associated with the making of better quality beers. In Sumerian texts, we find that as much as 40% of the grain crops were used for beer production. The use of different types of barley is mentioned for different qualities of beer. This means that selection was already going on. We know that eight different types of barley were grown in Sumer (eight types of wheat were grown as well with three types of mixed grains, although these may have been reserved to bread making exclusively). Sumarian texts refer to red beer, white beer, premium beer and beer with a head pointing to the variety of ingredients used in beer making.

The beer ration distributed to citizens of Mesopotamia varied from 2 to 5 liters a day depending on their importance and social status. While the beer may well have been used as currency as well as for personal use, this still points to a large per capita intake and stories in Greece point to the possibility that Mesopotamians were actually addicted to beer.

Beer is defined as a liquid made from fermented hop-flavored cereal product. Of course in Antiquity, beer was not flavored with hops. This started later in Egypt and Babylon and was institutionalized in Europe during the Middle Ages. This definition means that 2 processes had to be mastered before beer could be made:

-**Malting of barley to let the barley seed germinate**
The seed is a storage bin of energy for the germination: starches are polymerized sugars that will be broken back to sugars before energy is released. Yeast cannot use starches but can use sugars => germination which activates the enzyme that will release sugars from starch (amylase or diastase) must occur before fermentation becomes possible.

-Fermentation that will produce the alcohol
The first beer was made with unmalted barley 7000 BC. in Babylon. Malting, while it improves **digestibility** and **palatability** of the raw grain must have occurred later, again by accident. It eventually became a prerequisite to baking and brewing, probably around 5000 B. C

The malting and beer making processes
The malting process was originally developed in an effort to make the grains more palatable when mixed into gruels or baked into bread. Sumerians probably baked barley bread (bappir) twice to dry the barley thoroughly (for preservation because malted barley will be spoiled very rapidly by molds). The records attest to the wide use of <u>cakes</u> of crushed malt in water to provide a nutritious gruel or fermentable extract in Egypt as well as Mesopotamia. A classic beer recipe would call for a mixture of baked beer bread ("bappir" made of barley and/or

emmer), roasted barley, malted barley and honey. The mixture was placed in water and mashed at gradually increasing temperatures. During mashing the enzymes present in the malted barley would produce simple sugars from the starch of the roasted barley and the “bappir” since both the roasting and baking process had killed the enzymes needed for mashing. Honey and/or date juice was added for flavoring. After cooling a top fermenting yeast was added (from a previous batch of beer). This beer recipe produced an alcohol percentage of 3.5%. Malting was done in Egypt by burying barley grain in a vessel to let germination occur. Then it was crushed, made into dough and baked briefly (to kill enzymes). That cake was soaked in water (yeast must have been contaminating that water) until fermentation was complete. That beer was called Hek.

At least at first, yeast was added to the fermentable extract by the wind since the early brewer knew nothing about it. Indeed the Babylonians attributed the Transformation to the divine intervention of the goddess Nin-kasi (who presided over the beer making process) and her husband (or brother?)Siris. In Egypt, Osiris god of the nether world and Ra, the Sun-god, were prayed to for good beer making. It is interesting to note that in early Sumerian and Egyptian societies the brewing was left to women, the priestesses of Nin-kasi in Sumer devoted to beer making for ceremonial purposes. **WHY would that be????** Later yeast was added to the wort from a previous batch of beer.

In Egypt, as early as the Pyramid age, five types of beer were known to exist and some of them were probably a lot stronger than the beers that we drink - in this country at least. Strongly malted brews contained a lot of fermentable sugars and the mixtures of yeast probably allowed the near complete fermentation of these sugars. This is not the case today as the yeast in current use is engineered to produce only 3 to 5 % alcohol before they die.

Even though the Mesopotamians and Egyptians knew nothing of microorganisms in general and yeast in particular, examination of urns found at Thebes showed that the yeast cells, still there in dormant form after thirty to fifty centuries, showed improvements from 3400 to 1440 BC. These improvements in purity mostly imply that the Egyptians selected the better products rather than better yeasts (or mix of yeasts) for the inoculation of the next batch of wort but still ended up with improved yeast strains. These improvements were made possible by two principles that control the evolution of every species in nature.

The first principle states that changes occur in a population because the molecule (**DNA**) that holds the information about how an individual is built is sometimes reproduced with an error. We call that a **mutation** in the **genotype** or the DNA of an organism. This error will bring a change in this individual’s looks or functions (**phenotype**). This mutation can be **beneficial** if the individual functions better than others in the group, **deleterious** if the individual does not function as well as others in the group or **neutral** if the mutation does not affect the performance of the individual in the group. For example; the child of two white parents could be born with the ability to produce more of a skin pigment called **melanin** and therefore be of a darker skin color than the parents. Would that be a beneficial, deleterious or neutral mutation in our society? We would probably decide this is a neutral outcome because the child's survival would not be affected by this mutation. In our class for example we see a variety of eye color, hair color and even skin color, without any deleterious effects on those individuals.

The second principle states that while a large diversity exists in a population, changes in the environment may select for the survival of certain individuals who carry traits that are beneficial or neutral. Let's think back to the child who has darker skin. Let us suppose that the ozone layer becomes very thin in the atmosphere. The sun's ultra-violet radiations that are normally blocked by this ozone layer will then penetrate this protective layer, causing serious damage to the skin of people exposed to it. This damage eventually will lead to skin cancer and death. Individuals with darker skin will have a far better chance of survival in this new environment because their skin can protect them against the harmful effects of ultra-violet radiations. This child and others like he or she will survive and have children, also of darker skin color while the individuals with paler skins will be less likely to survive long enough to have children. After a few generations individuals with lighter skin will have disappeared from this population since they possessed a trait (light colored skin) that, in the new environment (intense ultra-violet radiations), proved deleterious. Only dark skin individuals will remain. This evolution of the population that brought about the survival of individuals who were fit therefore occurred because of **random mutations** in individuals and the ability that these mutations gave some individuals to survive their environment and procreate.

In the XIth dynasty at Thebes, the brewery and bakery were both present, pointing to the fact that both processes had important places in society. Further the two processes appeared separate by then. The importance of these processes in Egyptian society is underlined by the fact that they are mentioned in <u>religious</u> as well as other literature. Religious symbolism was given to alcoholic beverages and bread, probably because of the mysterious action of yeast during alcoholic fermentation in beer and wine and the **leavening** process in bread as well as the euphoric, God-like feelings that the alcohol generated in people.

While Sumerian hymns were written about beer making in Mesopotamia (Hymn to Nin-kasi written about 1800 BC) and have been used as a recipe book by modern beer makers the earliest known treatise on beer brewing was written by Zosimos of Panopolis in Upper Egypt in the 3rd Century AD.

Beer at that time was a strange brew of relatively bland, fermented barley. Flavoring herbs such as <u>**hops**</u> were added later but not by all cultures. Documents show their use in Babylon and Egypt at least. Also, filtration was not in general use. Mesopotamians did not use filters and so imbibed a thick cloudy fermented mash containing husks and all. Mesopotamian carvings show the use of drinking straws inserted in large communal vats from which the beer was drunk. These tubes enabled the drinkers to extract the beer from a brew made with husked grain and keep them from swallowing large particulates. The Egyptians however did use a filtering system made of horse hair. OOF! That left a lot of protein in the beer making it appear cloudy. It was a lot more nutritious however than the beer we drink nowadays. Today, protein is removed so that the product will be crystal clear.

In Egypt and Sumer beer was packaged in clay bottles with narrow necks and sealed with baked clay stoppers. Thus, the beer would still have a fizz to it when the Egyptians drank it.

In Egypt beer brewing technology remained exactly the same between the Vth and VIIIth dynasties. This is roughly 1000 years and attests to the importance of beer in Antiquity. In Egypt, beer was used to pay workers and was offered in sacrifices to the Gods. This again attests to the public and religious importance of this alcoholic beverage. The Egyptians brewed 50 to 60 different types of beer in facilities built at Palusium on the Nile delta and shipped these products as far as away India. For the ancient Egyptians, beer was so important that the hieroglyphic symbol for food was a pitcher of beer and a loaf of bread. In fact, pharaohs were buried with tiny model breweries, complete with miniature wooden brewers, to ensure a sufficient beer supply on the arduous journey to the afterworld.

In Mesopotamian society it is interesting to note that beer was subjected to:

1- legislation and quality control (Code of Hammurabi established around 1792-1686 BC)
2- taxation

This implies that the process was not a home process anymore.
CAN YOU EXPLAIN THAT CONCLUSION?

The Ancient Hebrews also made beer (machmetzeth) from stale bread, malt flour, and water. They may have learned the process from the Egyptians while in captivity but documentation on their beer making process is scant since the Hebrews were nomads and neglected to keep records of their civilization's undertakings.

It is probably the Egyptians who taught the Greeks how to make beer and they in turn taught the Romans. Both Greeks and Romans however considered beer a barbarian drink, most likely because northern people (Celts and Germans) drank beer in a tradition that predates the arrival of Roman legions. This is interesting because it implies that the beer making tradition among these people (Celts and Germans) could have been as old as in Mesopotamia and Egypt. It is indeed unlikely that people who used cereal production as a means of subsistence would not have discovered the malting process and beer and bread making. However there are no records confirming this. Beer (zythos) is only mentioned in passing by Greek writers and, while early Romans did drink beer early on (Plinius reported of the popularity of beer in the Mediterranean area during early Roman history) beer was later brewed only in the outer areas of the empire where wine was difficult to obtain. They however made one beer "cerevisia" in honor of Ceres, the goddess of agriculture & vis (strength in Latin).

C. WINE IN ANTIQUITY

It is possible that beer making is older than wine making. We have few records of the beginning of alcoholic fermentation. The archeological record shows the presence of Vitis vinifera in Anatolia (modern Eastern Turkey) around 9000 BC (Neolithic period). Anatolia would eventually become the land of the Hittites and their great (by reputation) if poorly documented wine making tradition. Wine making probably started 7,000 years ago in a region of what is now Northern Iran, although Georgia (east of the Black Sea), southeastern Turkey, northern Iran and Armenia have also been suggested as the point of origin of wine making. The wild grapes originally used were subsequently domesticated and cultivated. The bible relates in Genesis that after the flood Noah"s ark landed in the mountains of Ararat (in Anatolia) and that Noah became a **husbandman** (raised animals), planted a vineyard and made wine. While this story is revealing of the history of grape growing the problem is that the bible was written hundreds or thousands of years after events took place. This makes the timeline and the historical accuracy difficult to assess.

How do we know that something actually happened when we think it did? Archaeology studies human cultures and attempts to explain the origins and development of human culture and technology. It is a cross between history and anthropology. It relies on scientific analysis techniques, historical record and logical thinking to uncover the facts cited in this chapter. Let's pick a particular example. Archaeologists digging in Crete uncovered parts of an object that was dated to 1500 BC by carbon-14 dating. Digs in the area uncovered plant remnants dated to 1500 BC and shown to be from Vitis vinifera by DNA analysis. Searching the area archaeologists soon found caves where papyrus documents depicted this object in scenes of wine making. The text also described this object as a grape press. Chemical analysis of the wood in this press showed that it contained residues of grape juice. The "press" was replicated using the parts found at the digging site and the pictures in the papyrus document. It indeed performed as a juice press. Further research showed that people living on the island today still used an instrument very similar to this artifact to get the grape juice needed for wine making. Can we conclude that the culture made wine in 1500 BC? What if the press had been given to these people and never used? The papyrus did depict scenes of wine making and feasts where people did drink wine with multiple references to "oinos". Amphorae were also found on the site that tested positive for wine residues. This wealth of evidence supports the theory that these people did make wine in 1500 BC. Unfortunately evidence is often more scant and archaeologists are left with even more guess work and weaker conclusions.

First records of wine making are from 3000 BC in Mesopotamia (Irak/Iran). Yeast used for fermenting were wild yeast present on grapes and the first fermentation was probably accidental. But, since that was done in open containers, bacteria that make vinegar from alcohol would soon drift in on particles of dust. This meant that the wine had to be consumed very quickly after primary fermentation. Since wine is not very good at that stage, we gather that it must not have been consumed for its flavor but rather for its effects. We also know that in the barley growing regions of Mesopotamia the need for extensive irrigation made production of barley progressively more difficult. Eventually there was not enough barley produced to spare for beer making and the growth of dates supplanted that of barley. These dates were used in the production of a wine that slowly replaced beer.

In 1500 BC the Hittites (Middle East) called wine **uiian (or wee-an)**. It is the first mention of this alcoholic beverage associated with a n actual name. The Greeks called it **woinos** and later **oinos** and the Romans **vinum**. Today the English word is **wine**, the French call it **vin**, the Greeks **wiin** and the Hebrew word for wine is **vayin.** As you can see most languages in Antiquity and today used the Hittite root to describe wine.

It is interesting to note that viticulture in Egypt predated wine making by a large margin. The introduction of grape cultivation from the Levant (an imprecise geographical term historically referring to a large area in the Middle East south of the Taurus Mountains, bounded by the Mediterranean Sea on the west, and by the northern Arabian Desert and Upper Mesopotamia to the east.) to Egypt c. dates to 3000 BC. Wine was imported to Egypt for a long time and remained a drink for the rich (aristocracy, priests and royalty) while beer was reserved for peasants and workers. The making of wine was probably brought to Egypt from Canaan during the Early Bronze Age, starting from at least the Third Dynasty (2650 – 2575 BC). At first reserved for pharaohs a royal winemaking industry was established in the Nile delta. Indeed hieroglyphs dating from 1500 BC show grape harvest, crushing, fermentation and storage. But even then, the rich only drank wine in most of Egypt. In the Delta however, where grapes and wine were more common, both rich and poor drank wine. It was also taxed and producers diluted it to increase profits. This prompted the appearance of legislation controlling the quality of the wine sold for the same purpose as the Babylonian Code of Hammurabi had been established around 1792-1686 BC in Mesopotamia.

The deleterious effects of oxidation on wine quality were well known in Antiquity. Oxidation browns the wine and gives it a strong pungent oaky taste. Wines were therefore stored away from oxygen (O_2) by capping the storage urns. This also helped keep wines away from another great danger that can befall wines after they are made: spoiling by bacteria. Spoiling by bacteria usually means the formation of vinegar by <u>Acetobacter</u> which feeds on the alcohol produced by yeasts and therefore must grow after yeasts have converted the must to wine. These bacteria draw the energy needed for their growth by converting alcohol to vinegar. However since the most basic principles of hygiene had not been discovered yet, bacteria were introduced in the wine before capping the urns.

Capping was also far less than perfect and so oxidation and spoiling remained a frequent occurrence. Quality remained poor.

The Greeks took their wine seriously and it is rare to find in Greek texts references to grapes being grown only for eating. The Greeks also improved the process of wine making markedly. In the Odyssey and Iliad Homer describes the process in detail. After the fifth century BC Greece produced fine wines. After storage and preservation were improved, the trading of wines gained importance in the Greek economy. The appearance of trade with other cities and nations is an important clue as to the development of storage techniques because trade travel took time and the risks of spoiling during the journey were great. Throughout the Mediterranean basin the rich imported the excellent vintages of Lesbos and Chios. Since the climate of the region was very warm the harvested grapes contained more sugar than the yeast could metabolize into alcohol and those vintages were likely to have been very sweet. This prompted the practice of diluting wine with water in Greek and Roman times.

Interestingly, wines were blended with goat milk cheese, herbs, malt etc. This points to two possibilities:

- poor wine altogether
- Some or most wines were not stable in storage and oxidized rapidly. These herb mixtures probably prevented or masked the effects of oxidation.

Greek vintners for example fermented wine in vats smeared with resin that prevented oxygen from reaching the fermenting wine. This of course imparted a characteristic resin flavor to the wine. Wine could also be aged in smoke houses as smoking was thought to improve the wine. The high levels of carbon dioxide also prevented or reduced the effects of oxidation. These practices probably also covered up tastes caused by spoilage by Acetobacter.

The Bible mentions wine frequently: Noah’s story is one of the first mentions of wine making and drinking but that does not date the practice very precisely. The Bible was written much after the fact since the Hebrew’s were originally nomadic and thus did not have a written record of their history until they settled. The Bible therefore was originally a collection of legends that had been recounted as stories around the campfire during the nomadic period of their history.

Moses sent spies to the Promised Land who came back with grape clusters “too heavy for one man to carry”. Aside from this slight exaggeration this points to their interest in wine making. The bible also mentions the first bad effects of wine (Noah got drunk) and the Book of Proverbs moralizes extensively on the abuse of wine. Interestingly our society still battles with the widespread abuse of alcoholic beverages.

In the New Testament, wine becomes part of the religious ritual: Christ performed his first miracle at the feast of Cana. John 2: 10 reads: He (the steward) called the bridegroom and told him: "every one serves the good wine first and the poorer when men drunk freely; but you have retained the good wine until now". What does this imply? Very probably that there was very little good wine available and it was expensive. So hosts would serve the good wine while guests could still appreciate it and think that you were wealthy. Then the wine of inferior quality came out of the kitchens when the drunken guests could no longer tell the difference. This also implies that heavy drinking was a common occurrence at social events. **WHAT ELSE CAN YOU INFER FROM THIS PASSAGE?**

The New Testament is also a technical treatise. For example in Mark 2:22 we read : "Neither does one pour new wine into old wineskins, for the wine would burst the skin, with both wine and skins ruined. But new wine goes into new skins." And also in Luke 5:37: "No one pour new wine into old goatskins..."

Some versions of this story use the word “bottle” which means actually refers to goatskins. In Israel the more readily available containers for storage of liquids and solids were goatskins. This was also true in Greece where wine slated for local consumption was filtered in goatskins while wine to be exported was filtered in clay amphorae. New wine produced CO_2 that would stretch a new goatskin. The old one would not stretch as well and burst if a new wine was put in it. Thus it means that they were aware that a gas was formed in the early stages of fermentation. It also means that they referred to the fermenting grape juice as wine. Today the fermenting grape juice is called the **must** and the term **wine** is reserved for the finished, aged (and much better tasting) product. Today we would not ignore this distinction as we know that aging must occur before the final product is called "wine". In fact, the Hebrews agreed as well. As read in Luke 5:39: "And no one, used to drinking old wine, wants new wine right away, for he says:" the old is preferable". "

DID YOU KNOW:
Jesus agreed with the moderate consumption of wine and indulged himself.

Wine was appreciated in pre-Christian times for several reasons:

- Alcohol content was greater than in beer (as today) because grape juice contained more sugar; thus its effects came faster and were stronger than those of beer. The alcohol must have been a welcome escape from the rigors of life.
- Food: grapes growing in warm climate can accumulate enormous concentrations of sugar (up to 80% of weight or 40 to 50 brix). Since yeast cannot ferment that much sugar before they die of alcohol poisoning, these wines were very sweet (yeast die out after a maximum of 25 brix of sugar has been converted to alcohol). Therefore, wine was a better source of sugar and quick energy than beer was.

It is worth pointing out that humans are instinctively attracted to sugar. If you gave a choice to 100 volunteers to eat fruit, meat, vegetables and grains most would pick the former two. These two groups of foods represent the most readily available sources of sugar (in fruits), fat and protein (in meat). It is not surprising that while humans in general are far better fed than 5000 years ago, they still prefer fruit and meat. A surprising fact to add to this is that while we prefer these foods our very long **small intestine** (about 32 feet) is really more adapted to the digestion of foods containing a lot of fiber such as vegetables and grains. **WHY IS THAT?** Because, our simian ancestors were herbivores. This appears to be a contradiction but then again you have to admit that from an evolutionary standpoint we have been a fairly successful species despite massive numbers of inherent contradictions and flaws.

- **Wine was a source of vitamins** since they were drunk early and still cloudy from suspended yeast. Some of these vitamins are produced by yeasts and some came from the grapes.

- **Wine was also a sanitary drink** because the yeast prevented the growth of other organisms. Wine was safer to drink than water, especially in cities where sanitation was always poor. Imagine traveling to a country where water is not treated. What happens when you drink that water? Why are the inhabitants of this country not getting sick? Since wine was made with grapes only, the chances of contamination was non existent. That cannot be said for beer which is made with water.

- **The ingestion of wine** also took on religious significance in all cultures where wine was produced. In Greece for example, the cult of Bacchus and Dyonysos did associate the drinking of wine with orgiastic feasts as early as 700 BC.
- **WHAT CAN YOU INFER FROM THIS ABOUT GREEK SOCIETY?**

Around that time, (Pre-Christian) cults in Europe were importing Greek-made wine for their religious ceremonies. Even today in many Christian religions the use of wine is widespread in religious ceremonies. There are at least three reasons for this practice:

i Wine (especially sweet wine) is an energy source that revitalizes you.
Ii Wine has a red color, associated with blood. Therefore, the consumption of wine will give the drinker symbolic access to the blood of Christ for example.
iii Wine produces a feeling of exhilaration and power that was equated with the Gods. Thus the exhilaration of being drunk presumably made people

feel closer to God. The common drunkenness of the followers of Bacchus for example would be explained by this feeling.

D. ROME

Up until then, can you summarize what is known about the process of wine making from our study of Sumerian, Egyptian and Greek wine making technology???

The process of wine making was continually (and greatly) improved in roman times. Since Rome was founded by Greek colonists, they picked up where the Greeks left off. Roman vintages quickly supplanted Greek wines in quality and quantity and the Italian vineyards out produced Greek vineyards by a large margin. The first Greek settlers found grapes in Italy and for that reason called it Wineland (interestingly the Vikings visited America around 1000 AD and also called the continent Vineland; there are more species of grapes in America than on any other continent on the planet. None of them, however, produce high quality wines). The Romans were great technologists rather than scientists and were less interested in how Nature worked (the definition of Science) than in the making of tools and the taming of Nature (the role of technology). They used information from Greek scientific discoveries to make tools. We are ourselves, a technology-oriented nation. Of these two names which do you recognize immediately: Michael Faraday or Thomas Edison? Most of us recognize the latter. Michael Faraday was the scientist who discovered the principles of electricity (how nature works). Thomas Edison, the technologist, used that knowledge to build tools such as the electric bulb (technological tools). In that way we are very similar to the Romans in our outlook on science and technology.

In his Natural History, Pliny writes that Athenians showed how to cultivate vines and how the Romans expanded on that technology. The following advances in the processes of grape growing and wine making are attributed to the Romans**:**

1. Growing Grapes:

a- Pruning to increase grape production
b- Pruning knife - still used today
c- Fertilization
d- Classification of grapes by region, color, time of ripening, diseases, soil preferences and types of wine they produced.
e. While the press had been invented by the Greeks around 500 BC the Romans improved it and made it an integral part of wine making.

2. Wine Making:

a- While the amphora was still used, glass bottles did appear during the period for long term storage.
b- Other technological advances included wooden cooperage, i.e. barrels.

c- Romans still had the spoilage problems of the Greeks but they learned to heat the wine to reduce spoilage (pasteurization) and to add alkali to decrease acidity of spoiled wine.

Roman writers rave about a 100 year old wine. This implies that it was then possible for a wine to age that long without spoiling. Vintages such as the Opimian and the Falernian were sought after throughout the Mediterranean basin. Of course the majority of wines made for local consumption by commoners was still of poor quality and probably had to be consumed quickly.

Eventually the Romans introduced vine cultivation and wine making in Gaul, Spain, Germany and Britain and these wines rivaled Italy's best. The Greeks had planted the first vines around Marseille, France in 500 B.C. but the Romans spread the culture of vines North and East around 100 B.C., when Julius Caesar conquered Gaul and Germany. Today, vineyards of the Rhine use the **trellis method** of vine grapevine similar to Roman practices while the Moselle growers still use the **single stake method** of pre-roman times (See Fig.2.1). This implies that vineyards in the Moselle valley may have existed before the Roman invasion and that their wine making tradition pre-dates the Romans'.

After the fall of Rome (A.D. 400), vines were left unkempt and knowledge was lost. From the 5th to the 13th century, very little wine was made in Europe and what little was made was of very poor quality. Most wine was made for religious purposes by monks who kept the knowledge of wine making (and other technologies as well). Legend has it that these monasteries, free of marauding bandits and taxes, maintain the wine making traditions of the Roman era but innovated, taking advantage of the new grape varieties brought back from Arabia by Crusaders. A grape called Syrah (Shiraz), not to be confused with Petite Sirah, was believed to have its origin in the Middle East. It was used to make legendary wines such as Châteauneuf du Pape. Recent DNA analysis however has shown that it is a cross between two French grapes and is a native of the Rhone valley in France.

E. MIDDLE AGES AND WINE

Isolated from the turbulent Middle Ages, monks were able to recapture or maintain the Roman secrets and produce good wines which were aged for years in wood casks and exported. Trade flourished and regional wines were compared and classified: Bordeaux, Bourgogne etc. Perhaps the greatest contributions of the Middle Ages and the Renaissance to the practices of wine making were: trade, the middle class, **classification**, **standardization** and **wine quality control**. Let's see how these fit together. The growing trade in wines forced classification of wines as to their reproducible quality. This meant that if you liked a wine you just bought, you would want to buy more next week or next month or next year. You would know about the quality of the wine you liked because the making of wines had become standardized, i.e. a Bordeaux wine had to have been made in the region of Bordeaux from a very specific blend of grapes. To encourage repeat customers the manufacturer would have advantage in always producing the same quality of wine – one that his customers could recognize. The pressure on classification and standardization increased as more people joined the Middle Class late in the Middle Ages and the Renaissance and drank more wine.

During the 18th Century, fortified port sweet wines were developed. We will revisit these special types of wines in subsequent chapters. While bottling and aging remained common practice in the 1850's, still, 25% of wines spoiled during fermentation. Only the wines served by the Church and Nobility were of high quality. Luckily, Louis Pasteur was to change all that. We will come back how he revolutionized alcoholic fermentation in general and wine making in particular.

F. BEER MAKING FROM ANTIQUITY TO THE 19TH CENTURY

The Greeks considered beer barbarian (meaning foreign) and the Romans preferred wine even though their legions in England at least made a fermented beverage from spelt (wheat), rye and barley that would be the ancestor of what we now know as English beer. It seemed that even if the Romans and Greeks did not like beer, everybody else in Europe did (Germany, Gaul, Spain, Britain).

During the Middle Ages the process remained the same as described in ancient Egypt. **Barley** was malted to provide sugars that were then fermented. Horse hairs might be used to filter the particles out of the fermented brew but still it remained cloudy. Up until 700 A.D., herbs were used to flavor the beer: coriander, rosemary, bog myrtle. Monasteries used a secret combination of herbs including yarrow and bog myrtle called gruit. The first indication of the appearance of hopped beer came in 736. A mention is made of a hop garden providing **hops** for beer flavoring in Germany. This practice slowly spread to the rest of Europe, but not without opposition. In England, during the1600's the battle still raged. Hopped beer was considered a foreign drink by the proponents of "honest English Ale" (flavored with bog myrtle).

In 1847, the use of sugar in beer making was made legal in England and the practice spread throughout the rest of the world with the exception of Bavaria where, legally, only hops, malted barley and yeast could go into the making of a beer. This is still the legal definition of beer in several European countries. It is not in the US however.

DID YOU KNOW:
The Puritans loaded more beer than water onto the Mayflower before they left England for the New World.

George Washington, Benjamin Franklin, and Thomas Jefferson brewed or distilled their own alcohol beverages.

G. THE INDUSTRIAL REVOLUTON, MICROBIOLOGY AND LOUIS PASTEUR.

During the 1800's, the making of wine and beer was refined into the techniques known today. Still one problem remained: 25% of the fermented products turned to vinegar before the end of fermentation and nobody knew why.

At that time, it was still unknown that fermentation is due to a microorganism (**yeast**) that uses sugar for its own energy needs and leaves CO_2 and alcohol behind as waste products. In 1680, 200 years before Pasteur, Antonie Van Leeuwenhoeck, used his newly built **microscope** to observe yeast cells in fermenting beer, but the link between yeast and fermentation was not made and his observation forgotten. In 1837, three independent workers published the same observations and suggested a **causal relationship** that was *the presence of yeast caused fermentation.* They were ridiculed.

Louis Pasteur was asked by Napoleon III to look into the problem of spoiled wine (25%) and find a solution. The emperor was interested in the problem because he bought wine for the rations of his troops and lost a quarter of his purchases before the wine could reach his them. Pasteur verified that yeast was the microorganism responsible for alcohol and CO_2 production.

Two experiments were crucial to his conclusions**:**

a. Boiled grape juice would neither ferment nor spoil.
b. Boiled wine would spoil if exposed to air after boiling.

He concluded that**:**

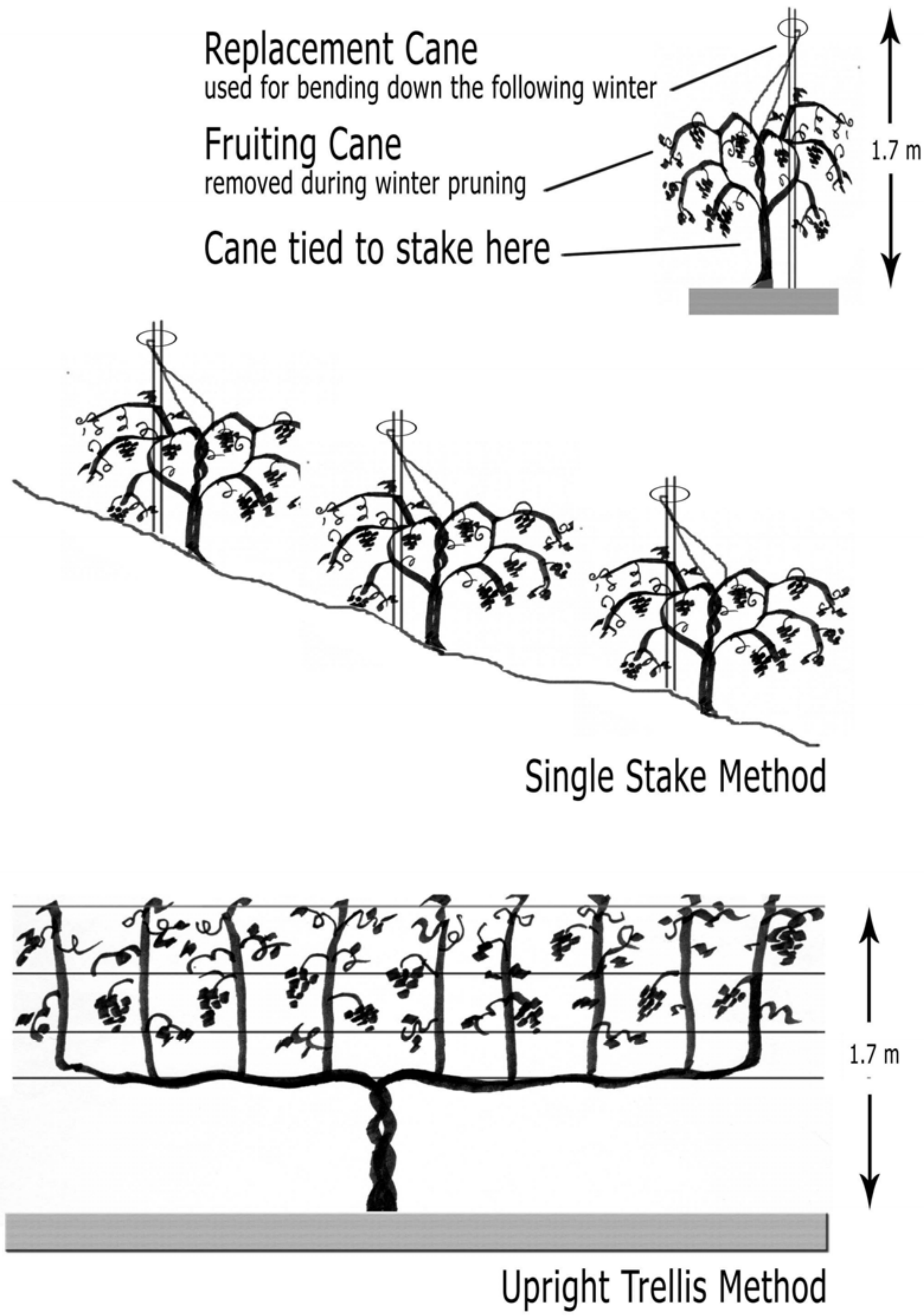
a. Living yeast cells caused fermentation when they were forced to live in the absence of air and that during fermentation, would convert sugar to alcohol + CO_2.
b. Spoilage was due to other organisms: **acetobacter** that reached the wine exposed to air and aerobically produced **acetic acid** (i.e. vinegar).

This also tells us that the yeast, called <u>Saccharomyces</u> <u>cerevisiae</u> was everywhere: on grapes, in the air etc. and that it came in contact with juice or malt "by accident" to ferment it. Pasteur suggested heating wine after it was fermented to prevent spoilage and thus opened the way for the **aseptic** conditions used today in wine making. Aseptic conditions mean that microorganisms are kept out of the fermentation vessels by thorough cleaning or boiling. We still call the process of the heating of foods to kill bacteria and fungi **pasteurization**. Keep in mind that pasteurization is different from sterilization. The former aims to reduce the number of harmful microorganisms in milk or wine so that they are unlikely to cause disease or an acidification of the wine. That is why the temperatures used for pasteurization are below boiling point and that pasteurized milk for example will still spoil after the expiration date as bacteria continue growing after the process.

In 1880 a Danish botanist started looking at **morphology** (shape and structure) and **physiology** (function) of yeasts and started finding differences. It seemed that some yeasts did better in alcohol production than others. For example, yeasts growing on grapes could be used in wine making but the wild yeasts growing on barley, hops or apples made bad beer or cider because these yeasts produced off flavors. He was able to separate the different "**strains**" of yeast into **pure cultures** or colonies containing only one type of yeast. While working at Carlsberg laboratories he promoted the use of pure cultures in brewing to increase quality. (Wine making however, at least in Europe, still uses the natural yeasts which grow on grapes since it is near perfect for wine making). Soon after, people started genetic experiments to improve yeast strains and for example, provide new strains that were cake hard at the bottom of beer bottles so that beer could be poured out without getting the **sediments** in the glass. These efforts have only been recent.
In 1883 the Carlsberg brewery of Copenhagen introduces pure cultures of yeast on the market. It becomes possible to brew a beer with only one type of yeast.
In 1897, the Buchner brothers showed that yeast cells actually contained elements that brought about fermentation when sugar was added: these **enzymes** present inside the yeast cells. Sugar had to be ingested by the yeasts for alcohol to be formed.

Later, 11 different steps (11 different enzymes) involved in the breakdown of sugar to alcohol were identified. Alcohol is a waste product and a poison for yeasts which get rid of it by excreting it into the medium where they live ; alcohol is also a poison for humans but since we can break it down (although slowly enough to call it a poison) it is also a food. It was determined that more energy could be extracted from alcohol if yeasts had **oxygen**: in these **aerobic** conditions 19 different steps are needed to breakdown sugar to carbon dioxide (CO_2) and water (H_2O). In **anaerobic** conditions (lack of oxygen), the breakdown of sugar stops at alcohol. Therefore, an organism can use oxygen to break down ingested alcohol to carbon dioxide and water. Humans use oxygen to extract energy from alcohol by this process.

Figure 2.1 Two methods used when growing grapes



One final event shaped the world wine industry into what we now know. Between 1850 and 1870, fungal epidemics powdery mildew, downy mildew and black rot brought from America had devastated the vineyards of Europe. In 1870, some American **root stocks** were brought to Europe's botanical gardens. Why? In search of improved varieties resistant to these fungi, scientists had turned to America and the Far East where most of the 50 varieties of grapes known to humans can be found. These roots however carried parasites called **Phylloxera (***Daktulosphaira vitifoliae***)**, small, pale yellow sap-sucking insects, related to aphids whose larvae feed on the roots of grapevines. American grapes and their tough fibrous roots were resistant or at least partially resistant to Phylloxera. The grapes grown in Europe however were all Vitis vinifera even though different strains existed within this variety. Their roots were softer and the louse penetrated them easily. Virtually, all vineyards in France were destroyed between 1870 and 1900 and most Spanish vineyards before 1910. Widespread damage also occurred in Austria, Germany, and Rumania. The solution was to use resistant American root stocks (V. labrusca is a favorite because of its high resistance to the louse) and **graft** European varieties onto them. When the best combinations were found, they were spread over Europe and only a limited number of varieties were replanted in European vineyards. That is in part why such uniformity exists today.

Finally the industrial revolution created the machinery for cultivating vineyards, hops gardens and barley fields, handling and crushing grapes, malting, fermenting tanks, filtration and pumping. The wine making and beer making process remained essentially the same but mechanization decreased costs. This created an expansion of sales and a broadening of the population segment that drinks wine and beer. Could you afford a $200 bottle of wine? Remember that wine was always considered a drink for nobility throughout history except where it could be produced cheaply as in Egypt's delta region. This was all changed by the industrial revolution as it lowered the cost of winemaking considerably.

Never before have populations enjoyed such high quality wines and beer at such a low cost as we do today. A $5.00 bottle of French, Italian or Rumanian wine is considered ordinary and yet would have been the best a Roman Emperor or a 14th Century French king could have gotten. People today are lucky, very lucky. Take lessons from History and maybe we will realize the incredible quality of the foods and drinks we consume.

a. Our growing methods are the best in history which means that:

   - Fruit produced is of the highest quality.
   - Insect and parasite damage is minimum.
   - Production per acre is at a maximum.
   - Varieties used today have been developed by centuries of selection by trial and error.

b. Wines are processed very rapidly, with nearly 100% success. Spoiling is practically unheard of. Beers are also processed very rapidly with equal success (i.e. no spoilage). The process has almost become a science.

Therefore, the products that end up on our tables (even the cheapest you can find on the market) are still of a quality far superior to the best products available to the nobility in Roman times or during the Middle Ages. Our power of observation and ability to learn from our experiences has enabled us, over the millennia, to develop the tools for quality products.

And yet, despite all the advances in **science** and **technology,** we still look at science with suspicion and fear. We tend to shy away from understanding the technology we use every day. CONSIDER THE FACT THAT A HUNDRED YEARS AGO, STUDENTS IN MOST COLLEGES HAD HALF THEIR CURRICULUM MADE OF SCIENCE CLASSES. Today you are required to take 4 to 8 credits in the sciences out of a total of 120 credits. WHY DO YOU THINK this change occurred???

How can we be seemingly so disinterested in science when we rely so heavily on the products of science, i.e. technology? Imagine trying to live without technology just one day. How would you fare? How would you manage without electricity, indoor plumbing, television, radio or cars? These are only a few of the technological tools that science has given us.

We are also asked to make collective decisions about science and technology such as disposal and storage of nuclear wastes or the use of genetically modified organisms (GMO). These decisions would better be made by an educated public, yet very few of us are aware of these technologies and the benefits or consequences of their use.

ANSWERS TO TEXT QUESTIONS:

p2: Why was the profession of brewer given to women in Babylon and Egypt? Remember the early days of bread fermentation. This “fertility” was thought of as a gift from the Gods. It is conceivable that the fertility of women (also thought of as a gift from the Gods) was, by association, what gave them the job.

p 3: Would a dark skin be a beneficial, deleterious or neutral mutation in our society? Probably neutral since the child’s survival would not be affected by this mutation. However in an area of the globe where the sun’s UV radiation are not filtered by our atmosphere dark skin would confer an advantage to those having it by protecting them from the damages caused by the radiations.

p 3: Recall first the definition of a neutral mutation i.e. a mutation that does not affect the survival of an individual. In our class we see a variety of eye colors, hair colors and even skin colors, that do not have any deleterious effects on the survival of those individuals.

p 5: How does legislation, quality control and taxation in Mesopotamian society help us conclude that beer making was not a home process anymore? It would have been impossible to police these government edicts and collect taxes if the process was not an industrial one.

p 7: What can you infer from John 2: 10? If good wine was very rare we can infer that it was served to impress, at the beginning of a feast. Serving good wine could then be a sign of wealth and status in that society. it can also be inferred that spoilage was still a very big problem in winemaking.

p 8: Imagine yourselves traveling to a country where water is not treated. What happens when you drink that water??? Untreated water is contaminated with *Shigella flexneri, Shigella sonnei, Shigella dysenteriae* and good old *Escherichia coli*, a bacterium that normally inhabits our own intestine together with Bifidobacteria and Eubacteria (E. coli is only 1% of the total number of organisms in our intestine). When you ingest these foreign bacteria they colonize your intestine and produce toxins that cause diarrhea. But if E. coli occupies our own intestine why should we get sick while traveling in South America? From one region to another the bacteria are a little different, in fact different enough to produce their own toxins. We are used to the toxins produced by our own E. coli and do not suffer from its potential effects on the intestine. But if somebody from a different country drank untreated water here they would experience the same symptoms you would experience if you drank untreated water in another country.

Why are the natives of those countries not getting sick? For the same reason that you are not getting sick here. They are used to their own intestinal flora as you are used to yours. Of course most industrialized countries treat their water to kill these bacteria before the population consumes it and that solves the problem.

How do we get those bacteria that colonize our intestine and what are they doing there anyway? In fact we are not born with E. coli in our guts. We acquire them by ingestion from our parents, from touching surfaces, from our food by touching them and then putting our fingers in our mouths. Breast feeding is a good one for that. These bacteria help ruminants (cows and goats for example) digest their food and also produce compounds that we need for our metabolism such as vitamin K. Without them we could not survive.

p 10: WHAT CAN YOU INFER FROM THIS ABOUT GREEK SOCIETY? In Greece for example the cult of Bacchus and Dyonysos did associate the drinking of wine with orgiastic feasts as early as 700 BC.

p 11: Up to Roman time, can you summarize what was known about the processes of grape growing and wine making from our study of Sumarian, Egyptian and Greek knowledge???

1. Grapes are grown on the single-stake method.
2. Pruning and fertilization are not used.
3. Wine is made in amphorae or animal skins.
4. Wine is not aged and consumed very quickly after primary fermentation.
5. The wood barrel and glass bottle are not used.

p 18: A hundred years ago most college students had to complete half their curriculum in the Natural Sciences. Today you are required to take 4 to 8 credits in the sciences out of a total of 120 credits. WHY DO YOU THINK this change occurred??? Open ended question.

EXERCISES.

1. Define beer.
2. Was beer always flavored with hops? Explain.
3. What were the critical ingredients of beer in Sumaria 5000 years ago?
4. List three possible ingredients you can add to the basic beer recipe for flavor.
5. How does malting affect the sugar and alcohol content of beer?
6. What role did gods and goddesses play in beer making?
7. Did yeast used in beer and wine making change from 3400 to 1400BC? Explain.
8. What are the effects of yeast mutations on beer?
9. What was the value of beer in Sumaria 5000 years ago?
10. When did the Egyptians begin filtering beer? How did they do it?
11. What were some of the nutritional effects of beer filtration?
12. How can a winemaker produce organic wine without using metabisulfite? If she does not use metabisulfite how does she kills wild yeast strains and other deleterious microorganisms?
13. Is wine easier to make than beer? Explain.
14. How did the industrial revolution affect the wine and beer making industry?
15. Describe the US tax system as it relates to alcohol production and consumption.

16. What is oxidation and how does it affect wine and beer?

17. Compare the quality of an ordinary table wine you purchased last week to the wines available to citizens of the Roman Empire.

18. Do you agree or disagree with the following statements? Explain your answers.

    a. Yeast with a tolerance of 3% for alcohol is more sensitive than a yeast with a tolerance of 10%.
    b. During malting the unwanted flavor compounds are transformed or degraded by yeasts.
    c. Irrigation limits wine production.
    d. The Romans called wine ulian.
    e. Vitis vinifera is the only grape grown in Europe.
    f. It is illegal to add diethylene glycol to wine as a sweetener.

19. What is grafting? How is it important to the history of grape growing?

20. What would happen to your broccoli production if you fertilized they plants every day of the growing season?
21. What is the advantage of using ceramic over glass bottles in wine making?

22. You are a winemaker on Seneca Lake and you have just planted your first grapevines. Which stake method will you use? Explain. What can you expect the first year of growing versus the second year?

23. What is pasteurization and how did it change wine production?

24. Describe the type of beer you are making in the lab.

25. You will not pasteurize your beer before bottling. How will you prevent spoilage of your beer?

26. How do plant breeders produce new hybrid varieties of grapes?

27. During the Roman Empire its soldiers conquered most of Europe. What was probably the best weapon they possessed? Explain.

28. How do you make “dry beer”?

29. How do you make “light beer”?

30. If you need yeast to make alcohol for your beer or wine why do we now have different types of yeast for beer and wine?

31. What were the ingredients used in the beer making process in Europe after standardization of the process in the Middle Ages?

32. Are you a Renaissance man/woman? Explain your answer.

33. List the possible types of mutations by their effects on the survival of an individual.

34. Referring to question 33, can mutations change type? EXPLAIN.

35. What is the rate of mutation during DNA replication? What could be the effects of introducing errors (mutations) in the DNA code?

36. Can you think of three mutations commonly present in the present human population that would have gotten the bearer killed very quickly 100,000 years ago?

37. Can technology render a deleterious mutation neutral in the human population?

38. Match the following: letters can be used more than once.

1. DNA:_____
2. Spore
3. Difference between 3000 and 1000B.C.:_____
4. Standards for beer making:_____
5. Natural selection:_____
6. Mutation:_____
7. Factor of survival:_____
8. Artificial selection:_____
9. Deleterious mutation:_____
10. Difference between 1000 B.C. and 1000 A.D.:_____
11. Artificial and natural selection:_____

a. Hammurabi code
b. Man decides
c. Deoxyribonucleic acid
d. Hibernation
e. DNR
f. 2000 years
g. Ten commandments
h. Decrease in brain size
i. Environment decides
j. Mating
k. Influence evolution
l. Neutral mutation
m. Affects survival
n. 1000 years

39. Order the following:

Increase the production of alcohol
malting
Increase sugar content

40. Describe yeast selection for beer making in Mesopotamia.

41. Give an example of two genes that would be beneficial for yeast to have when used in fermentation of beer or wine.

42. List the following from youngest to oldest date:

2005 A.D.
1400 B.C.
900 B.C.
1650 A.D.
1950 A.D.
200 B.C.
1 A.D.
1776 A.D.
1 B.C.
3600 B.C.

43. Fill in the story with the words below:
Phenotype
Natural Selection
Dominant
Beneficial
Mutation
Genotype

Bob has blue eyes. This ________________ is extremely beneficial since he gets many dates with many women. His children by many women have his ___________ and thus have blue eyes too. After many years of having children with many women, Bob had a child named Grace with blue and an amazing immunity to the flu. This ______________ allowed Grace to live to be 100 years old and bear 12 children. Only one of these children died at 5 years of age of the flu. The remaining children lived to be at least 90 years old and had at least 3 children each. None of these children ever caught the flu. This is an example of ________________________. The gene for flu immunity became ______________. And therefore this mutation can be considered _____________.

44. Write a story using the following words:
Beer
Artificial selection
Deleterious
Yeast
Tolerance
Aseptic

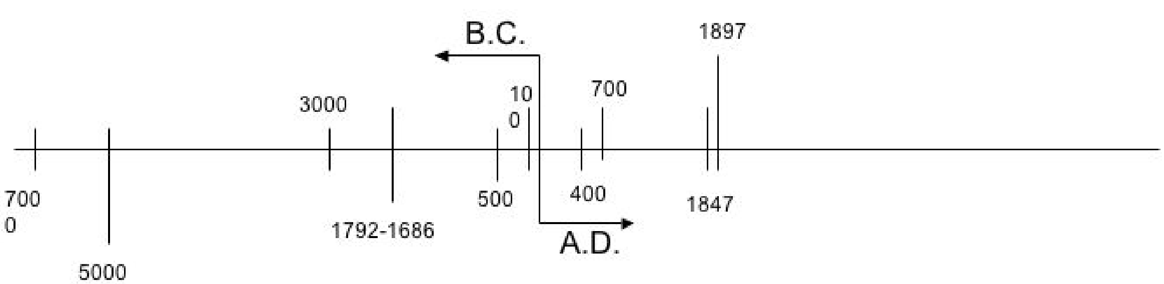
45. Explain the pros and cons of industrialization as it relates to brewing beer.

46. What is the significance of 1400 A.D.? How does this relate to fermentation?

47. Why would a wine have low haze? How could this term be applied to microorganisms?

48.

Put the following on a timeline and explain their importance to the history of fermentation:



A - yeast cells discovered to ferment beer

B – legal to use sugar in beer making

C – Malting used in beer making

D – First evidence of beer making

E – Hammurabi Code

F – hops used for flavoring

G - First evidence of wine making

H – grapes grown in France

I – Romans spread vineyard cultivation

J – Fall of Roman Empire

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APPENDIX 1.

REPRINTS OF TWO ARTICLES:

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Lesson 3. Grapes

6 Figures
1 Table
2 Illustrations

A. CLASSIFICATION

1. Introduction.

Grapes are unique in many ways. The term **"wine"** has come to mean grape wine which shows the importance of that particular fruit in our society. Grapes have characteristics that make wine making easier than with other fruits.

- Grapes have high sugar content (double that of apple). This gives
- wine a high alcohol content and good preservation characteristics
- Grapes have a high **nitrogen** content. Yeast cells grow well in grape juice without any supplements. That is not true for yeast growing in apple juice or other fruit juices.
- Yeasts naturally associates with grapes. The layer of “dust” that you often find on grapes is not dust. It is yeasts.
- Grapes have a low **pectin** content. Pectin will cloud the wine. Thus for most wines the product clears naturally without leaving a **haze**. This is especially true of red wines.

Today, these characteristics are not as critical to wine making because we can easily modify the juice to give it those characteristics by adding sugar, yeast and yeast nutrients. This process is called **adulteration** and is still the subject of some debate over its validity in the wine making process. In fact by law, in many countries and states, it is illegal to adulterate grape juice. These characteristics of grape juice however were crucial to the wine making process at the beginning of the history of wine making? **Why**?

Why talk about grapes? Because, grapes are responsible for all of the chemicals that you will see, smell, and taste in wine. For example, a grape vine that is less than 25 years old will not produce as good a wine as a 75 year old vine. **Why?** We don't know exactly why, but it is obvious that grapes from a 75 year old vine have a different chemical make up that will provide the wine with different chemicals which result in producing an ordinary wine or a great one. A grape vine will produce grapes good enough for wine production for about 100 years. It will live longer than that but after one hundred years the quality of the wine made from its juice declines.

You are probably aware that wines are available from a variety of grapes with different characteristics of color, aroma and flavor. This means that the growers have to know exactly what type of grapes they are growing. It would not be enough to say that a wine was made with red grapes. In order to determine which grapes are responsible for the results we wish to produce, a classification system was developed.

2. Criteria used for classification and Linnaeus.

A good classification system is invaluable. It enables us to see differences and similarities between objects or organisms. This close observation of organisms helps us conclude that Panda bears are actually not bears or that goats and deer are close cousins.

How can we classify things? We could use location to classify ourselves and other individuals. For example, you live in the Milky Way, in the solar system on the third planet from the sun. You can further classify yourself by saying that you live on the North American continent, in the USA. Can we classify further? You live in NY State, in Chemung County, in the city of Elmira. Street, house, apartment number can follow. This classification can give you an identity; a feeling of belonging. Unfortunately there is also a down side to classification. It is also a way to establish differences more clearly than similarities, and these differences give humans a sense of superiority that too often justifies aggression between nations. On the practical side it enables the postal service to forward your mail.

Now how do we classify a biological species? This time we will use anatomical and physiological features to establish similarities and differences between species. The system was designed by Carl Linnaeus in the mid eighteen century. He began with the broadest categories first; much as we did in our own classification using location. We started with galaxies while he started with kingdoms: bacteria (Monera), amoeba (Protista), plants, animals, Linnaeus had originally put fungi and plants together in one kingdom. Fungi were given their own kingdom later. His observations of the anatomy of individuals within each kingdom enabled him to pick the next broadest criteria (for example the presence or absence of a spinal chord in the animal kingdom). Here is an example of three organisms we should be familiar with by now and how **Linnaeus** classified them.

	HUMANS	**GRAPES**	**YEAST**
Kingdom:	Animal	Plant	Fungus
Phylum or Division:	Chordata	Spermatophyta	Ascomycete
Class	Vertebrates	Angiospermae or Magnoliopsida	
subclass		Dicotylidoneae or Magnoliopsida	
Order	Mammalian	Rhamnales (now Vitales)	
Family	Primates	Vitaceae	
Genus	Homo	Vitis	Saccharomyces
Species	Sapiens	varies	Cerevisiae
Variety	varies	varies	varies

The Division Spermatophyta refers to plants that produce seeds. Within the Spermatophyta Division there are 5 classes including the Angiosperms i.e. plants that produce flowers (Conifers are another Class that produce cones rather than flowers). A more modern classification ranks these groups as separate divisions (sometimes under the Superdivision Spermatophyta):

* Cycadophyta, the cycads
* Ginkgophyta, the ginkgo
* Pinophyta, the conifers
* Gnetophyta, the gnetae
* Magnoliophyta (Angiosperms), the flowering plants

The Magnoliopsida, often known as Dicotyledoneae or dicotyledons comprise seed plants that produce an embryo with paired cotyledons. When the seed germinates the two halves of that seed remain on the stem of the plant and may look like leaves. In Monocots such as grasses like barley, corn and wheat the seed does not split in half. The class comprises 6 subclasses, 64 orders, 318 families, and about 165,000 species.

The order Rhamnales is now referred to as Vitales. In older classifications this order had 3 families but reclassification has left only one, the Vitaceae, mostly tendril-bearing climbers (vines) with compound or lobed leaves, as in grapes (*Vitis*).

In the Family of Vitaceae there are 14 genera; grapes belong to the genus Vitis. The other 10 genera do not include food plants but rather ornamentals: Boston Ivy or the Virginia Creeper for example. Within the genus Vitis, there are 50 species of grapes. *Vitis vinifera* or European grape is the most common grape in Europe and California but is not a native to the North American continent.

Within each species there are also varieties: Asian versus Caucasian in humans, for example. For grapes 5,000 cultivars or varieties of *Vitis vinifera* are known to exist, each with different color, flavor, time of ripening, and resistance to cold or diseases. The roots of all vinifera however are sensitive to the louse **Phylloxera** and cold climates (of upper NY. State for example).

Grapes adapt wonderfully to their environment. For example, a variety of *Vitis vinifera* is known as Pinot noir, another as Pinot blanc. But, over the years, they have been grown in various areas and adapted to those areas, thus they look different from each another. We now have over 1000 different Pinot noir and 200 Pinot blanc varieties. The differences are obvious visually as well as by taste. Therefore, when the French make a wine from Pinot grapes, they actually have a bunch of different ones. That is called a **cépage** or a group of similar, closely related but not identical individuals sharing a common genetic background.

3. Diversity and evolution.

Why do species growing in different environments eventually look different? When each species reproduces, some errors may creep into their **DNA** so that the offspring do look a little different. These errors are called **mutations**. These mutations may be deadly to the individuals carrying them, in which case, these individuals disappear from the population. The mutations can also be neutral in that they do not give an advantage or a disadvantage to the individual. These mutations will remain in the population because they do not affect the ability of the individuals carrying them to survive in their environment. Can you think of a mutation in the classroom here that is neutral? Eye color or hair color could be neutral mutations. Would myopia be a neutral mutation in the tiger population? Finally the mutations can be beneficial to the individuals carrying them. They may give such individuals an advantage over their neighbors. These individuals survive better and will eventually supplant others in the population. After a few generations they make up the bulk of the population. Can you think of a beneficial mutation for tigers? Moths? Think here of what would help the survival of a predator like the tiger or a prey like the moth? This means that the environment, in a way, selects those individuals that are able to adapt and who are good survivors. If the environment changes (think of the glacial ages) individuals with different characteristics will survive in their new surroundings while others won't. We call this **natural selection**.

Humans have also performed a selection of certain individual plants and animals with characteristics that suited human purposes. They have selected plants that produce more fruit or dogs with shorter or longer legs. This is called **artificial selection** but works on the same principle i.e. survival in a given environment. If the characteristics of some individuals are not interesting to us we do not allow them to reproduce and these traits, these genes, are not passed along to the next generation. These types of selection allow animals and plants to evolve i.e. to change over long periods of time. This is the way that early hominids became modern humans and dinosaurs became modern birds. **Evolution is made possible by mutations and natural selection.**

4. Environmental changes, evolution and extinction.

The fossil record shows however that evolution is still continuing today. If species adapted to our environment thousands of years ago they should be stable by now. Yet they are not. Evolution is still taking place. One reason for this is the fact that mutations are still taking place. We can see the diversity that they create in our environment. There is also the fact that our environment changes. And those changes select within the diversity in a given population characteristics that will enable individuals to better survive the new environment. As the ozone layer is destroyed by human activity harmful UV rays are now penetrating the atmosphere is much larger quantity. These UV rays are more likely to damage the DNA of your skin than they were before when most of them were filtered out by the ozone layer. It is estimated for example that two thirds of Australians will experience skin cancer in their life time. It is also known that darker skin adds extra protection against UV rays. If the ozone layer were to completely disappear or drop down to only a fraction of what it once was would you expect that this would have an effect on the human population? What would happen if dark skin was not enough to protect humans against cancer? Dramatic changes in the environment may not give a population the time required to adapt. These changes may then lead to the extinction of populations. We know that dinosaurs were killed by the collision of an asteroid with earth 65 millions years ago. The collision sent billions of tons of debris into the atmosphere. This thick cloud prevented the sun from reaching the earth for a number of years. As plants died out from lack of sun and cold temperatures the dinosaurs saw their food supply dwindle and they disappeared from the surface of the earth. At least the cold blooded ones. We now have some evidence that warm blooded carnivorous dinosaurs (a flying theropod dinosaur called Archaeopteryx) may have evolved into what we now know as birds. At the time of the disappearance of dinosaurs small warm blooded furry mammal-like dinosaurs had started to appear on earth that were far better adapted to these dramatic climate changes than the dinosaurs were and they repopulated the earth. They evolved eventually into mammals. A branch of Archosaurs (a predecessor to dinosaurs and reptilians), ancestors to the present day crocodiles, alligators and caimans, was also able to survive the new conditions imposed on earth by the meteor collision and continue thriving to this day. A large number of species of dinosaurs disappeared because they were not adapted to the new conditions and they did not have the diversity in their population that could have given them tools to survive. But some of them did and they still exist today as mammals, birds and reptiles.

5. Evolution, diversity and cross-breeding.

The diversity that mutations bring to a species make it possible for a species to become more and more different from other species in the genus. As the differences accumulate a variety can become its own species and a species can become its own genus. It is important to note that two species of the genus Homo are more closely related than two genera of the family Primate**.** Homo sapiens is more closely related to Homo Neandertal than to gorillas. That is because Homo sapiens diverged from Homo Neandertal 600,000 to 750,000 years ago, long after it had diverged from its common ancestor with the great apes. Therefore it is false to say that humans descend from apes since apes and humans had a common ancestor from which both humans and apes evolved. It is now possible to measure how closely related two species are by looking at the similarities and differences in their DNA. The measure of percent homology tells us how similar the two species are. For example human DNA has a 98.7% homology with orangutan DNA. At the same time recent studies have shown that humans and Neandertals share 99.5% or their genetic makeup.

Interbreeding is a way to determine how closely related two species are. Dogs can interbreed with wolves and give viable offspring but they cannot do the same thing with cats. Since dogs and wolves are of the same species (Canis canis) one would expect a lot of homology in their DNA. One the other hand dogs can also interbreed with coyotes (Canis latrans) which are not of the same species. So can tigers and lions. Horses interbreed with donkeys (male donkey and a female horse produce a sterile mule; a female donkey and male horse produce a hinny, also sterile) but the offspring is sterile i.e. it cannot reproduce. From this would you say that dogs are more closely related to coyotes than horses to donkeys? It would appear so.

There is an ethical dimension to our efforts at crossbreeding different species. In doing this we are not concerned about the different habitat requirements that the hybrid offspring might have or the fact that the offspring is often sterile. Should we be concerned with these questions? Would it be advisable to attempt hybridization between humans and apes? Interestingly the level of chromosomal similarity in humans and apes is roughly the same as in horses and donkeys. Do you see any problems with this hybridization program?

Neandertals lived in Europe from 200,000 BC to 28,000 BC when they became extinct. Bone studies indicate that they were shorter and more stocky than contemporary humans. They also had larger brains. They hunted large animals but do not seem to have collected berries and roots They appeared to be less agile and dextrous and possessed fewer tools than Homo sapiens. Their life expectancy was also probably lower than that of contemporary humans. They were probably capable of speech since their DNA carried the same version of the speech-enabling gene FOXP2. The reason for their extinction is not known but likely to be a combination of rapid climactic changes, lack of adaptability and competition with numans.

Neandertal DNA has been recovered and a rough draft of its genome is known. This raises the possibility of bringing back Neandertals in Jurassik park fashion. This would enable us to study the species and confirm speculations from bone and DNA studies about Neandertal metabolism, muscle mass and speech capabilities among others. The question of cross-breeding with Homo sapiens could also be addressed (archeological data shows no evidence of this). While this is all going to be possible in the next 50 years the question is more likely to be: Should we? What do you think?

6. Distribution and diversity in grape populations.

The European grape was brought along by explorers and settlers to South America, Asia and California, etc. Today *Vitis vinifera* is the most produced wine grape in the world. Explorers found other grapes in North America and Asia and even named places for profusion of vines: Martha's Vineyard for example. *Vitis labrusca* is the most important food producer in North America. Coming from Mid-Atlantic states, it has a "foxy" flavor (it is also called fox grape). The “foxy” flavor is due in part to methyl anthranilate, a compound that is made today by organic synthesis and added to soda pop and other foods to give them a grape flavor. More than 2,000 varieties of *Vitis labrusca* have been developed in the 300 years since its discovery. Concord is typical to the species and produces table grape, jelly, juice and (unfortunately?) some wine. Other *Vitis labrusca* include Catawba, Delaware, and Niagara etc.

- *Vitis rotundifolia* is found in abundance in Southern Atlantic states
- Scuppernong muscadine grapes are used to make wine in Maryland and other states.
- *Vitis rupestris* or sand grapes
- *Vitis riparia* or river grapes

All of these grapes have flavors that most wine makers would consider unsuitable for wine making. However, they have several traits that Vitis vinifera lack such as disease resistance, resistance to the louse Phylloxera and adaptability and ruggedness to harsh soil and climate conditions. Because of these traits, American varieties resist humid conditions, very cold winters and diseases that would be deadly to European grapes.

These four American varieties have been used for:

a. Rootstock for vinifera. We have mentioned above that all vivifera grapes grown in Europe today have American grape rootstocks.
b. Crosses with *Vitis vinifera* to produce hybrids.

The problem here is that the American grape parent gives a foxy flavor to hybrid **offspring**. Thus, a tricky system of **backcrosses** to *Vitis vinifera* is needed to keep healthy characteristics of the rugged American grape parent but to get rid of the foxy flavor. The backcross is a system in which the offsprings of each generation are crossed to the original European parent. Each subsequent generation is selected for low foxy flavor and ruggedness and the selected vines are crossed again to the European parent. Varieties such as Baco, Foch, and DeChaunac (Seibel 9549) are some of those crosses (See Wagner Tables). The large numbers given to these hybrids give you an idea of the number of crosses that breeders make to create a hybrid grape suitable for wine making.

You can make wine by mixing juice from several varieties or even species. The product is then simply called table wine. In contrast, a varietal wine is a product where most of the juice comes from one variety of grape (51% or more by law in California, over 90% by law in New York). This wine is then called by the name of the grape: Seyval, Baco, Cabernet, and Sauvignon, to name a few. In contrast French and most European wines are given geographical labels (Bordeaux, St. Emilion, etc.) and reflect the specific blend of varieties of grapes grown in the region. When you buy a Bordeaux such as a $500 bottle of Chateau-Margaux 1964, you have a wine made of three or four different grapes including Cabernet, Merlot, Verdot and Petite Sirah; all different varieties of *Vitis vinifera*. Wine makers blend these different varieties according to their judgment of what will make a better wine, one that is consistent with the taste and bouquet expected of a wine of the region. It is not a varietal but an **appellation controlée**; a mixture of related and congenial grapes which are native to that climate and region and do well in it. They are fermented and aged according to the Bordeaux tradition. This method of making wine comes from the Middle ages when, as you will recall, each region standardized and controlled its way of making wine in order to acquire a distinctive character. The custom of naming a wine by the one grape it is made from is American and stems from the fact that America originally made good but not great wines. In recent years, however, and maybe because most European wines are good but not great, the **varietal wine** or **vin de cepage** has also emerged in Europe following the American lead.

B. GROWING WINE GRAPES

Factors affecting grape growing.

1. Climate

Climate is a major factor for the growth of grapes and the quality of wine produced. Figure 3.1 shows the climates acceptable for grape growing. If the average temperature is above 20^{O}C (78^{O}F) winters are so mild that leaf fall and frost (grapes need that) do not occur. The vines cannot grow good wine making grapes under these conditions. If the average temperature is below 10^{O}C (50^{O}F), winters are too long and cold, and the summer is too short for grapes to ripen. The roots may be killed by frost. Only a small portion of the world (with average temperature between 10 and 20^{O}C) is suitable for growing wine making grapes.

While these are the temperature limits for grape growing, it does not mean quality grapes for wine making can be grown everywhere in these regions. Within those limits, the warmer climates will produce raisins and currants but poor wines because sugar accumulates to high degrees in the fruit due to very warm summer climates. That, however, is far from enough to produce a good wine.

As temperatures get cooler we see the following progression:

hot	raisins	great yields, poor quality wine

warm	fortified wines table wine, reds	Best quality but lower yields
cool	white wines only	
cold	—	

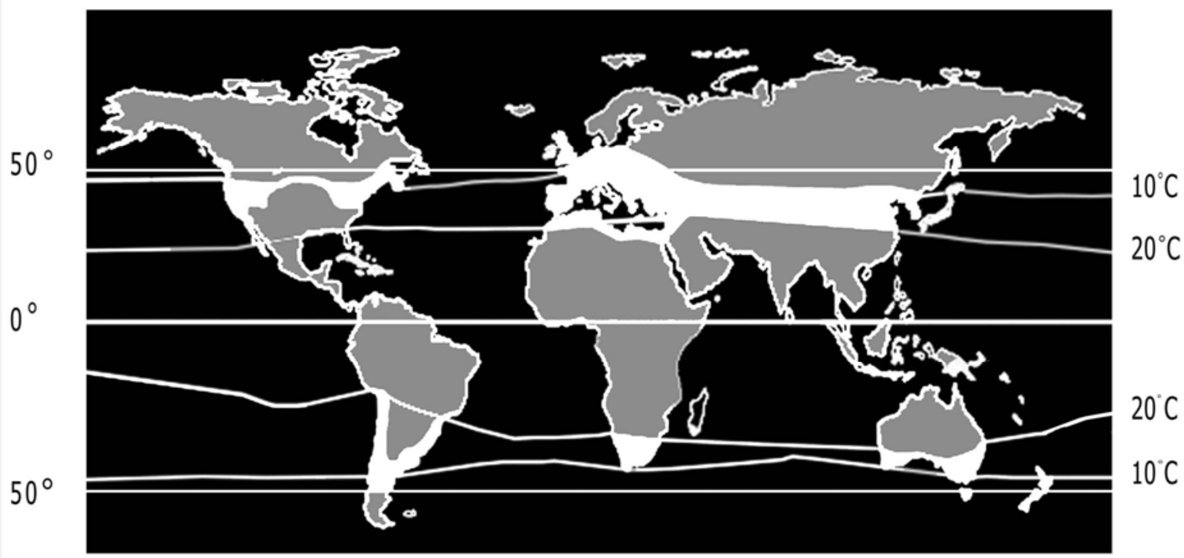


Figure 3.1. World distribution of viticulture. Almost all wine producing regions fall within the temperature zones between 10 and 20 degrees Celsius - average all year.

Therefore in the cooler climates where vines still grow (this applies to NY State) wine production is expensive because fall and spring frosts can damage the buds, winter can damage or kill root and cooler summers may slow down ripening. The result is that grape juice often does not rise above 22^{o} brix in sugar content and acid levels remain high. But, quality is usually excellent.

Why do cooler temperatures produce the highest quality wine? Most likely because cooler autumn temperatures are crucial. If temperature is too high, ripening occurs quickly and the whole metabolism of the grape is geared to sugar production. Acids and other chemicals that give wine its taste are not produced or are converted to sugars! A cool autumn slows down sugar production and gives enough time to the grapes to produce and accumulate other compounds that will give balance to the wine. In addition, long periods of ripening at warm temperatures lead to a decrease in acid concentration and produce an unbalanced wine.

How will you know if your area can grow grapes of a particular type for a given wine? There are several ways to figure that out using formulas.

a. Degree – days.

- Measure the average temperature for the month: mean daily max + mean daily minimum; divided by 2
- Subtract the base figure of $10^{O}C$ ($50^{O}F$) from it
- Multiply result by number of days in month.

Example:
av. $63.5^{O}F - 50^{O}F = 13.5\ ^{O}F \times 30$ days = 405 ^{O}F- days
A winter month might have a negative value.

- Add these degree-days units of heat for the growing season.
- Run a comparison of the last 20 years.
- Establish a determination of temperature over years for your area.
- Grow grape best suited for these conditions.

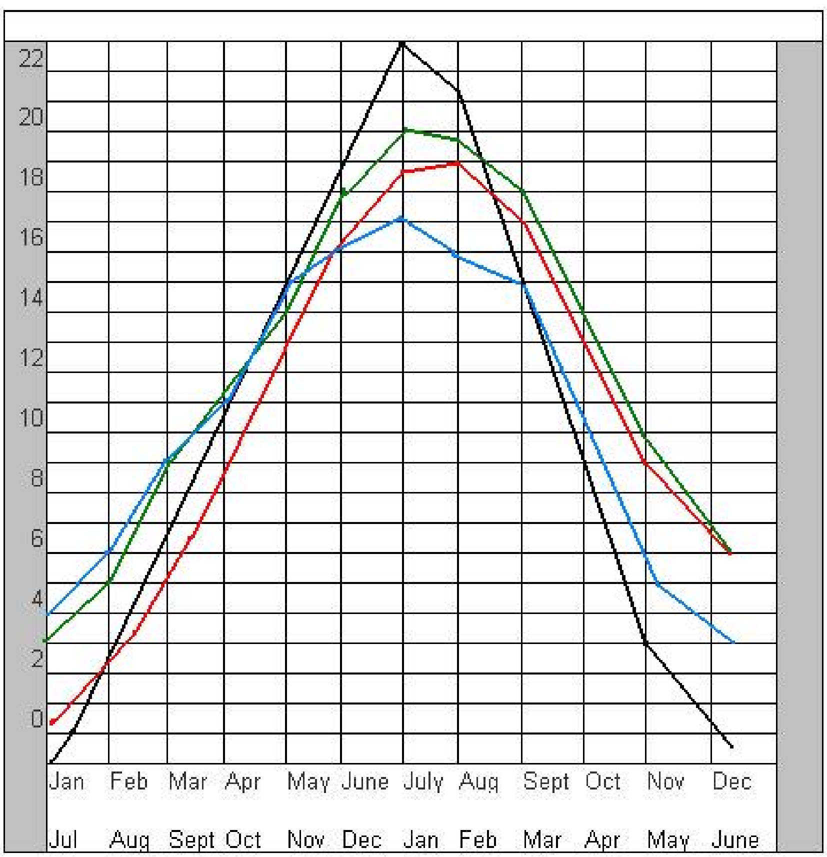


Figure 3.2. Mean monthly temperatures for four grape-growing districts:. Latitudes are shown for Bordeaux, France; Christchurch, New Zealand; Geisenheim, Germany; Prosser, Washington, USA.

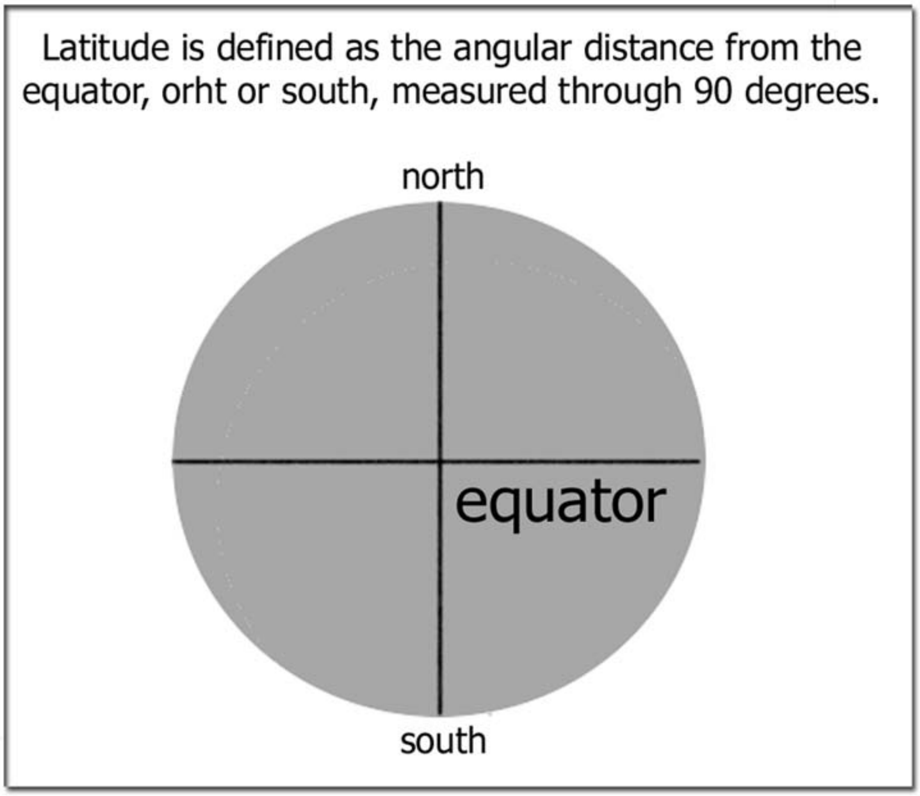
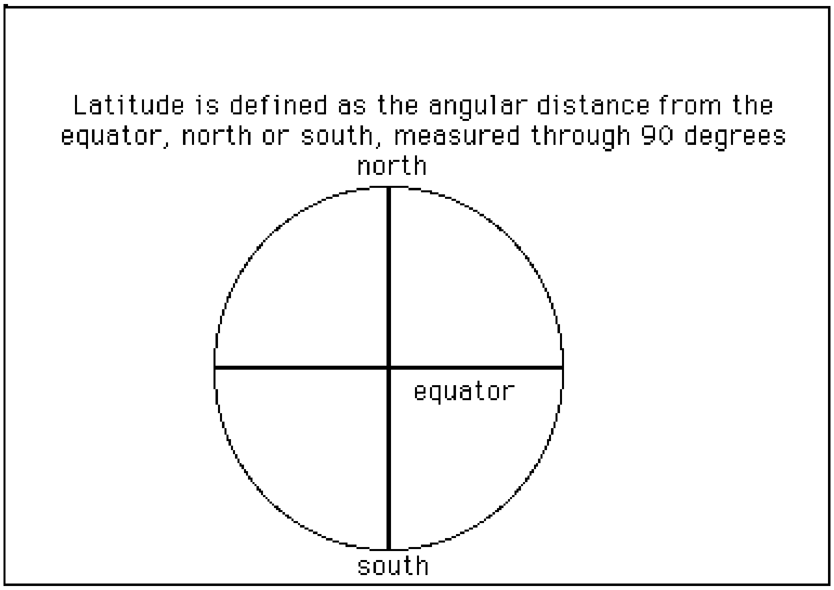


Figure 3.3


Latitude is defined as the angular distance from the equator, north or south, measured through 90 degrees
north
equator
south

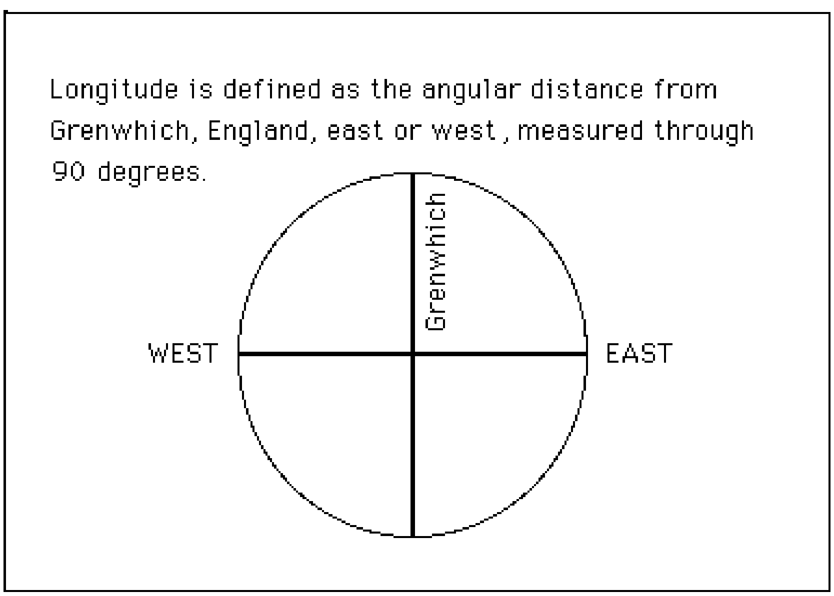
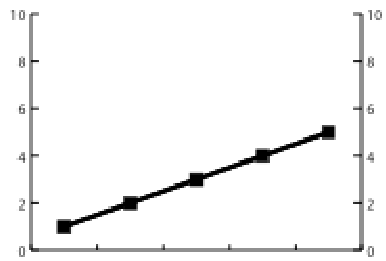

Longitude is defined as the angular distance from Grenwhich, England, east or west , measured through 90 degrees.
Grenwhich
WEST
EAST

Figure 3.3.

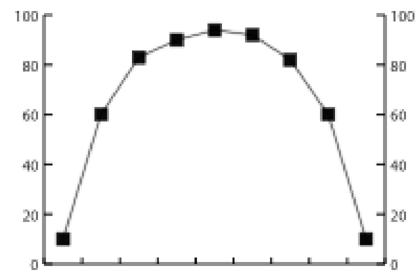
b. Mean temperature

Another method is to compare the mean temperature of the warmest month of growing season. For example Figure 3.2 shows four different localities at four different latitudes. Latitude is defined as the angular distance, north or south, from the equator measured through 90^{o}. (See Figure 3.3)

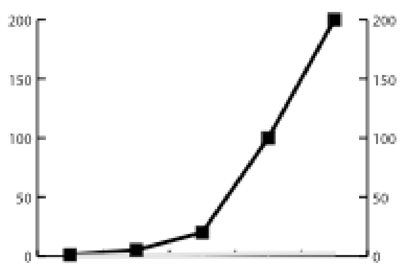
Let us go back to Figure 3.2. You have seen graphs before. They usually show two variables (x and y) on two axes placed at ninety degrees from each other (x axis or horizontal axis and the y axis or vertical axis). The curve shows the relationship between the two variables i.e. how the y variable changes when the x variable varies. The relationship or function is linear (a straight line) when y increases (or decreases) steadily as x increases or decreases. The function can also be hyperbolic, sigmoidal, parabolic or Gaussian (bell-shaped).



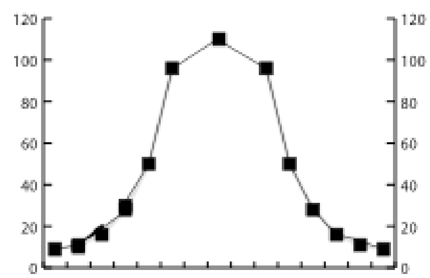
LINEAR CURVE



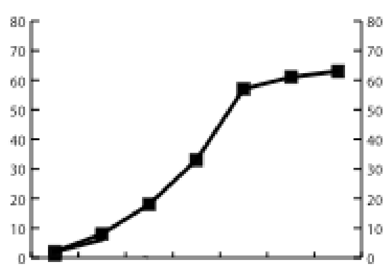
PARABOLIC CURVE



HYPERBOLIC CURVE

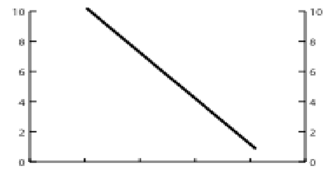


GAUSSIAN CURVE



SIGMOIDAL CURVE

Can you think of other types of curves? What about these next two? What is different with the models we have just seen? Is it still a linear or hyperbolic curve?



Let us go back to Figure 3.2. What is the function shown there? (Gaussian).

There are 2 x axes on Figure 3.2? Why? Growing season in the southern hemisphere (Christchurch is in New Zealand) is different than in the northern hemisphere; their summer is our winter and vice versa. Geisenheim and Prosser have a greater angle from the equator meaning that they are further north; this means that the growing season is shorter and the curves shown on the graph are steeper. Bordeaux and Christchurch are closer to the equator and therefore have more warm months and show less steep curves. On the other hand, Bordeaux and Prosser have the 2 warmest months while Christchurch and Geisenheim have colder maximum temperatures. Therefore Geisenheim has warmer mid summer temperature than New Zealand. But, New Zealand has a longer growing season. Thus New Zealand has greater ripening ability; in fact, as great as Prosser and almost as great as Bordeaux.

Which is more likely to give white wines? Geisenheim with low mean temperatures and higher latitude. Therefore Latitude is a better indicator of climatic suitability than degree - days or mean temperature.

c. LTI

The best indicator is probably **LTI** or **latitude - temperature index**

$$\text{LTI} = \text{mean temperature of warmest months} \times (60^{o} - \text{latitude}).$$

LTI will give you an excellent correlation between the type of grape grown and climate. Using this formula we find that while good table wines must come from a cool climate, there still remains a variable in climate which in turn results in a variable in quality.

What will give poor quality?

- Very warm summers result in a quick ripening, lots of sugar but bland taste because of low acid. In fact, it is not uncommon for California wine makers to add acid to the must during wine making to keep it above 0.6.
- Very cool summers result in grapes having too much acid which produce an imbalanced wine with low alcohol content due to low sugar content.

Grapes can be classified by climate: note here that LTI uses ^{o}C only.

- Cool climates mean low LTI (less than 190) and produce grapes such as Pinot Noir, Chardonnay (NY State grows Chardonnay very successfully) low alcohol, very dry, and acidic wines. Cool - warm means an LTI of 190 to 270. These conditions are ideal for growing Riesling and Pinot Noir.

- Pinot and Chardonnay are more full bodied than in Group I because the metabolism of the grape produce, aside from sugars, other compounds that bring flavor and more of it than in region A

- Warm LTI between 270 and 380 is where Cabernet, Merlot (a filler used extensively in California and France) and Sauvignon blanc will grow well. If these grapes are grown in zone B, the quality will decrease.

- Hot LTI above 380 is ideal for Grenache, Zinfandel, Thompson seedless. These are very sweet grapes. They are adapted to group D and will seldom ripen in region C. Region D gives dessert wines (sweet) and fortified wines (port) . Regions A through C give highest quality wines and do not grow grapes from region D

Enters global warming. The planet is warming up. The LTI calculated from Figure 3.2 is based on temperature data collected before the 1970s. If we use data from the past 20 years however we find much higher LTI.

	LTI before 1980	LTI 1980-2005	% increase
Bordeaux	288	394	36.8
Christchurch	281	380	35.2
Geigenheim	180	240	33.3
Prosser	296	436	47.3
Finger Lakes	210	497	137

So while traditionally NY state has been classified between group A and B, a study of the average temperature in the hottest month of the year over the last twenty years shows a warming trend that brings the LTI for this region to 400 AND ABOVE. This would make the Finger Lakes a region capable of growing the same grapes as Bordeaux and California. Unfortunately winters are still colder than in France or California and frost damage is still a concern. This points to a flaw of the LTI system for determining the suitability of an area to grow a particular type of grape.

Can we explain this warming trend? Over earth's recorded history warming and cooling trends have occurred. Periods of warm temperatures are followed by very cold periods. Some cold periods are very cold (major glacial ages) while others are not as cold (little or minor glacial ages). For example a little glacial age occurred between the 13th and late 19th century. Global temperatures dropped by 1-2 ^{o}C. While the earth did not become covered in ice, summers in the northern hemisphere were cold and rainy and started three weeks later than they would today. Crops failed and famine spread throughout Europe. It is very possible that the weakened state of the European populations made them more vulnerable to the plague that swept through the continent during that period, killing a third of the population.

We also know that the earth went through major glacial ages every 100,000 years and 40.000 years. During these periods the average temperature dropped significantly and ice sheets covered most of the North American and Eurasian continents. The last glacial ages occurred 20,000 years ago and ended 10,000 years ago. Scientists have hypothesized that these climate changes are related to several causes:

1. Carbon dioxide (CO_2) cycles between the air and solid and liquid (dissolved in ocean water) forms. CO_2 can exist in three different forms: as a gas in the atmosphere, dissolved in water or as a solid (carbonate). We know of dissolved CO_2 as the carbonation in soft drink and beer. What is interesting is that cold water has more dissolved CO_2 than warm water. Have you ever wondered why a cold soft drink fizzles so much when you put it in your mouth. It is simply because as your mouth warms the liquid CO_2 escapes from it since its solubility in the liquid has suddenly been decreased by the temperature rise. You can show this by putting a soft drink in two glasses and keep one in the refrigerator while this other is left at room temperature. After an hour the cold drink will has a lot more CO_2 than the warm one. It will "fizz" more in your mouth.

We also know that CO_2 in the air acts to trap heat into the atmosphere creating a "greenhouse" on earth. Since CO_2 can exist in the air and in the water there is a constant exchange between the oceans and the atmosphere. When there is more CO_2 in the atmosphere the temperature of the earth increases. When there is less CO_2 in the atmosphere the temperature of the earth decreases. If the temperature of the ocean decreased it would dissolve more CO_2 from the atmosphere and the temperature of the earth would decrease even further. That is caused a positive feedback loop. It the temperature of the ocean increased it would release CO_2 in the atmosphere which would then see its temperature rise.

But what would make the temperature of the ocean rise to begin with. Human activity can of course increase the amount of CO_2 in the atmosphere and increase the temperature of the earth, thus the oceans and this can start the cycle of ever increasing global temperatures. That is why more and more scientists are urging governments to decrease emissions of greenhouse gases. But there is also another factor that starts these positive feedback loops: the movement of the earth around the sun.

2. We all know that the earth gravitates around the sun in an elliptical orbit while rotating around its axis. We also know that the earth is actually tilted on its axis by about 24 ^{o}. This means that we have seasons. During part of its orbit around the sun the tilt exposes the northern hemisphere more to the sun. It is summer. The southern hemisphere being less exposed experiences winter. Six months later the same tilt is exposing the southern hemisphere more to the sun than the northern hemisphere and we have winter. But this tilt is not always constant. It changes from 22 to 24.5 ^{o} in a forty thousand year cycle (**Milankovitch cycles**). Currently this angle is 23.44 degrees and is decreasing. If the angle of the tilt is reduced the northern hemisphere is less exposed to the sun in the summer and summers are cooler. Winters are conversely warmer. It will still snow in the winter and that snow will stay longer in the summer. Since snow reflects the sun's energy more than water (we have all been blinded by the snow on a clear day) the earth will absorb less energy and will cool down. We have here another positive feedback loop. As the earth gets cooler more snow accumulates and more of the sun's energy is reflected back into space. The earth gets even cooler. But now we have another positive feedback loop entering the cycle. As the earth cools down more CO_2 is dissolved in the ocean leaving less in the atmosphere to retain heat from the sun and the temperature of the earth drops even more. That is how glacial ages start. Or this at least is one theory**.** **According to this theory can you explain how the earth gets out of a glacial age.**

Now how would the increase in atmospheric CO_2 due to human activity affect this cycle?

3. Another way to cool the earth is with volcanoes and meteorites. Both of these events project in the earth's atmosphere billions of tons of ash and debris which in turn obscure the sun. Volcanic activity has been suggested as one of the causes of the last minor glacial age while a meteorite hitting the Yucatan peninsula probably cooled the earth sufficiently and for a long enough time to kill the dinosaurs by depriving them of their food sources.

So where does that leave us today? To this day, the human race has burnt enough fossil fuel to put in the atmosphere about 1100 gigatons of CO_2. That is 1100 billions tons or 1.1 thousand billion tons (1 followed by 12 zeros). That is enough carbon dioxide to prevent the next glacial age. That might be good news for those of us who do not like winter. However warming the planet has other consequences that might be hard to live with.

Models of global warming suggest that the climate will become more extreme. Tornadoes and hurricanes will become more frequent and more violent. We could see heavy rains and flooding in Japan or California and, at the same time, severe drought in the Midwest. We seem to have seen that already happening over the last few years. Second the rise in temperature will melt the ice caps in the Artic and Antarctic. The flow of water in the oceans will raise sea levels and flood coastal areas that are currently just above sea level. It is expected that these levels could rise by 12 to 20 feet by 2050. That would put most of Florida and New York city under water. You may not want to buy beach front property on Cape Cod in the next few years. The water dumped in the ocean by the Artic ice cap will likely push the Gulf Stream away from the Eastern seaboard or interrupt it altogether. The Gulf Stream is a water current in the Atlantic ocean that brings warm water from the Equator to the eastern seaboard of the United States, Greenland and the Western approaches of England and Northern Europe. Water circulation in that stream was shown to have decreased by 30 % between 1992 and 2002 presumably because of the large input of cold fresh water from glacier melt. This could paradoxically bring a 1^oC to 6^oC drop in temperature to these regions, in effect creating minor glacial conditions in England, Northern Europe and the Northeastern United States. Also rising temperatures will likely make an arid area out of the Midwest as well as other subtropical regions of the world. What we now call a bread basket would become a desert. Finally rising ocean temperatures will reduce the solubility of carbon dioxide and methane and increase the release of these gases into the atmosphere thus increasing the greenhouse effect even further.

As grapes are subjected to warmer or cooler growing temperatures the characteristics of the juice produced from those grapes will change. The following table shows these changes. Can you explain them?

LTI	fruitiness tartness	alcohol content	dryness	sweetness	red pigment	acid
low	high	low	high	low	low	high
| V	| V	| V	| V	| V	| V	| V
high	low	high	low	high	high	low

2. Rainfall

Rainfall is of course crucial to vineyards; too much rainfall however is as deadly as too little. Grape growing areas must have below 750 mm (29.5 in) per year. Wet areas or areas where rain concentrates in the fall shorten the length of the growing season. Referring back to Table 3.I, rain fall above 800 mm means that your region grows grapes from one group below that given by the LTI.

HOW DO YOU CONVERT mm to inches? In science we use a method of solving problems that is referred to as dimensional analysis. This method uses the units rather than the numbers to solve a number problem. Here is a good example. You want to know how many inches there are in 760 mm. What you are given is 760 mm and what you need to know is how many inches that gives you.

1. Write what you have first:

760 mm

2. Further down the line write the units that you need:

760 mm = in.

3. Now convert the unit "mm" until you arrive at what you need. The first thing would be to convert to "cm". So divide by mm and multiply by cm:

$$760 \text{ mm} \times \frac{\text{cm}}{\text{mm}} = \text{in.}$$

4. Now put the numbers in: 1 cm is 10 mm. You know that from the metric system where units go in tens: 1 meter =10 decimeters=100 centimeters =1000 millimeters.

So, 1 centimeter =10 millimeters.

$$760 \text{ mm} \times \frac{1 \text{ cm}}{10 \text{ mm}} = \text{in.}$$

5. The next conversion will be from cm to inches. That is something that you need to remember: one inch = 2.54 cm. Remember that you need to get rid of cm and add inches to your solution. So multiply by "1 in" and divide by "2.54 cm".

$$760 \text{ mm} \times \frac{1 \text{ cm}}{10 \text{ mm}} \times \frac{1 \text{ in}}{2.54 \text{ cm}} = \text{in.}$$

6. Now look at the units. Which ones cancel out and which ones are left? If the units that are left match the unit that you where looking for in the answer, you are done. If not, keep converting.

This method can be used to solve any math problem. Try this: You are given a 350 inches of metal wire. How many feet do you have? How many centimeters do you have? Now try to do some of the exercises at the end of this chapter.

3. Chilling in winter

In Continental areas of Europe and America (California) winter frost will not be a damaging factor for grapes. In NY State, it is however. Grapes in groups 3 and 4 of our classification (see above) will be very sensitive to frost and they will not grow consistently well in NY State. Native grapes and Franco-American hybrids are more tolerant to cold and are used here in NY State. Some grape growers insist on using vinifera exclusively in

NY State and have to replace, every year a large percentage of their vines because of killer frosts (Frank vineyards for example).

If the mean temperature of the coldest month is below -1 ^{o}F (30^{o}C) or if the lowest temperature over the last 20 years reaches -20 ^{o}C (-4 ^{o}F) this area must be considered marginal for vinifera grapes; hybrids grow better in such an area and are the preferred grapes for growers.

An important factor here may be the cycles that we see in the weather. For example, we have noticed a 20 year cycle of relatively warm winters followed by 20 years of much colder winters. In 2007 it appears that we are in a 20-year warm cycle in NY State right now. How would that affect grape growing in NY State?

Summary: important factors in wine growing:

- length of growing season => latitude
- temperature during that season
- winter frost
- rainfall

What have we forgotten?

3. Soil conditions

Soils are actually a complex mixture of organic and inorganic materials. The inorganic material is a collection of minerals classified by the size of the particles. The smallest ones form clay, the medium ones form the silt and the larger ones form sand (the largest ones are called rocks). A soil formed mostly of clay retains water too well and prevents air from entering its structure. Sand drains too well and does not retain water. The best soils contain equal proportions of sand, silt and clay to retain enough water for the plants but permits air to penetrate its structure. This soil also provides plants with the minerals necessary for their growth. The last component of soil is the organic material made of decomposed plants and animals; we know it as compost.

Interestingly, grapes will grow in relatively rocky, sandy, infertile soils, unsuitable for many other crops as long as they drain well or are kept dry. The grapes have deep and extensive root systems and will withstand long droughts. While it is true that soil will change flavors in some wines, it is impossible to predict what type of wine a particular soil will produce. Heavy clay and poorly drained soils should be avoided. Choose deep soil with gravel or sand.

Fertile soil provides for heavy vegetative growth but less flowering in grapes. So growers prefer a less fertile soil to which they will add fertilizer only during the first 2 years to get vigorous trunks. The also prefer bare soil (without sod, i.e. grass) over the roots for less water retention and more heat absorption.

The selection of a proper site is often more important than soil the itself as shown in Figure 3.4:

- select a hill facing south so that the sun is on it all day; the vines will then catch more sun.

- Plant mid slope to avoid spring frost since cold air drops to the bottom of the valley.
- Wind shelter is also crucial as seen on Figure 3.4. If a vineyard faces the prevailing wind it will accumulate less heat in summer and the wind can damage the flowers. In this figure, site “a” is ideal . Which site would be ideal if the prevailing winds were reversed ?

Too much rain or poor drainage mean waterlogged soil; the danger of mildew is then greatly increased. In fact the absence of summer rain is often a favorable characteristic of grape growing regions. Rain during the blooming of flowers will damage and decrease setting of berries. Fog and high humidity during late ripening provide conditions for berry cracking and mildew. These facts all seem to point to dry conditions as ideal for grape growing. Why is that the case since all plants need water to grow well?

5. Example of grape growing conditions: NY State.
The Finger Lakes are one of 4 NY regions for wine making (see map on Figure 3.5).

a. Conditions. From climatic considerations, one would think that NY State is too cold for growing quality grapes for wine making. It is too far North and winters are too cold for V. vinifera to grow consistently well. But two points here make the Finger Lakes an area of choice.

- The Finger Lakes are very deep lakes that were dug by glaciers. They provide a vast reservoir of water that never freeze. This means that the temperature of these lakes never goes below 32 ^{o}F. If the temperature of the surroundings is 0 ^{o}F for example. He lakes will give off heat to the surroundings an increase the temperature of the surrounding hills where grapes are located. These lakes then temperate the climate in their immediate vicinity.
- The slopes provide shelter against the west to east prevailing winds. That is why grapes are planted mostly on the western slopes of the Lakes, facing east. Therefore even though the growing season is short (135-150 days or 1/3 shorter than California) it is dry and warm enough to allow grapes to ripen. The slopes provide good drainage and the temperate climate provided by the lakes prevents climatic extremes so that nearly every year is a vintage year.

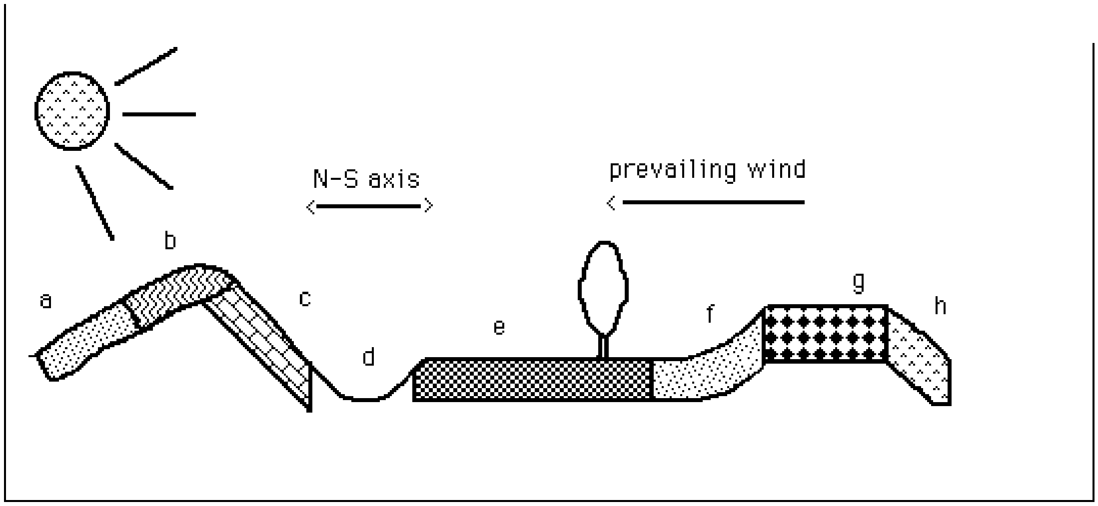


Figure 3.4. Microclimates of the Finger Lakes

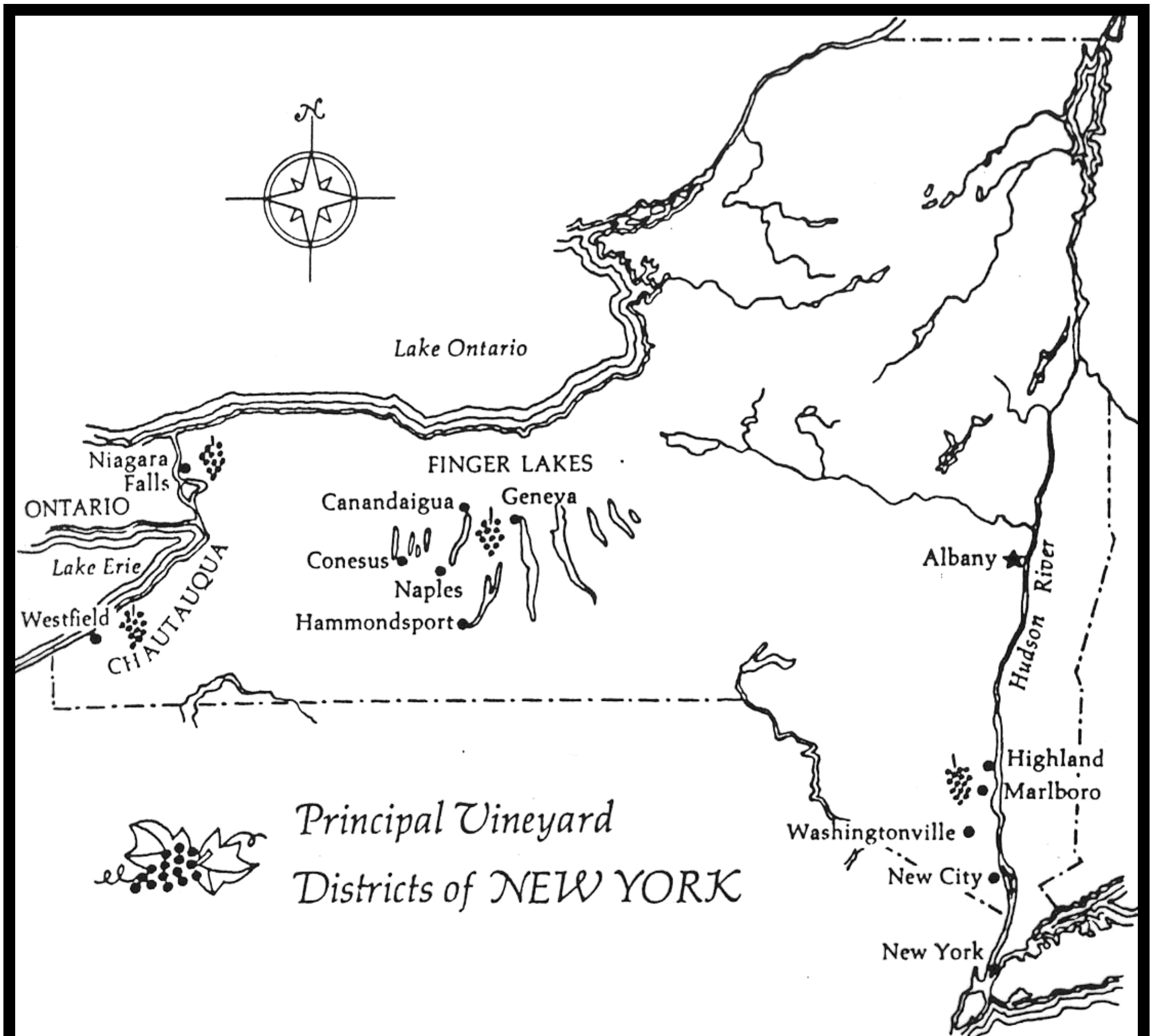
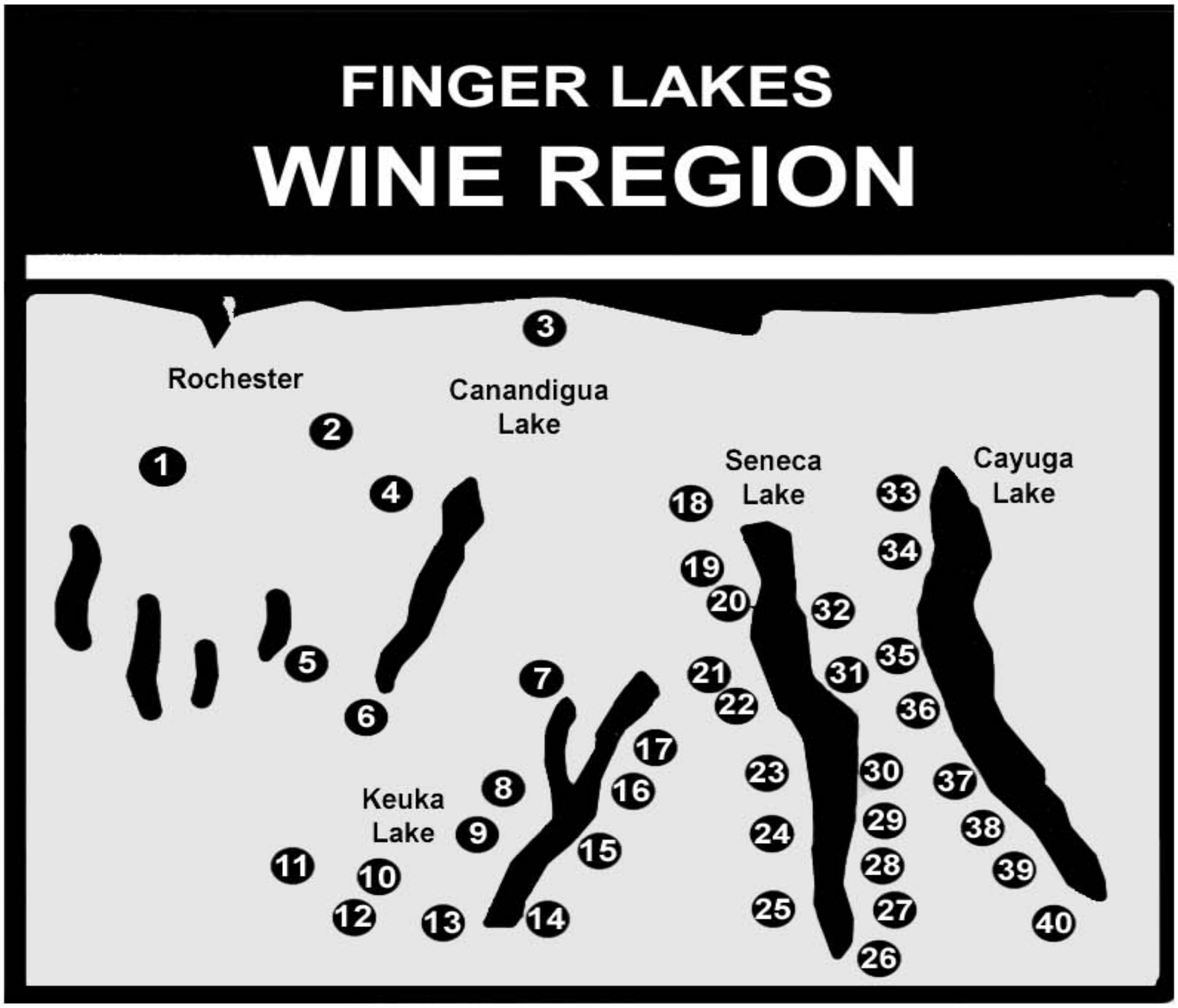


Figure 3.5

The snowy winters are too cold however for vinifera with temperatures often less than -20^{o}C. Only a few vinifera, when **grafted** to **American rootstocks**, can survive winter: Chardonnay for example. Chardonnay, Riesling was pure V. vinifera. Dr. Konstantin Frank began grafting on roots of Pinot grapes that had adapted to harsh winters in a convent in Quebec. After thousands of grafts were made, Dr. Frank and Charles Fournier of Gold Seal found that the grafts resisted a -25^{o}C winter better than some hybrids or some labrusca grapes.



Locations are approximate

1. Batavia Wine Cellars
2. Casa Langa Vineyards
3. Straubing Vineyards
4. Canandaigua Wine Co.
5. Eagle Crest Vineyards
6. Widmers Wine Cellars
7. Finger Lakes Wine Cellars
8. Dr. Franks Vinifera Wine Cellars
9. Heron Hill Vineyards
10. St. Waller de Bully
11. Bully Hill Vineyards
12. DeMay Wine Cellars
13. Taylor, Great Western, Gold Seal
14. Batavia Wine Cellars
15. Mc Gregor Vineyards
16. Ambrosia Farms
17. Keuka Spring Vineyards
18. Penn Yan Wine Cellar
19. Prejean Winery
20. Lake View Vineyards
21. Four Chimney Farm Winerey
22. Herman J. Weimer Vineyard
23. Barrington Champagne Cellars
24. Glenora Wine Cellars
25. Giasl Winery
26. Rolling Vineyards Farm Winery
27. Chateau Lafayette Reneau
28. Wickham Vineyards
29. Hazlitt Vineyards
30. Poplar Ridge Vineyards
31. Fair Haven Winery
32. Wagner Vineyards
33. Swedish Hill Vineyards
34. Lakeshore Winery
35. Knapp Vineyards
36. Planes Cauga Vineyard
37. Hosmer
38. Lucas Vineyards
39. Americana Vineyards Estate Winery
40. Frontenac Point Vineyards

During his life Dr. Frank also maintained that they are not harder to grow than hybrids, contrary to Cornell's Geneva Station's opinion. It is possible however that in some cases such wet summers V. vinifera are more sensitive to NY fungi and diseases (they do have to replace a large percentage of their vines every year). However, Dr. Frank's vinifera wines are the best in NY State and as good as European wines from similar types of grapes: Riesling, Pinot, and Chardonnay. Their prices also reflect that fact.

Most of NY grapes are American native grapes Vitis labrusca, grapes of strong, "foxy" flavor such as Concord, Catawba, Delaware, Niagara, Dutchess. These grapes resist cold weather but their wines retain a strong "grapy" flavor and, while good, will not rival in subtlety with European wines. Because of their hardiness these grapes are cheap to grow and their juice is inexpensive. As we said before experts argue that they are unsuitable for wine making because of their high concentrations of methyl anthranilate.

Thus far the 2 choices were to grow the strongest vinifera which would give good vintages but potential high loss (30% replanting/year) or grow American grapes and produce lower quality wines (Concord flavor is bitter sweet). Another alternative, which is becoming more popular, is to grow hybrids of the 2 that could show three characteristics:

- Cold hardiness
- Low levels of **methyl anthranilate** (and no or little labrusca taste)
- High complexity of taste that can rival the vinifera for better quality wines.

French horticulturists did that when American grapes were introduced in Europe; they were then called the French hybrids. Today, only France and the U.S. allow the use of hybrids in the wine industry but only 1/3 of France's wines are made of French hybrids." **Appellation controlee**" wines cannot contain French hybrids however, only "vins du pays", made for local consumption, are made of those hybrids. We have seen some of the history of hybrid development already. Remember than the French had to graft vinifera on American rootstocks (the result is not a hybrid) to get resistance to Phylloxera, however, they had to graft before planting and that was not convenient. So, they asked for direct producers i.e. hybrids made by sexual crosses. The first crosses tasted like the American parents with strong foxy flavors. But, by backcrossing (remember that backcrossing is the mating of the offspring of subsequent generations to one of the parents over and over again) to vinifera they selected and obtained Phylloxera-resistant grapes that tasted like V. vinifera grapes. As crosses continue today, more varieties emerge that have less and less "foxy" flavor of labrusca but retain resistance to Phylloxera, mildew, cold weather etc..

Here is a short list of the most commonly grown hybrids in NY:

Reds:

Baco #1
Chelois: Seibel 10878
Chancellor: Seibel 7053
Ravat Noir: Ravat #262
Marechal Foch: Kuhlmann #188-2
DeChaunac; Seibel 9549

Whites:

Aurora: Seibel 5279

Seyval : Seyve-Villard 5276
Villard Blanc 12375
Baco-blanc: Baco 22A
Ravat blanc: Ravat #6
Vignoles: Ravat #51

Controversy still surrounds the use of **hybrids** in Europe and the U.S., and, while France allows them, most other European countries have banned them. Here in the U.S., Dr. Frank would agree with such legislation since he claims that:

1. The quality of hybrids is inferior
2. V. vinifera can resist cold climates if grafted to proper rootstock

In contrast, Philip Wagner, who introduced hybrids in the U.S. says that they are more likely to give consistent crops and that they require less care than V. vinifera.

- Hybrids are being improved every year
- Good resistant -rootstock should be improved too
  however, if a cold summer is more the norm then the exception, the rootstock will not help fruit set on a V. vinifera that is not adapted to colder climate. If so, then maybe a hybrid is better for good, constant, year after year production. KEEP AN OPEN MIND.

b. Growth in NY

The first shoots appear on the vines in late May followed by leaves and later, flower clusters. Flowers open 6-8 weeks later (in early July). Pollination is made possible by light winds and does better in warm and dry weather. While flower set is reduced if the soil is too rich, it is also true that poor fertilization will cause the fall of the flowers, the fall of immature berries or no maturation of berries (they grow but remain green). At this point the grower cuts the tops of the vines to eliminate the **vegetatively** growing areas which compete with the fruits for nutrients. This yields larger crops of larger fuit.
As the fruit matures, it first increases in size and forms seeds. Then the fruit softens, sugar levels increase, acid levels decrease, chlorophyll concentration decreases and color increases. In the final stages of maturation tannins, flavor and aroma constituents develop. If rain or disease occurs in the final stages of maturation, or if the fruit is picked too early for fear of frost, loss of a potentially outstanding wine may occur. Can you explain why that is? While summers can be humid in NY State, fall is cool and dry and thus perfect for the final stages of ripening.

Harvest starts during the first week in September for the early varieties and continues into late October for late varieties. Some grapes (notably Riesling) are left on the vine late in the fall so that they grow sweeter as water evaporates from the fruits. Sometimes a mold develops on them (*Botrytis cinerea* also called **noble mold, noble rot** or pourriture noble) that will cause the grapes to shrivel and acquire a particular aroma and flavor. These grapes are pressed late in the fall and give a sweet wine that has exquisite bouquet and flavor: there are 4 types of such late wines:

- **Spatlese** means late picking: the grapes are picked after 2 extra weeks on the vine. They are not kept on the vine any longer due to the risks of freezing. They are harvested with or without mold. The yield of juice is less than if the fruits had been picked earlier but the sugar content is greater (about 23 ^{o}Brix) and thus they are more expensive.

A variation on this theme is ice wine, for which grapes are harvested later in the season; often after the onset of winter. Ice wine is made from overripe grapes that have frozen solid on the vine. They are harvested quickly and pressed while still frozen, so that only concentrated grape juice is extracted. Most of the water stays in the press as ice, so the resulting wine is very concentrated in sugar and.

- **Auslese** refers to a wine made from the selected picking of Botrytic infected grapes. The infection is just beginning at the time of harvest. Therefore the sugar content is about the same as in a Spatlese (up to 25 ^{o} Brix). The flavor is different however because of the mold infection. The word auslese means selection.

- **Beerenauslese** is a wine made from grapes more severely affected by the mold which draws moisture from the grapes. The water content is even less than for an Auslese and the sugar content is higher (26^{o} to 30 ^{o} Brix). The grapes are individually picked later in the season than for an Auslese. Botrytis also adds a honeyed flavor to the grapes.

- **Trockenbeerenauslese** is a wine made from selected dried berries that are further along in the botrytic infection; they are actually shriveled. This last wine is very expensive since very little juice is recovered from the pressing of the grapes and the selection of berries is done by hand. Those are harvested last in the season. Sugar content is extremely high (the must has to have at least 34^{o}Brix) and the wine produced contains a lot of residual sugar.

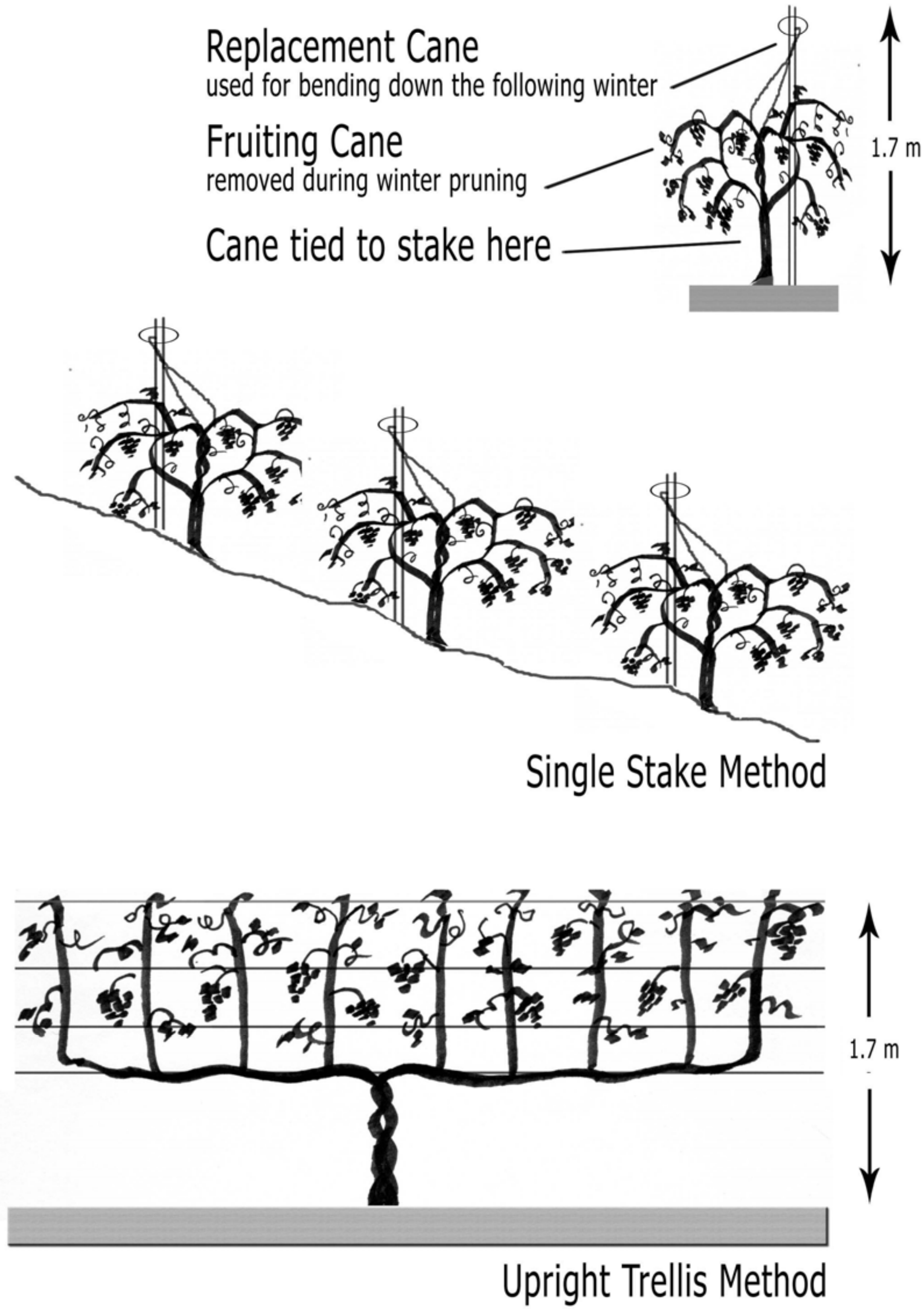
In NY State Widmer produces, when the climactic conditions cooperate, a Spatlese made from "NY Riesling" grapes. In fact, Riesling is not a V. vinifera but a cross of a V. labrusca with a V. riparia that gives a grape with a taste reminiscent of Riesling without the foxy labrusca taste. Chateau Esperanza also tried the same with a Ravat grape that had gone to 44 ^{o}brix. But that is unusual because the weather does not usually hold long enough for the botrytic effect to set in before winter.

Let's go back to harvest. It is done mechanically now except for the later Spatlese pickings. The machines straddle a row of vines, slap the vine to shake them and collect the falling berries in boxes; a machine does the work of 50 men but the grapes can be damaged in the process.

During the winter, 90% of the previous season's leaf growth is removed whether you are using the trellis method or the single stake method (Figure 3.6). Why? The Romans found out that pruning:

1. Spaces the shoots so that each will present maximum exposure to light next summer.
2. Spaces the shoots so that air circulation increases and humidity decreases.
3. Spaces the shoots for an adequate penetration of pesticide spray.
4. Selects the position of buds for best possible fruiting.
5. achieves appropriate bud number per plant for maximum yield and growth of maximum quality grapes.

Figure 2.1 Two methods used when growing grapes



Answers to chapter questions.

p.1: Why, at the beginning of winemaking were sugar content, nitrogen content, association of yeasts with grapes and pectin content crucial to the winemaker? Because high sugar content assures the production of enough alcohol to preserve the finished product. Nitrogen content is a nutrient for yeasts and yeasts associated with the grapes assured the fermentation of the juice.

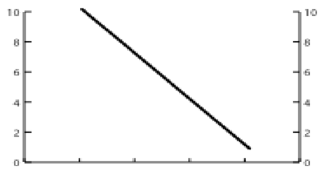
p.3: **Can you think of a mutation in the classroom here that is neutral?** Eye color, hair color could be neutral mutations because they do not affect our chances for survival.

p.3: Would myopia be a neutral mutation in the tiger population? This mutation could seriously hamper the capacity of an animal to find food and thus force the elimination of this individual from the population.

p.4: Can you think of a beneficial mutation for tigers? Moths? Think here of what would help the survival of a predator like the tiger or a prey like the moth. In both cases camouflage and ability to gather food would be great advantages. For the tiger, increased speed, agility and reflexes would be advantageous.

p. 10: Looking at the two curves shown below can you tell if they belong to any one of the categories described on page 3.14? What is different with the models we have just seen? Is it still a linear or hyperbolic curve?

The first curve is linear but is inverted. The slope is said to be negative compared to the positive curve of the model shown on page 3.14. The second curve is hyperbolic but is also inverted from the model on page 3.14.



LINEAR CURVE ?

HYPERBOLIC CURVE ?

p. 11: What is the function shown on Figure 3.2.? It is Gaussian.

p. 12: Of the four cities shown on Figure 3.2 which is more likely to give white wines? Geisenheim with low mean temperatures and higher latitude is more likely to produce white wines.

p. 13. **According to this theory can you explain how the earth gets out of a glacial age.**

p. 13. Now how would the increase in atmospheric CO_2 due to human activity affect this cycle?

C. EXERCISES.

1. Can you think of a way to classify yourself other than those mentioned in the text?
2. What is a mutation?
3. Are mutations all bad? Explain your answer.

4. What makes american rootstocks more suitable to grape growth?
5. Name three ways to grow quality grapes in North America.
6. What four factors must the grape grower take into consideration for good growth?
7. Convert the following: use dimensional analysis.
   a. 730 mm into inches
   b. 500 mm into meters
   c. 1500 mm into feet
8. Describe the major components of soils.
9. How does *Botrytis cinerea* contribute to the production of sweet wines?
10. What is pruning? Why is it done?
11. How do Europeans make wine varieties?
12. You need to bottle 10 gallons of beer (OK so you may have gone a bit overboard during the fermentation process). If each bottle contains 0.75 L and a gallon is 3.8 L, how many bottles will you need?
13. Give the complete classification (Kingdom, Family etc…) for the varieties Pinot noir and Concord grapes. Point to the differences and similarities between the two classifications.
14. Describe LTI and its importance to wine makers.
15. Referring to Figure 3.2 what would be a good location to grow Merlot grapes? Riesling?
16. Which of the four locations shown on Figure 3.2 have the longest growing season?
17. Calculate the LTI for a location with the growing season of Christchurch and the longitude of Geisenheim. Could it support the growth of grape varieties growing in Christchuch?
18. Two locations have respective LTIs of 292 and 325. Which one is closer to the equator?
19. How can you decrease LTI without changing temperature of latitude?
20. Draw a graph that describe the effect of grape vine age on wine quality.
21. Convert 272 ^{o}F to ^{o}C. Use dimensional analysis
22. Is it warmer at 14 ^{o}C or 14 ^{o}F?
23. Define latitude and longitude.
24. What climate produces in general poor quality wines? Explain.
25. Define organic and inorganic compounds. Can you name some of the inorganic compounds present in soil?
26. You are selecting a site for your grapeyard. What conditions will you be looking for?
27. Describe Dr. Konstantin Frank's contribution to the wine making industry in the Finger Lakes region.
28. What is methyl anthranilate and where is it found?
29. Why do grape growers remove some of the leaves from grape wines?
30. What must be done differently in grape growing to produce an ice wine?
31. Is an ice wine also a varietal wine?
32. What is a brix?
33. What is a cepage?
34. Are wines made from cepage in general good or bad?
35. You live in an area with a latitude of 46. The average rainfall is 850 mm. The average monthly temperatures during the growing season are shown below. What types of grapes are more suited to grow under these conditions?

Month	Ave. temperature(oF)
May	60
June	65
July	75
August	83
September	65
October	60

36. Match the following:

Celsius	Farenheit
100	98.6
37	212
22	32
0	71.6
-10	14

37. What would happen to grape production if pruning was not done?

38. Give 6 levels of classification (Broad to narrow) of Dr B. The first level is HUMAN.

Classify grapes.

39. Match synonyms:

____Gaussian
____cultivares
____biohazardous
____morphology
____grapes
____hydrometer
___homosapiens
___aseptic
___acid
___malting
___species
___critter
___ecology
___function
___linear
___hyperbolic

A. varietals
B. germination
C. bell-shaped
D. sterile
E. direct
F. vitis vinifera
G. Christian Waterman
H. saccharometer
I. relationship
J. exponential
K. tart
L. humans
M. appearance
N. pathogenic
O. breed
P. environmentology

40. Match the following:

______Boil to prevent spoilage
______Regulated mass production
______Apples that turn brown
______yeast producing CO_2 and alcohol
______acetobacter in wine
______Changes in DNA
______Natural or artificial
______sharpness of detail
______oil
______ocular and objective lenses on microscope
______microscope light source
______Opposite of oxidation
______removal of sediment

A. reduction
B. Oxidation
C. fermentation
D. immersion
E. illumination
F. magnification
G. contamination
H. pasteurization
I. filtration
J. standardization
K. selection
L. resolution
M. mutation

41. How are enzymes involved in fermentation?

42. Diagram the process of pasteurizing wine. What are the benefits and possible side-effects of pasteurization?
43. Are all enzymes catalysts and are all catalysts enzymes?
44. Are all yeast classified as Saccharomyces and are all Saccharomyces classified as yeast?
45. Are all Vitis vinifera hybrids and are all hybrids vitis vinifera?
46. Compare and contrast microbiology and biochemistry.
47. Fill in the spaces in the following LTI chart:

LTI	Sugar	Alcohol	Dryness	Acid	Red pigment
Lo	Lo	Lo	**(Hi)**	Hi	Lo
(Hi)	Hi	Hi	Lo	Lo	Hi

48. Classify the following wines from lowest to highest LTI using the above chart. **(1,3,2)**

i. `Wine 1: dry, white, 9% al;cohol

ii. Wine 2: sweet, red, 12% alcohol
iii. Wine 3: sweet, white, 10% alcohol

49. What are the parameters used to calculate LTI?
50. What conditions does LTI not account for? **Cold days of winter**
51. Arrange the following according to particle size: gravel, clay, rocks, silt, sand
    i. Want 1/3 of sand, silt, and clay = loam
    ii. Biggest – rocks, gravel, sand, silt, clay – smallest
52. How would the following soil conditions affect root growth?
    i. ½ rocks
    ii. ¼ sand
    iii. 1/8 clay
    iv. 1/8 gravel
    v. water flows easily and way from roots

53. What soil conditions (% rocks, gravel, clay, sand, silt) would cause the roots to be submerged in water for long periods of time? **Lots of clay**

54. Why are late harvest wines really sweet?
    i. Grapes mature and make more sugar
    ii. Grapes dry up and sugar is concentrated
    iii. Mold (Botrytis) sucks up water and so concentrates sugar

55. Normally winemakers get 150 gal of juice per 1 ton of grapes. Ice wines are bottled in 325 ml bottles and harvesting is late so only 60 gal of juice per 1 ton of grapes is collected. How many bottles of ice wine can be bottled if 4.5 tons of grapes are harvested in early Nov (1 gal = 128 oz and 1 oz = 29.6 ml)? **3147.6 bottles.**

56. Draw a graph of anything that is related to fermentation. Describe its function.

56. The latitude for Hammondsport, NY is 42 and the maximum temperature is 28^{o} C. The latitude for Istanbul, Turkey is 40 and the maximum temperature is 26^{o} C. Calculate the LTI for each region. Dr. Frank grows a varietal called Rkatsiteli. It originally came from Turkey about 5000 years ago. Dr Frank's is the only commercial supplier of this wine globally. Considering the LTI, describe how the wine in Turkey would taste as compared to the wine in NY.

58. Why are late harvest Rieslings called "Ice Wines"?

59. Describe the influence on each of the following on grape growing:

wind
rain
sun
rocky soil
clay soil
hills
valleys
proximity to large body of water

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Appendix 1.

Carl Linnaeus (1707-1778)

Carl Linnaeus, also known as Carl von Linné or Carolus Linnaeus, is often called the Father of Taxonomy. His system for naming, ranking, and classifying organisms is still in wide use today (with many changes). His ideas on classification have influenced generations of biologists during and after his own lifetime, even those opposed to the philosophical and theological roots of his work.

Biography of Linnaeus

Linnaeus was born on May 23, 1707, at Stenbrohult, in the province of Småland in southern Sweden. His father, Nils Ingemarsson Linnaeus, was both an avid gardener and a Lutheran pastor, and Carl showed a deep love of plants and a fascination with their names from a very early age. Carl disappointed his parents by showing neither aptitude nor desire for the priesthood, but his family was somewhat consoled when Linnaeus entered the University of Lund in 1727 to study medicine. A year later, he transferred to the University of Uppsala, the most prestigious university in Sweden. However, its medical facilities had been neglected and had fallen into disrepair. Most of Linaeus's time at Uppsala was spent collecting and studying plants, his true love. At the time, training in botany was part of the medical curriculum, for every doctor had to prepare and prescribe drugs derived from medicinal plants. Despite being in hard financial straits, Linnaeus mounted a botanical and ethnographical expedition to Lapland in 1731 (the portrait above shows Linnaeus as a young man, wearing a version of the traditional Lapp costume and holding a shaman's drum). In 1734 he mounted another expedition to central Sweden.

Linnaeus went to the Netherlands in 1735, promptly finished his medical degree at the University of Harderwijk, and then enrolled in the University of Leiden for further studies. That same year, he published the first edition of his classification of living things, the *Systema Naturae*. During these years, he met or corresponded with Europe's great botanists, and continued to develop his classification scheme. Returning to Sweden in 1738, he practiced medicine (specializing in the treatment of syphilis) and lectured in Stockholm before being awarded a professorship at Uppsala in 1741. At Uppsala, he restored the University's botanical garden (arranging the plants according to his system of classification), made three more expeditions to various parts of Sweden, and inspired a generation of students. He was instrumental in arranging to have his students sent out on trade and exploration voyages to all parts of the world: nineteen of Linnaeus's students went out on these voyages of discovery. Perhaps his most famous student, Daniel Solander, was the naturalist on Captain James Cook's first round-the-world voyage, and brought back the first plant collections from Australia and the South Pacific to Europe. Anders Sparrman, another of Linnaeus's students, was a botanist on Cook's second voyage. Another student, Pehr Kalm, traveled in the northeastern American colonies for three years studying American plants. Yet another, Carl Peter Thunberg, was the first Western naturalist to visit Japan in over a century; he not only studied the flora of Japan, but taught Western medicine to Japanese practicioners. Still others of his students traveled to South America, southeast Asia, Africa, and the Middle East. Many died on their travels.

Linnaeus continued to revise his *Systema Naturae*, which grew from a slim pamphlet to a multivolume work, as his concepts were modified and as more and more plant and animal specimens were sent to him from every corner of the globe. (The image at right shows his scientific description of the human species from the ninth edition of *Systema Naturae*. At the time he referred to humanity as *Homo diurnis*, or "man of the day". Click on the image to see an enlargement.) Linnaeus was also deeply involved with ways to make the Swedish economy more self-sufficient and less dependent on foreign trade, either by acclimatizing valuable plants to grow in Sweden, or by finding native substitutes. Unfortunately, Linnaeus's attempts to grow cacao, coffee, tea, bananas, rice, and mulberries proved unsuccessful in Sweden's cold climate. His attempts to boost the economy (and to prevent the famines that still struck Sweden at the time) by finding native Swedish plants that could be used as tea, coffee, flour, and fodder were also not generally successful. He still found time to practice medicine, eventually becoming personal physician to the Swedish royal family. In 1758 he bought the manor estate of Hammarby, outside Uppsala, where he built a small museum for his extensive personal collections. In 1761 he was granted nobility, and became Carl von Linné. His later years were marked by increasing depression and pessimism. Lingering on for several years after suffering what was probably a series of mild strokes in 1774, he died in 1778. His son, also named Carl, succeeded to his professorship at Uppsala, but never was noteworthy as a botanist. When Carl the Younger died five years later with no heirs, his mother and sisters sold the elder Linnaeus's library, manuscripts, and natural history collections to the English natural historian Sir James Edward Smith, who founded the Linnean Society of London to take care of them.

Linnaeus's Scientific Thought
Linnaeus loved nature deeply, and always retained a sense of wonder at the world of living things. His religious beliefs led him to natural theology, a school of thought dating back to Biblical times but especially flourishing around 1700: since God has created the world, it is possible to understand God's wisdom by studying His creation. As he wrote in the preface to a late edition of *Systema Naturae*: *Creationis telluris est gloria Dei ex opere Naturae per Hominem solum* -- The Earth's creation is the glory of God, as seen from the works of Nature by Man alone. The study of nature would reveal the Divine Order of God's creation, and it was the naturalist's task to construct a "natural classification" that would reveal this Order in the universe.

However, Linnaeus's plant taxonomy was based solely on the number and arrangement of the reproductive organs; a plant's class was determined by its stamens (male organs), and its order by its pistils (female organs). This resulted in many groupings that seemed unnatural. For instance, Linnaeus's Class Monoecia, Order Monadelphia included plants with separate male and female "flowers" on the same plant (Monoecia) and with multiple male organs joined onto one common base (Monadelphia). This order included conifers such as pines, firs, and cypresses (the distinction between true flowers and conifer cones was not clear), but also included a few true flowering plants, such as the castor bean. "Plants" without obvious sex organs were classified in the Class Cryptogamia, or "plants with a hidden marriage," which lumped together the algae, lichens, fungi, mosses and other bryophytes, and ferns. Linnaeus freely admitted that this produced an "artificial classification," not a natural one, which would take into account all the similarities and differences between organisms. But like many naturalists of the time, in particular Erasmus Darwin, Linnaeus attached great significance to plant sexual reproduction, which had only recently been rediscovered. Linnaeus drew some rather astonishing parallels between plant sexuality and human love: he wrote in 1729 how

The flowers' leaves. . . serve as bridal beds which the Creator has so gloriously arranged, adorned with such noble bed curtains, and perfumed with so many soft scents that the bridegroom with his bride might there celebrate their nuptials with so much the greater solemnity. . .
The sexual basis of Linnaeus's plant classification was controversial in its day; although easy to learn and use, it clearly did not give good results in many cases. Some critics also attacked it for its sexually explicit nature: one opponent, botanist Johann Siegesbeck, called it "loathsome harlotry". (Linnaeus had his revenge, however; he named a small, useless European weed *Siegesbeckia*.) Later systems of classification largely follow John Ray's practice of using morphological evidence from all parts of the organism in all stages of its development. What has survived of the Linnean system is its method of hierarchical classification and custom of binomial nomenclature.

For Linnaeus, species of organisms were real entities, which could be grouped into higher categories called **genera** (singular, **genus**). By itself, this was nothing new; since Aristotle, biologists had used the word genus for a group of similar organisms, and then sought to define the *differentio specifica* -- the specific difference of each type of organism. But opinion varied on how genera should be grouped. Naturalists of the day often used arbitrary criteria to group organisms, placing all domestic animals or all water animals together. Part of Linnaeus' innovation was the grouping of genera into higher taxa that were also based on shared similarities. In Linnaeus's original system, genera were grouped into orders, orders into classes, and classes into kingdoms. Thus the kingdom Animalia contained the class Vertebrata, which contained the order Primates, which contained the genus *Homo* with the species *sapiens* -- humanity. Later biologists added additional ranks between these to express additional levels of similarity.

Before Linnaeus, species naming practices varied. Many biologists gave the species they described long, unwieldy Latin names, which could be altered at will; a scientist comparing two descriptions of species might not be able to tell which organisms were being referred to. For instance, the common wild briar rose was referred to by different botanists as *Rosa sylvestris inodora seu canina* and as *Rosa sylvestris alba cum rubore, folio glabro*. The need for a workable naming system was made even greater by the huge number of plants and animals that were being brought back to Europe from Asia, Africa, and the Americas. After experimenting with various alternatives, Linnaeus simplified naming immensely by designating one Latin name to indicate the genus, and one as a "shorthand" name for the species. The two names make up the **binomial** ("two names") species name. For instance, in his two-volume work *Species Plantarum* (*The Species of Plants*), Linnaeus renamed the briar rose *Rosa canina*. This binomial system rapidly became the standard system for naming species. Zoological and most botanical taxonomic priority begin with Linnaeus: the oldest plant names accepted as valid today are those published in *Species Plantarum*, in 1753, while the oldest animal names are those in the tenth edition of *Systema Naturae* (1758), the first edition to use the binomial system consistently throughout. Although Linnaeus was not the first to use binomials, he was the first to use them consistently, and for this reason, Latin names that naturalists used before Linnaeus are not usually considered valid under the rules of nomenclature.

In his early years, Linnaeus believed that the species was not only real, but unchangeable -- as he wrote, *Unitas in omni specie ordinem ducit* (The invariability of species is the condition for order [in nature]). But Linnaeus observed how different species of plant might hybridize, to create forms which looked like new species. He abandoned the concept that species were fixed and invariable, and suggested that some -- perhaps most -- species in a genus might have arisen after the creation of the world, through hybridization. In his attempts to grow foreign plants in Sweden, Linnaeus also theorized that plant species might be altered through the process of acclimitization. Towards the end of his life, Linnaeus investigated what he thought were cases of crosses between genera, and suggested that, perhaps, new genera might also arise through hybridization.

Was Linnaeus an evolutionist? It is true that he abandoned his earlier belief in the fixity of species, and it is true that hybridization has produced new species of plants, and in some cases of animals. Yet to Linnaeus, the process of generating new species was not open-ended and unlimited. Whatever new species might have arisen from the *primae speciei*, the original species in the Garden of Eden, were still part of God's plan for creation, for they had always potentially been present. Linnaeus noticed the struggle for survival -- he once called Nature a "butcher's block" and a "war of all against all". However, he considered struggle and competition necessary to maintain the balance of nature, part of the Divine Order. The concept of open-ended evolution, not necessarily governed by a Divine Plan and with no predetermined goal, never occurred to Linnaeus; the idea would have shocked him. Nevertheless, Linnaeus's hierarchical classification and binomial nomenclature, much modified, have remained standard for over 200 years. His writings have been studied by every generation of naturalists, including Erasmus Darwin and Charles Darwin. The search for a "natural system" of classification is still going on -- except that what systematists try to discover and use as the basis of classification is now the evolutionary relationships of taxa.

The Linné Herbarium, at the Swedish Museum of Natural History, preserves some of Linnaeus's original plant specimens. The Museum also has an excellent, detailed biography of Linnaeus. You can also view Linnaeus's botanical garden and Linnaeus's manor home and garden at Hamarby, courtesy of Uppsala University, Linnaeus's alma mater. Uppsala University also maintains Linné On Line, a rich source of information on Linnaeus and his times (for those who can read Swedish).

Founded a few years after Linnaeus's death, the Linnaean Society of London is still going strong as an international society for the study of natural history. The Society preserves the bulk of Linnaeus's surviving collections, manuscripts, and library. The Strandell Collection of Linneana, at Carnegie-Mellon University, and the Mackenzie Linneana collection at Kansas State University, are major American collections of writings by and about Linnaeus and his associates. The Linnaeus Link at the British Natural History Museum, aims to make available electronic versions of Linnaeus's writings and documents.


Ordo I.

PRIMATES

Dentes primores superiores IV paralleli.
Mammae pectorales, binae.

1. HOMO nosce Te ipsum.

http://www.ucmp.berkeley.edu/history/linnaeus.html

Lesson 4.
12 Figures
no Tables

STRUCTURE OF PLANTS

A. THE PLANT BODY

There are two basic parts to most flowering plants: the **root** which grows underground and the **shoot** which grows above ground (Figure 4.1). The shoot has a main stem from which secondary stems start with leaves at the end. On Figure 4.1, we see two types of plant: a dicotyledon-type and a monocotyledon type plant. They look like the classic types you see everyday: a regular house plant and a grass-type plant (such as corn, wheat, timothy etc.) What would be a distinctive feature to differentiate the two? The leaves are round versus long and the angle to the stem is different. The other structures of the plants are similar in function although the form and shape may be different.

On the shoot you will note the presence of leaves and of other shoots. They must be connected to the vessels (called vascular tissue) carrying the sap (like our arteries carrying blood) in order to be fed by the roots. You see these vessels in light gray. The systems of vessels ((Figure 4.2) is laid around a core in the stem called the **pith**. The start of a new leaf's vascular system is called a **node**. You will also notice that if you look at the base of the leaf where there is a small bulge on the stem: that is called a **bud** and will lead to the development of a new stem. The Cotyledons also are shown: remember that they are seed leaves (two for **dicots** and one for **monocots**). They are better shown on the lower right diagram of a seed that has just started to develop into a root, a shoot and the cotyledons. (germinating seed on Figure 4.3). We will come back to this. NOW let's take a look at the smallest building block of the plant: the CELL.

B. THE CELL AND ITS COMPONENTS

An entire science is devoted to the study of the cell: **cytology**. WHY??? They are all alike aren't they. Yes and No. They do have some common structures and components but they <u>do not all</u> serve similar functions and they do it in ways that are similar sometimes and different sometimes.

Let's go back to the classification of living organisms for a minute. We said that there were 5 kingdoms, Animals, Plants, Fungi, Protista and Monera. The last two are simple organisms with single cells such as bacteria. Their cells are very simple in design and are called **prokariotic**. These organisms are known as **Prokariots**. The first three kingdoms are composed of multi-celled complex organisms. Their cells are also more complex and are called **eukariotic**. The organisms are **Eukariots**.

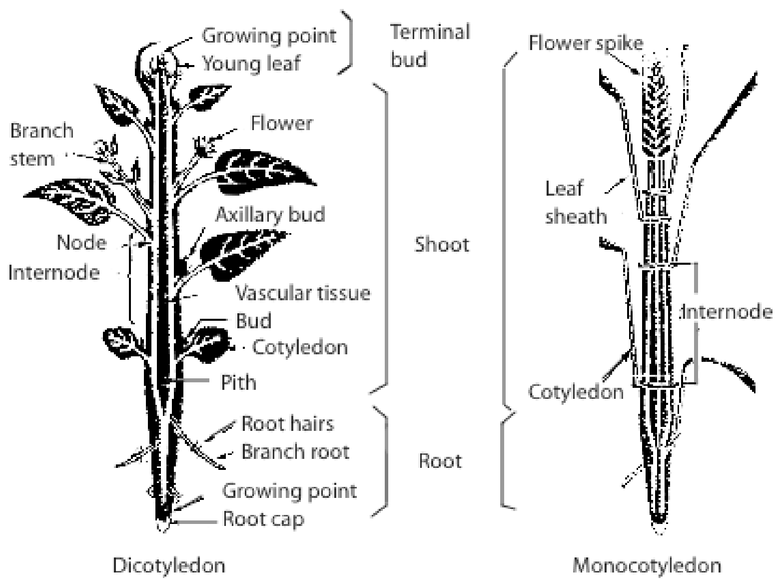


Figure 4.1. Anatomy of a plant. Compare monocots and dicots.

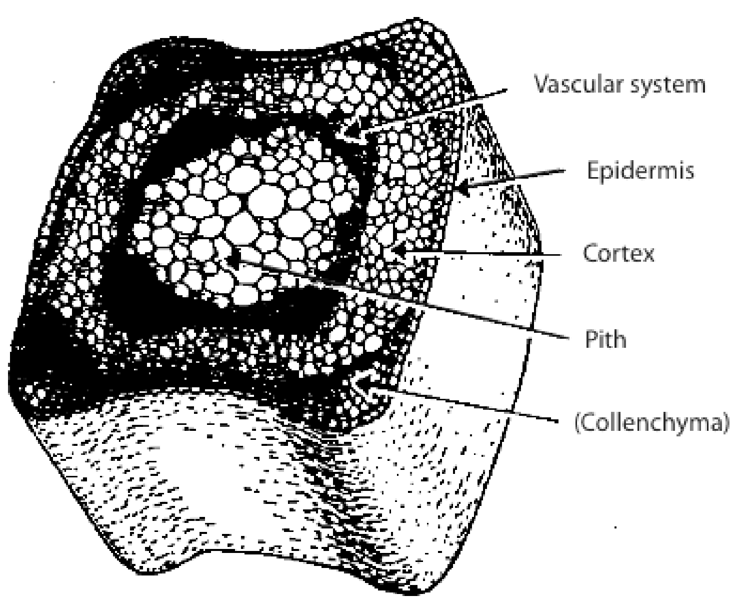
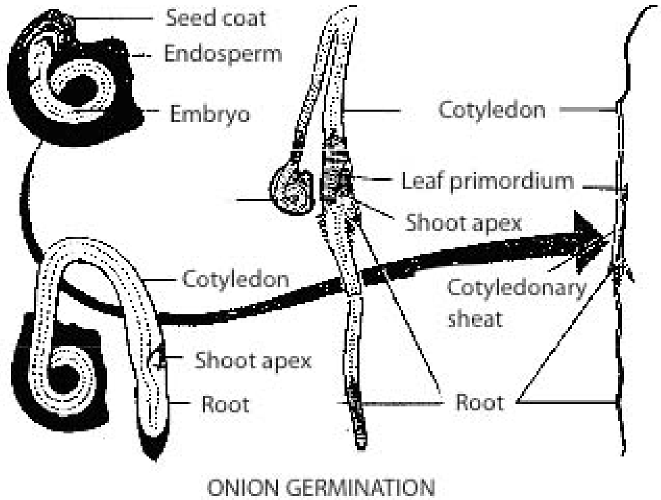


Figure 4.2. Cross section of a plant stem.



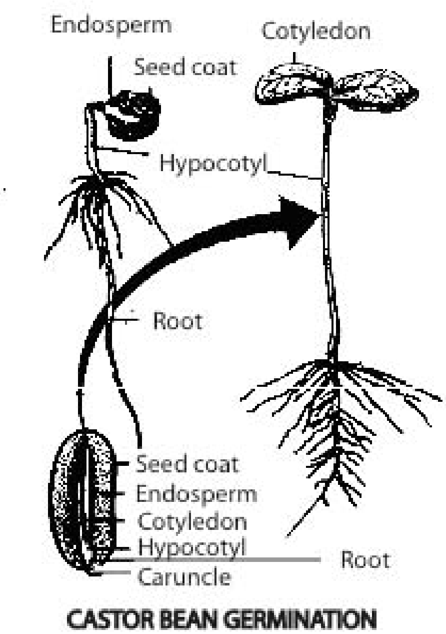


Figure 4.3. Seed germination in a monocot (onion) and a dicot (castor bean).

The basic plant cell is shown on Figure 4.4. Most higher plant cells look like this. They are about 0.025 to 0.25 mm in length but that varies a lot depending on the type (that is 0.001 to 0.01 inch). By contrast a prokariotic cell is about 2 micrometers (0.002 mm) in length.

1. More on the metric system

We have been talking about a lot of units that you may not be familiar with in a system that you have never used. So let's talk about this metric system for a little while. This system uses units that are very different from the ones in the British system. However realize that the US is about the only country in the world that is still using the old British system. What are those units?

1. length: **meter** (m). That is about a yard or 3 feet.
2. area: **meter ^{2}** (m^2)
3. volume: **meter^{3}** (m^3). We also use the liter or 1000 cm^3 (about a quart).
4. mass: **kilogram** (Kg). That is about 2 pounds (2.205 lb to be exact).
5. time: **seconds**. Now that is the same as in the british system
6. temperature: **kelvin**
7. amount of substance: **mole**
8. electrical current: **ampere**

The next feature of the system is the way its units increase in tens. In the British system we have 12 inches in a foot, 3 feet in a yard, 5260 feet in a mile. The metric system uses units of tens only:

1 micrometer:	1 millionth of a meter or 0.000001 m	(µm)
1 millimeter:	1 thousandth of a meter or 0.001 m	(mm)
1 centimeter:	1 hundreth of a meter or 0.01 m	(cm)
1 decimeter:	1 tenth of a meter or 0.1 m	(dm)
1 meter		(m)
1 kilometer:	1000 meters	(km)
1 megameter:	1,000,000 meters	(Mm)

All of these prefixes apply to any unit, be it length, volume, mass etc..... So a kilogram is 1000 grams. That is also 2.205 pounds. And 10 cm is 100mm.

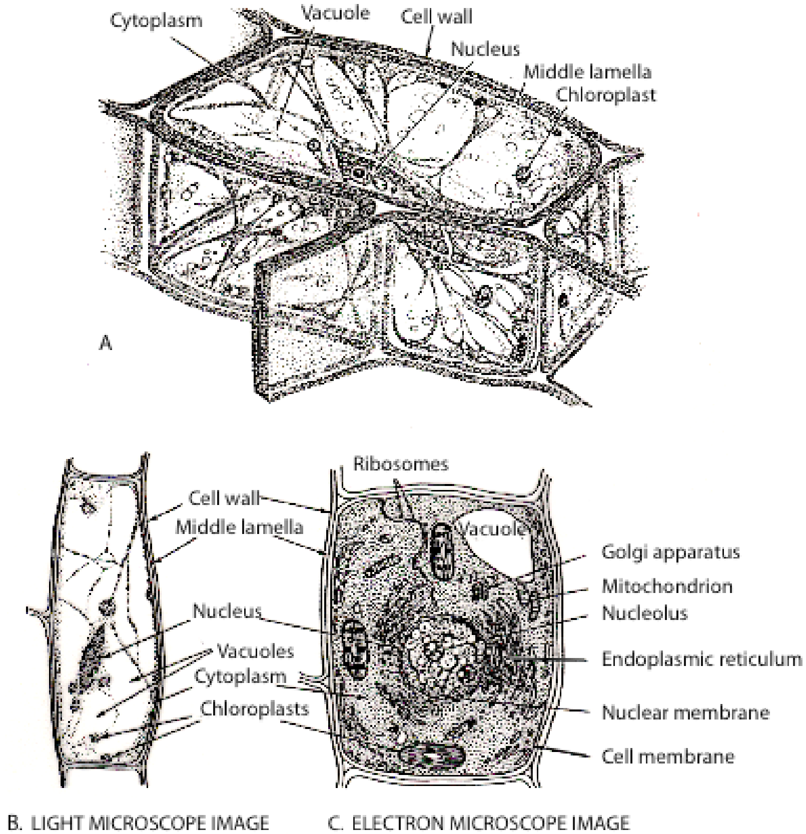


Figure 4.4. Structure of a plant cell.

You can also apply the principles of **dimensional analysis** to the conversion of these units.

$$10 \text{ cm} \times \frac{10 \text{ mm}}{1 \text{ cm}} = 100 \text{ mm}$$

Find how many micrometers there are in 10 cm. Use the **exponential notation** for this answer. When a number is very large (1,000,000) or very small (0.000001) we often use this notation: It consists of multiplying the integer or fraction in the number by an exponent (10^x) to reproduce the same number. Therefore 1,000,000 becomes 1×10^6 and 0.000001 becomes 10^{-6}. Use exponential notation to express 0.000025 meter(2.5×10^{-5} meter).

2. Cell components

a. Biological membranes

The inside of the cell has the consistency of beaten up jello; it cannot keep its shape and would leak out. So it is enclosed inside a **cell membrane**, the plasma membrane much like a human cell would. That membrane keeps things in. However the cell needs to be fed and needs to get rid of wastes; thus the membrane is semipermeable to let in and out only the food and the wastes. It is not enough to give it its shape however. A wall is actually added to the plant cell (a unique feature) to give it its shape and prevent it from exploding when water comes in. The wall is just a very rigid structure: that is what we call the wood of plants and trees. Now let's talk about this explosion business; that sounds serious because of a principle of biology called **osmosis**. Imagine that you have a reservoir in which you can keep salts, minerals, proteins and fats. The reservoir is made of a membrane through which water can go but nothing else. What would happen if the reservoir was put in water. Water would come in to try to dilute the salts, minerals, proteins and fats. That would expand the size of the reservoir considerably and possibly explode it. So we have already seen that plant cells avoid this fate by surrounding their cells with a rigid wall. This however gives plants a rigid appearance. How do animals do it? They have the same need to concentrate inside their cells a vast array of salts, minerals, proteins, sugars and fats. And yet they do not build cell walls. They instead bathe their cells into a solution that contains the same concentration of salts as the inside of the cell. The salts are different but their concentration is what is important. Have you ever tasted your blood when you cut yourself? It tasted salty because of this principle of animal cells osmosis. If the concentration of salts inside the cell is the same as outside the cell water will not rush inside the cell and explode it. The solution bathing the cell is then called **isotonic**.

b. Cytoplasm

The inside of the cell is called the **cytoplasm**. It contains a thick soup made of salts, proteins, fats and water that maintains the right conditions of acidity and salinity for the cell to survive. A lot of the reactions needed to build up the cell and keep it alive occur in the cytoplasm. You note other structures in the cytoplasm. Those are specialized structures with one defined role. All are surrounded by a membrane like the cytoplasm was.

c. Nucleus

The **nucleus** (in Eukariots) contains the DNA, a large molecule made of a repeating sequence of molecules to create a code; this code holds the information necessary for the **synthesis** of all the components of the cell. That is how the cell remembers what it looks like so that it will make an exact duplicate of itself when it reproduces. The DNA codes for your blue eyes, brown hair, yellow flowers, large or small leaves, tall and short plants etc etc. The DNA never leaves the nucleus except during cell division when the membrane of the nucleus dissolves. So how does it tell the cytoplasm what to make and how. It sends messengers out to the synthesizing machinery of the cytoplasm, the

ribosomes, that will make the proteins: the messenger, called **mRNA**, carries a copy of the code and transmits the information to the cytoplasmic ribosomes.

d. Mitochondria

The **mitochondria** (in Eukariots) are specialized organelles that transform the food into energy: they are little furnaces that break down food to produce the energy you or plants need to move and grow. The food you eat (sugar, fats etc.) must be broken down to produce another molecule called **ATP** that can be used in all the reactions of the cell requiring energy. ALL FOODS ARE BROKEN DOWN TO PRODUCE AN ENERGY MOLECULE, ATP. That is true in plants as well as animals.

e. Chloroplasts

Chloroplasts are present in plants (which are of course Eukariots) and algae (also Eukariots but classified as Plants or Protista depending on which biologist you talk to). They are what gives the plant its green color. While animals get their foods by eating other animals or plants, plants get their food by synthesizing it themselves from the energy of the sun and the CO_2 released by animals. They do that in the chloroplasts. (Figure 4.5) The green **chlorophyll** is the **pigment** that absorbs the light of the sun or artificial sources, like an antenna, and converts it into energy (ATP). That energy can be used to transform six molecules of water (H_2O) and six molecules of carbon dioxide (CO_2) into a molecule of sugar (glucose or fructose) and six molecules of oxygen (O_2). The conversion of sun energy into sugar is called **photosynthesis**.These sugars are then stored away in the form of **starch** or used by the mitochondria for energy production. Why should you care? Because plants keep you alive. They enable you to perform the reverse reaction where you burn one molecule of glucose in the presence of six molecules of oxygen to produce six molecules of carbon dioxide, six molecules of water and energy. Yeasts can perform a similar reaction where they convert glucose into ethanol of course.

Photosynthesis stats with energy from the sun. Energy must travel the great distance from the sun and it does in a wave. In fact all types of energy travel in waves. Waves can be characterized by their amplitude, wavelength and frequency.

Let’s look at a wave:

Waves are made of peaks and troughs. The length of a wave (**wavelength**) is the distance between two peaks. The amplitude is the height of the wave. Since the wave travels a certain distance over a certain period of time we say that the **frequency** is the number of times a peak goes by in a given amount of time.(it is also called a cycle). The wave can have various amplitudes and various wavelengths, Since all waves travel at the same speed shorter wavelength means a greater frequency. The type of energy is determined by the wavelength. Radio waves have a wavelength measured in meters. Microwaves are measured in centimeters, X-rays in nanometers, light or the visible spectrum in nanometers (10^{-9} meters) and gamma rays in picometers (10^{-12} meters). Those are very short wavelengths. Different instruments are used to capture the different waves. A radio can capture radio waves and your eyes capture light. The visible spectrum extends from 400 nm to 700 nm. Below that lay UV waves, above that infrared. You can see all wavelengths but is there a difference between these wavelengths? Each of them define a specific color for your eyes, from violet (400 nm) to red (700 nm). Further each wavelength carries with it a certain amount of energy. The shorter the wave the higher the energy it carries. The higher the energy the more dangerous it is to you. A radio wave has little energy compared to UV waves. Accordingly would you rather be subjected to radio waves or UV waves? Too much UV can cause skin burns but not radio waves. Similarly gamma rays can easily kill you. Nuclear weapons generate gamma rays as one of their means of destroying population centers.

Chloroplasts contain the pigment chlorophyll which absorbs all wavelengths of the visible spectrum except green (around 530 nm) which it reflects. That is why plants appear green to us. If a pigment absorbed all visible wavelengths it would appear black (absence of light). A pigment that reflected all visible wavelengths would be white.

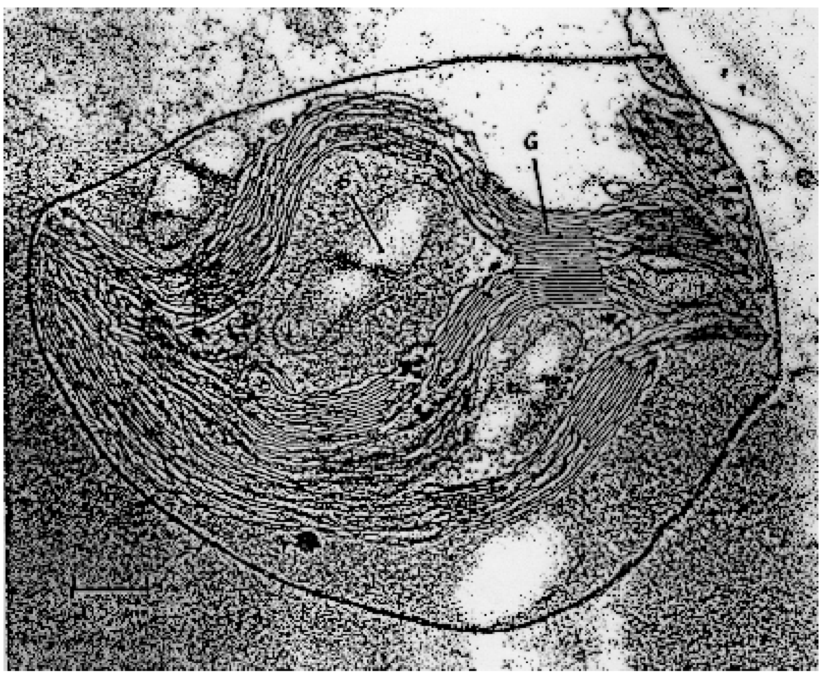


Figure 4.5. Electron micrograph of a chloroplast. The grana (G) appear throughout the chloroplast.

f. Vacuole

The last important structure in the cell is the **vacuole** (also unique to plants) that is a reservoir of salts and water that will be needed later in the cytoplasm. Salts and other small molecules are stored there for future use but they also play an important role in keeping the cell healthy and **turgid,** that is engorged with water. The vacuole may occupy as much as 80% of the volume of the cell.

Those are the most important but not the only structures in the cell.

3. Cell types
While plant cells all contain the basic components described above they also have to perform specialized tasks and are therefore specialized themselves.

a. Meristematic cells are undifferentiated cells. They have no central vacuole. Their role is to divide into specialized cells. This type of cell is found at axial and lateral buds, in the cambium and on some roots that can grow into whole plants (like potatoes).

b. Differentiated cells. These cells are specialized for specific roles. Epidermal cells are protective cells on the surface of leaves. Parenchyma cells are specialized in photosynthesis or storage or food. They have very large vacuoles. Collenchyma cells also have thick walls. They support areas of the plant that are growing quickly such as the stalk (petiole) of leaves. Sclerenchyma cells have very thick walls are give support to other cell types. Not surprisingly they are found in stems and leaf veins in areas of the plant that have stopped growing. These cells are dead at maturity but continue their supportive role. Xylem vessel cells and tracheid cells are dead cells with perforated, tapered ends that transport water throughout the plant. Sieve elements and companion cells are involved in the transport of nutrients throughout the plant (phloem). While sieve elements have no nuclei and only a few organelles, companion cells are fully functional and control transport through the sieve elements.

C. PLANT TISSUES

The cells are arranged into tissues that each serve a unique and needed role in the life or the plant. Think of the various tissues in your own body. The tissues that make up the skin are different from those that make up the eye or the heart or the liver. The same is true of plants.

Let's look at these tissues:

1. meristematic tissues. Figure 4.6.
Take a look at a bud that will form eventually a stem with its vessels, skin, leaves or flower. Will all these tissues be the same when the bud turns into leaves and stem and flower? No but for the moment they are all the same; the tissue making up the bud or the growing point of a root is called **undifferentiated**, or **meristematic**. The cells here are actively dividing and will eventually differentiate into specialized cells or the **permanent tissues**. Plants however maintain a fair amount of meristematic tissue around buds, roots and cambium.

This will help them reform a root or a branch or a whole organism (potato) if injured.This tissue and its cells enable a tree to grow over the years. In contrast we do not have as much undifferentiated tissue and so cannot reconstitute an arm if it is cut off.

2. permanent tissues. Two types here: simple and complex.
The simple tissues, made only one type of cells, are those making up the fleshy fruits, roots or the guard cells of the stomata. The complex tissues are made of more than one type of cells and play crucial roles such as transport of nutrients and water in the plant. For example we need to look at:

a. Vascular Tissue.

The **xylem** or what we would call wood is a collection of cells transporting water in the tree. Herbaceous plants also have xylem but there is less of it and this gives the plant a different appearance. A large portion of the xylem cells are actually single, long, dead cells (called **tracheids**); water moves up the tree through these cells arranged end to end. The pre-dates the second type of cells in evolution. Ferns and conifers contain only tracheid cells. The other cells are called xylem vessel cells. These cells form thick-walled tubes for water transport. At maturity the cells also die, the end walls dissolve and the cytoplasm disappears leaving behind only a large tube. The cells of the xylem also give strength to the trunk of trees are added to the tree year after year in layers and form the rings noticeable when a tree is cut.

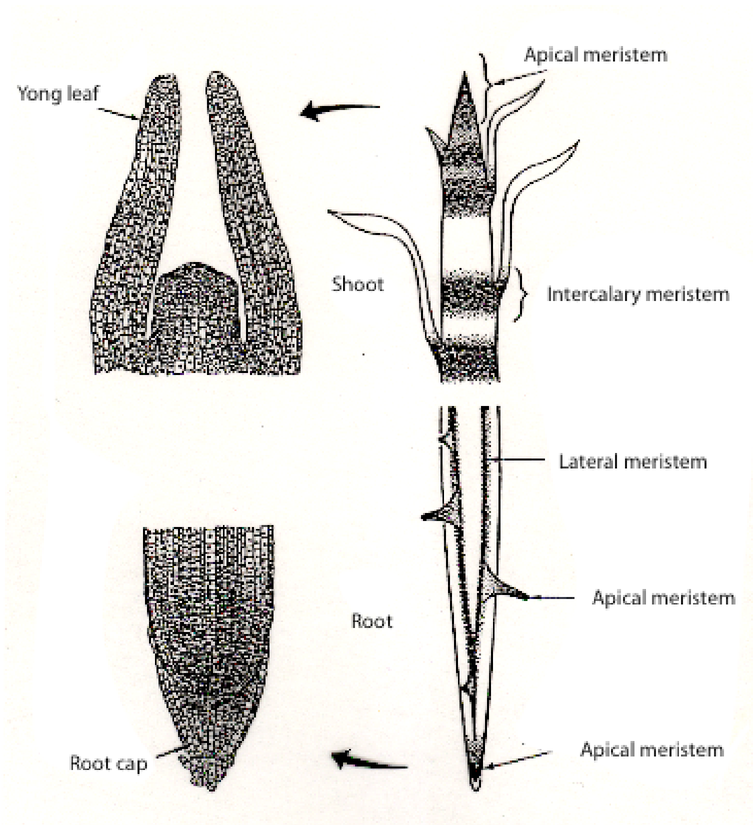


Figure 4.6. Anatomy and location of meristematic tissue in plant roots and shoots.

The **phloem** is the nutrient conducting system of cells that is part of the **bark**. It is made of two types of cells: sieve elements are very long cells that have sieve plates at the end to let nutrients through. The cells actually loose their nucleus once they mature and become hollow system through which nutrients travel up like a straw through which you suck up a milk shake). Their cell walls are reinforced with cellulose and lignin. Companion cells are still alive and control the flow of nutrients (sugar and amino acids) from the cells producing them in the leaves to the sieve elements for transport to the parts of the plant requiring these nutrients. Companion cells use "**active transport**" to take up these nutrients from producing cells and diffuse them into the sieve tubes. In active transport energy must be expanded to move compounds across a membrane or against a concentration gradient. In **diffusion** compounds move from a point of greater concentration to a point of lesser concentration.

- The xylem and the phloem are separated by a layer of meristematic cells that form the cambium. The cells of the cambium divide into specialized xylem (toward the inside of the stem) and phloem (toward the outside of the stem) cells whether the vascular system is continuous or discontinuous (Fig. 4.7).

b. Dermal tissue. Dermal cells form a protective layer covering the entire plant and function in a manner similar to your skin. This epidermis is covered by a layer of a waxy substance called the **cuticle** that is designed to prevent water loss.

c. Ground tissue. This tissue fills the interior of the plant. Its cells act as storage cells (parenchyma) or as support cells (collenchyma and sclerenchyma). The cortex, located between the epidermis and the vascular system in plant stems is made of storage parenchyma. Similarly the pith or central cylinder of stems is made of storage parenchyma (Fig. 4.7)

D. ANATOMICAL REGIONS

Let's put these tissues inside a plant to see how the plant lives. Figure 4.1 and 4.7 show a stem cross-section and the various tissues we mentioned. Anatomical regions would be similar to organs in animals where various tissues and their cell types are put together to form a working whole or organ. Muscles for example are made of muscle cells and nerve cells, ligament cells and tendon cells.

The phloem and the xylem are part of an anatomical region called **the vascular tissue** at the core of the stems, surrounding the pith. In Monocots, the vascular region is discontinuous but in dicots, it is a solid region. Around the vascular region we find the **cortex** that is there to protect the vascular tissue. Sometimes the cells of the cortex fill with a wax to form what we know as **cork**; the oak tree does that a lot and we use it to make corks.

The last region is the **epidermis** (equivalent to the skin in animals). More obvious in young plants and in succulents (which do not have a bark), it sheaths the entire stem. The hairs that you can feel on the surface of a stem or leaf come from the protrusions of the epidermis. The plant absorbs and releases gases (oxygen and carbon dioxide) from openings or pores in the epidermis called **stomatas** (like breathing holes). The cells of the epidermis also secrete a wax to the outside of the cell as a protective layer (**cuticle**).

E. MORPHOLOGICAL STRUCTURES.

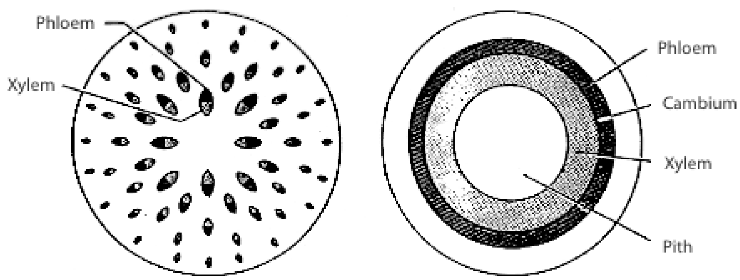
Let's now go back to the original drawing of a plant and look at the various parts of the plant (Figure 4.1)

1. The root.

Let's start at the bottom with the root. We never see that part of the plant but it is obviously terribly important. The roots can be a large part of the mass of the plant and dig very deeply into the ground; a corn plant can send roots 12 feet into the ground. The idea here is to increase the contact area of the plant with the soil to maximize the absorption of nutrients. To do this side roots branch out of the main roots and on and on. The branching pattern ends with tiny **hair roots** where the absorption takes place.

a. Two rooting systems.

- Many plants develop a central root that goes straight down into



A. Discontinuous vascular system of a monocotyledonous stem. Note lack of distinct pith.

B. Continuous vascular system of a dicotyledonous stem. Vascular system surrounds the pith

Figure 4.7. The vascular system of monocotyledonous and dicotyledonous plants.

the ground: called the tap root it anchors the plant down and allows water supplies from deep water sources (apple trees, hickory trees and carrots do that.). It is very difficult to transplant a taprooted plant because you end up breaking the tap root which leads to plant death. What you must do is to dig around the tree and use hormones to force a ball of roots to form by branching out; the next year you can break the taproot and transplant the tree which now can survive on the branched (**fibrous**) system. The taproot is never regenerated however.

- Other plants will instead have a system that branches a lot and remains shallow; no taproot is formed. Blueberries and a lot of weeds use this strategy to collect water and nutrients. This also anchors the plant and gives it the advantage of getting the rain water that stays at the soil surface during short downpours.

b. Types of roots

The roots of some plants are actually storage areas for starches. The carrot, potato and radish are edible types of roots that store starch in this manner. Some of those actually also carry types of tissues that are similar to the buds found on shoots (remember our first diagram). This meristematic tissue forms what we call the eyes of potato tubers. This meristematic tissue of potatoes enables the root to grow stems again and to propagate into whole plants. This type of propagation is asexual and often referred to as **vegetative reproduction**. Carrots cannot do that.

1. The shoot.
It is a central axis with side stems on it to hold the leaves and the flowers and the fruits. It supports those structures and transports food to them. The form or shape of the tree or plant will depend on how the side stems branches off of the main stem. The bud is the actively growing region of the stem that will develop into a new stem. Look at Figure 4.8. On a tree the most likely bud to develop into a flower, leaf or stem is the one furthest away from the roots and is called the **apical bud**. The ones below are called lateral and are less likely to develop. A bud present at the internode is called **adventitious** and results from an injury (a cut of the branches during pruning). Buds can be opposite or alternate on the stem (important for recognizing the type of plant you have to key out).

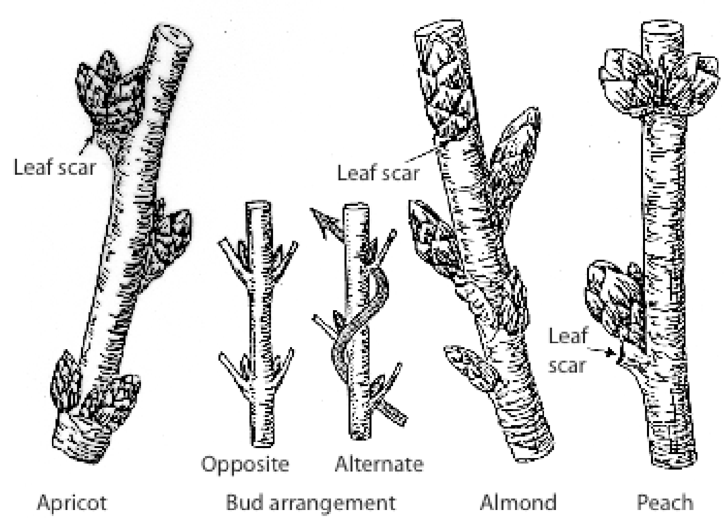


Figure 4.8. Bud arrangement on plant stems.

2. The leaf

The leaves are the antennae that collect the energy of the sun for the process of **photosynthesis**. The leaf **blade** is attached to the stem by a **petiole**.

You can see the vessels branching out on the leaf surface much as our system of arteries and veins. The size of the leaf will vary widely as its shape. Monocots have mostly very long narrow leaves while succulents have wider types of leaves. Some leaves are also very thick but most are relatively thin. The cabbage and lettuce heads are actually made of leaves rolled up in a ball. A lot of plants have edible leaves (such as lettuce, beets) but others do not (Rhubarb, tomatoes and potatoes for example) because they contain substances that are poisonous to humans.

3. The flower.
It is more complex in organization and is the location of sexual organs. See Figures 4.9 and 4.10 for pictures of a complete or perfect flower (Figure 4.9) and various types of flowers (Figure 4.10).

a. **Sepals** are an envelope of green leaf like structures that encloses the flower at the bud stage. When the flower opens they open up and remain on the stem below the petals.

b. **Petals** are the most obvious part of the flower. Brightly colored to attract insects that will spread the pollen grains to the female parts, they form the cup that contains the sexual organs. They produce perfumes and a sugary sticky substance (nectar) also to attract insects.

c. **Stamen**. Inside the "cup" we see the male parts around the female organ: the stamens are the combination of the long poles called **filaments** supporting a sac containing **pollen** grains (**anthers**).

d. **Pistil**. In the middle you find the female organ or **pistil** made of three parts:

- The **ovary**, a sac containing the **ovule** which will become the fruit when fertilized.

- The **style**, a long tube connecting the ovary to the stigma.

- The **stigma** on top of the style which will receive the pollen. Pollen then migrates to the ovule via the style to fertilize the ovule.

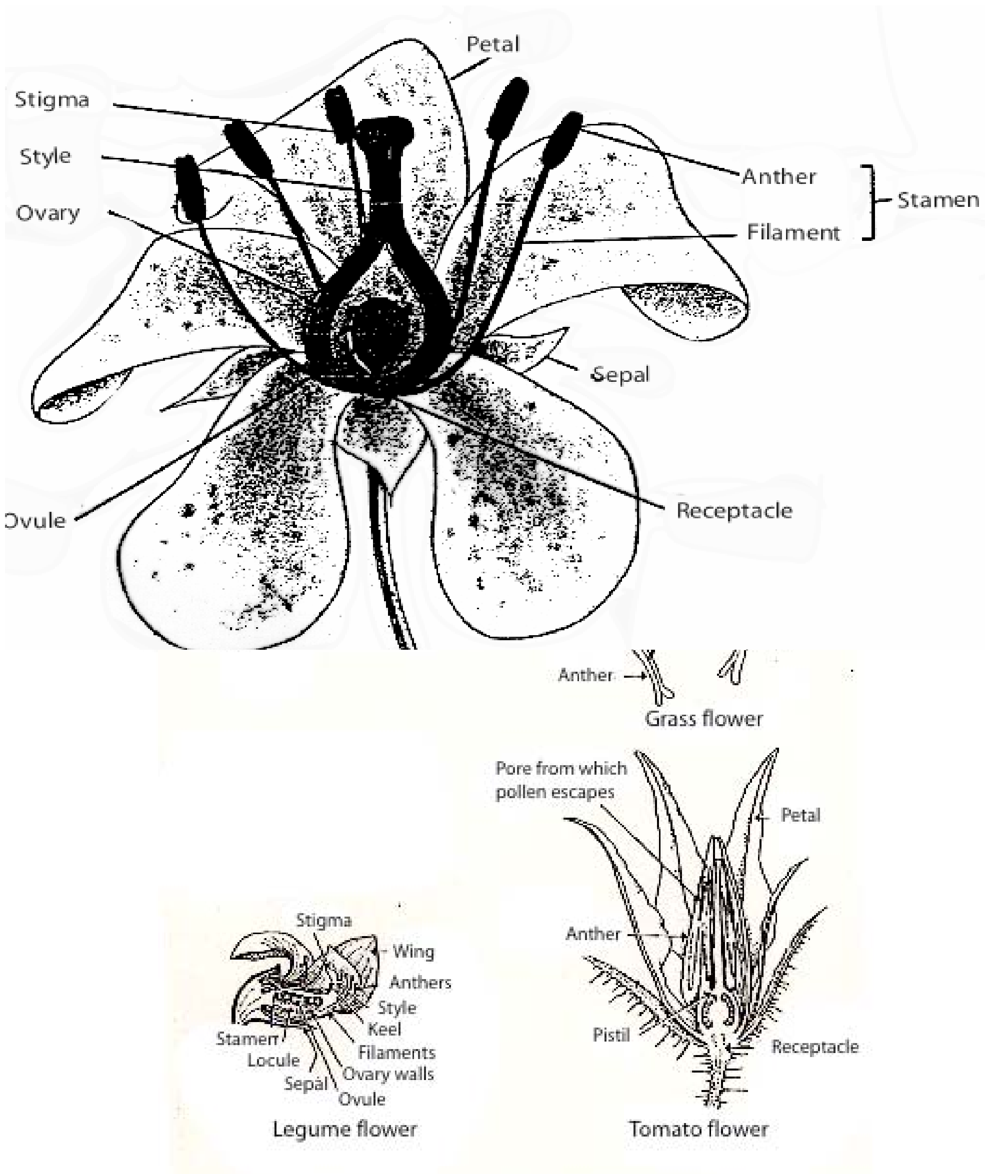


Figure 4.10. Examples of flowers in different plants.

Flowers with both male and female organs are called perfect. In some species some parts may be missing. That would be that case in plants where you have female flowers separate from male flowers, where the stamens or the pistil are missing; female flowers are called **pistillate** flowers; male flowers are called **staminate** flowers.

Figure 4.10 shows the different modifications to flowers from various types of plants. You see an enormous variety here despite the fact that the function of these flowers is essentially the same.

4. The fruit

It is essentially the mature ovary containing the seeds that appear inside. A simple ovary gives a simple fruit (muskmelon). If the flowers appeared in aggregates (with many pistils on a common receptacle) the fruit will also be aggregates (blackberry, raspberries). If the flowers were individual flowers but tightly clustered, the fruit is said to be multiple (grapes). Figure 4.11 shows these various types of fruit.

5. The seed

That is the equivalent of an offspring, a young etc.). The seed contains a source of energy (usually starches or simpler sugars) and the necessary equipment to start a shoot and a root. All this is wrapped into a **seed coat** that prevents damage to the content. The seed is usually very dry in order to preserve its content and also prevent germination when not desired. While the seeds may have many shapes and forms, they all have pretty much the same parts. Let's look at them.

There are 3 major parts to a seed (Figure 4.12):

a. The **seed coat** for protection.
b. The **endosperm** where the stored food is in. That occupies the bulk of the seed.
c. The **embryo** which is already differentiated into a tiny shoot and root and cotyledons or seed leaves.

That is all you need to know. There are other structures in the seed that are there as barriers or for other functions but they are not present in all seeds and are not really crucial to understand the basic function of the seed.

As the seed germinates, it will first **imbibe** that is gain water to start the various enzymatic systems needed for germination. An interesting phenomenon here is **dormancy** or the fact that the seeds produced in the fall will not germinate until spring even if water is available. The seeds have built in mechanisms to prevent germination until the right time (after winter is over).

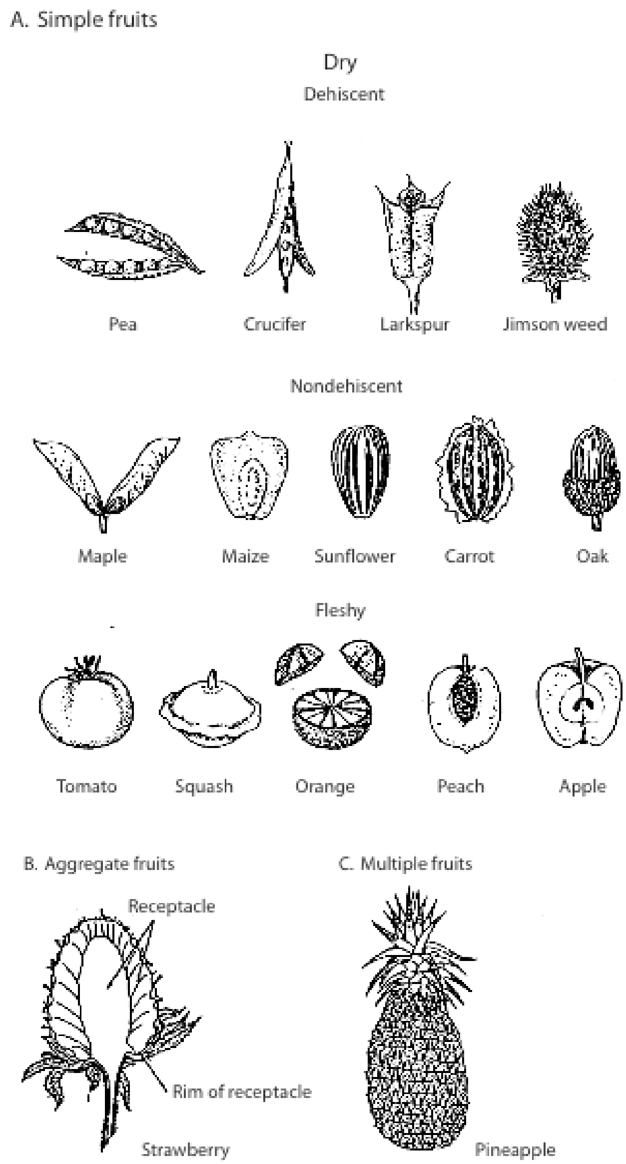


Figure 4.11. Types of fruit and examples.

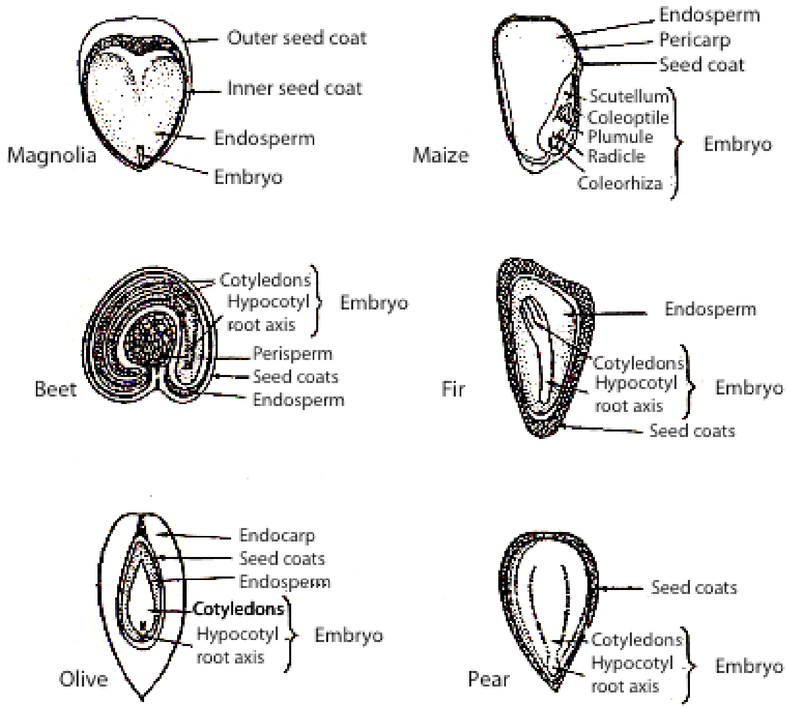


Figure 4.12. Seeds come in all shapes and sizes.

The mechanisms of dormancy vary and so does the manner in which you can break dormancy artificially:

a. Storage at cold temperature to mimic winter (called **stratification**). That can be done at cold temperature (in the refrigerator for a few days to a few weeks) for some seeds and warm temperature for others.

a. **Scarification** is the process of nicking the seed coat to let air in. You can do it mechanically or with strong acids. This lets air and water into the seed to break dormancy.

Figure 4.3 shows germination itself. The root appears first most of the time out of the embryo, using the storage food from the seed endosperm. The seed coat is broken by the growing cotyledons which then start collecting sun light right away. The stem then starts growing

F. EXERCISES

1. What is dormancy?
2. What would happen to a plant cell exposed to an environment containing less salt than that cell? EXPLAIN.
3. What would happen to an animal cell exposed to an environment containing less salt than that cell? EXPLAIN
4. Explain the principle of osmosis.
5. Convert:
   a. 0.25 mm into cm.
   b. 6.615 lb into Kg
   c. 125 lb into kg
   c. your own weight (in lb) into kg
   d. 905,782 seconds into days.

6. Use exponential notation to express:

   a. 25,000,000
   b. 0.0000654
   c. 8,954
   d. 0.025 mm into meters
   e. 1 metric ton (1,000 kg) into grams.

7. What does a eukariotic cell possess that a prokariotic cell does not?
8. As far as ATP is concerned what is the difference between mitochondria and chloroplasts?
9. What is the human equivalent of a plant seed?
10. Are mitochondria present in plants? What is their function there?
11. What is the function of ATP?
12.
13. Describe two types of roots.
14. What is the primary function of the roots?
15. What is the primary function of the leaves?
16. Explain the differences between pistillate and staminate flowers.
17. What does a pistillate flower requires that a perfect flower does not?
18. What is the relationship between the fruit and the seed?
19. What structure is critical for the exchange of gases in a plant cell?
20. Why is carbon dioxide a poison for humans but not for a plant?
21. What does a plant use carbon dioxide for?
22. Describe the female reproductive structures of a flower.
23. Describe the male reproductive structures of a flower.
24. What are the main differences between plant and animal cells?
25. What is the largest organelle in a plant cell? What is its function?
26. Describe two types of roots.
27. What is a bud?
28. What type of membrane surrounds the vacuole?
29. How is the water content of the vacuole regulated?
30. You have four objectives on your microscope: 4X, 10X, 40X and 100X. With which objectives could you see a prokaryote? Give an example of a prokaryote.
31. Referring to question #30 with which objectives could you see a eukaryote? Give an example of a eukaryote.
32. Referring to question #30 with which objectives could you see a eukaryote's organelle? Give an example of a eukaryote's organelle.
33. Compare apical and lateral buds.
34. How is a leaf connected to a stem?
35. What are the parts of a seed?
36. What is undifferentiated tissue
37. Give an example of undifferentiated tissue in plants.
38. Give an example of undifferentiated tissue in animals.

39. Match each item in column A with one or more items in column B.

A	B
1. 0.002 mm	a. Green color of plants
2. Fats/lipids	b. 1 x 10^{-4}
3. nucleus	c. Membrane
4. Converts solar energy to sugars	d. 1 x 10^{2}
5. Mitochondria	e. Chloroplasts
6. Chlorophyll	f. 25 µm
7. Stores salts and water	g. Makes proteins
8. Ribosomes	h. Vacuole
9. 1 x 10^{-5}	i. Cell wall
10. Animal cell	j. 1/10000
11. Plant cell	k. No cell wall
12. Animal or plant cell	l. Bacteria
13. 0.0001	m. Converts sugar to energy
14. 100	n. DNA

40. Compare and contrast the functions of human skin and plant epidermis.

41. You live in the Midwest. The area is known as Tornado Alley. You want to plant 2 trees on either side of your house, within 10 feet of the foundation. When you go to the nursery to purchase the trees, you notice that some of them have tap roots and some have fibrous roots. Which type of root system would be best and why? **Tap Root – very stable and less branching out into basement**

42. Your front lawn is a steep hill that is difficult to mow. So you decide to plant some "ground cover".
   a. Why or why not would you purchase plants that form rhizomes? **Yes to rhizomes**
   b. How do rhizomes form? **From meristematic tissue (not specialized)**

43. Why are some fruits classified as "single fruits"? **(one stem – apple)** What's the opposite of "single fruit"? **(Multiple fruit, pineapple)** Give an example of each.

44. Explain the potential benefit of scarification over stratification. **Faster**

45. Why did the blue Mexican corn seeds grow after 1000 years of storage in Indian caves? **Dormancy and stratification**

46. Draw a graph diagram of a plant cell, animal cell, and bacterial cell. What are the functions of the organelles that are present in one type and not the other? Why do eukaryotes need these extra organelles?

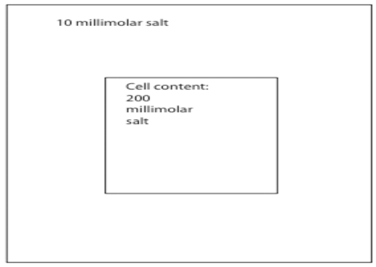
47. On a sunny day a plant cell absorbs a lot of light. List the steps for the conversion of light into energy. What are these steps called collectively?

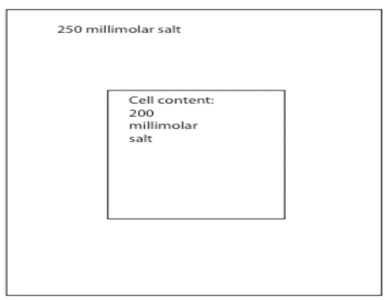
48. How is genetic information used within any cell?

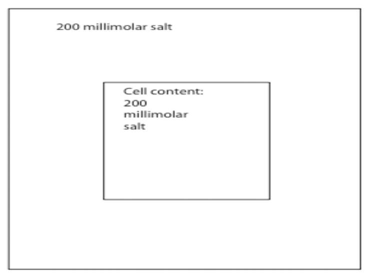
49. What are the benefits of a semi permeable membrane? What are the benefits of a cell wall?

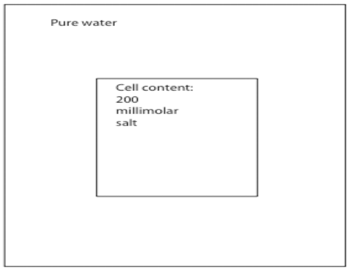
50. Why is equilibrium essential in a cellular environment? How is it achieved?

51. In the following diagram, each cell is surrounded by water or a salt solution. In which direction will osmosis occur for each cell? Draw arrows to indicate your answer


10 millimolar salt
Cell content:
200
millimolar
salt


250 millimolar salt
Cell content:
200
millimolar
salt


200 millimolar salt
Cell content:
200
millimolar
salt



52. Write directions for an experiment where you want to determine how much yeast is required to produce 10% alcohol in your wine. Use the following words in your directions.

Standard curve
% alcohol
amount of yeast
variation
days of fermentation
grape type
spectrophotometer
absorbance
graph

lesson 5. **Yeast**
4 figures
3 tables
8 appendices

A. Classification

Definition: Yeast are unicellular fungi which reproduce by **budding** or **fission**, a means **of asexual reproduction**. This means that **sexual reproduction**, while possible in yeasts, is the exception. Yeast reproduce mostly by division or **mitosis** rather than by mixing DNA from two different cells as in **meiosis**.

Yeasts are eukaryotic and thus their cells are larger and more complex than the prokaryotic bacterial cells. In fact yeast and bacteria belong to a different Kingdom altogether. Yeast are Fungi, like molds and mushrooms while bacteria are Monera. Let's find out where they stand in creation:

Kingdom: Fungi
Phylum or Division: Ascomycete although some people call it Class: there are 4 Phyli.
Other Divisions do have classes but in Ascomycetes there are no Class or Order because there are fewer groups.
Genus: Saccharomyces
Species: cerevisiae

Penicillium, Aspergillus, Morels mushrooms also belong to the Ascomycetes. Molds however are a different Phylum than the Ascomycetes.

There are 100,000 types of fungi and only 100 are pathogenic to us, mostly in the phylum Denteromycetes. And yet we tend to look at all fungi as repulsive and dangerous. Their appearance may have something to do with this perception.

B. Biology of fungi.

Fungi are the decomposers of our **biosphere** because a lot of them feed on dead plants or animals (they are called **saprophytes** for this reason), releasing CO_2 and simple nitrogenous compounds back to the soil. The picket fence or the deck that you just built will be attacked by fungi over the years and fall apart because of their action. These organisms are an integral and necessary part of our ecology, operating under the principle that every organism has a role to play, is dependent on other organisms and other organisms are dependent on it. For example a deer feeds on vegetation and is in turn eaten by predators of its species. So it occupies an important place in the food chain. It also needs the oxygen produced as a waste by plants and produce a waste, carbon dioxide, that plants need. The extinction of such a species would therefore create a serious disturbance in the ecology of the planet. So even if we find fungi repulsive they have an important role to play in the **ecosystem**. The science of **ecology** studies these interactions between species and tries to understand how each species is dependent on others. Yet human activity is responsible for the **extinction** of an estimated 1500 to 2000 species of plants and animals every year. Could we survive if we destroyed most species on this earth except the ones that we find cute? How many species of animals and plants do we need to survive?

Fungi are also very interesting in that some of them can grow in conditions where other organisms could not (no O_2, high alcohol concentration) and some produce interesting compounds that keep other organisms away: alcohol, antibiotics etc.. Another example of interesting fungal products is cyclosporine, a compound produced by a soil fungus that suppresses the human immune system; it is used in organ transplants to slow down the efforts of the recipient's immune system to destroy the transplanted organ. We do not know however what its original function is in fungi. Read the paper accompanying this chapter for more information.

A lot of fungi live in association with other plants or bacteria: a lichen is a **symbiotic system** where a fungus lives in association with algae or cyanobacteria. Symbiosis may be more common than we thought. While we said that each species depends on others, in a symbiotic relationship, the dependence is more immediate. For example humans need the bacteria living in their intestine (Escherichia coli) for the nutrients that they produce while the bacteria use the food in the intestine to live on. The survival of each partner of a symbiotic relationship depends directly on the other.

A lot of plant and animal diseases are fungal in origin: some fungi attack living rather than dead organisms to feed and grow from the nutrients derived from the killing of living cells. Mildew, rots, skin infection (yeast infections etc.) are examples of such fungal diseases even though only about 100 of the100,000 types of fungi are pathogenic to us.

Fungi and thus yeast are unicellular organisms like bacteria. However their cells are more complex and have larger cellular dimensions than bacteria:

BACTERIA	FUNGI
no nucleus; DNA floats in cytoplasm attached to outside wall	a nucleus contains the DNA
DNA is about 4-6,000 base pairs long; only one chromosome	much larger DNA; several chromosomes
no organelles to carry out specialized functions; those functions all take place in the cytoplasm	several organelles with specialized functions
2 micrometers in length	much larger in size

about the size of an organelle	than a bacterium

Fungi are unicellular but what about mushrooms. The cells make long filaments called **hyphae** through which they send their nuclei during sexual reproduction. Put a lot of those filaments together and you get a **mycelium**, a structure that we know as a mushroom. So when you eat a mushroom you are eating some cells but mostly a lot of long tubes or filaments.

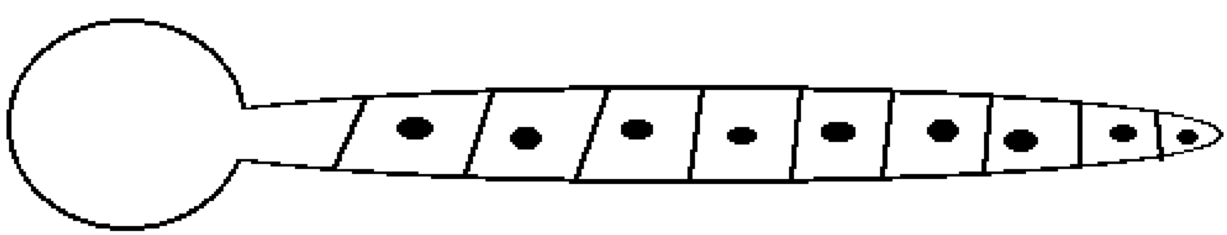
Yeasts make only short filaments or **hyphae**. Because these filaments are very short you never see the mushroom shape. In fact this is an important feature to distinguish between the Divisions within the Fungi kingdom: the shape of the mycelium and the structure of the hyphae separate the various Divisions

- Zygomycetes: possess many nuclei found in cytoplasm, with short or no hyphae.

- Ascomycetes and Basidiomycetes possess hyphae where nuclei are separated by walls with perforations.

DIAGRAM 1.

HYPHAE: long hair-like structures made of compartments separated by walls with perforations. These compartments contain nuclei.



C. Reproduction.

1. What is DNA and where is it stored?

DNA is information, stored as a code, that contains the building blueprints for proteins and other biological material. DNA is the reason why you have blue eyes or brown eyes, why you are 6 foot tall or 4 foot tall, why you are going to be bald or not. DNA controls every aspect of your biological life. In Eukariotic cells the DNA is located in the nucleus.
The nucleus is a membrane bound organelle that contains the genetic information in the form of chromatin, highly folded ribbon-like complexes of **deoxyribonucleic acid (DNA)** and a class of proteins called **histones**.

When a cell divides, chromatin fibers are very highly folded, and become visible in the light microscope as chromosomes. During interphase (between divisions), chromatin is more extended and is not visible under the microscope.
The DNA of chromatin is wrapped around a complex of histones making what can appear in the electron microscope as "beads on a string" or **nucleosomes**. Changes in folding between chromatin and the mitotic chromosomes are controlled by the packing of the nucleosome complexes.

There are three types of molecules in DNA proper: a sugar (**deoxyribose**), a phosphate and a base. DNA or **d**eoxyribo**n**ucleic **a**cid is a large molecule structured from chains of repeating units of deoxyribose and phosphate linked to four different bases abbreviated A, T, G, and C. We will later show how the simple structure of DNA contains the information for specifying the proteins that allow life. DNA is arranged in two long chains or strands of repeating bases. DNA is said to be double-stranded. The two strands are linked by interactions of bases on each strand. The base A interacts with T, C with G. A segment of DNA would look like this:

T - A
T - A
A - T
A - T
A - T
C - G
G - C
C - G
C - G
G - C
T - A
T - A
G - C
A - T
T - A
G - C
G - C
C - G
G - C
T - A
C - G
C - G
C - G

You can see that the two strands complement each other. For this reason the first strand is called the template and the second the complementary strand. The two strands are twisted around each other in a helix called he double helix that gives it strength. The helix in turn is wrapped around histones to form the chromatin.

DNA is going to be involved in two different processes: **replication** where it will double itself for mitosis or meiosis and **transcription** where it will produce messages transmitting its codes to the cytoplasm for the synthesis of proteins. We are interested in replication here as mitosis is about to begin.

```
          T  -  A
          T  -  A
          A  -  T
          A  -  T
          A  -  T
          C  -  G
          G  -  C
          C  -  G
          C  -  G
          G  -  C
          T  -  A
          T  -  A
          G  -  C
          A  -  T
          T  -  A
          G  -  C
       G              C
     C                  G
   G  -  C         G  -  C
   T  -  A         T  -  A
   C  -  G         C  -  G
   C  -  G         C  -  G
   C  -  G         C  -  G
```

The process of replication is designed to insure that exact copies of the DNA in chromosomes are passed on to daughter cells. To do so enzymes first separate the two strands of DNA from each other. Then using the existing strands as templates new strands are formed that complement the existing ones. The result is two identical double helices, one for each of the two daughter cells that will be made during mitosis. Finish the replication of this DNA and satisfy yourselves that the two DNA fragments you have synthesized are identical.

2. **Mitosis.**

A mammalian cell replicates every 12 to 24 hours, a plant cell every 6 to 8 hours. Compare this to the 20 minutes it takes for a bacterial cell colony to double its cells. The main mode of reproduction in yeast as implants and animals is vegetative or asexual. The process is called **mitosis** (Figure 5.1). Here the cell duplicates all of its internal components before splitting in two, thus producing two daughter cells that are identical to each other and to the original or mother cell. Cells normally contain two sets of each chromosome. We call them 2n or **diploid**. During cell division, the cell will first duplicate all of its DNA to become 4n before dividing into two 2n identical cells. Mitosis is a process where the following steps take place:

a. **Interphase**. Cells spend most of their time in interphase. In onion cells the division process itself takes 80 minutes but cells go though division only every 12 to 24 hours. In other words interphase would last 11 to 23 hours and all other steps of mitosis combined would take up 80 minutes.
Interphase is not really a step in mitosis but rather describes the time before mitosis when the cell grows, replicates its DNA and builds up its protein and fat content in preparation for division. The replication of DNA doubles the amount of nucleic acid present in the cell which then becomes 4n (tetraploid). Interphase also has 3 stages: G1 (first gap), immediately following mitosis, S phase in which replication of DNA occurs and G2 (second gap) immediately preceding mitosis. We often describe the cell cycle in terms of these steps and in the following order: G1, S, G2 and M (mitosis).

b. **Prophase**. In the nucleus chromosomes begin to condense and becomes visible in the light microscope.

c. **Prometaphase.** The nuclear membrane dissolves, marking the beginning of prometaphase. Microtubules attach at the center of each pair of chromosomes and the chromosomes begin moving to opposite ends of the cell. .

d. **Metaphase.** Spindle fibers align the chromosomes along the middle of the cell. This line is referred to as the metaphase plate. This organization helps to ensure that in the next phase, when the chromosomes are separated, each new nucleus will receive one copy of each chromosome.

e. **Anaphase.** The paired chromosomes separate and move to opposite sides of the cell.

f. **Telophase**. Chromosomes arrive at opposite poles of cell, and new membranes form around the daughter nuclei. The chromosomes disperse and are no longer visible under the light microscope. The spindle fibers disperse, and cytokinesis or the partitioning of the cell may also begin during this stage.

g. **Cytokinesis.** In animal cells, cytokinesis results when a protein called **actin** contracts the cytoplasmic membrane around the center of the cell pinching the cell into two daughter cells, each with one nucleus. In plant cells, the rigid wall requires that a cell plate also be synthesized between the two daughter cells.

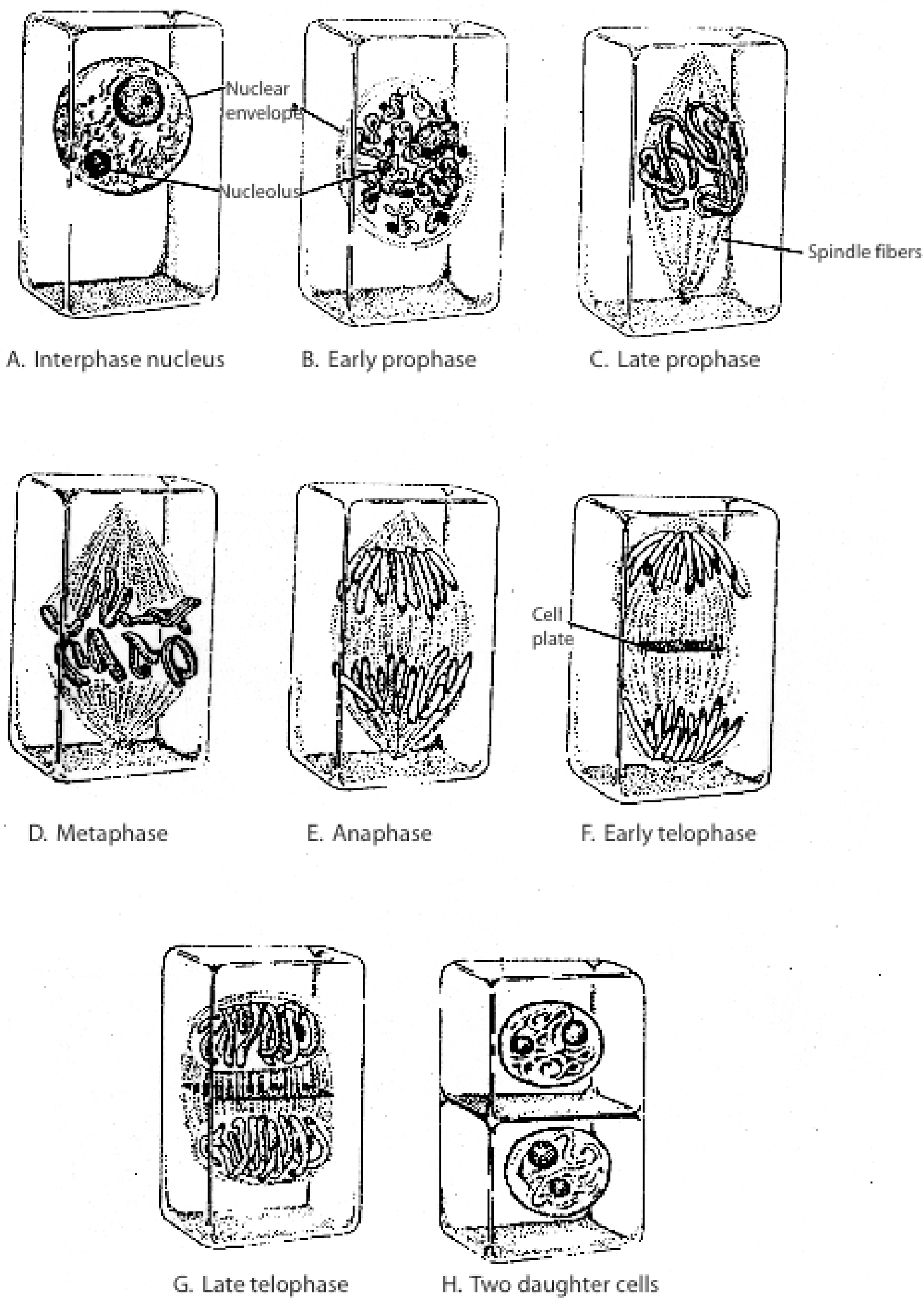


Figure 5.1. Mitotic stages of mitosis in onion root tip cells.

Is the daughter always identical to its parent? Most of the time. Occasionally (0.001% of the time) the replication of the DNA of the mother cell produces an error, what we call a **mutation**. A mutation will bring a change in the composition of the DNA (genotype) and therefore a change in the characteristics of the organism (**phenotype**). These changes could be beneficial to the survival of this organism or, be neutral and not affect its potential for survival. These two types of changes will remain in the population. On the other hand if the changes are deleterious to the survival of the organism it will disappear from the population. Remember that the DNA of all organisms is divided in sequences called **genes**, each gene coding for one protein of this organism. The DNA however exists in duplicate genes so that each gene has 2 copies or **alleles**. This insures that if a copy is not functional the protein coded for by the gene can still be synthesized with the information contained in the other allele. One copy comes from one of the two parents, the other from the other parent. If one copy is defective and does not produce a viable protein (it is called **recessive**), the other copy will (it is called **dominant**). If both copies are functional or if both copies are non-functional the organism is called **homozygous**. If only one copy is functional and the other is not the organism is called **heterozygous**. If heterozygous the organism will have characteristics of the dominant or functional copy of the gene.

3. Meiosis.

In sexual reproduction DNA from 2 different individuals is mixed. The process is called **meiosis** (Figure 5.2). Here a **diploid** cell goes from 2n to 4n to 4 **haploid** cells (n) called **gametes** each with n chromosomes. These gametes carry only one set of DNA. In animals they are the egg and the sperm. In plants they are the ovule and the pollen grain.

Each haploid gamete must unite with a gamete from another individual to form a **zygote** that is again 2n or diploid. Therefore the zygotes formed are different from each parent since they are combinations of each parent's chromosomes.

A yeast cell does reproduce asexually mostly. It goes into sexual reproduction only when under stress. We can argue that for a lot of other organisms including humans sexual reproduction is a consequence of stress or at least enhanced by stress. Look at baby booms in war times and see that we may not be that different from yeast. Other Ascomycetes do reproduce sexually and the mushroom is a way for hyphae from different individuals to fuse in order to put 2 gametes or spores together so that DNA from 2 different individuals is combined in one cell The nuclei fuse and the mixed DNA goes through the first and second meiotic division that yields 8 individuals.

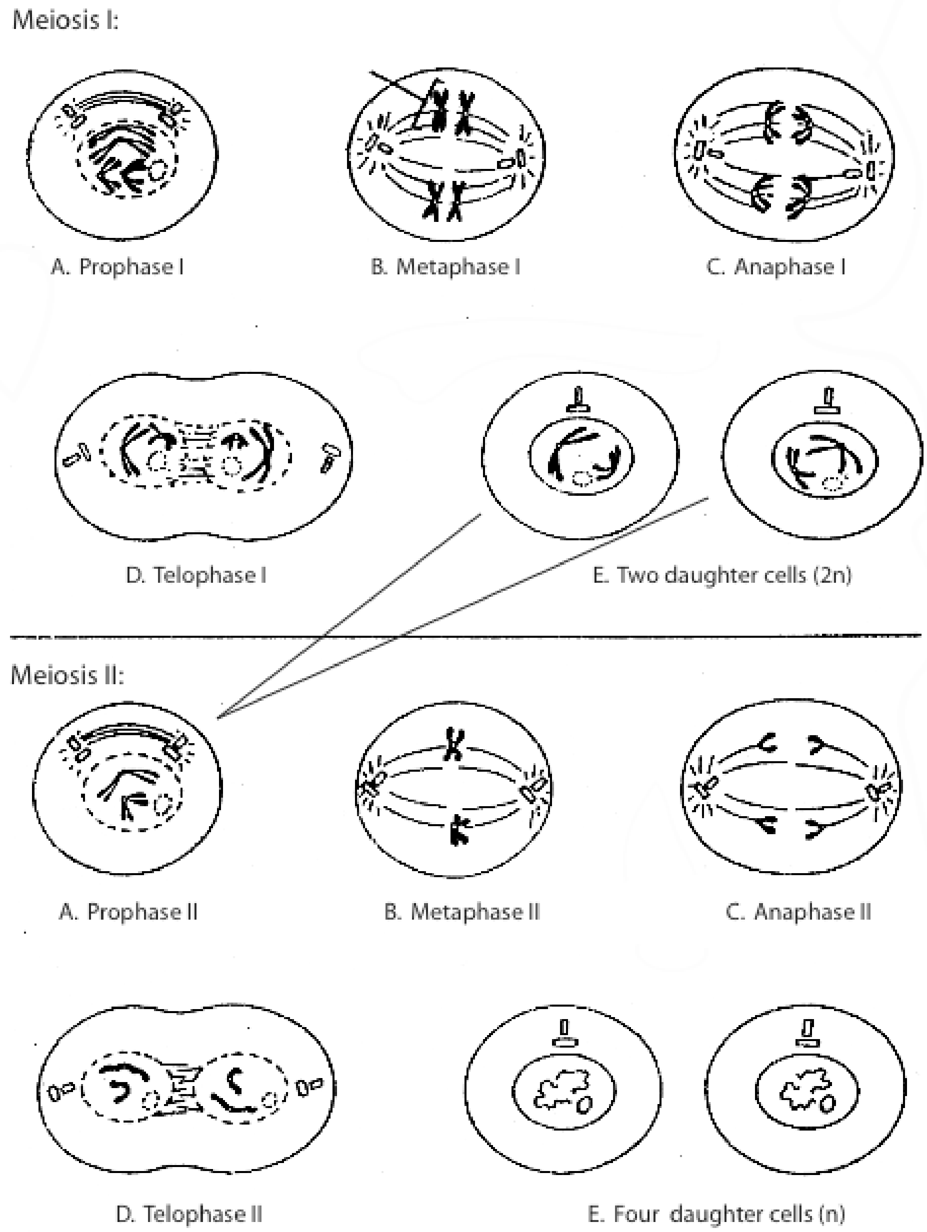


Figure 5.2. Meiotic cell division.

The process of meiosis is longer and more complex than mitosis since two meioses occur one after the other. In the first meiosis the diploid cell doubles its DNA and following the

same steps as mitosis splits into two diploid daughter cells. The difference is that in Prophase 1 the chromosomes exchange pieces of DNA in a process called cross over. Remember that we each carry two sets of chromosomes (we are 2n), one set from our mother and one set from our father. If your mother had blue eyes and your father brown eyes the Piece of DNA coding for eye color coming from your mother will .be different from the piece of DNA coming from your father. So each n in the 2n is different from the other. Now imagine that the DNA from your mother and the DNA from your father started exchanging pieces during Prophase 1. The result is that the two daughter cells will end up with DNA for different characteristics. They do not have identical DNA.
In the second meiosis the two diploid cells do not replicate their DNA and therefore split into a total of four haploid cells. Those are the egg and the sperm (or pollen grain) formed in plants and animals for example. These cells are not viable since they are haploid and must fuse (one egg with one sperm) to form a viable **2n zygote**. If yeast reproduces sexually, it will not form hyphae. Instead the haploid cells fuse to give a 2n zygote that will then do one of 2 things

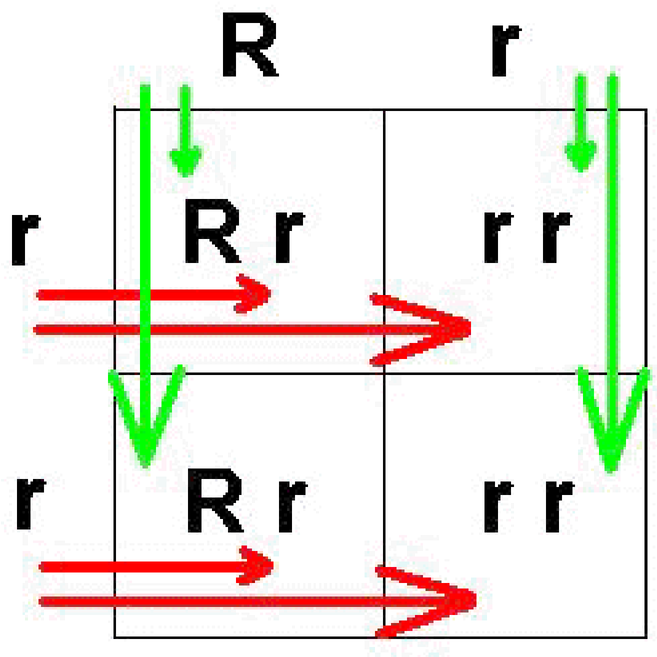
- produce diploid buds
  or
- go through meiosis --> 4 haploid individual cells
  + mitosis

Yeasts can sometimes reproduce sexually. This implies that two organisms mix their DNA to create hybrid offspring. In meiosis the two parents split their diploid DNA into haploid **gametes**, i.e. reproductive egg and sperm that contain only one copy of each gene. The egg and the sperm must then fuse to make a complete (diploid) offspring. This is where it gets interesting. This offspring possesses two copies of every gene, each copy coming from a different parent. As we have said above if the two copies are different the offspring is said to be heterozygous. If they are the same the offspring is homozygous.

4. Inheritance of genes in offsprings.

We can see the inheritance of genes in offsprings as described in **Mendelian genetics** with the use of **Punnett squares**.

We will assume that genes are represented by letters. Capital letters represent dominant genes. The genes of one parent are given on the top horizontal line while the genes of the other are given on the left column.



The results here show that if a homozygous recessive parent and a heterozygous parent are crossed half of the offspring will be homozygous recessive and half will be heterozygous. The baggage of genes that an organism carries is called the **genotype**. But the characteristics that each organism displays are referred to as the **phenotype**. For example if R is functional and gives violet pigment to grape vine flowers (violet flowers) and "r" is defective and gives no pigment to flowers (white flowers) you can see first that the mutation (no pigment) will not affect the survival of the plant and that is why we can see and count offspring that have white flowers. Now offsprings that are RR or Rr will both have violet flowers. They will have different genotypes but similar phenotypes. Imagine now two parents both heterozygous for purple flowers.

a. What is the color of their flowers?

b. Draw Punnett squares for their offsprings.

c. How many different genotypes exist in the offsprings?

d. Give the percent of offspring for each different genotype.

e. How many different phenotypes exist in the offspring?

f. Give the percent of offspring for each different phenotype.

One can see the usefulness of Punnett squares in showing how sexual reproduction can increase the genetic diversity in a given population. In the example above we can see that a population of RR and another of rr would give rise to Rr individuals which did not exist before. That is the advantage of sexual reproduction over asexual reproduction.

D. Fermentation

How do yeast cells grow? Like any organism, they require energy and they get this energy from food. A yeast cell cannot generate its own energy: like us it has to acquire it. What organisms can generate their own energy? Plants do. They take the energy from the sun and store it in the form of sugars, molecules made with CO_2 from the air and H_2O. Sugars store the energy from the sun in a form that can be used by the plant itself but also by animals or fungi that eat the plant.

Plants can also link up these simple sugars one after the next into a huge molecule called **starch**. That molecule must be broken down into simple sugars again however before animals or fungi can use it for energy. Energy can be stored as simple sugars as in fruit and berries or a complex sugars (starch) in grain for example. You and I and animals can break down complex sugars into simple sugars and then break the simple sugars into carbon dioxide, water and energy; thus we can use starch as a source of energy. Yeast cannot. They can use simple sugars and go as far as break the combination of 2 simple sugars: **sucrose** for example is table sugar and made of 1 **glucose** and 1 **fructose**. Yeast can use all 3 of these but cannot break more complex sugars. They lack the enzymes that do that. As we said fruits contain mostly fructose. This means that yeasts can grow easily on fruit juices. Grapes are ideal for yeasts since they contain fructose, some glucose and all vitamins needed for healthy growth (Yes, yeasts need vitamins too).

DID YOU KNOW:
When yeast cells run out of sugar they do not die. They simply go into a dormant state.

1. How do animals use sugars?

They extract the energy of the sugars by breaking them down completely back to CO_2 + H_2O: but they need oxygen to do that. In fact they cannot survive without oxygen; they are known as **obligate aerobes**. Yeast can do the same thing that animals do and if given O_2 will break down simple sugars to CO_2 + H_2O. Oxygen enables them to free up all the energy that the plant has put in the sugar. The energy is released in steps during the process. Out of 19 steps needed to break down a simple sugar to carbon dioxide and water, 9 steps extract that energy.

But if the yeast is deprived of oxygen, it can use only the first 11 steps of the process which do not require O_2 and still extract some energy (only 6%). The final product then is alcohol + CO_2.

Alcohol then is formed when O_2 is absent (under anaerobic conditions). When you first put yeast in a source of energy, lots of CO_2 is produced but no alcohol because O_2 is present, dissolved in the juice. After O_2 runs out the yeast produces alcohol and CO_2 under anaerobic conditions. You can see from this that, as opposed to animals who can live under **anaerobic** conditions only for very short periods of time, yeast can live for extended periods of time without O_2.

2. Alcohol

The alcohol produced by the yeast is a particular type called **ethyl alcohol or ethanol**. This type of alcohol is not toxic in small quantities because our bodies can break down small quantities of it; in larger amounts it is a deadly poison. Other types of alcohols are **methyl alcohol (methanol)**, deadly to us even in small amounts (but not to rats for example) because we cannot break it down. It is found in windshield washer fluid for example. Isopropyl alcohol or **isopropanol**
(also called **rubbing alcohol**) is also deadly for the same reason.

What are the differences and the similarities between these alcohols?

CH_3-OH — 1 carbon: methyl alcohol

CH_3-CH_2-OH — 2 carbons: ethyl alcohol

```
CH3
  \
   CH-OH
  /
CH3
```

3 carbons: isopropyl alcohol

Wine has between 8% and 18% ethyl alcohol. This variability depends on the amount of sugar available for fermentation and the sensitivity of the yeast to alcohol. For example we cannot make a 30% alcohol wine with fermentation. That is because the yeast will die at 12 to 14% alcohol. For them alcohol is a waste product and as most waste products, it is toxic. CO_2 is a waste product for you and also toxic. Luckily it is a gas that diffuses away from the organism that produces it.

3. Measurement of sugar.

We now know all the reactions necessary to go from sugar to alcohol. So by using chemical mathematics called **stoichiometry**, we can measure how much sugar is in solution and therefore calculate how much alcohol will be produced from this sugar (Table 5.1). We can then find out how much sugar to add to the juice in order to produce a certain amount of alcohol (Table 5.2).
There are 3 common ways of measuring the sugar:

a. The **hydrometer** (or **saccharometer**) measures the density of a solution. Because it is denser a solution of sugar floats a weight more easily than water (Figure

5.3). You know that it is easier for us to float on salt water than fresh water because of the same principle. You will normally read the sugar content in brix unit or % Brix or ^{o}brix. Sugars are not the only solids that will increase the density of a solution. Proteins, vitamins, salts also increase the density of a solution. The hydrometer measure the density of a solution and therefore all solids dissolved in that solution

Let us take a look at some equivalent units for this method.
^{o} Balling = ^{o}Brix = % dissolved solid in liquid (w/v)
^{o} Baume' is a French measure that we don't use much anymore. The corresponding Beaume is given by : (^{o} Brix /2) + 1 = ^{o}Beaume. So 18 ^{o} Brix = 10 ^{o} Baume'.
The German scale of Degree Ochsle (^{o}O) is based on the difference in weight (in grams) by which a liter of must is heavier than a liter of water. The first three figures after the decimal point of the specific gravity are equivalent to Ochsle. Thus 1.075 S.G. = 75 ^{o}O).

b. The flotation method uses the principle that the presence of sugar in water makes it heavier. One quart of honey is heavier than 1 quart of water. We use a unit of **% (w/v)** (= weight in grams of sugar in a certain volume of liquid) to measure the amount of sugar in a solution. A 40 % (w/v) sugar solution has

Table 5.1. Relationship between specific gravity, ^{o}Brix (Balling), percent sugar and potential alcohol in a must.

specific gravity at 15 ^{o}C	Brix Balling	%sugar (W/V)	potential alcohol (%, V/V)
1.050	12.4	10.3	6.0
1.052	12.7	10.8	6.3
1.054	13.3	11.4	6.7
1.056	13.7	11.9	7.0
1.058	14.2	12.4	7.3
1.060	14.6	13.0	7.6
1.062	15.1	13.5	7.9
1.064	15.4	14.0	8.2
1.066	16.0	14.6	8.6
1.068	16.5	15.1	8.9
1.070	16.9	15.6	9.2
1.072	17.4	16.2	9.5
1.074	17.8	16.7	9.8
1.076	18.3	17.2	10.1
1.078	18.7	17.8	10.5
1.080	19.2	18.3	10.8
1.082	19.6	18.8	11.0
1.084	20.0	19.4	11.4
1.086	20.5	19.9	11.7
1.088	20.8	20.4	12.0
1.090	21.4	21.0	12.3
1.092	21.8	21.5	12.6
1.094	22.3	22.0	12.9
1.096	22.7	22.6	13.3
1.098	23.2	23.1	13.6
1.100	23.6	23.6	13.9
1.102	23.9	24.0	14.2

Note. The German scale of Degree Ochsle (^{o}O) is based on the difference in weight by which a liter of must is heavier than a liter of water. The first three figures after the decimal point of the specific gravity are equivalent to Ochsle. Thus 1.075 S.G. = 75 ^oO). ^{o} Baume' is a French measure that we don't use much anymore. The corresponding Beaume is given by : (^{o} Brix /2) + 1 = ^{o}Beaume. So 18 ^{o} Brix = 10 ^{o} Baume'

Table 5.2. Correcting for sugar

specific gravity	^{o}Brix	sugar (lbs/gal)	potential alcohol (%v/v)	sugar to add for 12% alcohol (in grams per liter)	(in pounds per gallon)
1.039	10	0.69	4.5	121	1.01
1.044	11	0.78	5.1	110	0.93
1.048	12.2	0.87	5.7	99	0.83
1.052	12.7	0.96	6.3	89	0.74
1.056	13.7	1.05	7	78	0.65
1.061	14.9	1.14	7.8	66	0.56
1.065	15.8	1.23	8.4	56	0.48
1.069	16.7	1.33	9	45	0.37
1.074	17.8	1.42	9.8	32	0.28
1.078	18.7	1.52	10.5	22	0.19
1.082	19.6	1.61	11	10.5	0.10
1.087	20.7	1.71	11.9	0	0
1.091	21.6	1.81	12.5	0	0
1.095	22.5	1.91	13.1	0	0
1.099	23.4	2.01	13.8	0	0

NOTE: the amount of sugar to be added refers to table sugar.

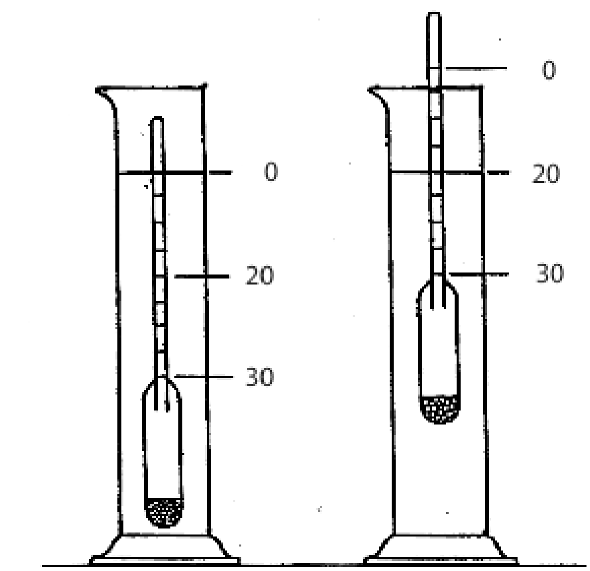


Figure 5.3. Saccharometer and hydrometer jar.

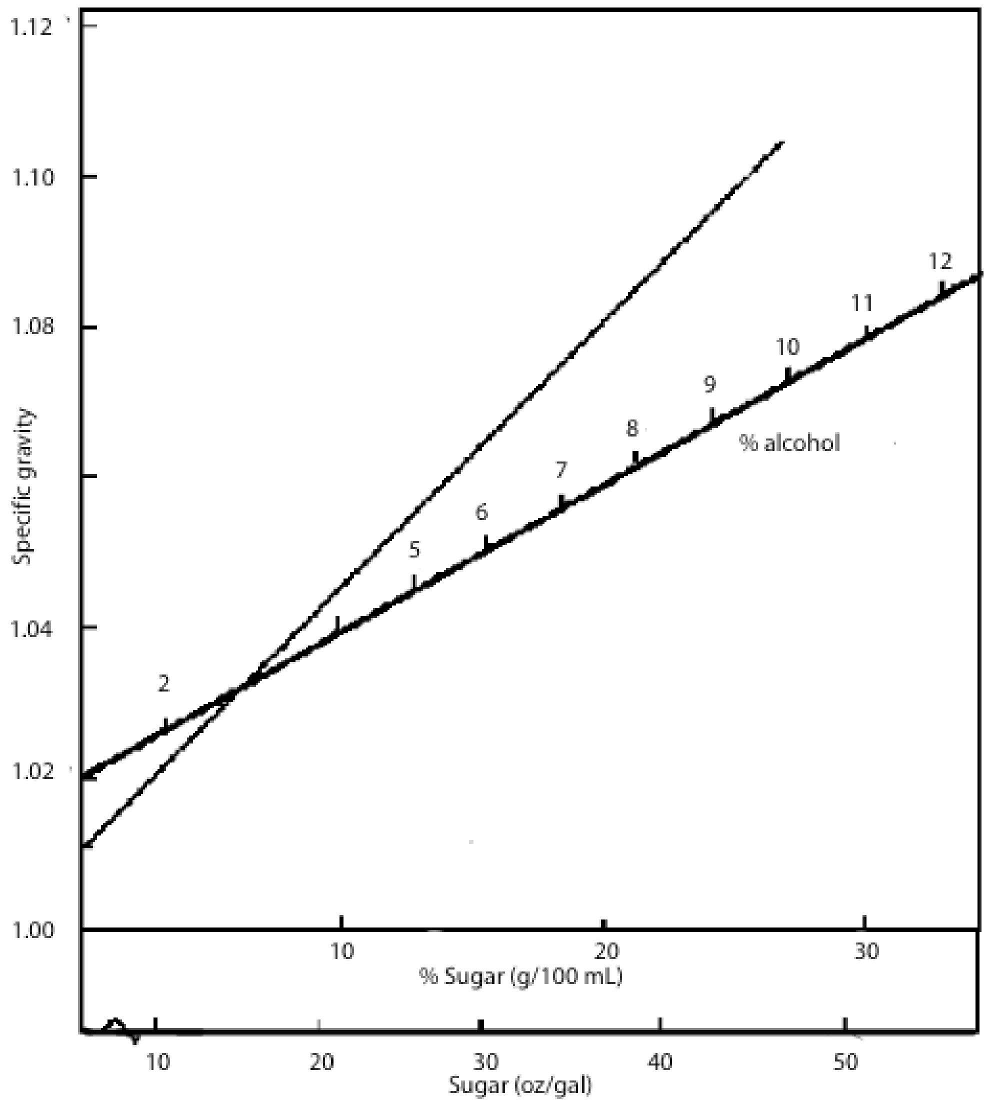


Figure 5.4. Sugar to alcohol conversion graph. The data from Table 5.1 was used to build this graph.

40 grams of sugar in each 100 mLs of solution. That unit is not limited to a mass per volume; it can be w/w, w/v or v/v. In a 40 % (v/v) solution of alcohol you have 40 mLs of alcohol for every 100 mLs of solution. The unit varies because of the state in which the dissolved **solute** comes: a v/v unit may be more useful for the measure of alcohol in water because alcohol is a liquid; on the other hand you could not measure a 40% (v/v) solution of sugar because sugar is a solid.

c. Specific gravity: the sugar dissolved in water makes the solution heavier than water alone. We say that sugar solutions are more dense that water. You can then measure the mass of a certain volume of solution of sugar in water and the weight of the same volume of water alone by using a device called a **pycnometer**. Divide one by the other for a ratio also called **specific gravity** that is the density of solution/density of water. Notice that this measure has no units.

Knowing the amount of sugar present in a juice will tell you how much alcohol the yeast will produce if all the sugar is used. You obtain the % alcohol produced (this is approximate by the way) by dividing the ^{o}brix by 2. Another way to give the alcohol content is to give the proof. Simply multiply that % alcohol you have just obtained above by 2 to get the **proof**: 80 proof = 40% alcohol. Table 5.1 gives a more accurate conversion than this simplistic method to determine the % alcohol and proof. Simply find the amount of sugar in the juice and find the specific gravity and the alcohol potential. Or if you have the specific gravity find the % sugar and the alcohol potential. Why is ^{o}Brix higher than % sugar. Because other solids are dissolved in the juice and contribute also to increasing the specific gravity.

The same results can be achieved with a graph (Figure 5.4) that plots the specific gravity as a function of the sugar content of the juice. Note here that the sugar content is given in % and in ounces per gallon as well. There are two ways to use this graph:

If you have measured the specific gravity as 1.080 for example use this value on the vertical axis and slide to the angled axis to find the alcohol potential. You should find 11 % alcohol. Note that table 5.1 shows 10.8 %. These two results are very close. You may have the sugar content in ounces per gallon or in percent. Then find this value on the horizontal axis (20 % for example) and determine the specific gravity by using the steeper line on the graph. You find 1.085 specific gravity. Now that you have the specific gravity go to the % alcohol on the angled axis. You find 11.6 % alcohol. The table gives you a value of 11.9 %. Again results by the two methods agree.

4. CO_2.

The production of CO_2 bubbles means that the yeast cells are working and producing alcohol. This means that sugar is still present and alcohol is below 12-14%. The production of CO_2 is seen very simply by using a water trap called an **air lock** that forces the CO_2 through a tube on top of the fermentation vat. The tube is filled with water to prevent air from coming into the vat. You see bubbles escaping through the tube when yeasts are consuming sugars because the CO_2 produced builds up a pressure inside the bottle that pushes the gas out. When no CO_2 is produced, the pressure inside equals the pressure outside and the levels of the water are equal in the two chambers of the air lock.

CO_2 is a waste product for the yeast but as a gas can dissolve in the liquid and escape through the airlock. A sparkling wine, champagne and beer are effervescent because the CO_2 that was dissolved in the liquid escapes as a gas when the bottle is opened and the pressure over the liquid is released.

The waste products of biological functions are usually **poisonous** to the organism producing them. Carbon dioxide is a poison to us and oxygen is a poison to plants. Of course we don't think so because we use oxygen in respiration reactions. But then again carbon dioxide is a food for plants and a poison to us.

A poison is defined as a substance that interferes, by its very nature or simply its concentration in the tissues, with the body’s functions and that cannot be metabolized or eliminated. A poison can be a substance that our body needs (vitamin A) when taken in too large a quantity; it can also be an ingested compound that our body does not need (dioxin, strychnine, cyanide). Can you name other poisons? Our bodies use what they can from the food we ingest and destroy or eliminate the rest, often using the liver to do so. In fact the reason we can eat just about any type of food (we are **omnivores** which is unusual in the animal kingdom) is that we have a large liver that **detoxifies** our blood stream constantly. So what we call a poison then will depend on the compound and the organism involved and how well the organism can detoxify the compound. Therefore a poison is defined by:

a. **Dose.** Carrot juice is not considered a poison and yet a few years ago a man in England died after consuming 4 quarts of it a day for months. So the dose is an important factor in considering whether a substance is a poison or not.

b. **Body weight**. A few years ago in Baltimore girls in a high school died after consuming in rapid succession 3 or 4 shots of 151 (proof) rum. Their body weight was small (about 90 pounds) and the concentration of alcohol in those small bodies rose above the lethal range. A large person (200-300 pounds) would have survived the experience. This points to the notion of **concentration**. The alcohol was more concentrated in the blood of these girls than it would have been in the blood of a larger person.

c. **Retention** in the body: We can never die of an overdose of vitamin c because the gut absorbs only what we need (about 160 mg per day). The rest is eliminated in the feces. But other substances can become soluble in the fat tissues and accumulate in the body to the point where they become dangerous. That is why we say that it is possible to overdose on vitamins that are fat soluble but not on vitamins that are water soluble. Poisons can also be eliminated in sweat, urine and expired breath. Methanol is a case in point. It cannot be broken down (we lack the enzyme to do so) but can be eliminated in sweat, urine and expired breath.

d. **Habituation**. We get used to things that are not good for us. For example kings in Mesopotamia were in constant fear of being poisoned by their subjects. So they consumed daily dosages of poison to get used to them so that if they were given poison they would only get sick but not die unless the subject were aware of that and gave the king a large dose of the poison.

e. **Sensitivity**. Some of us are more sensitive to certain compounds than others. Some people cannot drink coffee because they are more sensitive to caffeine and get an instant headache while others can drink 12 cups a day without any effects.

We can see that the dose and the concentration of a substance as well as its nature are important in determining its lethality but that not everybody will react to it in the same way we use the measure of LD_{50} to define the toxicity of a substance. It refers to the amount of a substance in grams, milligrams or nanograms per kilogram of body weight that killed 50% of the test subjects (goats, pigs, rats etc). Oral LD50 values for methanol in animals are 0.4 g/kg
in the mouse, 6.2 to 13 g/kg in the rat, 14.4 g/kg in the rabbit, and 2 to 7 g/kg in the monkey. We can see that some species of animals are clearly more susceptible to methanol than others. Mice and rabbits for example are affected very differently by methanol. Rabbits appear to be 40 times more tolerant to methanol than mice.
The LD_{50} for dioxin (a contaminant in herbicides) is 0.02 mg/kg of body weight for a rat and 0.001 mg/kg of body weight for a dog, i.e. the rat is twenty times more tolerant than the dog. Compare the LD_{50} data for methanol and dioxin. What do you notice?

5. Yeast and Fermentation.

So, we know that yeasts produce an alcoholic fermentation. Where do humans use this? There are three basic categories:

Bread making
Wine making
Beer making

Then why isn't bread alcohol ridden? For bread making, the yeast produces CO_2 that will lift the dough and create the airy form of bread in the leavening process. The structure of breads shows it to be full of holes that were CO_2 pockets. Why isn't alcohol formed? Because O_2 is present in the dough. Remember that the bread is kneaded repeatedly during leavening. Very little alcohol is formed in the process of yeast growth; the only by-products are CO_2 + H_2O. If some yeast cells find themselves without oxygen during the process of leavening alcohol is formed but then evaporates during baking.

Is the same yeast used for all 3 processes? In Babylon and Egypt the same yeast was used for baking, brewing and wine making. But people started selecting for better beers in Egypt (and therefore selected for modified yeast) and we saw that the yeast found in amphorae dating from 1500 BC. were different (i.e. improved) when compared to those found in amphorae from 5000 BC. In the late 1800s, people started using a more scientific selection of specific yeast for beer making and isolated strains that were sufficiently different from Saccharomyces cerevisiae to warrant a different name, Saccharomyces carlsbergensis or Saccharomyces uvarum. How did that happen? As you grow the same batch of yeast over and over again, mutations will appear. Some are bad and kill the yeast , others bring new characteristics to the yeast and therefore to the wine or beer. Now remember that Egyptians and Sumerians continually selected for a better beer or wine. If a new batch is terrible, you throw it out, but if it is great, you keep it and save that yeast to produce the next batches. In the process you select those mutations that are advantageous to you. And if you do that for 6000-8000 years as humans have, you eventually obtain brewing yeast that are very different than those that make bread or wine because you will be selecting for different characteristics in your bread versus your beer.

What do you select for in a beer making yeast for example?

a. Efficient alcoholic fermentation of sugars. As we said other microorganisms use sugar for energy but end up producing acids instead of alcohol. You do not want your yeast to start doing that! You also want the yeast to convert that sugar quickly in order to increase the concentration of alcohol fast so that other organisms cannot start using the sugars.

b. **Alcohol tolerance**. What if your yeast died in 3% alcohol? Could you make wine? WHY? In fact the several yeast types that are involved in wine making show varying tolerances to alcohol. While most yeast types used today in wine making die around 12% alcohol. Champagne yeast can resist up to 18% alcohol.

c. Production of **organoleptically** desirable compounds. As we said, the yeast modifies compounds present in juice so that wine tastes very different from grape juice. But what if your yeast produced a high concentration of alcohol as well as compounds that tasted like putrefying cabbage? Over the millennia brewers and wine makers have been selecting for yeasts that produce the most pleasing tastes in alcoholic beverages.

d. **Strain stability**. Mutations are good for they bring new characteristics but a strain too susceptible to mutations would lose these good characteristics too quickly so that a balance is necessary. Once you have found a good yeast that gives your beer all the characteristics that you desired you want it to keep giving you a stable product.

e. **Flocculation**. Toward the end of fermentation yeast tend to agglomerate. The process is called flocculation and when it occurs the large clumps of yeast settle at the bottom. Sugar may be left at the top and this leads to an incomplete fermentation. So by screening for yeasts that will use all the sugar, you screen, and obtain eventually, yeasts that flocculate less rapidly during the fermentation process. On the other hand it is easier to separate the alcoholic product from the yeast if it accumulates at the bottom. Thus you will not want to completely eliminate the flocculation process at the end of fermentation but rather retard it until all simple sugars have been consumed.

So we now know several different types of S. cerevisiae that are specialized and give the best results in the production of wines, sherries, beer and bread: Let's look at some examples.

- Wine: these yeast types have been growing on the grapes of Europe for thousands of years. They have evolved into types that are perfect for wine making and so fermentation is done with these natural yeast types. This simplifies matters greatly since no sterilization and addition of "proper" yeast strains are needed before fermentation. In the US, that sterilization process is done to kill the natural yeast strains (which are unsuitable for wine production) before European yeast are added to the wort. That is because we also have "wild" yeasts on the fruit and they are not as desirable as their European counterparts. Some of these European yeast strains include Montrachet for example, sometimes called Saccharomyces ellipsoideus because it is different enough from Saccharomyces cerevisiae. Sometimes Saccharomyces oviformis is used after cerevisiae has died to increase the alcohol level to 18% if enough sugar is present. This yeast is also called champagne yeast.

- Beer: 2 types of yeast are used here. The first one, a top fermenting yeast, tends to float in the early stages of fermentation and form a head. Later, it will sink: That's Saccharomyces cerevisiae and was used to make ale, an alcoholic beverage fermented at warm temperature. This yeast is also the major yeast used in wine making. Two others however also contribute, Saccharomyces apiculata is the first one to start fermentation but will die out at about 3-4% alcohol and Saccharomyces cerevisiae var. ellipsoideusthen that takes over carrying the fermentation to 12% alcohol.

- Bottom fermenting yeast or cold fermenting Saccharomyces carlsbergensis (called also Saccharomyces uvarum) a yeast that settles more easily and earlier in fermentation . It is used to make lagers, the most common type of beer in the US today. Within each species of Saccharomyces you will find multitudes of strains that are still called cerevisiae or carlsbergensis but vary slightly between from one to another. The question is to determine when two varieties of the same species (for example cerevisiae) have become sufficiently different to warrant the classification into another species. That is evolution in action.

f. Contamination

In vineyards and cellars where wine has been made for centuries, the proper concentration of the right yeast is very high and inoculation may not even be necessary. However there are other microorganisms in the air that are spoilage organisms. Example: Acetobacter that transforms alcohol to vinegar. In fact the lore is that you cannot make vinegar and wine in the same house because you end up making vinegar only. But yeast has 2 advantages that help it overcome other organisms:

- It grows in the very acid conditions offered by the grape juice
- It grows anaerobically while most spoilage bacteria and yeast require O_2 or less acid.

Still 4 rules are very important:

- Hygiene: wash everything before and after wine making. Never leave residues where microorganisms will grow. Clean out all equipment.

- Do not let air in your fermentation. The air lock is used for that reason. During early fermentation when CO_2 is produced, that gas will keep O_2 away from the surface of the fermenting liquid; therefore open containers can be used. But once fermentation has slowed down, the container must remain closed to minimize contamination.

- Use sulfur dioxide for sterilizing. Add a compound called metabisulfite that forms the gas SO_2 in contact with water. The gas inhibits the growth of harmful microorganisms. It is used to sterilize juice, container and equipment. Luckily while yeast are also affected by the gas wine and beer yeast are less sensitive to SO_2 than harmful organisms.

- Because of the presence of wild yeast that give off flavors (taste cider that was left at room temperature to ferment to get an idea of what I mean) you need to inoculate with the proper yeast and make sure that they take over the fermentation container; if they duplicate so fast that they don't let other yeasts (or other organisms) the chance to grow, the fermentation will be successful. Therefore the fermentation should be carried out at optimum temperature (68^{o}F). Some people start it at even higher (70^{o}F) for a few days and later lower it to 50^oC for 1-2 months.

Remember also yeast must have nutrients and vitamins. While grape juice provides all necessary nutrients and vitamins other fruit juices such as strawberry, apple, pear etc. lack some of these nutrients and vitamins. Additions of such compounds may become necessary (Table 5.3).

Table 5.3. Major components of grape juice.

Category	Compound	% range (w/w)
Sugars	glucose	8 to 13
	fructose	7 to 12
Organic acids	tartaric acid	0.2 to 1.0
	malic acid	0.1 to 0.8
	citric acid	0.001 to 0.005
Tannins	cathecol, chlorogenic and caffeic acid	0.01 to 0.1
Nitrogenous compounds	amino acids and proteins	0.03 to 0.17
Minerals	phosphates	Traces
	sulfates	Traces
B vitamins	thiamine	Traces
	riboflavin	Traces
	pyridoxine	Traces
	nicotinic acid	Traces
Vitamin C (Ascorbic acid)		Traces
Volatile compounds		Traces
Color constituents		Traces

Answers to chapter questions:

p. 2: Could we survive if we destroyed most species on this earth except the ones that we find cute or useful? How many species of animals and plants do we need to survive? Open discussion.

p. 10: Imagine two parents both heterozygous for purple flowers.

a. What is the color of their flowers? Since purple is dominant their flowers will be purple

b. Draw Punnett squares for their offsprings.

	R	r
R	RR	Rr
r	Rr	rr

c. How many different genotypes exist in the offsprings? Genotypes represent the DNA of the individuals. In this case there are three different combinations of genes: RR, Rr and rr.

d. Give the percent of offspring for each different genotype. One out of four is RR (25%), two out of four are Rr (50%) and one out of four is rr (25%)

e. How many different phenotypes exist in the offspring? Since purple is dominant RR and Rr will be purple. Only rr will be different (white). The answer is two.

f. Give the percent of offspring for each different phenotype. Three out of four types will carry at least one dominant R gene and be purple (75%). The last type (recessive) will comprise 25% of the total population.

p.12: What are the differences and the similarities between the following alcohols?

CH_3-OH	1 carbon: methyl alcohol
CH_3-CH_2-OH	2 carbons: ethyl alcohol
CH_3 \\ CH-OH / CH_3	3 carbons: isopropyl alcohol

All of them carry the groups –OH and for that reason are all called alcohols. The number of carbons on each molecule is different however. Note that the human body can only break down the molecule carrying two carbons.

p. 20: Can you name other poisons? If you look around the house, cleaners are poisonous. So is gasoline, paint and paint thinners.

p. 21: Compare the LD_{50} data for methanol and dioxin. What do you notice? Dioxins are far more poisonous to rats than methanol.

p. 22: Could you make wine with a yeast that had a low tolerance to alcohol? WHY? You could but the wine could not be stored for extended periods of time since at least 9% alcohol is required for preservation of the wine.

EXERCISES.

1. What is a saprophyte?
2. Describe two compounds produced by fungi that are useful to humans.
3. What is the net result of mitosis in yeast? Animals?? Plants???
4. What is the net result of meiosis yeast? Animals?? Plants???
5. What is the role of actin in mitosis?
6. How many strands of DNA are there is a DNA duplex?
7. Describe three ways in which humans eliminate poisons. One of these three ways is to store the poisons in fatty tissues.
8. From a structural point of view what are the differences between methanol and ethanol?
9. For humans what is the major difference between methanol and ethanol? Explain.
10. What are the qualities that a brew master looks for in yeast strains?
11. What important rules must you follow while using a fermentation process?
12. Differentiate the types of yeast used to make lager and ale.
13. What does LD_{50} measure?
14. What does a low value of LD_{50} means?
15. How would you build a tolerance to cyanide? Define tolerance in your answer.
16. Your grape juice gives a reading of specific gravity of 1.060. How much sugar must you add to it (in g/100 ml) in order to obtain an alcohol level of 12%?
17. Describe how fungi fit in our ecosystem.
18. List the differences between <u>Saccharomyces</u> <u>carlsbergensis</u> and <u>Escherichia</u> <u>coli</u>.
19. What structural feature divides the Fungi Kingdom into Divisions?
20. What types of molecules make up a strand of DNA?
21. Explain what makes a gene recessive or dominant.
22. What is the biggest benefit for an organism that reproduces sexually?
23. What is (if any) the advantage of reproducing asexually for a population?
24. Why do wine makers prevent oxygen from reaching a fermenting juice?
25. What is the chemical difference between methyl, ethyl and isopropyl alcohol?

26. Which of the three alcohols listed in question 24 is (are) poisonous to humans?Explain.
27. What is an airlock and what is its purpose?
28. What is needed for a wine maker to produce a Chardonnay with 11% alcohol?
29. Two men go to a bar and both consume 10 beers in one hour. One is taken to the hospital with alcohol poisoning and the other is not. What are the possible explanations?.
30. Is flocculation desirable in wine making? Explain
31. What is an organoleptically pleasing compound?
32. Explain a situation where cyclosporine is beneficial. What might be the side effects?

33. Circle all that apply. Gametes are:

2n
haploid
eggs
grow outside the body
combine to form 4n embryo
result from mitosis
sperm
diploid
4n
hair follicles
result from meiosis
combine to form a 2n zygote

PROBLEMS

1. In pea plants, spherical seeds (S) are dominant to dented seeds (s). In a genetic cross of two plants that are heterozygous for the seed shape trait (Ss x Ss), what fraction of the offspring should have spherical seeds?

A. None
B. 1/4
C. 1/2
D. 3/4

2. In Mendel's "Experiment 1," true breeding (SS) pea plants with spherical seeds were crossed with true breeding (ss) plants with dented seeds. (Spherical seeds are the dominant characteristic). Mendel collected the seeds from this cross, grew F1-generation plants, let them self-pollinate to form a second generation, and analyzed the seeds of the resulting F2 generation. The results that he obtained, and that you would predict for this experiment are:

A. 1/2 the F1 and 3/4 of the F2 generation seeds were spherical.
B. 1/2 the F1 and 1/4 of the F2 generation seeds were dented.
C. All of the F1 and F2 generation seeds were spherical.
D. 3/4 of the F1 and 9/16 of the F2 generation seeds were spherical.
E. All the F1 and 3/4 of the F2 generation seeds were spherical.

3. A genetic cross between two F1-hybrid (Ss) pea plants for spherical seeds will yield what percent spherical-seeded plants in the F2 generation? (spherical is dominant over dented)

A. 100%
B. 75%
C. 50%
D. 25%
E. 0%

4. A genetic cross between two F1-hybrid pea plants having yellow seeds will yield what percent green-seeded plants in the F2 generation? Yellow seeds are dominant to green.

A. 0%
B. 25%
C. 50%
D. 75%
E. 100%

5. When true-breeding tall stem pea plants are crossed with true-breeding short stem pea plants, all of the _________ plants, and 3/4 of the ___________ plants had tall stems. Therefore, tall stems are dominant.

A. F1, F2.
B. G1, G2.
C. parental, F2.
D. F2, parental.
E. P1, P2

6. To identify the genotype of yellow-seeded pea plants as either homozygous dominant (YY) or heterozygous (Yy), you could do a test cross with plants of genotype ________.

A. y
B. Y
C. yy
D. YY
E. Yy

7. A test cross is used to determine if the genotype of a plant with the dominant phenotype is homozygous or heterozygous. If the unknown is homozygous, all of the offspring of the test cross have the ___________ phenotype. If the unknown is heterozygous, half of the offspring will have the ___________ phenotype.

A. dominant, incompletely dominant
B. recessive, dominant
C. dominant, epistatic
D. co-dominant, complimentary
E. dominant, recessive
The test cross was invented by Mendel to determine the genotype of plants displaying the dominant phenotype.

ANSWERS TO PROBLEMS

1. D. 3/4

One fourth of the offspring will be homozygous dominant (SS), one half will be heterozygous (Ss), and one fourth will be homozygous recessive (ss).

2. **E.** All the F1 and 3/4 of the F2 generation seeds were spherical.

All of the F1 plants were true hybrids with a phenotype of Ss. The recessive trait reappears in the F2 generation.

3. **B.** 75%

Only 25% of F2 plants will have the recessive phenotype.

4. **B.** 25%

Among the F2 plants of a Yy x Yy cross, 25% will be yy with the recessive, green-seeded phe

5. **A.** F1, F2.

The F1 plants are all Tt hybrids. The recessive trait (tt) reappears in the F2 generation in about 25% of the plants.

6. **C.** yy

A cross with the homozygous recessive (yy) is a test cross. If the parent of unknown genotype is heterozygous (Yy), half of the offspring will have the recessive trait. The unknown genotype could also be determined by a cross with a known heterozygote (Yy).

7. **E.** dominant, recessive

The test cross was invented by Mendel to determine the genotype of plants displaying the dominant phenotype.

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APPENDIX ONE: DNA Basics

What is DNA and where is it stored?

The nucleus is a membrane bound organelle that contains the genetic information in the form of chromatin, highly folded ribbon-like complexes of deoxyribonucleic acid (DNA) and a class of proteins called histones.

When a cell divides, chromatin fibers are very highly folded, and become visible in the light microscope as chromosomes. During interphase (between divisions), chromatin is more extended.

The DNA of chromatin is wrapped around a complex of histones making what can appear in the electron microscope as "beads on a string" or nucleosomes. Changes in folding between chromatin and the mitotic chromosomes is controlled by the packing of the nucleosome complexes.

DNA or **d**eoxyribo**n**ucleic **a**cid is a large molecule structured from chains of repeating units of the sugar deoxyribose and phosphate linked to four different bases abbreviated A, T, G, and C. We will later show how the simple structure of DNA contains the information for specifying the proteins that allow life. The process of mitosis is designed to insure that exact copies of the DNA in chromosomes are passed on to daughter cells**.**

APPENDIX TWO: The Cell Cycle

Stages of the cell cycle

The cell cycle is an ordered set of events, culminating in cell growth and division into two daughter cells. Non-dividing cells not considered to be in the cell cycle. The stages, pictured to the left, are G1-S-G2-M. The G1 stage stands for "GAP 1". The S stage stands for "Synthesis". This is the stage when DNA replication occurs. The G2 stage stands for "GAP 2". The M stage stands for "mitosis", and is when nuclear (chromosomes separate) and cytoplasmic (cytokinesis) division occur. Mitosis is further divided into 4 phases, which you will read about on the next page.

Regulation of the cell cycle

How cell division (and thus tissue growth) is controlled is very complex. The following terms are some of the features that are important in regulation, and places where errors can lead to cancer. Cancer is a disease where regulation of the cell cycle goes awry and normal cell growth and behavior is lost.
Cdk (cyclin dependent kinase, adds phosphate to a protein), along with cyclins, are major control switches for the cell cycle, causing the cell to move from G1 to S or G2 to M.
MPF (Maturation Promoting Factor) includes the CdK and cyclins that triggers progression through the cell cycle.
p53 is a protein that functions to block the cell cycle if the DNA is damaged. If the damage is severe this protein can cause apoptosis (cell death).

1. p53 levels are increased in damaged cells. This allows time to repair DNA by blocking the cell cycle.
2. A p53 mutation is the most frequent mutation leading to cancer. An extreme case of this is Li Fraumeni syndrome, where a genetic a defect in p53 leads to a high frequency of cancer in affected individuals.

p27 is a protein that binds to cyclin and CdK blocking entry into S phase. Recent research (Nat. Med.3, 152 (97)) suggests that breast cancer prognosis is determined by p27 levels. Reduced levels of p27 predict a poor outcome for breast cancer patients.

APPENDIX THREE: Mitosis

What is (and is not) mitosis?
Mitosis is nuclear division plus cytokinesis, and produces two identical daughter cells during prophase, prometaphase, metaphase, anaphase, and telophase. Interphase is often included in discussions of mitosis, but interphase is technically not part of mitosis, but rather encompasses stages G1, S, and G2 of the cell cycle.

Interphase & mitosis

Interphase
The cell is engaged in metabolic activity and prepares for mitosis (the next four phases that lead up to and include nuclear division). Chromosomes are not clearly discerned in the nucleus, although a dark spot called the nucleolus may be visible. The cell may contain a pair of centrioles (or microtubule organizing centers in plants) both of which are organizational sites for microtubules.

Prophase
Chromatin in the nucleus begins to condense and becomes visible in the light microscope as chromosomes. The nucleolus disappears. Centrioles begin moving to opposite ends of the cell and fibers extend from the centromeres. Some fibers cross the cell to form the mitotic spindle.

Prometaphase

The nuclear membrane dissolves, marking the beginning of prometaphase. Proteins attach to the centromeres creating the kinetochores. Microtubules attach at the kinetochores and the chromosomes begin moving.

Metaphase

Spindle fibers align the chromosomes along the middle of the cell nucleus. This line is referred to as the metaphase plate. This organization helps to ensure that in the next phase, when the chromosomes are separated, each new nucleus will receive one copy of each chromosome.

Anaphase

The paired chromosomes separate at the kinetochores and move to opposite sides of the cell. Motion results from a combination of kinetochore movement along the spindle microtubules and through the physical interaction of polar microtubules.

Telophase

Chromatids arrive at opposite poles of cell, and new membranes form around the daughter nuclei. The chromosomes disperse and are no longer visible under the light microscope. The spindle fibers disperse, and cytokinesis or the partitioning of the cell may also begin during this stage.

Cytokinesis

In animal cells, cytokinesis results when a fiber ring composed of a protein called actin around the center of the cell contracts pinching the cell into two daughter cells, each with one nucleus. In plant cells, the rigid wall requires that a cell plate be synthesized between the two daughter cells.

APPENDIX FOUR: Meiosis 1 & 2

What is Meiosis 1?
In Meiosis 1, chromosomes in a diploid cell resegregate, producing four haploid daughter cells. It is this step in Meiosis that generates genetic diversity.

The phases of Meiosis 1 & 2

Prophase I

DNA replication precedes the start of meiosis I. During prophase I, homologous chromosomes pair and form synapses, a step unique to meiosis. The paired chromosomes are called bivalents, and the formation of chiasmata caused by genetic recombination becomes apparent. Chromosomal condensation allows these to be viewed in the microscope. Note that the bivalent has two chromosomes and four chromatids, with one chromosome coming from each parent.

Prometaphase I

The nuclear membrane disappears. One kinetochore forms per chromosome rather than one per chromatid, and the chromosomes attached to spindle fibers begin to move.

Metaphase I

Bivalents, each composed of two chromosomes (four chromatids) align at the metaphase plate. The orientation is random, with either parental homologue on a side. This means that there is a 50-50 chance for the daughter cells to get either the mother's or father's homologue for each chromosome.

Anaphase I

Chiasmata separate. Chromosomes, each with two chromatids, move to separate poles. IN A WAY YOU CAN SAY THAT Each of the daughter cells is now haploid (23 chromosomes), but each chromosome has two chromatids.

Telophase I

Nuclear envelopes may reform, or the cell may quickly start meiosis 2.

Cytokinesis

Analogous to mitosis where two complete daughter cells form.

Meiosis 2

Meiosis 2 is similar to mitosis. However, there is no "S" phase. The chromatids of each chromosome are no longer identical because of recombination. Meiosis II separates the chromatids producing two daughter cells each with 23 chromosomes (haploid), and each chromosome has only one chromatid.

APPENDIX FIVE: Comparing Meiosis and Mitosis

* Chromosome behavior
1. Mitosis: Homologous chromosomes independent
2. Meiosis: Homologous chromosomes pair forming bivalents until anaphase I

* Chromosome number- reduction in meiosis

1. mitosis- identical daughter cells
2. meiosis- daughter cells haploid

* Genetic identity of progeny:
1. Mitosis: identical daughter cells
2. Meiosis: daughter cells have new assortment of parental chromosomes
3. Meiosis: chromatids not identical, crossing over

APPENDIX SIX: Meiotic errors

* Nondisjunction- homologues don't separate in meiosis 1
1. results in aneuploidy
2. usually embryo lethal
3. Trisomy 21, exception leading to Downs syndrome
4. Sex chromosomes
1. Turner syndrome: monosomy X
2. Klinefelter syndroms: XXY

* Translocation and deletion: transfer of a piece of one chromosome to another or loss of fragment of a chromosome.

APPENDIX SEVEN: Terms to know in Mendelian Genetics

alleles
The different forms of a gene. Y and y are different alleles of the gene that determines seed color. Alleles occupy the same locus, or position, on chromosomes.
autosomal
A locus on any chromosome but a sex chromosome. Not sex-linked.
co-dominant alleles
Two different alleles at a locus are responsible for different phenotypes, and both alleles affect the phenotype of the heterozygote. For example, consider the situation where there are three alleles A,B, and O that determine human blood type. Three possible genotypes are AA, BB, OO that correspond to the phenotypes of blood type A, B, and O respectively; Two other genotypes are AO and BO that correspond to blood types A and B, respectively

because the O allele is recessive, The remaining genotype is AB, corresponding to blood type AB. Both the A and B alleles contribute to the phenotype of the heterozygote. Thus the alleles A and B are said to be co-dominant.

complete linkage.

Complete linkage describes the inheritance patterns for 2 genes on the same chromosome when the observed frequency for crossover between the loci is zero.

dioecious

Organisms produce only one type of gamete; i.e. humans

dominant trait.

A trait expressed preferentially over another trait.

Drosophila melanogaster

The fruit fly, a favorite organism for genetic analysis.

epistasis.

One gene masks the expression of a different gene for a different trait.

F1 generation

Offspring of a cross between true breeding plants, homozygous for the trait of interest

F2 generation

Offspring of a cross involving the F1 generation.

genotype

The genetic constitution of an organism with respect to a trait. For a single trait on an autosome, an individual can be homozygous for the dominant trait, heterozygous, or homozygous for the recessive trait. Yellow seeds are dominant, but yellow seeded plants could have a genotype of either YY or Yy.

hemizygous

If there is only one copy of a gene for a particular trait In a diploid organism, the organism is hemizygous for the trait, and will display a recessive phenotype. X-linked genes in fly or human males are hemizygous.

heterozygous

Differing alleles for a trait in an individual, such as Yy.

homologous chromosomes

The pair of chromosomes in a diploid individual that have the same overall genetic content. One member of each homologous pair of chromosomes in inherited from each parent.

homozygous

Both alleles for a trait are the same in an individual. They can be homozygous dominant (YY), or homozygous recessive (yy).

hybrid

heterozygous; usually referring to the offspring of two true-breeding (homozygous) individuals differing in the traits of interest.

incomplete dominance

Intermediate phenotype in F1, parental phenotypes reappear in F2. The flowers of the snapdragon plant can be red, pink, or white. Color is determined at a single locus. The genotype RR results in red flowers and rr results in white flowers. The heterozygote genotype of Rr results in pink flowers. When the heterozygote has a different, intermediate phenotype compared to the homozygous dominant or homozygous recessive individuals, this is said to be incomplete dominance.

lethal alleles.
Mutated genes that are capable of causing death.
linkage.
genes that are inherited together on the same chromosome. Three inheritance patterns are possible: non-linkage, Partial linkage, and complete linkage.
mendel's law of independent assortment of alleles.
Alleles of different genes are assorted independently of one another during the formation of gametes.
mendel's law of segregation
Alleles segregate from one another during the formation of gametes.
monoecious
Organisms produce both male and female gametes; i.e. garden pea.
monohybrid cross.
Cross involving parents differing in only one trait.
mutation
Change in the DNA sequence of a gene to some new, heritable form. Generally, but now always a recessive allele.
non-linkage.
Non-linkage describes the inheritance patterns for 2 genes on the same chromosome, when the expected frequency for crossover between the loci is at least one. The observed inheritance patters for non-linked genes on the same chromosome is the same as for 2 genes on different chromosomes.
partial linkage.
Partial linkage describes one of the inheritance patterns for 2 genes on the same chromosome, when the expected frequency for crossover between the loci is greater than zero but less than one. From partial linkage analysis we can learn about the order and spacing of genes on the same chromosome.
phenotype
The physical appearance of an organism with respect to a trait, i.e. yellow (Y) or green (y) seeds in garden peas. The dominant trait is normally represented with a capital letter, and the recessive trait with the same lower case letter.
pleiotropic.
A single gene determines more than one phenotype for an organism.
recessive trait.
The opposite of dominant. A trait that is preferentially masked.
reciprocal cross
Using male and female gametes for two different traits, alternating the source of gametes.
sex chromosomes
Sex determination is based on sex chromosomes
sex-linked.
A gene coded on a sex chromosome, such as the X-chromosome linked genes of flies and man.
test cross
Generally a cross involving a homozygous recessive individual. When a single trait is being studies, a test cross is a cross between an individual with the dominant phenotype but of unknown genotype (homozygous or heterozygous) with a homozygous recessive

individual. If the unknown is heterozygous, then approximately 50% of the offspring should display the recessive phenotype.

true-breeding

Homozygous for the true-breeding trait.

wild-type allele

The non-mutant form of a gene, encoding the normal genetic function. Generally, but not always a dominant allele.

APPENDIX 8. Reprint of: Cyclosporin to the rescue.

LESSON 6 WINE MAKING.
3 Figures
2 Tables
1 appendix

A. INTRODUCTION.

1. Definitions.

Wine is the product of the fermentation of sound, ripe grapes without additions or subtractions except such as may occur in the usual cellar treatment (chemical reactions during fermentation and aging). This is the strict definition of wine as expressed in the laws of several countries in the world. This has interesting ramifications as we shall see shortly.

a. The word wine thus implies the exclusive use of grapes. While any fruit juice that contains sugar can be fermented these products cannot be called wine by law. In fact other names are used to describe the other alcoholic products: fermented barley is beer or ale of course, fermented apple juice is cider, fermented pear juice is perry, and fermented honey is mead. Cherries, rose hips, strawberries and dandelions are also fermented and the products are called wine but they are not technically even though they do not have their own designations.

b. While the definition states that nothing can be added or taken out it is often the case that there is not enough sugar in the juice to produce more than 9% alcohol (the minimum needed for preservation of the product). This is especially true in cold climates like New York where the growing season is often too short for that amount of sugar to form in the fruit (about 24 ^{0}brix is needed to produce 10-11 % alcohol).

c. This definition also means that the wine cannot be filtered after fermentation as is often the case in American wines because the filtration prevents the chemical reactions necessary for proper aging. A filtered wine will taste, 100 years from now the same as when it was bottled as long as it is kept away from air (oxidation). On the other hand an unfiltered wine could improve tremendously upon aging for 100 years. Conversely it could also deteriorate to the point of being undrinkable.

Those you might say are technicalities but they have been the basis for famous wars in the past, the most recent being the battle between Walter Taylor and the Taylor Wine Company. That company was started by Walter Taylor's grandfather (Walter Taylor) in the 1880s in Hammondsport, NY. During the Prohibition the company stopped making wine and sold grape juice only in order to survive those hard years. In the early 1970s the company went public and was run by a board of directors. One of the descendants of the founder was Walter Taylor, one of the directors. He became dissatisfied with the drop in the quality of the company's products and started a campaign for improvement. His major concern was the fact that in order to sell a reproducible product the company was heavily modifying the must every year and using filtration. While this gives you a consistent product year after year it also reduces the quality and prevents the production, occasionally, of a truly great wine. Taylor publicly criticized the fact that for example the company used large proportions of California juice to blend in what they still called NY wine. He also complained about the addition of sugar to the must and the addition or removal of acid (again this is done in order to produce a consistent wine). He eventually quit the company and established himself on the original vineyard where his grandfather had started. He produced a wine that was supposedly unadulterated and used this fact to great advantage in his advertisement. However, he still needed, at time, to add sugar or to dilute the acid with water. He also started importing grape juice from Long Island (instead of California) to blend with Finger Lakes juices in order to produce a more consistent wine and still be able to call it NY. While he markets his wines as " produced without guilt" he still needs to blend and adulterate his juice but to a lesser extent than the parent company.

2. Types of wines.

There are several types of wines available on the market today and here are some of them.

a. Red wines: Often said to be the best wines in the world, they are very complex in bouquet and flavor, especially those produced in the regions of Bordeaux and Bourgogne in France. Their color comes from red pigments contained in the skin of the grape. Those pigments are extracted by pressing the grapes or sometimes by fermenting the juice with skins. A red grape can actually produce a white wine if pressing is very light since the pigments are contained in the skin. Other components of the skin are extracted as well that confer to the wine its body, firmness and astringency (caused by tannins contained in red but not as much in white skins). A red wine can be dry or sweet depending on how much sugar was originally contained in the fruit or how much sugar was added to the must prior to fermentation and how much sugar is left in the wine after fermentation has stopped. Aging can greatly improve the quality of red wines presumably because of the chemistry of a variety of skin-related compounds during the aging process. The chemistry of aging is still only poorly understood.

b. White wines are made from red or white grapes. This fact creates a vast range of flavors. Bordeaux type white wines can be very dry with one basic flavor while German Rhine wines are very light, sweet and fruity. The juice is separated from the skins very rapidly and fermented without them. This eliminates a wide range of skin compounds from the wines and usually gives a more fragile product that lacks the astringency of red wines. It is usually not aged before consumption because of its fragile nature and consumed soon after bottling.

c. Rose wines are made by two possible routes:

1. by crushing red grapes and fermenting first with the skins but removing the skins shortly after fermentation has started. This will prevent fermentation from removing very much of the pigments and other compounds from the skin. You can see here that the process is intermediary between a red wine fermentation (where skins

remain in the fermentation vat for a few days to a couple of weeks) and white wine fermentation using juice from a red grape where skins are not present at all during fermentation.

2. Another possibility is to crush deep pigmented red grapes and press to remove the skins before fermentation. The pressing is done in a way that will provide the desired color in the must.

The Rose produced in "1" is closer to a red wine in character, astringency and stability because more of the skin's chemical characters are extracted. Interestingly the region of Bourgogne was originally producing mostly rose wines. The regions switched to the production of red wines only recently (2-300 years ago).

d. Natural sweet wines.

These wines are produced with juices that contain more than 24 ^{o}brix of sugar. This is possible if the grapes are overripened or if more sugar is added to the juice. Several vineyards in Germany and France use a mold (Botrytis cinerea) to extract water from the grape as it continues to ripen. The result is a juice that can contain as much as 45^{o} brix of sugar. These German white wines are known as Trockenbeerenauslese. The most famous French sweet white wine is Chateau d'Yquem. These wines are extremely expensive to produce because so little volume of juice is recovered from the pressing of the grapes: a normal harvest of non-botrytic grapes would produce about 300 gallons of juice per ton of grapes. However botrytic grapes yield only a fraction of that volume (sometimes less than 80 gallons).

Finger Lakes wineries produce such wines only occasionally because the climactic conditions are not always favorable. When they do they use a hybrid grape called Ravat (also known as Vignoles). In general the production of a sweet wine is difficult since great care must be taken to prevent other organisms from using the residual sugar to grow and spoil the wine.

It is also possible to stop the fermentation of a wine before the yeast die out in order to retain some of the sweetness of the juice. Of course the alcohol level will be low (less than 12%). These wines are often fortified with added alcohol (in the form of brandy) to produce a stable product (see fortified wines).

e. Fortified wines.

There are two major types:

- Sherry (white wine with an brownish, oxidized color and a baked taste): produced originally in Spain from Palomino and Pedro Ximenez grapes it is made by letting the grapes dry before crushing (this increase the sugar content to 26-28 ^{0}brix). The product is then left in large casks outside to pick up from the environment a special local yeast (flor) that will cover the surface of the vat and for over a year impart to the wine the characteristic flavor of a sherry. Alcohol levels reach about 15%. During blending sugar and alcohol are added to obtain the characteristic final product. In summary sherry is fortified and sweetened (if needed) after fermentation.

- Port is made in Portugal and is a sweet, fortified red wine. The fermentation of crushed grapes is stopped with an alcohol level of about 8% leaving a lot of sugar in the must. This must is then blended with brandy to yield a wine of about 20% alcohol and 8% residual sugar. The wine is then aged in half-filled casks like sherry. In summary port is made by adding brandy to unfinished wine so that the sugar content of the port actually came from the original grapes.

B. THE INITIAL STEPS IN WINEMAKING

1. Introduction.

Wine is very easy to make. It is the result of a very simple process and requires complicated operations only if you are out to make an outstanding wine. Wild yeast would ferment the juice naturally but might impart off-flavors to the wine (there are as many as 500 different varieties of wild yeast out there). For this reason it is recommended to sterilize the juice by adding SO_2 to it in the form of sodium metabisulfite to kill microorganisms. After sterilization it is then possible to inoculate with a pure strain of cultured yeasts which is more resistant to the metabisulfite than the wild yeast originally present in the must.

In this chapter we are going to look at the basic steps and precautions necessary to make wine. This is by no means inclusive and you will find that experimentation will help you develop your own wine making techniques

2. The Grapes.

The grapes are harvested when they have reached a balance of acidity and sugar content. Figure 6.1 shows the increase in sugar and the decrease in acids in the last eight weeks of ripening in a Cabernet-Sauvignon grape grown in the Bordeaux region. Sugars are accumulated in the fruit to provide energy to the seeds when they are going to start growing. Acids decrease because of two reasons that are connected to sugar content: they are going to be used up by cells as a source of energy but they are also going to be converted to sugars. There are other changes that occurring during grape maturation but the first two are more intimately connected to the fermentation process.

You can see the rapid changes taking place in the levels of these two measures. Consequently it is easy to see that a late a harvest means that the grape will contain a lot of sugar and too little acid for a nice balance of taste. It may come as a surprise that acid is a contributor to taste but it is true. Think of yogurt or even Pepsi as foods that rely heavily on acid for taste. If the harvest if done too early the acid levels are too high (with very low sugar) and again the taste will not be well balanced. In the Finger Lakes region, grapes are often harvested too early for fear of damaging frosts. The juice obtained from these grapes is rarely above 22^0 brix and the acid level is high (above 1). This means that sugar must be added to the juice to produce alcohol levels above 10%. The grape

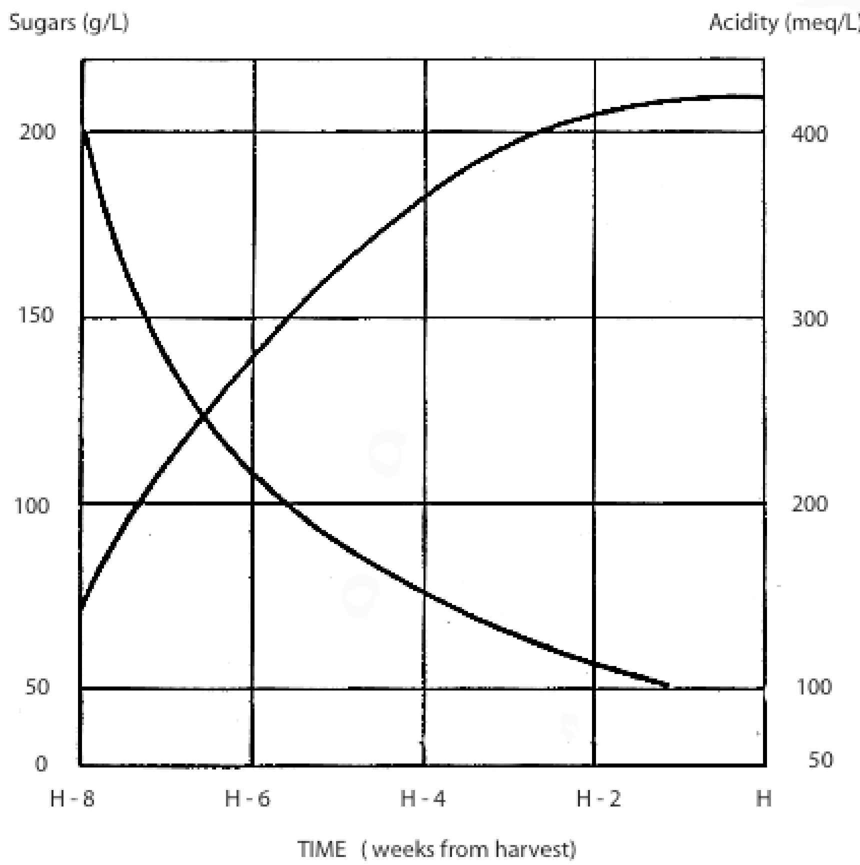

Figure 6.1. Sugar and acid content of grapes during the last eight weeks of ripening of Cabernet-Sauvignon vones in Bordeaux climate. The time scale is relative and expressed in weeks from harvest (H).

growers monitor the sugar content of grapes every day and listen to the weather forecast in order to harvest as late as possible. An article attached to this chapter by Gary Blonston will give you an idea of how growing conditions are related to wine quality and flavor via the biochemistry of grapes.

An instrument used to measure the sugar content is called a refractometer. It measures by how much light is refracted when it hits a solution. The greater the refraction index the greater the concentration of solids or solutes in the solution. Let's see how the refractometer works. You are familiar with the fact that if you look in the water at the end of a pier and see a fish the fish is not really where you think it is. It is because the light was bent (or refracted) when moving from the water to the air. The refraction index measures the angle of the bent light. The index increases with the concentration of solute in the water. Thus we can measure the % solids in the grape juice by using a refractometer. Of course we will make the assumption here that the % sugar is the same as the % solids. It is not true in fact because there are other solids in the juice such as pigments, proteins etc. that will also contribute to the increase in refraction index. However since the sugars make up the vast majority of the solids present in the grape juice we use this value as a good approximation of sugar content.

Another measurement of sugar content can also be made using the hydrometer. The principle used here is that as more and more solids are dissolved in water, the density of the solution increases. The hydrometer therefore floats higher and higher on that solution as more and more sugar is added. A calibration (in ^{0} brix or units of specific gravity) is used on the side of the hydrometer to give a value to the density of that solution.

Acidity can be determined also by a method called titration. But before we talk about titration we need to understand what an acid is in chemical terms. And before that we need to talk about elements and compounds. All compounds that we deal with every day are made of elements. You see those elements on a periodic chart. You may recognize some of them already: Iron, magnesium, aluminum etc. Each line on the chart shows a layer into which negative electrons will come gravitate around the nucleus to balance the positive charge of said nucleus. Those electrons are arranged in layers (or shells) and each layer can take only a set number of electrons. When a shell is full the electrons start occupying another layer. Look at the way those elements are positioned relative to the last column on the chart. All the elements of group 1 for example have 1 electron in the valence shell. They all have other shells that are full of electrons but they have all opened a brand new shell (called the valence shell) and put 1 electron there. Interestingly the last column is called the noble gases or rare earth elements. These elements are the only ones in the periodic table to have a filled valence shell. Therefore the positioning in relation to the rare earth elements (group 8) indicates how many electrons are surrounding the nucleus of these elements. Group one elements have one electron on their valence shell, group 2 elements have 2 electron on their valence shell etc… What is fascinating about chemistry is that all the elements are going to attempt to get a full layer of electrons by either loosing or gaining electrons in chemical reactions. Looking at the chart you can see that chlorine can either loose 7 electrons or gain one in order to acquire the electronic configuration of a noble gas. Which do you think is the easier way???

Gaining one. On the other hand sodium can either gain 7 electrons or loose one in order to acquire the electronic configuration of a noble gas. Loosing one is a lot easier. Usually elements that would gain more easily than loose electrons will pair up with elements that would more easily loose than gain electrons. So sodium will pair up with chlorine in a one to one ratio so that the number of electrons lost by sodium is equal to the number of electrons gained by chlorine. If chlorine reacts with magnesium, two chlorine atoms will pair up with one magnesium atom because the two electrons lost by magnesium will go to two chlorine atoms. The compounds made in such a way are called ionic because electrons are actually gained or lost by the atoms forming them. These atoms are then called ions. We also call these compounds “salts” or **ionic compounds**. For the elements in the middle like oxygen and nitrogen to mix together neither will be able to pull hard enough on the other's electrons to actually take them away; thus they will end up sharing these electrons; such is the case for carbon dioxide for example. The compounds made here are called **covalent compounds**.

But let's go back to the ionic compounds, those made from elements on the extreme ends of the periodic chart. When you put these compounds in water they dissolve and then dissociate into the elements that make them. But then those elements do not give up or re-acquire the electrons that were lost or taken. They remain in the ionic state: when you put salt (NaCl) in your soup, the ions present after the salt dissolve are Na^+ and Cl^-. You do not see Na metal and Cl gas.

When one of the two elements making up an ionic compound is hydrogen, the compound can also dissociate in water and put H^+ (hydrogen ions) in solution. So hydrochloric acid (HCl) in water becomes H^+ and Cl^-. The compounds that dissociate to H^+ in water are called **acids**. Compounds that can dissociate into an ion called hydroxide (OH^-) are called **bases** (NaOH for example). When H^+ encounters OH^- the two combine into a new compound called water (H_2O). That is why we say that mixing an acid and a base neutralizes the acid and gives a salt and water. Now it is interesting that all acids have a sour taste and all (or most) bases have a bitter taste. That in fact was the first way for humans to identify acids. And we have already seen that acids are an important additive to foods because they enhance their taste.

Let's go back to our titration now. Assume that you want to mix an acid and a base together. The acid has acid characteristics before it is mixed and the base, basic characteristics. But when they have reacted completely to one another, the water left will have neither characteristics and is said to be neutral. That is why the reaction is called a neutralization reaction. The acid neutralizes the basic characteristics of the acid and vice versa. Let's see if we can write this in the form of an equation.

$$HCl + NaOH \text{ ---> } H_20 + NaCl. \qquad (1)$$

Or more appropriately:

$$H^+ + Cl^- + Na^+ + OH^- \text{ ---> } H_20 + Na^+ + Cl^-. \quad (2)$$

Since the ions Cl^- and Na^+ are not changed in the reaction we can write a final version of the equation (net ionic equation):

$$H^+ \ + \ OH^- \ \text{--->} \ H_20. \qquad (3)$$

You can see that in water, one molecule of HCl will dissociate to give one ion of H^+ and one molecule of NaOH will form one ion of OH^-. The combination of these two ions will give one molecule of water. The proportions of the two must be exact. If 99 molecules of HCl are mixed with 100 molecules of NaOH, the resulting solution will have what kind of characteristics? Neutral, acid or basic? Interestingly any acid mixed with any base will always give the same net ionic equation that is the formation of water. Can you write the equation for the mixing of KOH and HI? (use the equation above as an analogy):

(4)

This is very important because if we have a solution containing an unknown number of molecules of acid (such as a grape juice) we can maybe neutralize it with a solution of base, measure how many molecules of base we needed for the neutralization and then know how many molecules of acid we had. But first we need to know exactly when the acid has been neutralized. We do that with molecules called indicators which change color depending on the acid content of the solution. For example a molecule of phenolphthalein is red in basic solutions and colorless in acid solutions. If we slowly add our base solution to the acid in the presence of this indicator the solution will barely starting to turn pink when the same amount of base and acid are present. The solution is then neutral and the titration has reached equivalence, the point where there is neither acid nor base left in solution. We can then calculate the amount of base used and therefore know the amount of acid present in the grape juice.

How do we calculate those amounts of acid present? We first need to know that we do not look at individual molecules of acid or base because they are too small to weigh out. So we look at bundles of those like we look at a dozen eggs for example. That bundle is called a mole and that is a pretty big number (6.022×10^{23} oranges or pencils or atoms or molecules). It is very useful because it corresponds to the mass of elements, in grams, shown in the periodic chart. Let's look at sodium chloride for example. The chart says that a mole of sodium has a mass of 22.9 grams and the mass of one mole of chlorine is 35.4. So by adding the two I find that the mass of one mole of sodium chloride is 58.3 grams. So if I weigh out 58.3 grams (that is about two ounces or 1/8 of a pound) of sodium chloride I have one mole of the salt. You can see from this that the mass of a mole of NaCl is going to be different than the mass of 1 mole of HCl or $MgCl_2$. It is because the mole is a number of copies of the same molecule not the mass of their individual atoms. That is the same thing with a dozen eggs and a dozen baseball bats. In both cases you have one dozen but the former weighs less that the latter.

So now let's say that I needed 40 grams of base (NaOH) to neutralize the acid contained in my grape juice. How many grams of acid did I have??? Remember that these conversions must always be made in moles. So what is 40 grams of NaOH??? 1 mole. So how many moles of acid could one mole of base neutralize??? One mole. Thus we had one mole of acid or 36.45 grams of HCl in the juice that we titrated. Therefore I can write that 40 grams of NaOH are needed to neutralize 36.5 grams of HCl. How many grams of HI could be neutralized by 40 grams of NaOH?

Now one last thing about titrations. That mole of acid was contained in say a liter of grape juice (about one quart). If that same mole of acid had been placed in half a liter of juice what would that mean??? It would mean that the second solution is more concentrated than the first by a factor of two. That is the concept of concentration (the more solute is present in solution the more concentrated the solution is). The same is true for KOOLAID: to make it more concentrated you must add more powder to the same quart of water. Conversely ten spoonfuls of KOOLAID in a quart of water is more concentrated than to spoonfuls of KOOLAID in ½ quart of water. The second solution tastes more sweet and looks darker but they both contain the same amount of KOOLAID. What would happen if you doubled the powder added to a quart and then added another quart of water. Would the KOOLAID taste more concentrated then? The same is true in chemistry. The unit for concentration here is expressed as molarity (number of moles of solute per liter of solution) and is read as M ; in this example a 0.1 M solution contains 1/10 of a mole per liter of solution. So let's go back to our titration. Assume that you were titrating a solution of HCl (100 ml or 1/10 of a liter) with 100 mL of a one molar (one mole per liter) solution of base. How many grams of acid were present in 100 mL the HCl solution??? Again we are going to figure out the number of moles of base involved here.

You needed 100 ml of 1 M base. How many moles is that?

$$100 \text{ mL} \times \frac{1 \text{ liter}}{1000 \text{ mL}} \times \frac{1 \text{ mole base}}{\text{Liter}} = 0.1 \text{ mole of base}$$

You know that one mole of base is required to titrate one mole of acid. Therefore the number of moles of acid is:

0.1 mole of base = 0.1 mole of acid (HCl)

Finally the number of grams of HCl is:

$$100 \text{ mL} \times \frac{1 \text{ liter}}{1000 \text{ mL}} \times \frac{0.1 \text{ mole of HCl}}{\text{Liter}} \times \frac{36.5 \text{ grams of HCl}}{1 \text{ mole of HCl}} = 3.65 \text{ grams of HCl}$$

Let's try an exercise. What is the concentration of HCl if 100 ml of the acid is titrated with 25 ml of 0.25 M NaOH ?

The concentration of acid is usually what we need to know rather than the number of grams of acid present because the concentration of acid can be changed in a grape juice more easily than the total amount of acid present (just think of what evaporation of water could do here). The concentration of acid can be lowered by adding water; but the total amount of acid can be changed only by adding a base. Both are done in wine making but they adulterate the grape juice. The former also dilutes the sugar so that more sugar needs to be added if water is added.

After this rather long parenthesis let's go back to our grape harvest. If you crush grapes, you will get a mixture of :
5% stems
20% skins
75% pulp
5% seeds.

These proportions are approximations (that is why the percentages do not add up to 100%) will of course vary from grape to grape. The pulp is where the juice is going to come from for the most part. It is called the pulp however because if you have ever eaten a wine grape you know that behind the skin is not just juice but rather a pulp from which juice is extracted. Flavor and astringency will come from the skins but also from the stems.

3. Choice of grapes.
In NY state the variety of grapes available for wine making is not as great as in Europe or California but nonetheless considerable. This state grows very few vinifera grapes except, most notably, Chardonnay and Riesling;. Remember that vinifera vines are fragile and that on average 20-30% of the vines die every year; this brings a high cost to the maintenance of the vineyard. The majority of grapes grown in NY are hybrids that can witstand low winter temperatures and the dreaded Phylloxera louse. The vinifera hybrids are developing into varieties (Leon MIllot, Baco, Chancellor, Chelois, Marechal Foch, DeChaunac are reds while Cayuga, Seyval, Aurora and Ravat blanc are whites) that are devoid of the anthranilate flavor and have enough lightness and complexity to make wines that rival their vinifera counterparts. Pure American grapes (Vitis labrusca or Vitis riparia) can also be used for wine making and because of their abundance and ruggedness. These labrusca grapes, such as Concord and Catawba, have the distinctive flavor of methyl anthranilate, the strong, "foxy" taste that carries in the wine. Some American hybrids such as Ives, Diamond, Dutchess and Delaware do not have the foxy flavor of Concord but are still too strong tasting to give a subtle wine. Some like it however.

Also other fruit juices can be used such as peaches, pears, apples, strawberries, raspberries and dandelions (with oranges). Since these juices lack the vitamins and the sugar of grape juice their use in wine making never became widespread; these deficiencies are easily remedied nowadays and we have seen a resurgence of fruit wines in home wine making.

4. Pressing.
Here the wine makers have a decision to make. The pressing may or may not be done depending on the type of grape they have and on the type of wine they want to make. As we have discussed before, the skin of red grapes contains substances that will give the wine richness of flavor and taste but also confer to it more stability and astringency.

Fermenting the juice with the skins (no pressing is involved) will bring these substances into the must and give the wine a richer, fuller body as well as deeper color. That can be done with a red or a white grape actually but mostly with reds. Fermentation with skins can last from 2 days to 2 weeks but usually pressing is done when residual sugar reach 2%.

If a light pressing is done before fermentation on a red grape, a rose or white wine will be obtained. A harder pressing (where more pressure is applied) before fermentation will yield a light red (called claret).

Finally some farmers in the past would ferment with or without the skins, press the juice and add water to the skins. This would then be boiled to extract even more color and tannins. The result was called a " blood wine" with extremely sharp, astringent flavor and, as the name indicates, very deep dark red color. This is rarely done nowadays.

There are also a lot of vineyards trying all kinds of new ways to make wines. For example Bully Hill sold a Baco red for a long time while other wineries tried to market a Baco rose. An ice rose has been introduced on the market recently where the grape juice is fermented at very low temperatures. The slow fermentation gives the final product a very mild flavor because at low temperatures the yeast produces less flavor compounds.

Yet another fermentation technique involves leaving the uncrushed or slightly crushed grapes in a container to ferment. The mass of the grapes will crush some of the bottom fruit and fermentation will start. The juice that escapes from the grapes is called the free run. It is collected separately from the pomace which is subsequently pressed for a darker stronger flavored product. The free run is produced in this region by a company called Heron Hill, on Keuka lake nearby. They make for example two Chardonnay: a free run Chardonnay and the pressed Chardonnay. The free run has a lighter flavor and is considered superior. While this technique is uncommon in the US it is the technique of choice in France. The pressed wine is considered inferior there and is often sold to distilleries to make brandy.

Finally a last interesting variation on the theme is called **carbonic maceration**. Here again unpressed grapes are placed into a container and the O_2 is replaced by N_2 or CO_2. The fermentation that occurs is not due to Saccharomyces because it cannot have access to the juice inside the grape. Instead, an internal fermentation occurs that turns a little sugar into ethanol (about 2% alcohol is formed). At the same time acid levels drop 25 to 50% and malic acid levels are lowered even more significantly. Color intensity and tannins are reduced 30%. Some aromatic compounds also appear that will give different flavors to the wine.

In the mean time some fruit are crushed by the weight of grapes on top and a free run is formed with the characteristics described above. Yeast will start fermenting that free run present on the surface of the skin. Before mechanical pressing occurs, the free run is collected and can account for as much as 75% of the total volume. During that maceration, 20% of the grapes will be undergoing normal fermentation (because they were crushed early in the process and therefore could not undergo the internal maceration), 20% will undergo only the maceration (because they remain intact all the way to pressing) and 60% undergo both processes depending on when they get crushed in the tank by the weight of other grapes. The process gives a lighter tasting wine with less acid and characteristic and complex secondary aroma. “Maceration carbonique” is best used in areas that normally make harsh tasting, acid wines. Usually these wines do not age well because they have not extracted from the skin all the compounds that would normally favor aging processes.

Let us go back to our juice. We obtained the juice for our fermentation from the producer, already crushed and pressed. If fermentation with skins is desired skins can be obtained from the producer (they always save them for this purpose). You are also given two pieces of information with this juice: acidity and sugar content. Both are crucial to help you make a NY state wine because of the variability in growing season length and consequently sugar and acid content. That information will enable you to adulterate your juice in order to produce a more balanced wine.

a. Sugar content is given in ^{o}Brix. Division by two will approximate for you the % alcohol possible: 20 ^{o}Brix means that roughly 10% alcohol will be produced. If sugar is to be added corn sugar, table sugar (cane sugar) or fructose can be used. The former is a simple sugar, glucose, the next, sucrose, is made of two molecules of simple sugar put together (fructose and glucose), the last is a sugar found in fruit. Yeasts can ferment all three.

TABLE 5.2 in the previous chapter gives you the corrections for addition of sugar. You can start with a refractometer reading (or the reading from the producer) or a hydrometer reading (giving the specific gravity) to approximate the potential alcohol. For example a Balling (same as a ^{o}Brix unit) measure of 14 (14 ^{o}Brix) will give a wine that is 7.2% alcohol. To make it 12% alcohol, 0.65 pounds of sugar need to be added per gallon of juice. That is a lot but then again 14 ^{o}brix is extremely low. More characteristic of NY state would be a juice that is 18 ^{o}Brix. How much sugar must be added to this juice to make it 12% alcohol??? 0.28 pounds per gallon. The sugar is normally dissolved in a small volume of the juice brought to near boiling point then cooled and added to the juice. What do you do if the sugar content is 21 ^{o}Brix or above??? Add water possibly, mix this juice with another juice containing less sugar or be ready to drink a sweet wine.

b. Acidity. As we said too much or too little acid in a wine will give an unpleasant product. The unit most often used to measure acid content is in grams of acid (as tartaric acid) per 100 ml of juice. The range for California grapes is 0.4 to 0.85 (grams/100 ml). Below 0.6 grams/100 ml the juice will probably need to be adulterated otherwise the wine will be tasteless; tartaric acid or a mixture of citric (20%), tartaric (50%) and malic (30%) acid are often used in these cases.

In NY state and especially in the Finger Lakes the range is 0.8 to 1.8 grams/100 ml. Above 1.2 grams/100 ml, again the juice may need dilution or treatment. If dilution is done with water, sugar levels have to be corrected but flavor will be diluted by the added water.

More frequently the acid in the juice is diluted with another juice of lesser acidity to maintain flavor.

These corrections to the acidity of a wine are often done after fermentation because as we will see later a special type of fermentation (called **bacterial malolactic fermentation**) is possible with juice of acid levels between 0.9 grams/100 ml and 1.2 grams/100 ml. This fermentation will lower the acidity by as much as 30%.

c. The commercial wine maker also wants to know the organic material content of the juice before fermentation. These organic materials (other than salts) are compounds that contribute, with the sugar content, to the ^{o}Brix in solution; those are proteins, DNA, lipids. All compounds that contain the element carbon are in fact called organic compounds. This information is obtained by ashing the juice. The juice is first dried to solid material which is then weighed and burned to ashes (hence the name of the technique). Ashing will get rid of all organic materials which escape as CO_2. The remainder is made of the salts and minerals. The ashes are weighted again and the mass difference between the two weighing is the mass of organic materials. These organic materials will drive the fermentation because they include the vitamins needed for yeast growth. Usually a grape juice contains 2% organic material. If it were also 18% sugar the ^{o}Brix would then read 20 ^{o}Brix.

5. Sterility and the use of sulfur.

As we have seen the juice needed for fermentation comes from crushing and pressing the grapes. But the juice contains wild yeasts and bacteria. Before a cultured yeast can be added to the juice other microorganisms present in the juice must be killed or slowed down. To do that our ancestors burned sulfur candles in the barrels where the juice would be fermented into wine. The sulfur as it burned would combine with oxygen to form a gas called sulfur dioxide (SO_2). This gas kills bacteria and molds but only stuns yeasts. It also acts as an antioxidant to prevent oxygen from turning wine brown and strong tasting.

Today we use a compound called sodium (or potassium) metabisulfite, which combines with water to produce SO_2. Metabisulfite comes as a powder or in preweighted tablets called Campden tablets. One gram of metabisulfite produces 1/2 gram of SO_2. So if a cleaning up protocol calls for a concentration of 1 gram of SO_2 per 10 gallons you must add 2 grams of metabisulfite to these ten gallons.

The $S0_2$ formed evaporates with time and so will not stay in the wine for it would give it a distinctive and undesirable flavor. Some people are in fact allergic to SO_2 and even the little left in wine will produce a major anaphylactic reaction (swelling of lung tissues that hampers or even prevents breathing) which can be lethal. Often metabisulfite is added to finished wines to prevent the growth of bacteria that use alcohol for food and produce acids as waste. Such an organism is Acetobacter which makes vinegar. The problem is more acute in white wines because they lack the skin compounds that increase the acidity of red wines to prevent the growth of such organisms. Other skin compounds are modified by yeast to produce potent antibacterial agents which of course will be lacking in white wines.

This is the reason why the white juice that can be obtained from a producer contains 80 to 100 ppm of SO_2 to 40 ppm for a red juice. NOTE here the unit of ppm, a concentration unit meaning parts per million; forty ppms means 40 parts per million parts. These parts can be any unit of mass such as grams, milligrams, micrograms etc. per million grams, milligrams, micrograms.

6. Fermentation.

a. Preparing a yeast starter. There is no such thing as a "no yeast" wine recipe. At the start of fermentation, the yeast added to the must may have to compete with strains of wild yeast that the SO_2 has only slowed down and not killed off. Therefore it is to your advantage to add to the must or juice yeast cells that are already actively growing and dividing.

The wine yeast from the store comes in a dried form. This means that if these cells are added directly to the must or juice, it will take them a certain amount of time to take up water and begin their active life. So, even the full content of the packet were added to the juice, these 20 billion cells will be inactive for the first 24 hours.

There are however two possible alternatives to the addition of dried yeast to the juice. The first one involves drawing a quarter cup (60 ml) from the juice or must, boiling it to kill microorganisms and adding it to the dried yeast after it has cooled down; after stirring for 20-30 minutes to insure a more rapid rehydration of the yeast cells, the suspension of cells is then added to the juice for fermentation.

The second method consists of preparing a yeast starter a few days before fermentation. Here, the dried yeast cells are added to a medium that will support their growth for a few days. You can then add the starter to your juice or must at a time when the yeast cells are at their peak of activity. The yeast then has a much better chance of succeeding since it is in full activity. This medium can be made of grape juice (beware of Concord grape juice however since a cup of it in 5 gallons of another juice is enough to impart the methyl anthranilate flavor to the wine) or any fruit juice (oranges for example). This starter medium however will not support the yeast forever. After a few days, the nutrients will start to disappear and the cells will become dormant again. Addition of a dormant starter to you juice would not accomplish the quick start fermentation since the yeast will have to come out of dormancy again once they are in the must or juice.

The following protocol is a reproduction of a lab protocol for the preparation of a yeast starter.

NOTE:

1. Half-fill a clean small neck quart bottle with grape juice or a pint of water (about 200 ml) and the juice of two lemons or two oranges. Add one ounce (30 grams) of table sugar and yeast nutrients if oranges or lemons are used.

2. Plug the opening with cotton wool. Boil 20 minutes. Let it cool while you perform steps 3 - 6.

3. Empty the content of a packet of yeast in a clean cup, cover with an ounce of warm water.

4. Wait for the yeast to take up the water.

5. Add more water and let stand 20 minutes.

6. Stir with a clean spoon to break up the clumps.

7. Split the content of the cup in two inocula for addition to the cooled juice. Shake well to disperse the yeast completely. Put the plug back in place.

8. Let stand at room temperature for 2 days.

9. At the time of inoculation of your must or juice the starter should be in full ferment. If it is not you should discard it and use dried yeast for an inoculum.

b. Starting wine fermentation. The following protocol is a reproduction of a lab protocol for the start of fermentation.

NOTE: RECORD EVERYTHING YOU DO IN A NOTEBOOK.

1. If using a dry yeast starter, mix the dried yeast in one ounce of lukewarm water and let stand for a few minutes. Add a half cup of juice and let stand 20 minutes.

2. Using a saccharometer, measure and record the sugar present in the juice.

3. Correct for sugar content by adding table sugar or corn sugar.

4. Record the acidity reading.

NOTE If you are using a white grape see note at the bottom.

5. Add the yeast starter to your 1 gallon bottle. One packet of yeast or your wet starter is enough for a 5-gallon quantity of must or juice. Consequently, each starter is sufficient for 4 students.

6. Adjust the level of juice in the container to minimize air space, but at the same time leave enough space for the foaming to occur. This means filling the bottle to 5/6 of its volume with juice.

7. Put the airlock in place. Fill the airlock with water and add a few grains of metabisulfite to prevent bacterial growth.

8. Let the container sit undisturbed for a month at temperature between 60 and 70 ^{o}F.

NOTE:

If using a white juice the following steps can replace steps 5, 6 and 7:

5'. Add pectic enzyme to the juice before adding the yeast. This will help clear the pectin from your wine.

6'. Adjust the level of juice so that a large air space is present on top of the jug (5/6 full).

7'. Do not use an airlock just yet but cover with gauze. Shake the bottles every day to keep air in the ferment.

8'. After heavy fermentation is over (2-3 days), combine several bottles so that the level of liquid is brought up to full.

9'. Put an airlock on. Let secondary fermentation proceed (1-2 months).

The reason for this is that the white wine is more susceptible to bacterial contamination than the red. Therefore air is added to the ferment in greater amount to promote the growth of the yeast. However enough of an anaerobic condition prevails in the ferment to create the alcohol. After the sugar is gone, the airlock is put on and the secondary fermentation allowed to proceed.

c. The must is another name for the juice that has started fermenting. If you have 1000 grams of must (about 2 pounds) you have 750 grams of water 200 grams of sugar, 10 grams of organic acids, 3 grams of salts and minerals and 1 gram of protein.

If crushed grapes (or juice to which skins have added) fermentation is started in an open container. Very soon the skins rise to the surface and form a cap on top of the juice. A foam may form on top of that if the fermentation is going very (because of the oxygen still present in the juice). Temperature also increases and where large vats are used in wineries these vats must be cooled to maintain a lower temperature. The cap is broken 2 times a day to mix the skins into the juice so that fermentation can release these compounds from the skins and to mix in oxygen so that the yeast can get a strong start.

Sugar is fermented at a rate of 4% per day. This means that fermentation can be over in 5 days. Usually however temperature is lowered so that fermentation can proceed a little more slowly. When fermentation has slowed (because of lack of oxygen) and about 2% sugar is left in the must, the wine (free-run) is separated from the pomace (skins). A home producer will simply siphon off the juice and leave the pomace in the open container but wineries will press the pomace and extract another 25% of the volume for subsequent mixing with the free-run or separate sale to a distillery.

The secondary fermentation is completed in casks at the winery or in 5-gallon glass carboys at home. That secondary fermentation takes a long time since little sugar is left and no oxygen is present. During this period which lasts months the lees also settle at the bottom of the carboy (or cask) as the wine clears.

d. The differences between red wines and white wines.

- red wines contain more skin extract for color, nutrients, tannins, flavor, antibiotics.
- red juices require less SO_2 for sterilizing because of their content in natural antibiotics.
- red wines clear more easily because their high tannin content precipitates the haze-producing proteins more readily.
- red wines ferment faster because the red juice contains more nutrients.
- white wines require more SO_2 for sterilizing.
- white wines are fermented at higher temperatures in order to establish the wine yeast more rapidly.
- white wines often require the addition of pectic enzyme to the must to destroy the haze producing protein, pectin before the start of fermentation.
- white wines are cleared and racked in a shorter period of time than reds.
- white wines are usually not aged since they are more fragile.

This is a summary of the characteristics of red and white musts and wines. Let us now apply this information to the practical steps in the making of red and white wines.

e. Making red wine.

1. A red wine can be fermented from grape juice concentrates, frozen juice or fresh juice. The use of a starter is not necessary; dry yeast can be added to the must to start fermentation. The start of fermentation is a little slower however than if a started has been used. Sugar corrections should be made before fermentation starts because it is easier to measure the sugar content and make corrections before the sugar starts disappearing.

2. Skins may or may not be used in the primary fermentation. The skins may come from the grapes prior to pressing or may be added after pressing; in the latter case skins from a different grape variety may be used to add complexity or color or both to the wine.

3. Acid corrections are done later in the process of wine making and should not be undergone at this point.

4. Fermentation temperature is not as critical as in white wine fermentation. Notable here is the fact that higher temperatures (about 70 ^oF) will confer a stronger taste (and color if skins are used in the fermentation) to the wine. Cooler temperatures (45 ^oF) will produce a milder tasting wine. This difference exists even when juice is fermented without skins. This indicates that yeast cells produce different compounds at different temperatures that affect the taste of the wine.

The primary fermentation is usually done in a container with a wide opening for easy access and clean up. The container is never filled to the top with must for fear of foaming. The top is fitted with an airlock or gauze.

5. Tasting the wine at this point of the fermentation is not a good idea. You will not like it.

6. After a week the fermented must is transferred from the primary fermentor to the secondary fermentor, a glass carboy with an airlock. It will stay there for up to one year with rackings occurring 3 to 4 times during that period. Keep at warm temperature in case malolactic fermentation initiates.

f. Fermenting white wine. The juice you bought had over 120 ppms of SO_2 in it. That is to keep wild yeast, molds and bacteria from growing. The juice is also refrigerated when purchased.

1. As you let the temperature rise, correct the sugar levels and add pectic enzyme to destroy pectin. This will help the wine clear after fermentation is completed. Do not correct acid levels.

2. Fermentation can be started directly in a glass carboy but it is advised to use a starter instead of dry yeast in order to start the cultured yeast as soon as possible.

3. White wines ferment more slowly than red wines because the yeast may lack some of the nutrients present in red musts. At least initially ferment at high temperature (70 ^oF)
again to start the cultured yeast as soon as possible. Add oxygen to the must by mixing several times over the next two days.

4. Airspace should be left at the top of the carboy to aerate the yeast and also to prevent overflowing in case of foaming.

5. The airlock can go on after 3-4 days. The lack of oxygen will slow down the yeast but hopefully by then the cultured yeast will be established and no other organism will grow in the must.

6. Fermentation should be done in 2-3 weeks. Check for sugar content from time to time to make sure that fermentation has not arrested. After a week fill the carboy completely (consolidating 5 containers in 4 for example or using a bottle of finished wine to top off the carboys).

7. Cool the temperature to 40-45 ^oF (unless acid levels are high and you want malolactic fermentation, in which case the temperature should be raised to 75 ^oF) and let the wine rest on its lees for another 2 weeks.

8. Rack into a fresh carboy, add SO_2 (every time you rack a white wine) and let rest at 40 ^oF for a month. IGNORE this step if you want malolactic fermentation. WHY?

9. If the wine is clear at this point, bottle. If not rack another time, add SO_2 and let stand another 2-3 weeks.

7. Secondary fermentation: malolactic fermentation.
At this point the fermentation is over and no sugar is left in the wine. The wine is simply resting on its lees and clarifying. In 12% alcohol solution the yeast die and the dead yeast as well as the protein is sedimenting (i.e. falling to the bottom) and that is why the wine is clearing.

Sitting on its lees, the wine will sometimes crystallize tartaric acid especially if the wine contains a high level of acid. Those crystals will fall to the bottom with the rest of the lees. The tartaric acid is only one of two major acids in the wine; the other is malic acid which is present in high concentrations in NY grapes because they are most often not ripe. This acid can disappear from the wine in a process called malolactic fermentation. The process is accomplished by bacteria that convert malic acid into another acid (lactic acid) that is not as strong an acid of the former. The process is shown below:

$$C_4H_6O_5 \;\; \text{--->} \;\; C_33H_6O_3 \;\; + \;\; CO_2.$$
$$\text{1 gram} \qquad \text{0.67 gram} \quad \text{0.33 gram}$$

The resulting product, lactic acid, is a weaker acid, that frees less H+ in solution). The result is a softer wine with less harshness to it. In fact degrading malic acid into lactic acid can cut the acidity by a factor of 2.

The bacteria responsible for this process may be present in the wine during fermentation. If so it will not grow because the yeast have taken over early. If enough are present (you need at least one billion cells per liter) malolactic fermentation will start after the yeast die. However if sugar is still present at this point the bacteria will use sugar rather than malic acid and produce other very unpleasant acids. In fact this bacterium is in the same family (Leuconostoc) as those involved in the production of sauerkraut.

Let's look at the steps involved in malolactic fermentation.

a. first the wine must be very dry (i.e. no residual sugar)
b. the temperature must be relatively high (75 ^oF)
c. SO_2 should not be used after primary fermentation is over.
d. Acid levels should be around 1.1 for the bacteria to start feeding on malic acid. Levels of acid much above 1.1 discourage bacterial growth. To reduce high acid levels $CaCO_3$ (chalk) may be used (do not mistake chalk for gypsum ($CaSO_4$). However chalk binds mostly tartaric acid and thus the result may be that malolactic fermentation will convert too much malic acid into lactic acid. This might actually lead to too little acid in the wine.
e. If all these conditions are met malolactic fermentation may occur. It is also possible to add the bacterial cultures to the wine but growth is not guaranteed since the other conditions must also be in place before growth occurs.

Malolactic fermentation is in vogue now in NY state because of the often high acid levels present in the juices.

Let's take a look at an example of acid levels in a wine during fermentation.

	Acid levels	
step	red wine	white wine
crushed grapes	1.36 (9/24)	
pressed grapes	1.18 (9/28)	1.03 (10/5)
new wine	1.18 (11/12)	0.93 (11/12)
+ chalk	0.92 (12/4)	no chalk
after M-L ferm.	0.72 (2/4)	0.69 (12/16)
bottled	0.72 (2/4)	0.68 (1/26)

From the earlier chemical equation you see that CO_2 is formed in the process of malolactic fermentation. That is why bottling too soon may result in explosions in the cellar. Especially in NY state let the wine rest on its lees for the times indicated in sections 6c and 6d.

If you are fermenting a must of low acid levels it is wise to do what California wineries do which is to remove the wine from the lees immediately after primary fermentation, add SO_2

and filter. The wine is then ready for bottling after only a few weeks rather than a few months.

8. Racking.
The word simply means to separate the wine from the deposit at the bottom (the lees). That is done by siphoning the wine out of the carboy and placing it into a clean one. Racking can be done without (Figure 6.2) and with (Figure 6.3) oxygen from the air mixed into the wine.

After malolactic fermentation the wine is chilled and SO_2 may be added to white wines. The chilling will accelerate sedimentation and racking may be done shortly after. The first racking is done while the wine is still cloudy but a clear sediment has appeared at the bottom of the carboy.

Be careful not to introduce too much oxygen in the wine as you rack; make sure that the hose is delivering wine to the second, lower positioned carboy while staying under the liquid level. Top off the carboy so that no oxygen is left in it; SO_2 is added to white wines here especially.

The first racking should occur after malolactic fermentation. If a wine was started in September the first racking would occur in December for a white and January for a red. Over the next few months (up to one year) a red wine can be racked two more times (or until clear) while a white should be bottled no later than February (after two rackings).

The long periods of sitting at low temperature for red wines (up to one year) will yield a wine that is more oaky flavored. Nowadays however people prefer light fruity reds and whites. Thus the practice of bottling very quickly (after the second racking) has become more popular. So it is up to your tastes. Experiment to find the way to produce your favorite wines.

The racking is done (FIGURES 6.2 and 6.3) by placing the first carboy higher than the second. Put the siphon in (6 feet of rubber tubing will do; more fancy siphons are available as well; make sure that the siphon has been sterilized with SO_2). Pull on the siphon with your mouth or a pipette but make sure that the siphon does not touch the lees in the upper wine container while you do that. Let the wine flow through the tubing to the second container making sure that the tube is under the surface of the liquid (to prevent the introduction of too much oxygen

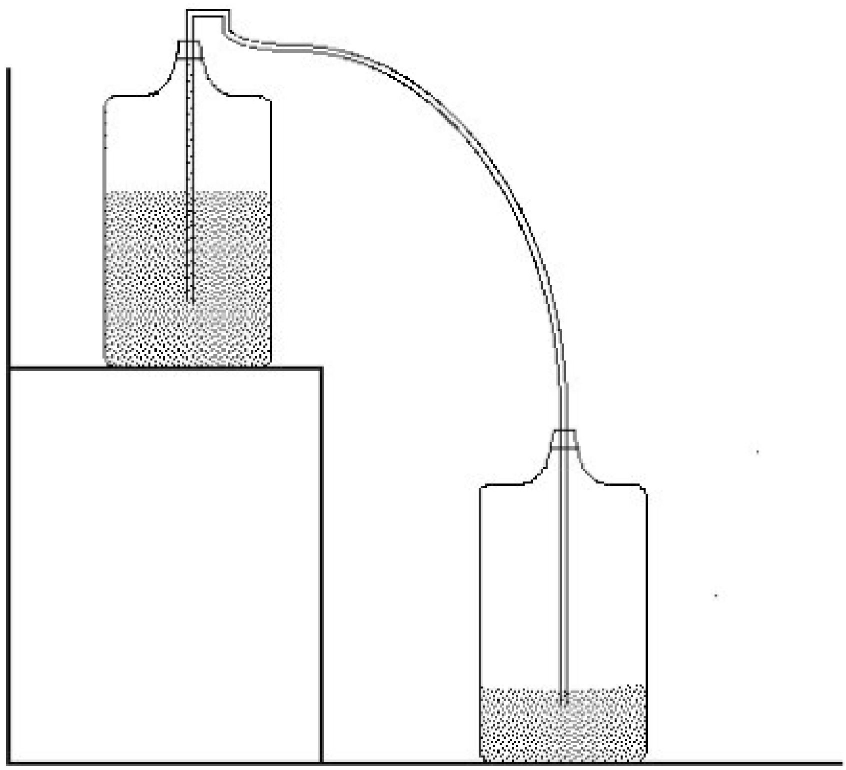

Figure 6.2. Racking without aeration.

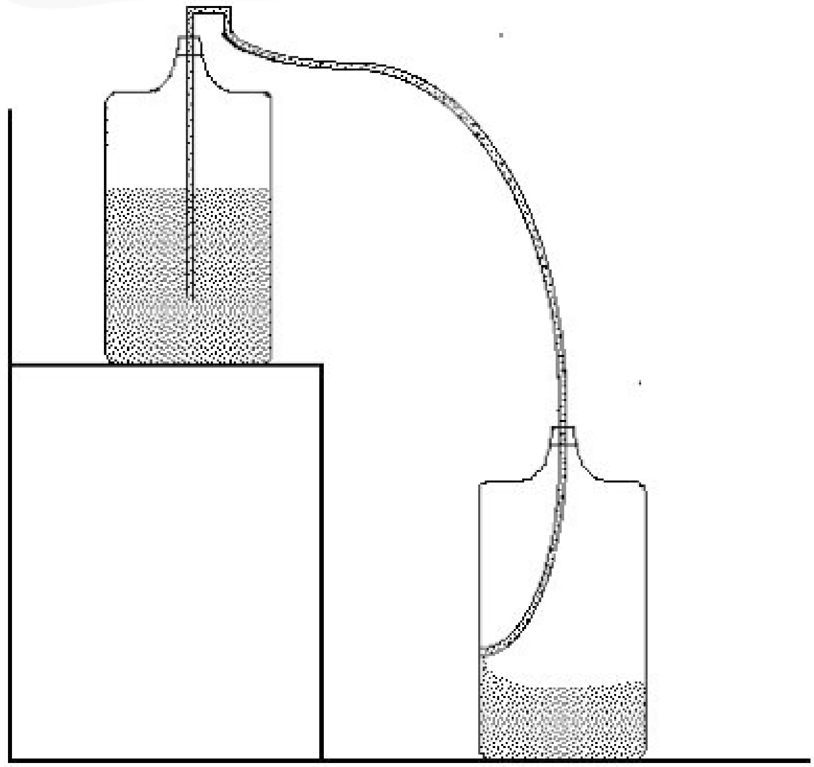

Figure 6.3. Racking with aeration.

which would oxidize the wine). Top off the second container to 1/4 inch from the top; use a good finished wine to do so.

When using several carboys it is then possible to top off the carboys with a little from the next one to be racked and top off the last one with finished wine. Use an airlock when racking is completed. Metabisulfite may be added here (1/8 teaspoon) while racking, especially in white wines. After racking the airlock (Figure 6.4) is carefully placed back on the carboy.

The wines are then chilled in preparation for the next racking. Bottling can occur as soon as the wine is clear. If not wait a while or you may opt here to do a fining.

9. Fining.

Fining is the last resort if a wine does not clear of its own accord. These hazes can be due to proteins or other small particles that are too small to fall to the bottom and too large to be invisible. Often also large particles are kept from binding to each other (and consequently precipitate) because they both carry negative charges on their surfaces. The charges will repel other particles keeping them from binding. They are called colloids in chemical terms. The haze is due to the fact that when light hits the particle it is deflected because of the size of the particle and so you see less light going through the solution; it thus appears turbid (cloudy) to you.

The fining material will bind these particles into large aggregates that will then deposit at the bottom; we call that coagulation. The problem is that often the fining agents will either leave a flavor in solution or take away from the wine a flavor molecule; either way the taste of the wine may be changed.

The best fining agent is gelatin, from your local grocery store. It will bind the colloids that you want to get rid of but also tannins
(which will reduce the astringency of the wine.). In fact if you use gelatin in white wines you will need to add tannins (1/4 teaspoon (2.5 g)/5 gallon) with the gelatin to help it precipitate.

Mix the gelatin (1/4 teaspoon/5 gallon) in warm water to soften it and add to the wine while stirring. If tannins are added as well they should go into the wine before the gelatin. Chill the wine again and wait for the clearing. If the haze persists, add more tannin.

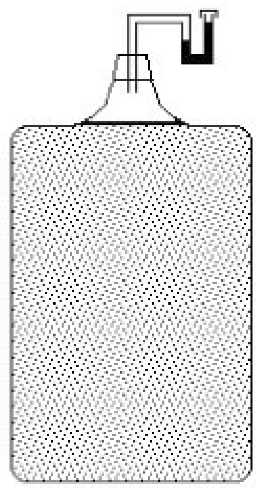

Figure 6.4. Illustration of an airlock.

Other finings are more dangerous because of risks of changing wine flavors. They include bentonite (clay), normally added at a rate of 5-10 grams/5 gallon.

10. Filtration.
The technique is very popular in the US and equipment has been designed for the home wine makers as well. It is an alternative to fining. However you must keep in mind that filtering removes all microorganisms from the wine so that the wine cannot age anymore. The filters used are expensive because they need to have small enough pores to prevent microorganisms from going through (about 20-40 microns in pore size; a micron is 0.000001 meter). Your coffee filter will not cut it here.

11. Tasting and blending.
Before bottling the wine maker may want to alleviate some problems in their wines. Alcohol level may be a little low or acidity may be a little high. To compensate they may want to blend in other wines. They may also want to mix varietals to complement flavors or add complexity.

In blending wines however there are some warnings and rules to follow.

a. A labrusca wine that has too much foxy flavor cannot be mixed with a milder flavored wine. The labrusca flavor is too strong to be masked. If you were to mix one part of Concord with ten parts of Baco for example the product would still taste like Concord.

b. A wine gone bad cannot be salvaged by blending. The result is wasting the good wine you blended in.

c. Try the blends in small quantities first so that the results can be tasted before large scale blending takes place.

d. Always blend high alcohol levels with low alcohol levels, high acid levels with low acid levels and strong color with light color.

e. Never blend sweet wines with dry wines.

12. Bottling.
A wine that is not producing gas anymore (no bubbling), has completely cleared and has been chilled to remove as much tartaric acid as possible is ready to bottle. Corks made from the bark of trees are normally used because their porous nature allows a small amount of air in the bottle during the aging process to allow the wine to mature.

DID YOU KNOW:

Cork production is actually sustainable. The trees are not hurt by the harvest of their bark. A cork oak (***Quercus suber***) can live up to 250 years and must grow 10 t0 12 years between harvests.

The largest known cork oak tree in Portugal can produce, during one harvest, enough cork to bottle 100,000 bottles of wine.

In Portugal it is illegal to cut down a cork oak.

White wines are more fragile than red wines, they are more sensitive to oxidation and they do not age well. Red wines can be aged in cooperage (or carboys) where they acquire an oaky taste that complements well their basic flavors. For these reasons the white wines are usually bottled earlier than red wines. Wines will continue to age in the bottle but in a much more subtle and slow way. That is why white wines are consumed quickly. Of course if the wine has been filtered whether it be red or white aging will not be possible.

The aging process is still very mysterious and we do not fully understand what happens here. We know that during aging the natural fruitiness of a young wine will be reduced, its bouquet will improve and the wine will soften in general. These improvements will take years but the time course for these improvements varies from wine to wine. Some red wines do not age well at all (Beaujolais for example). Conditions of aging are also very important. Corks must be used on the bottles rather than screw caps to facilitate exchanges between the inside and the outside of the bottle. Temperature and relative humidity also appear to play an important role in the aging process. We also know that these improvements reach a peak after which the wine starts to decline. In NY state the peak is reached after 6-9 years while some French wines are still improving after 500 years. But the brand of wine is no guarantee of such improvements. Some 200 or 300 year old bottles of wine from famous wineries are often auctioned these days for tens of thousands of dollars. And yet that wine might have peaked 50 or 100 years ago,

At home, it is recommended that the carboy be moved to where bottling takes place the day before to allow the lees to settle again if they were disturbed while moving the carboy. Make sure that the bottles and the corks (# 9 corks 1 and 3/4 inch long) have been washed in metabisulfite for at least 20 minutes. A siphon can be used to fill the bottles. Fill each bottle to within no more than an inch of the top to minimize the amount of oxygen in the bottle. Use a corker to place the cork in the bottle. Let the bottles stand upright for a couple of days after bottling to balance internal and external pressures. Store the bottles on their sides at 50-60 ^{o}F. Avoid extremes of temperature.

13. Equipment for making wine at home.

   a. If you wanted to start with your own grapes you can actually buy a small crusher and a small press. They are relatively expensive ($200-$300) and it is a lot easier to buy juice, either frozen, concentrated or fresh.
   b. A saccharometer (hydrometer) is essential to measure sugar levels at different times before and during fermentation.
   c. If you decide to ferment the juice with the skins, it is better to

acquire a plastic tub with a lid and an air lock, They are very inexpensive ($15) and easy to clean.

d. Secondary fermentation (or primary fermentation of juice without the skins) is done in a glass carboy ($15) with an air lock.
e. Siphon and tubing for racking and bottling.
f. Used wine bottles are easy to acquire; clean thoroughly before bottling. The screw type variety is useless however.
g. A good corker ($20) and new corks will be needed in bottling. Do not reuse old corks because they have shrunk and will not fit the bottle well enough to prevent oxidation.
h. Metabisulfite is used for cleaning equipment.
i. You will also need funnels, measuring cups and utensils for various measurements.
j. Finally a thermometer will come in handy.

Interestingly our society still allows individuals and families to make alcohol (but not for sale); quantities are limited to about 200 gallons per year for a family.

And remember that experimentation is half the fun in making wine at home.

D. Additional fermentations

1. Sparkling wines.

a. Introduction. Sparkling wines contain dissolved $C0_2$ above a certain minimal level. The Government defines it for tax purposes as:

- Still wine (taxed at $0.70/gal) : below 0.392 g CO_2/100 ml (<1 atm CO_2)
- Sparkling wine: above 0.392 g of CO_2/100 ml, two categories are defined:
- Natural yeast carbonation: 4 atm $C0_2$ => tax = $4.30/gal
- Artificial carbonation: 2 atm $C0_2$ => tax = $ 2.50/ gal

DID YOU KNOW:
The pressure in a Champagne bottle (90 pound per square inch) is 3 time that in a car tire.

One would think that the latter would be a popular wine, cheaper to produce and cheaper to buy. However champagne is viewed as a luxury by the public and the government. When buying a luxury, people prefer to buy the more expensive wine. Further the carbonation lasts longer in a naturally carbonated wine. The reasons are:

- No haze is present in naturally carbonated wine that would favor the formation of large bubbles and therefore reduce the time a wine stays carbonated in the glass.

- Other gases in artificially carbonated wines favor the formation of bubbles.

- Presence of amino acids and small peptides in naturally carbonated wines favors the formation of small bubbles. These amino acids and peptides are present because of yeast autolysis (self-destruction).

The use of CO_2 in wine is not just a fad or a trend. It actually has a basis in our physiology. Two factors play a role in the effect of CO_2 in beverages. The first one has to do with the way we taste foods. Figure 8.1 shows that our tongue is subdivided in areas that carry specific types of taste buds specialized in the perception of salty, sweet, bitter and sour. Since the nose also plays a role in tasting we look for foods that have strong aromas as well as tastes. We also try to enhance flavors with the use of a variety of chemicals. Can you name one? Salt, monosodium glutamate, acids and yes CO_2. Carbon dioxide can fool your taste buds into thinking that there is more flavor there. We actually put CO_2 in more than just wine. We find it in sodas, water, beer and wine. It is interesting that a lot of people like the taste of carbonated water; but of course water is tasteless. And yet the carbonation seems to give it a taste.

The other factor in the attraction that we have for carbonated wine is the fact that CO_2 increases the rate at which alcohol is absorbed through the stomach liner. This means that the effect of the alcohol is more immediate and also stronger.

b. History of champagnization. Dom Perignon, a monk in a Benedictine abbey in the region of champagne discovered the principle by accident some time between 1668 and 1717. The accident must have occurred when sugar fermentation was stopped in the barrel because of cold weather that fall. It resumed in the spring in the bottle, thus forming the carbonation. The fermentation in the bottle is the basis for the methode champenoise of making sparkling wine. However only wines made in the region of champagne in France can be called champagne. All others (even those made by the methode champenoise) can only be called sparkling wines.

c. Making champagne. First select a dry white wine with moderate alcohol levels (9.5 - 11.5 % alcohol). Acid levels should be about 0.7 to 0.9 percent. A very light straw color is essential. No CO_2 must be present because the yeast must grow again. Champagne is usually a blend of Chardonnay and Pinot noir and that is why you normally do not see a varietal designation on a champagne bottle. The only indication sometimes is "blanc de blanc" meaning that white grapes were used (conversely 'blanc de noir' means that red grapes were used to generate white wine. HOW?). The blend is done to achieve the right mix of acidity, balance and fruitiness rather than a distinctive varietal flavor. In fact the champagne wine has a very neutral flavor by itself.

The still wines will be racked, blended, clarified, stabilized and fined before the champagne is made. Yeast is then added to the 'cuvee' along with sugar and yeast nutrients (ammonium phosphate). The yeast must have the ability to grow under low T, high pressure and high alcohol levels; it must also form hard clumps when it dies and go to the bottom of the bottom (or the top actually in the case of champagne).

The yeast starter, sugar and nutrients are added to the cuvee and mixed: 24 grams of sugar per liter of wine will produce 6 atmospheres of CO_2 (that is 20 pounds per 100 gallons). Oxygen is also mixed in to encourage yeast to grow.

Here we split into two possible methods:

- Methode champenoise. The mixed wine is drawn off and bottled in thick walled bottles. The corks used are also thicker than usual and they are held by wire. Bottles are stacked horizontally at 60 ^oF (15.5 ^oC). The cold temperature is needed to prevent too rapid a fermentation that would result in excessive pressure before the CO_2 produced has a chance to dissolve; bottles would then burst. However this means that the fermentation is very slow (it occurs over a period of weeks). The batch (tirage) is then stored at 10 ^oC for a year to four years. This lets the yeast settle and die. The flavor of the champagne is given by this slow aging, as the yeast cells die and lyze (burst).

  The bottles are then placed neck down at a steep angle, in racks so that the yeast can deposit at the top. The "remuage" then starts which means that at intervals of a day for a week to a month, the bottles are rotated 1/8 turn sharply and jolted so that the yeast that is on the sides can settle in a layer on the cork with no other deposit anywhere on the walls of the bottle.

  The plug of lees must then be removed from the bottle in a process called "disgorging "(degorgement). The bottles are brought to near freezing point (that lowers the pressure in the bottle) and the plug is frozen solid by dropping the bottle in a subfreezing bath. The bottle is then turned 45^o from upright, opened quickly so that the pressure pushes the plug out and the bottle is held on a spring loaded plug to minimize the loss of carbonation until it can be quickly recorked. In the meantime more wine is added to replenish losses and a "dosage " is also added. A dosage is a syrup added to adjust the sweetness of the wine. The following table shows how sweet a champagne is:

brut (the driest) :	0.5 to 1.5% sugar.
sec (dry)	2.5 to 4.5% sugar

demi sec (semi-dry: 5.0% sugar

doux (sweet): 10.0%.

Note that even the BRUT has sugar. Without it the wine would taste sour. The dosage is made of a syrup consisting of 60 grams of cane sugar per 100 ml of white wine. The dosage does not ferment because the pressure of $C0_2$ and the alcohol levels are too high for fermentation to start again. After the dosage has been added further aging is no great advantage. Can you think of the reason for this?

This type of operation is labor intensive and cannot be automated.

- The transfer process. Here the wine is fermented in the bottle but then the wine is emptied out into a large tank with the sediment. The wine is kept under pressure in the tank to keep the CO_2 in the wine. The wine is filtered and poured back into the washed bottle to which the 'dosage' has already been added.

  This process saves time and labor and offers a more uniform product (Why?). There is more of a chance of air getting in however and a darker, oxidized product, is more likely. SO_2 would then have to be added with the risk of taste changes.

- The third method is called the bulk method. It is the cheapest and produces a lower quality product. Also known as the charmat method it consists of fermenting the champagne from a still wine in a stainless steel tank rather than a bottle for a time of one to two months (compare with 1 to 4 years in the methode champenoise). To do it that quickly the fermentation must be done at higher temperature (13 ^oC). The flavor of this product is different from that of a methode champenoise product because the yeast is not allowed to break down. The wine is then stabilized in the cold (to remove tartaric acid) and filtered under pressure. The wine is then bottled in bottles already containing 'dosage' and sulfur to prevent the possibility of fermentation of the 'dosage'. Chances of oxidation are even greater in this process and the wines usually are darker and sharper in flavor. Thus a sweet or semisweet "Charmat method" wine is not unusual on the shelves. The addition of $S0_2$ becomes imperative here with the distinct possibility of an off-flavor in tasting.

  Red and pink sparkling wines are also available and are made in methods similar to those described above. Re-fermentation is harder because of possible higher alcohol content and higher tannin content. Because reds are harder to clear, filtration of the bulk is a more likely process to be used in the making of red sparkling wines.

- Finally a really cheap sparkling wine can be made by pumping $C0_2$ directly into the still wine and bottling it under pressure. The product is the equivalent of making soft drinks (without the alcohol of course). The champagne flavor that comes from the second fermentation with the yeast is totally lacking and the wine is usually not well balanced. The prices reflect this fact. This wine should in fact be referred to as carbonated rather than sparkling.

E. CONCLUSION.

One last point about making wine. The bulk of wines produced in the world are " ordinary " wines (vin ordinaire as the French call them; we might call them here "jug wines"). These wines are very good but will never be classified as great wines. The truly great wines (called Grand Cru in France for example) are a minute fraction of the volume of wine produced in the world. If you ever decide to make wine during your life it will be of the ordinary sort but, join the club, that is what most wines are. It will of course have the distinction of being your wine and thus have a unique quality.

Answers to chapter questions.

p. 9: If 99 molecules of HCl are mixed with 100 molecules of NaOH, the resulting solution will have what kind of characteristics? Neutral, acid or basic? 99 molecules of HCl will react with and neutralize 99 molecules of NaOH since the two compounds react one on one. This leaves one molecule of NaOH which is basic by definition. Thus the solution will be basic.

p. 9: Can you write the equation for the mixing of KOH and HI?

KOH + HI -→ KI + H_20

The ionic equation is:

H^+ + I^- + K^+ + OH^- ---> H_20 + K^+ + I^-. (2)

Since the ions K^+ and I^- are not changed in the reaction we can write a final version of the equation called the net ionic equation:

H^+ + OH^- ---> H_20.

Note that regardless of the acid and the base mixed together the net ionic equatio is always the same.

p. 10: How many grams of HI could be neutralized by 40 grams of NaOH? 40 grams of NaOH is one mole of NaOH. And one mole of NaOH can neutralize one mole of HI. Since H has a mass of 1 gram per mole and I has a mass of 127 grams per mole 127 grams of Hi can be neutralized by 40 grams of NaOH.

p. 10: What would happen if you doubled the powder added to a quart and then added another quart of water. Would the KOOLAID taste more concentrated then? They would both taste the same because both solutions have the same amount of KOOLAID and water.

p. 11: Assume that you were titrating a solution of HCl (100 ml or 1/10 of a liter) with a one molar (one mole per liter) solution of base. You required 100 ml of base for the titration. How many grams of acid were present in the HCl solution?

You know that one mole of base is required to titrate one mole of acid. The number of moles of base is:

$$100 \text{ mL} \times \frac{1 \text{ liter}}{1000 \text{ mL}} \times \frac{1 \text{ mole base}}{\text{Liter}} = 0.1 \text{ mole of base}$$

You know that one mole of base is required to titrate one mole of acid. . Therefore the number of moles of acid is:

0.1 mole of base = 0.1 mole of acid (HCl)

Finally the number of grams of HCl is:

$$0.1 \text{ mole of HCl} \times \frac{36.5 \text{ grams of HCl}}{1 \text{ mole of HCl}} = 3.65 \text{ grams of HCl}$$

p. 11: What is the concentration of HCl if 100 ml of the acid is titrated with 25 ml of 0.25 M NaOH ?

The number of moles of base is:

$$25 \text{ mL} \times \frac{1\text{Lliter}}{1000 \text{ mL}} \times \frac{0.25 \text{ mole base}}{\text{Liter}} = 0.00625 \text{ mole of base}$$

You know that one mole of base is required to titrate one mole of acid. . Therefore the number of moles of acid is:

0.00625 mole of base = 0.00625 mole of acid (HCl)

Since the concentration of a solution is the number of moles of compound per liter of solution:

$$\frac{0.00625 \text{ mole of acid}}{100 \text{ mL}} \times \frac{1000 \text{ mL}}{1 \text{ Liter}} = \frac{0.0625 \text{ mole acid}}{\text{Liter}}$$

p. 23: Why should you not add SO_2 to a wine that you hope will go into malolactic fermentation? The SO_2 will kill the bacteria responsible for malolactic fermentation.

p. 35: How can red grapes be used to generate white wine? The pulp of red grapes does not contain the red pigmentation. Pressing the grapes very lightly will not extract the red pigments from the grapes and therefore produce a white juice.

p. 37: After the dosage has been added further aging is no great advantage. Can you think of the reason for this? Bacteria present in wine are responsible for aging, at least in part. Remember that carbon dioxide is a poison and the high concentration of it in champagne kills the bacteria responsible for aging.

p. 37: The transfer process saves time and labor and offers a more uniform product. Why? Bottles are not manipulated individually and this saves time. Uniformity again comes from the fact that bottles are not treated individually.

EXERCISES.

1. What is the strict definition of wine?
2. Why are grapes the perfect fruit to make wine with?
3. Can you make a white wine with a red grape? EXPLAIN.
4. Describe two ways to make a rose wine.
5. What are the differences in the making of port and sherry?
6. Is it possible to harvest grape juice that is very high in sugar and acid? Explain.
7. Explain what a hydrometer does.
8. Atoms react together to form molecules (i.e. compounds). How many atoms of sulfur must react with two atoms of hydrogen to form 3 molecules of H_2S?
9. Why do atoms on the left hand side of the periodic chart tend to loose electrons when they react?
10. Why do atoms on the right hand side of the periodic chart tend to gain electrons when they react?
11. Why do atoms on the left hand side of the periodic chart tend to react with atoms on the right hand side of the periodic chart?
12. Weigh the pros and the cons of growing vinifera grapes in the Finger Lakes for wine making.
13. What is a mole?
14. How would Magnesium mix with nitrogen to form a molecule?
15. Organize the following according to size: molecule, atom, electron, compound and element.
16. What is the mass of one mole of Gold (Au)?
17. If I gave you 2.2 pounds (1KG) of gold how many moles would I give you?
18. Calculate the mass of one mole of Al_2O_3.
19. How many atoms of sodium are there in 58 grams of sodium chloride (NaCl)?
20. How many moles of chlorine atoms must react with one mole of magnesium atoms to form 5 moles of $MgCl_2$.
21. Circle the acids and X the bases: HCl, H^+, H_2O, NaOH, H_2SO_4, KOH.
22. What is the pH of a solution that contains 0.001 mole of hydrogen ions per liter?
23. What is the pH of a solution that is 0.1 molar in hydrogen ions?
24. How can you determine the presence of H^+ in a solution?
25. What ion is used to neutralize hydrogen ions?
26. Write the chemical equation for the reaction of NaOH and H_2SO_4.
27. What unit of measure do you use to determine the amount of acid in a solution?
28. If 99 molecules of HCl are mixed with 100 molecules of NaOH, the resulting solution will have what kind of characteristics? Neutral, acid or basic?
29. A solution has a hydrogen ions concentration of 0.05 molar. The titration of this solution is done with 25 ml of NaOH that if 0.1 molar. What was the original volume of the acid solution?
30. A solution has a hydrogen ions has a pH of 3. The titration of this solution is done with 10 ml of NaOH that if 0.01 molar. What was the original volume of the acid solution?
31. What are the conditions necessary to achieve malolactic fermentation?
32. How does malolactic fermentation lower the acidity of a wine?
33. Describe two other ways to lower the acidity of a wine.
34. What are the main differences between red wine and white wine?
35. Given 5% stems, 20% skins, 75% pulp, 5% seeds, where does the juice and flavor of the wine come from? Where does the color come from?
36. In figure 6.1 what does the x axis represent?
37. In figure 6.1, at what time does acidity equal sugar?
38. If you have a juice with 32 ^{o} brix what will be the alcohol content of the wine made from this juice?
39. If a beer is 8% alcohol what was the original ^{o} brix in the must?
40. What is free run and pomace? Describe what Heron Hill uses free run for?
41. How and why is a refractometer used by the wine maker?
42. Why is sterilization important in wine making?
43. What are the advantages of using metabisulfite for sterilization>

44. Is taste affected by the use of metabisulfite? Explain.
45. What do the skins of red grapes contain that simplify the making of a red wine over a white wine?
46. What is carbonic maceration? How is it different from free run?
47. If a wine has a complex “nose” how will this affect the taste?
48. What methods exist for adding yeast to juice?
49. Compare filtered and non-filtered wines.
50. What are the main differences between white and red wines? Which can be aged for long periods of time?
51. There are 2 types of Port, ruby and tawny. What could be the difference between them?
52. What happens to grapes during the growing season that gives Trockenbeerenauslese its characteristic flavor?
53. Describe the four methods used to make sparkling wines.
54. In question 53 which method(s) enable(s) the makers to sell their sparkling wine as a Champagne?
55. Name two effects carbon dioxide has on the human body that makes it a good addition to sparkling wine.

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LESSON 7. BEER MAKING.

5 Figures
1 Table

A. INTRODUCTION.

The strict definition of beer is that of a malted, fermented cereal grain (Anheuser Busch uses rice and barley to make beer). In some European countries, by law (Reinheitsgebot or German purity law in the Duchy of Bavaria), beer can only be made of malted barley, hops and water. Enacted in 1516 it was a way to prevent competition between brewers and bakers for wheat and rye.

"Furthermore, we wish to emphasize that in future in all cities, markets and in the country, the only ingredients used for the brewing of beer must be Barley, Hops and Water. Whosoever knowingly disregards or transgresses upon this ordinance, shall be punished by the Court authorities' confiscating such barrels of beer, without fail.
Furthermore, should there arise a scarcity and subsequent price increase of the barley (also considering that the times of harvest differ, due to location), WE, the Bavarian Duchy, shall have the right to order Curtailments for the good of all concerned."

That is the modern definition of beer. However, over the centuries the fermented malted barley was supplemented with various flavoring agents until the use of hops was generalized. “

Today we use two basic types of fermentation processes to make beer:

a. Ale: the original type, this beer is made at higher temperatures (65-70 ^{o}F) with a top-fermenting yeast (S<u>accharomyces</u> <u>cerevisiae</u>). This yeast tends to float at the surface of the **wort**. The higher temperatures give the ale a stronger taste and aroma.

b. Lager: a relatively new development (it is probably only 700 years old), the process uses a bottom-fermenting yeast (<u>Saccharomyces</u> <u>uvarum</u> (Carlsbergensis)) at very low temperatures (35-50 ^{o}F). The process takes a lot longer but yields a beer that has a much milder aroma and taste than ale.

All the different beers that have been made in the past and are made today belong to one of these two groups. The differences arise from variations in the basic brewing processes. Some examples:

a. dark beers: where the barley is roasted after malting to give a darker color and stronger flavor to the beer.

b. Kraeusen beers: advertised by the Miller Brewing Company a few years back it referred to an old German process where sugar is not added to the finished beer to provide carbonation. Instead some foaming wort is added to obtain a more natural carbonation. Since lagers are stored at cold temperatures for extended periods of time the yeasts will become dormant.

When comes time for carbonation the dormant yeasts may not revive as well or as fast as is needed. Adding foaming wort with its high content of yeasts solves this problem. And since the wort still contains sugar no extra sugar is needed. **Gyle** is another word for fermenting new beer (or fermenting wort) used for priming (carbonating) the finished beer before bottling.

c. Pilsener is a lager developed in 1842 originally in the city of Pilsen. The characteristics of this lager were a result of better control of the temperature during the kilning process that allowed for a lighter .

d. Bock beer: where more malt was used to generate a higher percentage of alcohol. This was needed in those days to help preserve beer for shipment over great distances. The beer trade flourished when this goal was attained.

d. Indian pale ale: with a higher level of alcohol and hops to prevent spoiling during long storage periods this beer could be made in England and shipped to India for consumption by the troops of occupation.

f. Porter is a sweet dark beer made with roasted barley and incompletely fermented so that sweetness remains in the beer.

g. Stout is a dark, dry beer also made with roasted barley.

h. North American type beers are all lager types. However contrary to European beers they are made with large amount of **adjuncts**, such as wheat or rice (instead of barley) for the source of malt. This gives a clear product that is much lighter in taste than its European counterparts and a lot cheaper to produce. The carbonation is greater than in European beers to compensate for the weaker taste common to beers made with adjuncts.

All these beers were developed for economic, cultural or practical reasons; or just curiosity. The variety in the world today is rather mind boggling. It is estimated that over 20,000 different beers are made today worldwide. We will come back to this diversity at the end of this chapter after we know more about the process of beer making and some of the possible variables.

Before Prohibition the US enjoyed the same sort of diversity. Small towns could have as many as 15 to 20 different breweries. There were 2300 breweries in operation in this country in 1880. Needless to say the male population consumed vast quantities of beer. Given the hardships of life for workers living in an excessive capitalistic system where social measures were practically non-existent the use and abuse of alcohol led to violence, injuries and sometimes death. The town of Tombstone for example had a murder rate equivalent to that of modern day New York city. The **Women's Christian Temperance Union** (**WCTU**) was formed by women fighting against the violence that they were too often the victims of. Its efforts focused on the connection between alcohol abuse and violence and finally persuaded the American Congress to vote a law prohibiting the production and sale of alcohol in the US (The Volstead Act or 18th amendment to the Constitution was the law of the land from 1920 to 1933). The thirst of the American public for alcohol was too great however and organized crime quickly moved in to fill the need. It rapidly controlled the manufacture of alcohol in the US and the traffic of contraband alcohol from Canada as well as its distribution in clandestine bars called "speak easies". Since it has been argued that Americans drank more alcohol during than before Prohibition it is easy to see the vast sums of money generated by the manufacture and sale of contraband alcohol and the riches that organized crime accumulated during this period. It is clear that Prohibition enabled organized crime to build the financial basis that fueled its rapid expansion during and after this period. Even after 1933, when Prohibition was repealed, the strong anti-drinking sentiments of the WCTU forced the newly reopened breweries (Of the 478 breweries operating in the U.S. in 1919 only 160 remained at the end of Prohibition.) to put out a diluted, paler and lower alcohol product. To produce such a beer, the pilsener recipe was modified by the addition of adjuncts.

DID YOU KNOW:
The WCTU was still in existence 10 years ago, with a membership of 25,000.

During Prohibition cruises became very popular because passengers could start drinking as soon as the ship entered international waters. Those ships would go in circles for a certain time before returning to port. The cruises were advertised as cruises to nowhere.

Shortly after the Russian revolution Prohibition was imposed throughout the country.
The term "rule of thumbs" comes from the time before thermometers were invented. Brewers then dipped a thumb in the wort to check its temperature before adding yeast

The advent of WW II brought a lot of women to the factories (as men were drafted in the army) and in the bars after work. Thus women became major consumers of beer. The rationing caused by the war also forced the breweries to look for alternative grains and use sugar The result was that beer became even milder, clearer and lower in alcohol content. The product appealed to women and set the way for the production of that type of beer after WW II and to this day. Also, because of the extensive use of adjuncts, sugar and water, all cheap ingredients, the beers were extremely cheap to produce. This was not without appeal to the capitalistic concerns running the breweries. Therefore the use of cheaper adjuncts which had started as a means to please women became the fuel for capitalistic greed and price wars. The result was that breweries had to become very large to compete more effectively with one another and these small breweries of the turn of the century quickly amalgamated to create the three or four national giants that exist today. The age of the microbrewery (producing less than 10,000 gallons of beer) was over and very few of the thousands of brewers who had flourished in the early part of this century remained in this competitive atmosphere.

Another result of this intensive war was that all breweries arrived at the same conclusion as to what type of beer would please the consumer. And that beer was also the cheapest to produce. Therefore people have argued that the US (and Canada) produce only one type of beer, the diluted lager. The differences between brands are not significant enough to warrant a different designation.

Over the last five decades however a lot of people have become bored with that same product and have started looking for variety. They have found it in the resurgent microbreweries, started in the 1960s. There were 60 microbreweries in 1983, 280 microbreweries in 1990, 366 in 2003 and 1,449 in 2009. They produce less than 15,000 barrels a year (< 465,000 gallons). Compare that to Budweiser's yearly U.S. production of 105 million barrels. That's is not much. Over the years the definition of microbreweries has shifted from production size to traditional and experimental approach to beermaking with an emphasis on customer service. It is therefore not surprising to see that Sam Adams brewed 1.2 million barrels in 1996 and yet is still considered a microbrewery (more specifically a regional brewery) by many. Those beers are also not pasteurized and so do not travel very long distances. **Pasteurization** is the heating of the beer to kill microorganisms. The process "kills" microorganisms that could spoil the beer but can also destroy a lot of the taste of the beer. Microbreweries also use the original definition of beer and do not, aside from experimental purposes, use adjuncts or added sugar. The products are therefore more expensive but far more flavorful. In 2009 microbreweries and regional breweries accounted for only 3.8% of production in the U.S. but had a yearly growth rate above 10%.

It is also very easy to make one’s own beer. Interestingly it is nearly impossible to make a wine to rival the best of Europe's but it is relatively easy to make a beer that will rival even microbreweries' products. It is also legal in the US (except in Arkansas, Utah and Oklahoma) (since 1978) and Canada to make up to 400 liters (100 gallons) per person per year (800 liters per family).

The popularity of beer in this country and the world today is undeniable. It is in fact the most popular alcoholic beverage in the world today. World total production was less than 80 million barrels in 1972. In 2005 it had increased to 1,300 million barrels a year (40 billion gallons) or a 17-fold increase in 35 years. In this country alone the average per capita beer consumption is 23 gallons per year compared to 2 gallons of wine and one gallon of distilled sprits. This thirst has led to the rise of giant brewing companies. The Anheuser Busch company, the largest brewer in the US, produced 126 million barrels (105 million barrels in this country) of beer in 2006 or 8% of the entire world production. Assuming 31 gallons per barrel how many six-packs of Bud are Americans drinking every year? Of course Anheuser Busch is not the only brewer in the US. In all US brewers produced 24 billion liters (6.3 billion gallons) of beer in 2004. This means that every man, woman and child drank 269 bottles (45 six-packs) of beer that year and Anheuser Busch produced 138 of those bottles (23 six-packs). Amazingly Americans ranked only 13th in beer consumption in the world in 2004 at 24 gallons per capita. The Czech Republic was first with an annual beer consumption of 42 gallons per capita. That is 1.2 bottles per day for every man, woman and child in the country. And yet it is still below the staggering 54 gallons of beer consumed per year in medieval Belgium.

In 2007 Anheuser-Busch employed nearly 31,000 people and had revenues of 16.7 billion dollars It is interesting to note that the giants of American beer making industry have attracted international attention. In 2008 Anheuser-Busch was sold to a Belgian company called InBev for 52 billion dollars, making it the largest beer manufacturer in the world. Number 2 American brewer Miller Brewing Company was sold in 2002 to South African Breweries or SAB for 5.6 billion dollars, forming. The third-largest brewer in the U.S., Coors Brewing Company with revenues of 5 billion dollars merged in 2004 with Canadian brewer Molson to form MolsonCoors. Molson Coors Brewing Company is the fifth-largest brewer in the world with 42.1 million U.S. beer barrels sold in 2006 and revenues of 6.7 billion dollars Finally, in 2007, SABMiller and Molson Coors Brewing Company announced a joint venture to be known as MillerCoors for their U.S. operations that will market all of their products. The trend toward the formation of ever growing megabreweries has given the ten largest breweries in the world producing 55% of all the beer consumed world wide.

This restructuring of beer makers leave the following rankings.

1. Inbev
2. SABMiller
3. Anheuser-Busch
4. Heineken International
5. Molson Coors Brewing Company

B**.** THE BEER MAKING PROCESS.

1. Malting.

This process will convert the unfermentable starches of the barley into fermentable simple sugars. During grain maturation the sugars made by the plant for storage in the grain (or seed) are "**polymerized**" into long chains of glucose. That chain is called a **starch** and is a very efficient way of packaging energy into a minimum space, second only to the energy packaging of fats. Starch cannot provide energy unless it is first broken back down to simple sugars however. The problem is that yeast cannot do that. It lacks the enzymes to do it and can feed only on simple sugars. (NOTE: we have now engineered yeast strains that can break down starches and later simple sugars to produce a very dry beer. More on this later)

But when the seed is planted in the ground these enzymes missing in the yeast will be activated in the seed and will start breaking down the starches to provide the seed with the glucose it needs to generate the energy needed to grow roots and shoots. How can the beer maker fool the seed (or grain) into thinking that it needs to break down these starches? Simply by putting them in water and letting the grain germinate (for a while) (the process is called **malting**). When enough of these starches have been broken down yeast cells are added and the fermentation can begin.

During malting another category of enzymes will contribute to the way beer looks and tastes. These enzymes will degrade proteins in the grain, proteins that form **hazes** in the fermented product. We will come back to this point later.

Let's look at the steps in malting.

a. Grain selection. Barley was originally a grain similar to wheat and rye (they are all considered grasses like corn). Figure 7.1 shows the two rows of grains on the stalk of the plant. The rows refer to rows of fertile flowers on the stalk. The flowers have the potential to turn into grains if they are fertilized. The 2-row variety has 6 rows of flowers but gathers sunlight less efficiently and only 1/3 of the flowers become fertile thus producing 2 rows of fertile flowers and two rows of barley grains. Thousands of years ago however humans began to breed the barley plant for bigger production and developed types that were more efficient at converting the energy of the sun and produced 6 rows of grains (the 6-row barley). A quick comparison shows that 2-row barley yields about 80 **bushels** (a bushel is an agricultural measure of weight equivalent to about 60 pounds or 30 Kg) per acre while the 6-row barley can yield double that amount. This has traditionally made the former more expensive for the breweries to purchase. Modern breeding techniques have bridged the gap however and new varieties of 2-row barley produce close to 160 bushels per acre.

Let us look at the differences between the grains in the six-row and the two-row barley. The grain in two-row barley is rounder, larger, with a thinner **husk** (outside envelope that is a protective barrier with no nutritive value). Since the husk is thinner, there are less tannins, giving a milder tasting beer. It is however harder to filter a beer made with 2-row barley because the husk acts as a natural filter to retain small particulate matter. Two-row barley has less enzyme potential than its 6-row counterpart but contains more starch (and therefore more potential for malt production). However here again breeding techniques may change that in the future with the development of 2-row varieties with increased enzyme potential. The 6-row varieties have more husk and embryo weight per total weight but their enzyme potential is such that they can convert 40% more starch to simple sugar than the 2-row varieties. And while the higher husk content of the 6-row variety makes filtering easier during sparging, it yields less extract per weight and therefore less flavor.

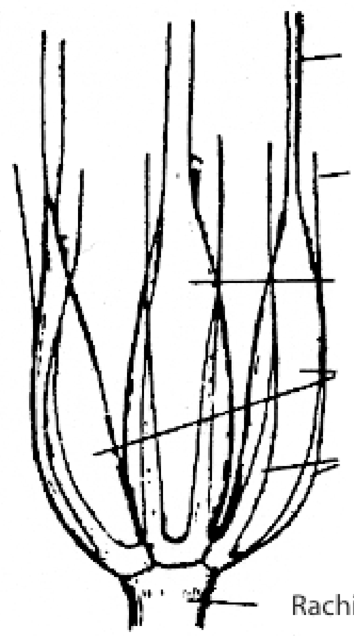

Figure 7.1. Three spikelets of six-row barley at a single rachis node.

To summarize, 2-row barley has more starch, greater extract potential, produces more flavorful beers, less bitterness but less enzyme potential. So it would seem that there are pros and cons for the use of both types of barley in beer making. However traditional beer makers (in Europe for the most part) focus on flavor and use 2-row barley because it produces a tastier, although more expensive beer. In fact 6-row barley is considered unsuitable for beer making in Europe.

So why haven't we come up with a variety that has the good characteristics of both types. It is a good question that addresses a fundamental problem in plant breeding: you cannot get everything you want. Plants have limited energy resources and must focus these resources on accomplishing a limited number of goals. If energy is going to be concentrated on producing 6 rows of grains each grain will have to contain less starch because the plant cannot produce any more. Instead it will produce a thicker husk that is cheaper (from an energy point of view) for the plant to produce than starch. Other examples of these energy limitations include the famous failure to develop potato varieties that produce high yields as well as defense mechanisms to repel insects that attack them (such as aphids). Breeding experiments involved two type of plants: some that could defend themselves very well against those insects but produced very small tubers and plants that could produce very high yields but could not defend themselves very well. The result or **offspring** always possessed intermediate yields between the two parents and intermediate defense potentials between the two parents. Again those plants like all plants lacked the energy reserves to build both an effective defense mechanism and a high yield. They could accomplish one of these two tasks well or both poorly. Some might argue that humans beings often have the same problem.

b. Malting process. The grain is essentially an **embryo** with energy reserves (the **endosperm**) in the form of starch (Figure 7.2). With those energy resources the embryo is able to survive long enough to grow roots and shoots that will be able to extract nutrients from the soil, from the sun and from the air.

The malting process accomplishes four (4) objectives:

- Produce enzymes that degrade starches and proteins.
- make soluble the walls of the endosperm and degrade starches.

- Degrade proteins to supply amino acids to the growing embryo. Of course yeast cells take advantage of this food for their own growth needs.

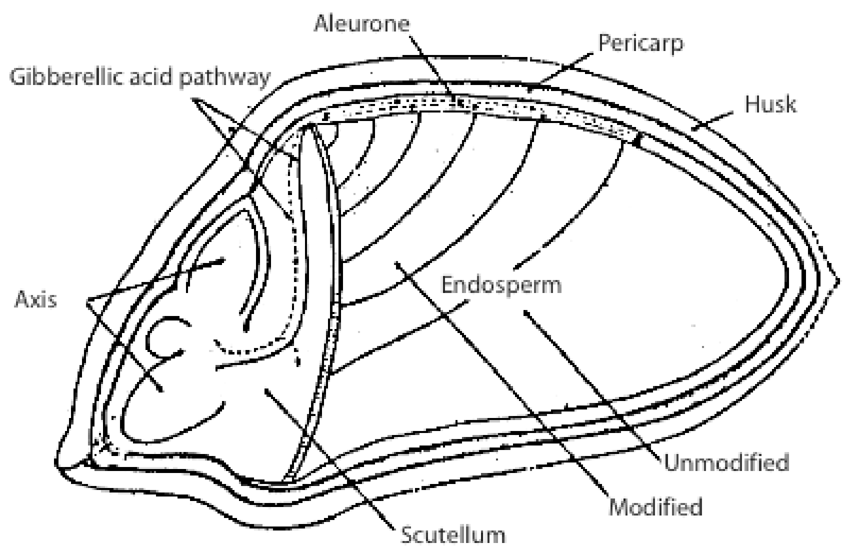

Figure 7.2. Longitudinal section through a barley grain. After Palmer (1969)

- Accomplish the three steps above as quickly as possible.

In the first step of malting the beer maker will sort the grain by size because the time course of germination is dependent on size. This will ensure that all grains germinate at the same time, over a 48 hours following **imbibition (**accumulation of water by the seed and its subsequent swelling).

During the first 24 to 30 hours the grain imbibes. Enzyme synthesis starts (Figure 7.3) during that time. Starch-degrading enzymes have to be **synthesized** because they cannot be stored in the seed. There are two types of starch-degrading enzymes here. The first one is called **alpha-amylase** and makes internal cuts in the long chains of sugars that make up the starch. In essence the enzyme cuts down the starch into molecules called **dextrins** (the center of the starch molecule, called **amylopectin**, made of branched glucose molecules that amylases cannot break down). The second enzyme, **beta-amylase**, cuts the starch and the dextrins to produce molecules of the simple sugar glucose. We see here that the first amylase speeds up the action of the second one by creating a large number of sites available to the beta-amylase.

Other enzymes called **proteases** are formed to break down **proteins** into their individual building blocks, called **amino acids**. The head on a glass of beer is actually created by amino acids and smaller **peptides** (smaller than a protein but larger than an amino acid). If larger proteins remain in the beer they form the haze that we have talked about before. That is why heavily malted beers (British ales for example) are rarely clear.

We can see a dilemma arising here. The beer maker wants to wait as long as possible so that the enzymes have a chance to accomplish their tasks as well as possible. However longer malting periods result in full germination and thus loss of nutrients for yeast growth. That is because the seed starts to use energy from the breakdown of starches and amino acids to grow roots and shoots.

After 3-4 days, the grain is dried in a **kiln** to stop germination. At this point not all proteins and starches have been broken down; nonetheless germination has reached the point of diminishing returns and must be stopped. The drying also kills microorganisms but about 75% of the enzymes survive if the temperature does not exceed 150 ^{o}F (65 ^{o}C). Beta-amylase is the most sensitive of these enzymes to heat. Drying at that heat will darken the grain and if a light malt is desired, rapid air movement over the grain will help dry it and reduce the kilning period so that the grains do not roast. Longer kilning will result in a darker malt

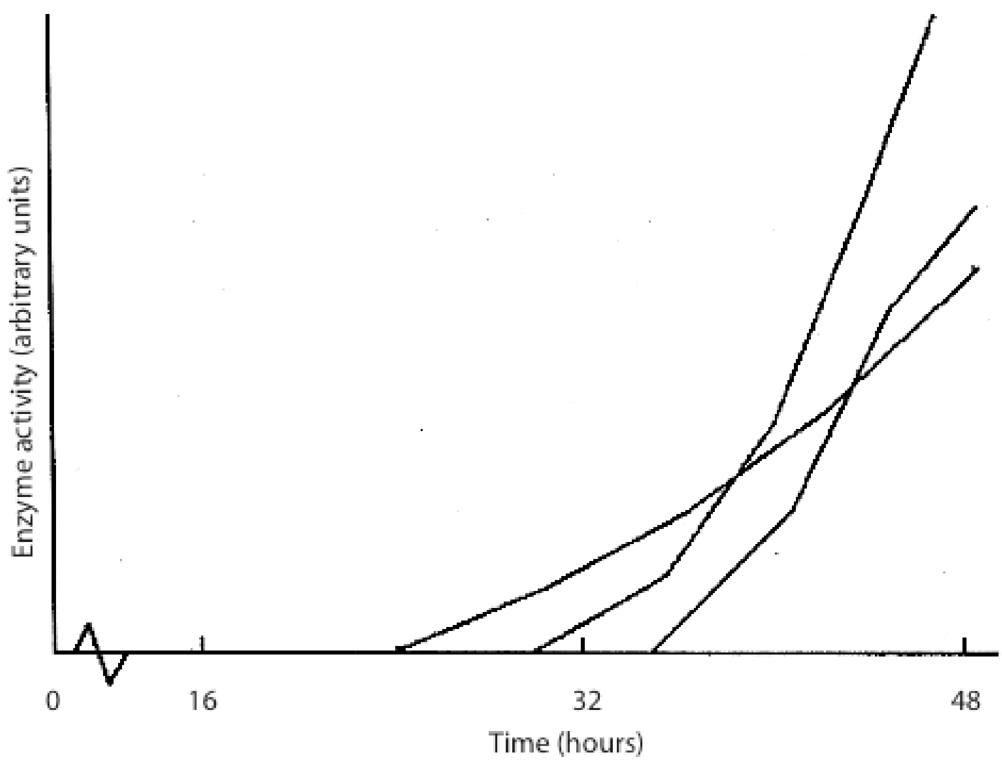

Figure 7.3. Appearance of three hydrolytic enzyme activities after the start of imbibition.

and a darker beer. The enzymes will have died during longer kilning periods and lighter malt (where the short kilning has not killed the enzymes) will have to be mixed with the dark for the mashing process to proceed.

2. Mashing.

During **mashing** water is added back to the grain in order to allow the enzymes to continue working now that the danger of germination has been eliminated by the kilning. When water is added again the grain is called the **mash grist**. Here the composition of the mash may be changed in order to:

a. Maintain the correct acidity. This will keep the enzymes functioning at maximum efficiency.

b. Maintain the correct balance of minerals that will contribute greatly to the activity of the enzymes and to the taste of the beer. We know that breweries were originally established in cities (Dortmund, Pilsen) where the mineral balance of water was perfect for beer making (Table 7.1). Today of course water processing has eliminated this need. A common mineral addition is gypsum or $CaSO_4$. Often added to precipitate phosphate ions from solution (as calcium phosphate) and free the three H^+ associated with the phosphate it lowers the acidity level of basic mash to a constant pH 5.4. We can see here that adding **gypsum** to a mash that is around pH 6.0 will lower the pH to 5.4. Calcium carbonate can also be added to increase the pH; can you describe how? Do not confuse the two compounds as they have very different effects on the pH of a solution.

A pH of 5.4 is ideal for maximum enzyme activity (Can you explain why?). Remember that yeast operate at a pH of 3 in wine making. This means that maximum enzyme efficiency varies from enzyme to enzyme (or group of enzymes in the case of yeast). The plant enzymes involved in germination work best at a pH of 5.4 while yeast enzymes work best at pH 3. That difference of 2.5 pH units by the way is a 400 fold difference in H^+ concentration.

Now while ions are necessary for proper enzymatic function they also impart a taste to the solution they are in (remember drinking “hard” water, a solution of high concentrations of calcium and magnesium). Too much calcium would give the beer a thin taste. Other ions impart different flavors to beer: magnesium is associated with a sour/bitter flavor, sodium, a sour-saline note and chloride a sweeter note. Further, some ions, such as nitrate, iron, are to be avoided because they are toxic to yeast or they impart strong, undesirable flavors.

Table 7.1. Mineral composition of water in various cities known for their beer making tradition.

Mineral (ion)	Pilsen	Munich	Dublin	Dortmund	Burton-on -Trent	Milwaukee
Calcium (Ca)	7	70 to 80	118	260	260 to 352	35
Sulfates (SO_4)	5	5 to 10	54	283	630 to 820	18
Magnesium (Mg)	2 to 8	18	4	23	24 to 60	11
Sodium (Na)	32	10	12	69	54	?
Chloride (Cl)	5	1	19	106	16 to 36	5

Values are expressed in parts per million (ppm)

- change the flavor, color, clarity or cost of the final product by the addition of adjuncts. The commercial beer maker has to take into consideration the cost of making the beer as well as the taste preferences of their customers. Barley is expensive even in the 6-row variety. Other sources of starch can be substituted in countries where these substitutions are legal (they are not in most European countries). Rice, wheat, oats, **triticale** (a cross between rye and wheat), sorghum (millet), corn and potato can be used as adjuncts to provide the starch that barley enzymes will break down in the mashing process. These adjuncts have added advantages beside their lower cost. Because of their relatively low protein content they give the beer a high level of clarity, a lighter color and a milder taste. Thus the brewer can now use cheaper barley that would have for example an unusually high protein level and would be unusable in beer making if it were not for the adjuncts added to dilute this high protein content.

Corn and rice are the most used adjuncts because they are cheap, have no taste, and, give a lighter color and a crisper aspect to the final product. They also stabilize the beer and increase its shelf life because they clear better and therefore leave less protein and other compounds in solution for spoiling microorganisms to live on.

When all additions have been made the barley (with or without adjuncts) is ground up and the mashing starts under controlled conditions of pH (= 5.4) and temperature (65 ^{o}C). This ensures the best conditions for enzyme activity. During mashing 75% of the starch is broken down to fermentable simple sugars (the rest are **dextrins**). The finer the grind the faster these simple sugars are produced and become soluble in the liquid (starch granules are not very soluble in water; remember what corn starch looks like in water). Protein breakdown is not a factor here as most of it occurred in malting. Other reactions do however occur during mashing such as extraction of vitamins, inorganic ions, tannins and lipids from the ground up barley grains.

As we said the temperature and pH of the mash give its character to the beer. A higher temperature produces less alcohol but more flavor because the enzymes breaking down the starches work less efficiently at higher temperatures but the high temperatures extract more flavor compounds from the barley. The Europeans use a 4-stage process in mashing with the temperature going from 100^{o}F to 175 ^{o}F. This first stage gives proteolytic enzymes the lower temperatures needed to finish degrading their **substrates**, the starches and the proteins. The subsequent higher temperatures are needed to extract more flavor compounds. In North America, a quick 2-stage mashing process is used: the first at 145 ^{o}F, the other at 175 ^{o}F. This accelerates the process and is adequate because protein degradation by proteolytic enzymes is less necessary because of the addition of adjuncts (Can you explain why?).

Mashing does not take very long (less than one hour). The longer process is the sparging or filtering of the mash to leave particles behind and obtain a relatively clear liquid. Sparging is done at 175 ^{o}F in order to kill all enzymes and extract as many flavor compounds as possible from the residual material that will be left behind. Can you convert these temperatures to the Celcius scale?

3. Post mashing.

The liquid obtained by sparging is the **wort**. This wort is placed in the **brew kettle** and boiled for three hours to evaporate water and concentrate flavors. Hops are also added to the boiling wort to give its flavor to the beer. The boiling also sterilizes the wort, dissolves added sugar (if necessary) and precipitates proteins that might be left in the wort. Color development also occurs as the wort becomes more concentrated and oxidizes. Steam is used as a source of heat for the boiling (hence the advertising gimmick: steam beer) although a popular beer maker in the US advertises a beer that is fire-brewed. This of course should make no difference but makes for good advertising.

Why would sugar be added here? If enough barley malt has been added to the mash there is no need for more sugar. But sugar is cheaper than malt and thus is substituted for malt to give a lighter color and flavor to the beer for the same alcohol content. This also boosts profits enormously because sugar is cheaper than barley. If too much sugar is added to compensate for too little malt however, the concentration of amino acids for yeast growth can be too low and the result can be a poor fermentation. Some also claim that the addition of sugar gives the beer a more bitter taste but I do not know that this has been shown.

The hops are added here to give the flavor that is now characteristic of beer. The best hops come from Czechoslovakia; they give a good balance between flavor and bitterness. American hops are usually more bitter and less balanced. You can grow hops in your backyard actually (that is very easy to do) and these European variety are available. Hops are of the genus <u>Humulus</u> and the family Cannabinaceae. Another genus within this family is <u>Cannabis</u> of marijuana fame. Interestingly marijuana, while an illegal crop in the North America is the biggest money maker as a cash crop, ahead of corn and wheat.

The part of the hops used for flavoring is the cone or flower that contains oils and resins. They give the beer its flavor and bitterness. However during the boiling the aroma compounds evaporate and all that is left in the wort is the bitterness. That is why more hops are added at the end of the boiling period to extract the aroma compounds into the wort. Some breweries use a pelletized hops extract for flavoring but it is considered a compromise in quality.

After boiling hops are removed with the "**trub**" or precipitated proteins by filtration or centrifugation. The wort is then rapidly cooled to 50 ^{o}F (15 ^{o}C) by cooling systems built into the kettle.

4. Fermentation.

   a. Yeast selection.

At this point yeast cells are added. We have already said that a choice is made here by the brew master as to the type to be used.

- S. cerevisiae is known as ale yeast and will float on top of the wort more easily and longer than other varieties. It will form a thick head on top of the wort. Ale fermentation is done at higher temperatures and lasts a very short time.

- S. uvarum or carlsbergensis is a lager yeast that ferments at colder temperatures, for a longer time and does not float on the beer but sinks more rapidly. It can also ferment a trisaccharide called raffinose while the ale yeast can only ferment a third of it.

Yeast cells are selected for what they can do for the brewer. Only 25% of the breweries surveyed in 1960 in England used pure cultures. The others used mixtures of up to three different strains. The reason for this is that the brewer is looking for very specific characteristics in their beer and no pure culture can provide them.

In their search for the perfect yeast brewers look for: (Figure 7.4)

- High yeast count at racking: this tells the brewer how fast the yeast cells grew and established themselves over other microorganisms. It also tells the brewer how well they survived the changing conditions inside the wort during fermentation.

- The pH value is a measure of what the yeast cells did with the

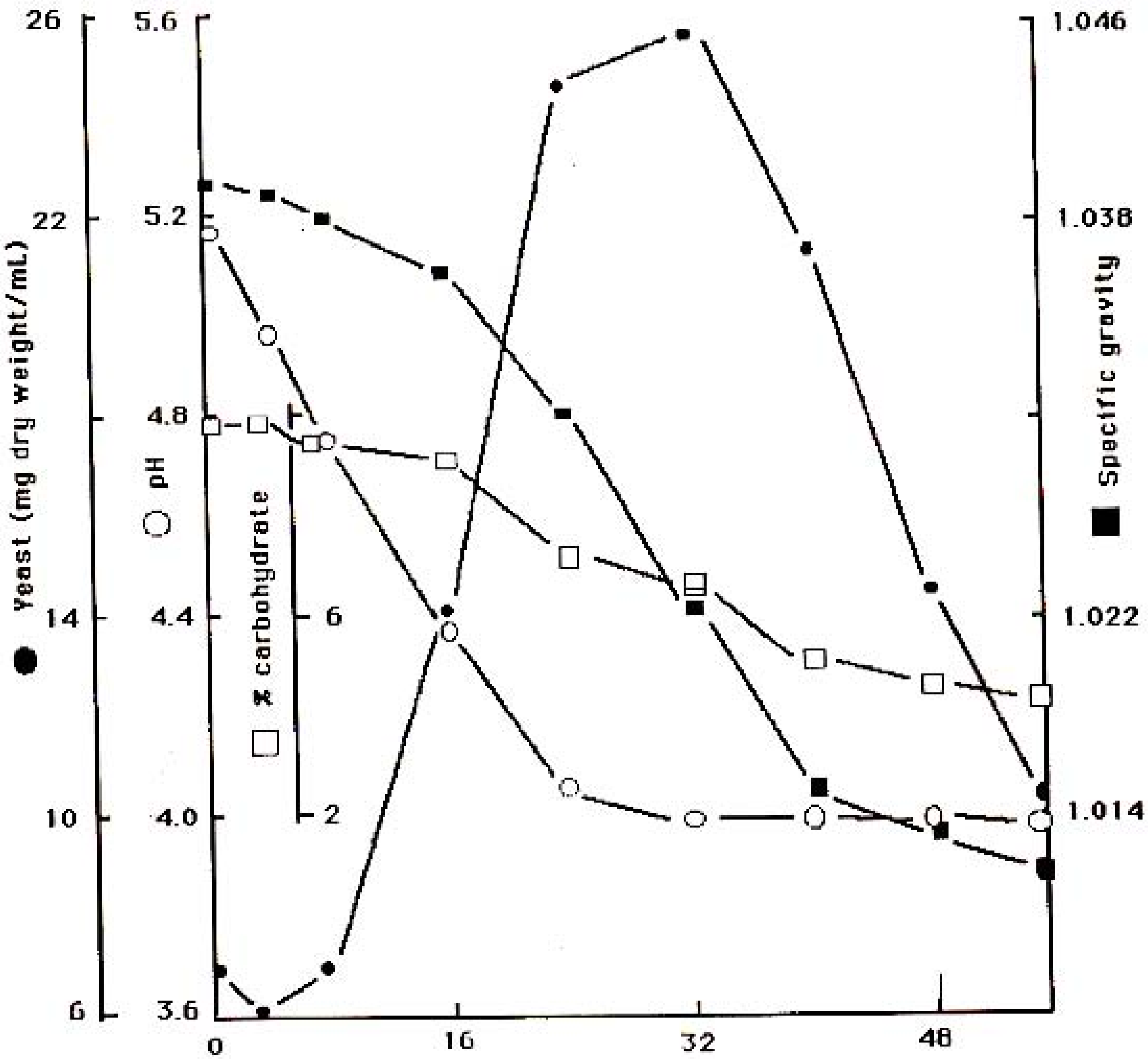

Figure 7.4. Changes in wort chemistry during fermentation. Measured parameters were: number of yeast cells in suspension ( ), pH value (o), wort gravity (■) and total carbohydrates( ).

sugar they consumed. Acids are formed by yeast which increase flavor but again a balance must be reached for too much acid means an imbalance in the flavor of the beer.

- Balanced production of flavor compounds such as ethyl acetate, fusel alcohols etc. The compounds included in fusel alcohols are chiefly: 1-propanol, 2-propanol, butanol, amyl alcohol and furfural. Fusel alcohols are formed when distillation occurs at higher temperatures and lower pH. Excessive concentrations of these fractions may cause off flavours, sometimes described as "spicy," "hot," or "solvent-like." Some beverages, such as whiskey, Siwucha (homebrew vodka) and traditional ales and ciders, are expected to have relatively high concentrations of fusel alcohols as part of the flavor profile. In other beverages, such as vodka and lagers, notable presence of fusel alcohols is considered a fault.
  Very high concentrations, usually caused by incompetent distillation, can cause acute illness, including headaches, nausea, vomiting, clinical depression, or coma.

- We already talked about how quickly yeast cells are established but the brewer also wants to know how quickly they will metabolize sugars. Here they talk about **attenuation power** or the ability of these yeast cells to reduce the concentration of sugar in the wort down to zero (What could prevent them from doing that?).

- **Flocculation** properties. The word means the tendency of yeast cells to clump together and form visible chunks that sink to the bottom (lager process) or float to the top before sinking (ale process). We must realize here that attenuation is reduced by yeast cells that flocculate very early in the process of fermenting the wort. On the other hand flocculation helps in removing yeast cells from the fermented wort. Therefore they have to flocculate after most of the sugar has been fermented. Flocculation is desirable only at the right time.

  Yeast cells are categorized into 4
  different classes based on their flocculation properties.

  - Class I: does not flocculate. The cells will stay in suspension until they die of starvation. These achieve maximum attenuation.

  - Class II: start flocculating after 2/3 of the sugar is fermented. The rest of the sugar is fermented as the yeast cells complete the flocculation and the net result is a clear, dry product.

- Class III: starts flocculating at the same time as class II but so fast that it leaves fermentable sugar behind.

- Class IV: starts flocculating very early, floats to the top to Form a strong head and produces an incomplete fermentation (for Porter).

- Head formation. Class II would appear to be ideal for the modern beer maker. However a century ago, the beer maker needed to collect yeast cells from a batch to start another one. If these yeast cells were collected after fermentation was over (i.e. at the bottom of the fermentation tank) they would be for the most part dormant and therefore slow to start growing in a new wort. That is why brewers all used ale yeast cells that, while flocculating, attached to carbon dioxide bubbles and floated to the surface, still fermenting actively. This meant the formation of a strong head on the fermenting wort. A fraction of this head (**barm**) could easily be harvested to start a new batch. Therefore flocculation was very important and had to start early enough during fermentation to yield a head made of actively fermenting yeast cells.
  Because lager yeast showed poor adhesion to carbon dioxide bubbles they could only sink as flocculation started. Thus these yeasts were not useful to the ale brewers who relied on previous batches to inoculate new ones.

- Haze level: hazes are more pronounced with yeast cells that do not flocculate easily. In most cases these are undesirable. However remember that British ales for example are "muddy" to start with because of their high levels of malt. In this case therefore the appearance is taking a back seat to flavor. It is also important to remember that the head on top of the finished product is a result of proteins left behind. Again a good balance must be struck to obtain a clear beer with a good head.

- Haste test; whatever the brewer had in mind.

5. Fermenting systems.
We will take a look at three different fermenting systems.

a. The traditional ale fermenting system. The original system was an open vat called also a skimming system. Today the vessel is enclosed which makes it harder to skim but then again the inoculating cultures are bought instead of recycled. The system also brews beer one batch at a time which is less efficient than the modern continuous fermentation systems. We will come back to that. A lot of microbreweries in England and North America are still using the old skimming system for the sake of tradition. The cost is higher but is reduced by the use of centrifugation to get rid of the yeast cells after fermentation. Air must be blown in the wort continuously in order to keep the highly flocculent yeast cells into suspension so that the sugar is all fermented. Fermentation of ales is done at high temperatures and is completed in 60 hours (less than 3 days).

b. The lager system. The word lager means to store. It originated in Germany around 1800. At low temperatures (as low as 10 ^{o}C) the fermentation is done over a long period of time. At the low temperature of fermentation it takes a long time to ferment all the simple sugars available to the yeast cells. After an initial fermentation (called primary) of 40 hours the wort is transferred to a new vessel leaving the sludge behind. The beer is stored at very low temperature (down to 35 ^{o}F sometimes) for periods of up to 3 weeks. This is often called a secondary fermentation even though it is not really and refers to the simple storage of the beer at a colder temperature for finishing. No persistent head is formed because the yeast cells do not adsorb to carbon dioxide bubbles very well. Aging or conditioning follows this secondary fermentation at near freezing temperature (for up to months) during which the body and flavor of the lager develop. The alcohol content of the lager is lower than that of an ale (3 to 4 % versus 4 to 6%) because the low temperature of fermentation has eliminated the need to put extra malt in the wort.. These cold conditions give the beer a milder flavor.

c. Continuous fermentation system (factory-made beer) (Figure 7.5). Here the wort is pumped in a first vessel under controlled conditions to keep the yeast cells growing at maximum efficiency. In this first vessel 1/2 of the sugar is fermented. The beer is then transferred to a second vessel where sugar is fermented further. In the third vessel the beer is cooled in order to flocculate and settle the yeast cells. Here then the yeast cells have to flocculate only after full attenuation (class I). Beer will float on top of the heavier wort and is pumped to the next tank, to the next, to the next. As fermented beer is pumped out of a tank into the next one, fresh wort is pumped in. Once in a while dead yeast cells and sediments are pumped out of the bottom of the tanks. This allows for continuous fermentation.

Fermentation then lasts a very short time in the case of ales to a very long time in the case of lagers. Only the first 12 hours however are aerobic to allow for subsequent alcohol formation.

6. Maturation and conditining.

a. Lagers. Traditionally, after fermentation, lagers are transferred to casks at 2 ^{o}C for up to nine months (the **lagering** process). This removes unwanted volatiles created during fermentation as casks are vented periodically. It also clears the lager by flocculation, clears the hazes and finishes the fermentation of residual sugars to provide the beer with carbonation.

b. Ales are conditioned (or aged) more rapidly so that finings must sometimes be added to clear them. Ales are carbonated by the addition of sugar or wort (Kraeusen). Volatiles evaporate quickly because of the high temperatures. After conditioning the ales are stored at -2 ^{o}C for three days to eliminate the haze and the remaining floating yeast cells. Even so a traditional ale is more cloudy than the lager because of the rapidity of the fermentation process which prevents complete precipitation of suspended solids and the fact that more malt was used thus increasing the concentration of protein in solution. Are there other reasons???

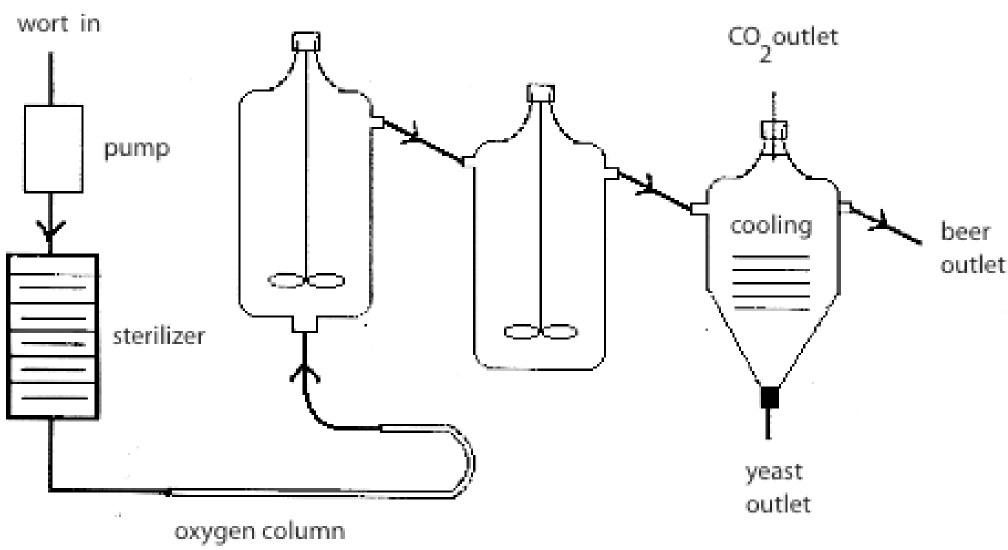

Figure 7.5. Schematics of a continuous fermentation system.

c. Today conditioning and maturation of ales and lagers are done chemically or mechanically. Clearing is done by centrifugation and hazes are eliminated with finings or enzymes. Lagering time can be cut down by chemical and mechanical means. Carbonation is done by pumping $C0_2$ into the beer. So though the beer you buy at the store is called a lager it really is not except in its taste, reminiscent of that of a lager. It would be more accurate to simply call it a "**ten-day-beer**", the time it now takes to produce it. Interestingly not all large scale beer makers use this process. Anheuser Busch for example ferments its beer for 5 to 7 days at low temperature and subsequently lagers it for 2 days also a low temperature.

7. Pasterurization.

The filtering that a beer goes through removes particulate matter but not microorganisms. In most cases modern factory-made beer is pasteurized before shipping. Pasteurization simply means the use of heat to kill microorganisms. Of course pasteurization is very likely to change the taste of beer and give it a caramelized flavor much like boiled, no-alcohol beer. However beer makers now use a technique called flash pasteurization in which the beer is very quickly heated up to 70 ^{o}C (160 ^{o}F) and kept there for a total of 20 seconds. The beer is then bottled and heated again. That is enough to kill bacteria, molds and yeast. This ensures stability and allows for long transit times during shipping. Of course special (and expensive) filters can replace pasteurization. That is why we have witnessed on the airwaves an advertisement battle between heat pasteurized and cold filtered products. But whether the beer is filtered or pasteurized its flavor has been changed because the yeast cells were removed from the beer so soon after fermentation that the aging process was not allowed to foster exchanges between the yeast and the beer as the former slowly settles out of the solution. Microbreweries use traditional beer making techniques and do not pasteurize. This means that their products must be consumed relatively quickly and this limits the range of distribution to local or regional areas. But their products are usually far more flavorful than Budweiser or Molson.

One final note here on bittering (making the beer more bitter). This is sometimes done by putting hops in the beer after rather than before fermentation. The reason for this is that the pH of the beer after fermentation is more favorable to the bittering process. This means that more of the hops' bitterness can be extracted under the conditions present in the beer after fermentation.

III. EVOLUTION AND THE BEERMAKING PROCESS.

Now that we are familiar with the process of beer making it is a little easier to look at the evolution of beer making and the various advances in the process over the millennia.

Up until 1850 the fermentation of beer was done by top-fermenting yeast cells that rose to the top with carbon dioxide bubbles during fermentation. The brewer would take yeast cells from previous batches and add them to the new wort to start a new fermentation. The brewer used an open vat which he kept in a cellar to maintain a relatively constant temperature. The temperature in this cellar would rise however because fermentation produces a lot of heat and refrigeration was unknown at that time. The challenge was to keep other microorganisms, especially acidifying bacteria that grow well at higher temperatures, from competing with yeast. The key to winning this battle was to heavily malt the beer so that yeast cells could have a rapid initial growth phase. This of course produced ales with high alcohol contents. At that time hops had not yet been introduced in England and the definition of ale was that of a malt liquor with various herbs and barks added as preservative (bog myrtle was a popular addition to ales). In contrast beers made in the rest of Europe used hops as a flavoring agent and preservative. After hops were finally accepted in England in the making of ales as well as beers, the distinction between beers and ales became simply a question of the quantity of malt used in the process and therefore the alcohol content.

While the process of lagering had been known in Germany since the 15th century, in the early 1800s the Germans found that the control of fermentation and of bacterial contamination depended in great part on the temperature and the length of fermentation. **Acidification** (a sign of bacterial contamination) was prevented at temperatures below 75 ^{o}F. Ideal temperature for beer making was between 55 ^{o}F and 60 ^{o}F. In those days these temperatures could be achieved only in the winter time. In summer the best temperatures attained were between 60 ^{o}F and 75 ^{o}F. Therefore bottom fermenting yeast became popular in Germany because its low fermentation temperature (40 ^{o}F to 45 ^{o}F) made it possible to limit spoilage by other microorganisms but only in beers made in the winter. It also produced a beer that was much milder in taste, again because of the low temperatures used during fermentation. These beers were made during winter, the only time of the year when cellar temperatures reached 40 ^{o}F to 45 ^{o}F. During the rest of the year ales were produced. This even became a law in Bavaria where lagers could be produced only from October to February.

In the US in 1870 the invention of refrigeration eliminated the distinction between winter lagers and summer ales. The making of lagers became possible all year long and the public demanded more and more of this milder tasting product.

The original lagers were brewed in Germany with top fermenting yeast cells, in winter only. The only difference with ales then was the lagering process used because the lagers were still heavily malted and hopped. The product was still lighter in color and flavor than the ales produced at the time. Later refinements replaced the top fermenting yeast cells with a bottom fermenting ones and reduced the amount of malt and hops used in the recipe. Further refinements especially in the US added adjuncts to further lighten the color and flavor of the beer.

a. German lagers.

The lagers were to become extremely popular in Germany and the rest of Europe and several types were developed. These are only a few of the types developed:

-Dortmunder
-Pilsener
-Vienna
-Munich
-Octoberfest
-Bock
-Weiss

Today the term Pilsener has come to signify lager but as we have said before the term referred to a lager made in the town of Pilsen and showing characteristics defined more by the water used for the beer making (In which stage of beer making is water used?) than anything else. The term Dortmunder is not used anymore but then meant a lager made in that town.

Vienna lagers used less hops but more malt than Pilseners and Dortmunder and showed a darker color and stronger malt flavor.

Munich was a dark lager, full-bodied with a sweet malt flavor and light hops taste; the recipe was similar to that of a Vienna except for the addition of roasted barley. Nowadays caramelized sugar is often used to darken a beer at a cheaper price. The alcohol content of the original Munich was high at 5% reflecting the addition of extra malt to the wort.

Octoberfest was a light lager made in September only (in order to be ready for October harvest festivities) with a high alcohol content. How was that possible?? Can you come up with a hypothesis?

Bock beer came from the town of Einbeck and was originally a heavy dark lager beer with strong malt and hops flavors (and a high alcohol content). It was brewed in winter with a top-fermenting yeast for consumption in the spring. It had been originally developed as a high alcohol beer (10%) for export purposes. Why would the high alcohol content be related to export???

Weiss lagers were also made in Einbeck as the first prototype of lager beers made with adjuncts. Weiss means white because in contrast with its darker contemporaries, Weiss was made with a mixture of wheat and barley.

b. Other lagers.

Today in North America you will encounter beers with names like Vienna or Bock but these beers share with their ancestors in name only. The modern products are made by a totally different method (continuous fermentation, no lagering time, use of adjuncts) and with very different ingredients (6-row barley versus 2-row barley). These beers are all made by the same process and the differences between Miller and Budweiser or Molson are more in their advertisements than in their beer.

Before Prohibition however true variety existed in North America. An example of this is the steam beer made on the West coast. Because of ice shortages the beer was made at 60 to 68 ^{o}F, with barley malt only (i.e. no adjuncts were used) and fermented for 5 days. The beer was then put in barrels with Kraeusen and finings and capped. After 4-6 days the pressure in the barrels or "steam" was released (up to 50 psi or pounds per square inch, a measure of pressure. Compare that to the 32 psi in an average tire) and the barrels shipped. At the saloon the barrels had to be left to settle for a while before use (Why?). The beer was tapped directly from the barrel and for this reason called a “draught”. Today we use the terms “on tap” or draught” or “draft” to refer to a beer poured directly from the barrel.

Of course the resurgence of microbreweries nowadays adds a whole lot of diversity to the offerings available to the public. However in 1995 microbreweries held less than 5% of the North American market.

c. The latest lagers: light beer, dry beer and ice beer.

Light beer was first introduced by the Rheingold brewery in 1967 for the diet conscious beer drinker. Since then the light beer market has grown to about 40% of the total beer market. Light beer contains less dextrins than regular beer to reduce the number of calories. This can be achieved in several ways:

- add water to the beer. This will lower the concentration of dextrins. It will also lower the alcohol content and the flavor of the final product.
- Add alpha amylase to the mashing. Alpha amylase is already present in the mashing but the added enzymes insure complete degradation of the starches to dextrins. This means that more alcohol can be produced with the same amount of malt. It is then possible to reduce the amount of malt used and still produce a high alcohol content. However using less malt means reducing the flavor of the final product.
- It is possible to add another enzymes to the mash that can break down dextrins into simple sugars. This reduces even further the amount of malt needed to make beer but also reduces further the flavor of the beer.

Regardless of the process used, light beer has less flavor than regular beer since it has been diluted or the amount of barley malt used has been reduced.

Ice beer is based on the old German eisbock beer. Developed in the early 90s by Canadian brewers it was meant as an alternative to dry beer for flavor and microbrews for alcohol content.

After the lager beer has been fermented it is cooled to 24-28 ^{o}F and stored for several days to allow the water in the beer to form ice crystals. During this time compounds that give its bitterness to the beer stick to the ice crystals. These crystals are then removed by filtration. The result is a less bitter product with an increased alcohol content. The alcohol content is 12% higher than in regular beer because 12% of its water content was removed in the form of ice. Since 1994, the year of its introduction, ice beer has captured 5% of the North American market

As we have said earlier in this chapter yeast cells lack the enzymes necessary to digest starches completely. A lot of dextrins are left in the beer after fermentation is done. However since the advent of biotechnology it has been possible to add to the genome of yeast cells the genes coding for those enzymes that can break down dextrins. What is the difference then between dry beer and the light beers produced by the third process shown on page 27? In the mid 1980s Japanese brewers used these engineered yeast cells to brew a beer that contained less dextrins and therefore less calories (10% less calories). The product also had less of an aftertaste. It was called dry beer by analogy with wine which is said to be dry when it contains little or no sugar. This product was popular in the late 80s and earlier 90s but has been in decline ever since. It may have just been a fad but who knows.

d. Other types of beer.

- English bitters are brewed from pale malts with rice or corn added to make the beer lighter in color. Extra hops are added to make a truly bitter beer.

- English brown ales (Whatney's, Bass etc.) are heavier in malt and produce high alcohol levels.

- Stouts are made from roasted unmalted barley (which differentiates it from a porter) and roasted malt (malted barley) added to normal malted barley to give the beer an intensely dark color. They were also heavily hopped to balance the strong burnt flavor of the roasted malt.

Porter is the ancestor of stouts and was made from brown barley malt (malted barley), that was kilned over wood fires and added a brown color and a bit of a smoky flavor to the beer and mild hops. No barley grain was used. Robust porters were black in color and had a roast malt flavor but no roast barley flavor. These porters had the sharp bitterness of black malt without a burnt/charcoal flavor. The final product had a sweet malty flavor and mild, fruity hop aroma and flavor. The sweet flavor came from the fact that a lot of starch was left in the beer. The enzymes in your mouth (alpha-amylase) hydrolyze the starch into simple sugars giving porter a sweet flavor. Brown porters were paler in color. They lacked the roast barley or strong burnt malt character but still had a sweet flavor along with medium hop bitterness. Porters were a big seller in Europe in the 18^{th} century and a favorite of George Washington who developed his own recipe. The robust porters evolved into the Stout (without the malty sweetness) while milder porters were eclipsed by brown ales. In fact the stout was at one point called Stout Porter.

- Malt liquor today refer to beers with an alcohol level higher than 5%. The law regulates very closely the percent of alcohol in beers for taxation purposes. A product designated malt liquor will be taxed more heavily than a beer. In the US a beer has an alcohol level of 3.4 % (for premium beers) or less. In Canada most beers have 5% alcohol but malt liquors have more (Brador, a popular malt liquor is 6.2% in alcohol). In Europe some malt liquor have up to 8 or 9% alcohol but such high levels of alcohol often affect adversely the taste of these products*.*

- Sake is a Japanese germinated rice ferment. Based on a tradition that dates to before 800 BC the Japanese have substituted malted rice for malted barley and produce a high alcohol beverage that is consequently considered more a wine than a beer.

Answers to chapter questions.

p. 12: Calcium carbonate ($CaCO_3$) can also be added to increase the pH of a solution; can you describe how? A high pH means a low concentration of H^+. The carbonate ion in Ca CO_3 is in fact the dissociated ion of carbonic acid (H_2 CO_3). When carbonates are placed in solution they will try to reform H_2 CO_3 by combining with free H+ ions:

$$2\,H^{+} + CO_3^{--} \rightarrow H_2CO_3$$

This reaction lowers the concentration of free H^{+} in solution and therefore increases its pH.

p. 12: A pH of 5.4 is ideal for maximum enzyme activity. Can you explain why?

Enzymes are sensitive to the presence of ions such as H^{+} in solution. They function best in specific concentrations of these ions.

p. 15: In mashing why is protein degradation by proteolytic enzymes less critical when adding adjuncts? The presence of protein will add an undesirable haze to the beer. Hence the necessity of proteolysis during mashing. However since adjuncts contain less protein that barley, this process becomes less critical.

p.15: Can you convert 175 ^{o}F to the Celcius scale?

Remember the steps of dimensional analysis.

1. Write down first what you have and what you are looking for:

$$175\ ^{o}F = ?\ ^{o}C$$

2. Remember the conversion:

$$\frac{(^{o}F - 32\ ^{o}F) \times 5\ ^{o}C}{9\ ^{o}F} = ?\ ^{o}C$$

3. Make sure that the units cancel out to leave you with the units you want (in this case, ^{o}C)

4. Finish the operation:

$$\frac{(175\ ^{o}F - 32\ ^{o}F) \times 5\ ^{o}C}{9\ ^{o}F} = 79.4\ ^{o}C$$

p. 18: What could prevent yeast cells from reducing the concentration of sugar in the wort down to zero ? Yeast cells could die from their low tolerance to alcohol or lack of nutrients.

p. 21: Are there other reasons that can explain the cloudiness of ales? The presence of yeasts in the ale could, at least in part, explain the cloudiness. Because the processing time for ales is so short it is possible that not all yeasts precipitated.

p. 25: In which stage of beer making is water used? During mashing the malted grain is ground in water to enable enzymes to finish breaking down starches and proteins.

p. 26: How could you produce a lager with a high alcohol content? Start with more barley or adjuncts to increase the sugar content of the wort or, add sugar to the wort.

p. 26: Before refrigeration beer produced for export (Bock or India pale ale) had a high alcohol content. Can you explain why? An alcohol content of 9% or higher was necessary to insure preservation of the beer during the long trips overseas.

p. 26: When steam beer arrived at the saloon the barrels had to be left to settle for a while before use. Can you explain why? Since the beer had been made in the same barrels used for shipping it contained yeasts which had to settle before use.

p. 28: What is the difference between a dry beer and a light beer produced by the third process shown on page 27? Both beers are made with an enzyme that can break down dextrins. In the case of light beer the enzyme is added to the mash or the fermentation by hand and dextrins are broken down during mashing or fermentation. For dry beer this enzyme is actually produced by the engineered yeasts during fermentation.

EXERCISES.

1. Distinguish a wort from a beer.
2. What distinguishes a porter from a stout?
3. Did women cause Prohibition? Explain.
4. Compare the use of 2-row and 6-row barley in beer making.
5. Organize the following according to size: starch, glucose, polysaccharide.
6. In question 5 which can yeast cells use as food?
7. What does boiling the wort accomplish?
8. What is scientifically common to hop-s and marijuana?
9. Where does beer flavor come from specifically?
10. How can you make a dark beer?
11. Explain why the American lager beer evolved into the light-colored, light-tasting product we know now.
12. Why can't yeast cells use starch as a food source?
13. Describe the malting process.
14. Why do brewers sort grains before malting?
15. Give an example of an enzyme that degrades starch. What types of enzymes degrade proteins?
16. In Figure 7.3 when is the activity of endo beta glucanase equal to that of peptidase? What is the difference in time between that and when the activity of alpha amylase equals that of endo beta glucanase?
17. Do all enzymes work best at pH 5.4?
18. Why not add enzymes to the mash before kilning?
19. Why would you want to change the composition of mash?
20. Explain the meaning of “high attenuation power”.
21. Summarize the characteristics that brewers look for in yeast.
22. What are the advantages and disadvantages of using other types of grain in the mashing process?
23. What would cause a beer to be hazy?
24. Is pasteurization of beer the same as pasteurization of wine? Are the results the same?
25. Why is malting necessary in the beer making process?
26. Explain the differences between 2-row and 6-row barley.
27. What is an adjunct? Name some adjuncts.
28. For what reasons are adjuncts used in the beer making process?
29. Why are hops added twice during the beer making process?
30. Why is beer fattening?
31. Name some of the qualities a brew master looks for in yeast.
32. Why is a mix of yeast strains used to make beer?
33. Describe what in our view would be an organoleptically pleasing beer. How can you control the production of organoleptically pleasing compounds?

34. Describe the four types of flocculating yeast.
35. Describe the continuous fermentation system.
36. In which way does sake qualify as a beer? In which way does it qualify as a wine?
37. In the 1920s and 1930s immigration to the US was at its highest in history. How could this have played into the governmental decision to impose Prohibition?
38. Name two local microbreweries and their location. Based on the information given in the first pages of this chapter classify: Michelob extra, Guinness, Saranac light and Dos Equis dark.
39. What type of fermentation would you use to make beer for your family? If your first batch turned out well would you switch to a different system in order to make larger quantities? Explain.
40. Why did beer change with the seasons in the 18oos?
41. Based on the definitions of different types of beer given in this chapter what type of beer do you drink more often? Why?
42. If money was no object what beer would you choose to drink? Explain.
43. Describe the characteristics of yeast that are important in producing beer versus wine.

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LESSON 8. LIQUORS, SPIRITS AND DISTILLATION

No Figures
1 Table
1 Illustration

A. INTRODUCTION.

Beers and wines are limited in their alcohol content by the natural ability of yeasts to resist the poisoning effects this waste product of their own metabolism. However it is possible to obtain alcoholic beverages that have higher alcohol contents. Do you know how it is possible? The processes employed to obtain such high alcohol levels must all be capable of concentrating the alcohol. One of these processes uses the freeze and thaw method to concentrate the alcohol in beer (ice beer) and cider (apple jack). We have mentioned these two products in previous chapters. The other process is called **distillation** and we will learn more about it in this chapter. A fermentable material must be used first to produce an alcoholic beverage. Grains, fruits or vegetables, concentrated sugar such as molasses or honey, or milk provide this starting material. As we can see the variety of available distillates depends in great part on the material used for the original fermentation.

Yeast then ferments this material to provide a solution of between 3 and 12 % alcohol. This solution is then heated in a process of distillation to evaporate the alcohol preferentially over the water. This increases the alcohol percent to a maximum of 95 % (190 proof), even though most of these beverages are sold at 40% alcohol content (80 proof). The term “proof” refers to a technique used originally to confirm the high alcohol content of different spirits. The alcohol was mixed with gunpowder and lit. If the mixture flashed the alcohol content of the liquor was “proved”.

Can you name the starting material used to make:
Rum
Whiskey
Bourbon
Vodka
Dry gin
Sour mash
Calvados
brandy

The Encyclopaedia Brittannica lists in its article on 'Alcoholic Beverages' the following dates and places of origin of several distilled alcoholic beverages

TABLE 1. Chronology of distillation products appearance in human history.

DATE	MATERIAL	FERMENT	DISTILLATE	ORIGIN
800 BC	rice millet	Tehoo	Sautchoo	China
	rice molasses palm sap	Toddy	Arrack	Ceylon India
	mare's milk	Kumiss	Arika	Tatars
	mare's milk	Kefir	Skhou	Caucasus
	rice	Sake	Sochou	Japan
500 AD	Honey	Mead	Distilled Mead	Brittain
1000 AD	Grape	Wine	Brandy	Italy
1100 AD	Oats Barley	Beer	Usqubaugh	Ireland
1200 AD	Grape	Wine	Aqua Vini	Spain
1300 AD	Grape	Wine	Cognac	France
1500 AD	Barley	Beer	Whisky Aqua Vitae	Scotland

As we can see from this table the process of distillation has been used by humans for almost 3,000 years. We know of the distillation of sake in China around 800 BC. Documents show that distillation existed in Europe in the 12th century. A drink called uisge beatha , made from the distillation of fermented oats and barley, the ancestor of whiskey in name and in practice, already existed in Ireland when the Norman invaders came in the 12th century.

In the US in 2003 the sale of spirits was a 45 billion dollars a year business. In the early colony people distilled fermented extracts of pear, apple, cherry and grains such as rye and barley and, sugar cane imported from the Caribbean islands. When England stopped the import of molasses to the US farmers started distilling whiskey from grain mash fermented from barley, corn, rye. Georges Washington produced rye whiskey at Mount Vernon from 11 pot stills.

In 1792 a tax was raised by the government against all distilled spirits in an effort to raise revenues. Rebellious farmers took refuge in Kentucky where corn was plentiful and the water, rich in minerals but with very little iron, was perfect for fermentation. Corn was crushed into a grist and rye, barley or wheat added. The sour mash produced by fermentation was distilled. This distillate was aged for 2 years in charred wood barrels. The charring produced 150 flavor compounds, six of which similar to vanilla. The end product was called bourbon. Today bourbon must be made in the US, from at least 51% corn and aged at least 2 years in charred oak barrels to be used only once. Since different barrels age differently the content of these barrels is blended before bottling.

Tennessee whiskey is similar to bourbon except that the distillates are steeped with charcoal before bottling.

Scotland produces a spirit from fermented barley and calls it Scotch. In single malt scotch barley is spread on the floor and allowed to germinate. The germinated grain is placed in a kiln where peat is burned to dry the grain, stop germination and introduce smoke flavor into the grain.
The dried barley is then ground and mixed with water for fermentation. Water is critical to germination and flavor as we have seen in chapter 7. After distillation the product is aged in barrels (often from US bourbon distilleries) for 12, 15, 30 or 40 years. Single malt scotch is unblended whiskeys made from 100% barley while 90% of all scotches are blended from unmalted barley, malted barley and corn whiskeys.

DID YOU KNOW:

In 1992 a bottle of 50 year old Glenfiddich wiskey sold for $79,552.

Agave is a cactus that grows in Mexico only. Its fleshy leaves cover the pineapple-shaped heart of the plant, or pina, which contains a sweet sticky juice. The blue agave can take up to 12 years to mature. At maturity leaves are cut away from the pinas which are then cooked for 24 hours to soften the flesh and produce sugars from starches. The juice is then extracted from the pinas, fermented and distilled into Tequila. Only blue agave can be used in the production of tequila and the juice from an average size agave plant can produce 9 bottles of tequila.

Gin is a British tradition that dates to the 17th century even if it was originally developed in the Netherlands. In 1733 11 million gallons of it were produced (6 times more than beer) in England or 14 gallons per capita, attesting to its popularity.
A mash of corn, wheat, barley is first fermented. The product is distilled once and the base spirit is then steeped for 24 hours with a mixture of juniper berries and seeds, coriander, bitter orange peel, lemon, almond, licorice, angelica roots, orris roots (a type of iris), cinnamon and cassia bark, or, any combination of the above and then distilled again, usually in a column still. Dry gin simply refers to the distillation process and not the flavoring.

B. DISTILLATION.

1. THE STATES OF MATTER.

Let's look at three substances from our fermentation experiments: sugar, grape juice and carbon dioxide. At room temperature the first is a solid, the second is a liquid and the third is a gas. On the other hand if we vary the temperature we can change the state of these substances. For example I could boil the grape juice and make a gas out of it. So it seems that at least two factors can affect the state of matter: temperature and a property intrinsic to matter. Let's see how these two interact to produce liquids, solids and gases.

a. Molecular attractions. We notice that it is easy for us to walk through a gas. We can walk through a liquid with more difficulty but cannot walk through a solid. The reason is that in a solid, molecules stay very close to one another and keep a rigid configuration in relation to one another. In a liquid the molecules are also close together but are not kept in a rigid structure and can roll over one another. In a gas molecules are actually very far away from one another.
Molecules attract each other in a variety of ways. For example ions that are charged positively attract ions that are charged negatively. These ionic attractions are actually the strongest forces of attraction and keep substances in rigid structures that we call solids. Other forces are less powerful and can keep molecules close together but not in rigid structures; we call these liquids . And some others are even less powerful and produce gases.

There are four basic **forces of attraction** between molecules and they are different from one another. In water all molecules are under the influence of the same attraction forces. In gasoline the same is true but these forces are different than those holding water molecules together. And because these forces in water and gasoline are different water and gasoline are not attracted to each other and do not mix. Gasoline actually floats on top of water. On the other hand ethanol and water have similar attraction forces and therefore attract each other. For this reason ethanol and water mix together. We call this mixture beer or wine for example. We can then generalize and say that if two substances have similar forces of attraction, they will blend together. We say that these substances are **miscible**.

b. Temperature. When temperature is increased molecules start to move faster. When temperature is decreased molecules move more slowly. Now the strength of a given attraction force never changes but an increase in temperature can increase the movement of particles and counteract the effects of these attraction forces. Therefore a liquid can be heated to become a gas. Conversely a decrease in temperature will not counter the effects of attraction forces which will make a solid out of the substance being cooled. Liquid water can be cooled into solid ice.

2. VAPOR PRESSURE AND EVAPORATION

A glass of water left on the counter top will evaporate over time. If the temperature is raised evaporation will occur faster. But now let's assume a liquid that is not held together by strong attraction forces like water is. Would it evaporate faster than water? The answer is yes. Can you describe what accelerates the evaporation?

The gas formed on top of the liquid that evaporates creates a pressure, a vapor pressure. The **vapor pressure** increases when the temperature rises. As the temperature rises the vapor pressure rises until it reaches the same level as the atmospheric pressure, the pressure created by all the molecules of air in the atmosphere pushing down on things. The liquid is then said to be boiling. The temperature at which a liquid boils changes depending on the strength of the attraction forces. If the attraction forces are strong the liquid boils at higher temperatures. So if I said that ethanol boils at 78 ^{o}C while water boils at 100 ^{o}C what could you conclude from this information?

3. DISTILLATION

Distillation uses the fact that different liquids boil at different temperatures to separate them from one another. Let us take a mixture of ethanol and water consisting of 5% ethanol. If the temperature of the mixture is raised to 78 oC the ethanol will boil away but not the water. The alcohol vapors can be collected in a cooling column and cooled down to a liquid. The alcohol concentration in the collected liquid is now much higher than in the original solution. At this point only the alcohol has been collected. It is too concentrated for safe consumption and does not have any flavor. The flavor compounds and the water have been left behind. It is then the practice to raise the temperature further in order to boil and collect water and flavor compounds.

Remember that we said there were two ways to concentrate alcohol from a solution. The other way is the opposite of boiling it, that is freeze it. As we have said that different liquids have different boiling points, they also have different freezing points. Ethanol freezes at a lower temperature than water. If we freeze a solution of ethanol and water the water on the outside of the container will freeze first and push the ethanol molecules toward the middle. The process will continue until a small volume in the middle of the container has accumulated most of the alcohol, flavor compounds and some water. Apple cider is treated this way by farmers in upper New York state to make apple jack.

4. TYPES OF DISTILLATES.

a. Brandy and Cognac. One of the best known drinks in the world, the distilled spirit of wine is known as brandy. It is also called Cognac if the brandy was made in the region of Cognac in France. Let's look at the making of Cognac as the prototypical brandy.
As early as the 12th century the region of Cognac was known for its white wines. As we have seen in chapter 2 this region had already standardized its wine making practices and grew a white vinifera grape called Ugni blanc and produced a wine that was known as far away as England. Between the 12 and the 14th century the practice of distilling this wine became more and more widespread. The wine used for distillation was usually between 8 and 10% alcohol and the brandy produced was 75% alcohol. Distillation was (and still is) performed in copper pots and cooling tubing and was performed twice. The reason is the presence of other alcohols and esters present in the wine as a result of yeast metabolism. These compounds are responsible in part for the hangover we experience after drinking. As the ethanol is concentrated by the distillation process so are these alcohols. The

process of distilling twice eliminates some of these compounds and makes the consumption of these distillates more pleasurable. These distillates re then aged in oak barrels for decades sometimes in order to infuse color and flavors into the brandy.

b. GRAIN DISTILLATES. As we have seen in chapter 7 grains can also be a source of sugar and nutrients for yeast. The resulting beverages are loosely called beer. While barley has traditionally been used in this endeavor other grains can be used as well. When the beer is to be distilled it is not flavored with hops or other herbs. After malting and mashing the grain mash is fermented much as beer would be. The resulting solution is about 5% alcohol. It is then distilled into whiskey (wheat), rye (rye), bourbon (corn), a 40% or higher alcoholic beverage. The distillate is often aged in oak barrels for up to twenty years. Blending is also used to produce similar quality products year after year. A notable exception is the single malt whiskeys of Scotland and Ireland which come from the same batch of grain.

c. OTHER DISTILLATES. As we have seen in the introduction a wide variety of sugar containing products are also used to produce alcohol by fermentation. Potatoes, molasses, honey, berries and milk are fermented and used to distill well known types of liquor. The most famous are vodka, rum and gin but the list is by no means limited to these.

d. Liqueurs and Cordials. The terms refer to distilled brandy or whiskey in which fruit or spice flavors and sugars have been dissolved. Do not confuse the term liqueur with liquor, the latter referring to the distilled alcohol only. Literature on these drinks appeared in13th century Europe as the work of alchemists. These drinks were believed to have medicinal properties and their recipes God inspired. Royalty and the very rich initially consumed them, first in Italy and then in France but their use eventually spread out to the middle class. From the 13th to the 17th century monks and alchemists produced these potions and elixirs and recipes appeared throughout Europe. Benedictine, Chartreuse, Carmeline and Trappastine were only a few of these liqueurs dispensed by monks as elixirs of life. It is ironic since some of these drinks contained plant extracts that were quite poisonous. Oil of bitter almonds is now known to contain cyanide and Anisette and oil of wormwood contain compounds that cause brain damage. Today it is illegal to use these ingredients and they have been replaced by less toxic ones. The recipes were secrets jealously guarded by the monasteries and to this day only a handful of people in the world know the recipes to some of these liqueurs.
By early 17th century the monks had started to lose their control of the market and several distilleries were producing their own liqueurs.

Flavor compounds are extracted from fruits, herbs, spices and other plants by one of three methods: maceration, distillation and percolation.

In maceration the starting material is steeped in water or alcohol for a period of time. The flavor compounds, often referred to as the essence, are dissolved in the solvent which is then added to the brandy or whiskey to be flavored. The flavor compounds can also be distilled from the macerated starting material. This permits the isolation of the pure essence from other compounds and the solvents thus producing a very strong flavored extract. In the last method the solvent (water or alcohol) is dripped repeatedly over the starting material or its steam in passed over the material, condensed, re-evaporated and passed over the material in a series of cycles until the essence has been extracted.

The type of solvent used and the method will extract different flavors from the starting material. For example the use of alcohol as a solvent will permit the extraction of more oily essences which would not be soluble in water. It is interesting to note that the essence used to flavor orange liqueurs is an oil extracted from the rind of the fruit and not the water-soluble flavor compounds of the fruit itself.

Amaretto (almond taste but actually made from apricot kernels), Benedictine (herbs), Drambuie and Irish Mist (herbs and spices), Grand Marnier (orange), Crème de Menthe (mint), Chartreuse (herbs), Crème de Cacao (chocolate), Ouzo (anise), Sambucco (anis), Southern Comfort (herbs) are a few of the liqueurs in use today. Do you know of others?

5. Production and distillation of vinegar. Vinegar results from the contamination of an alcoholic beverage with a bacterium called Acetobacter This organism is one of the spoilage bacteria of wine that we have talked about in previous chapters. While yeasts use sugar to produce alcohol in the absence of oxygen, Acetobacter uses alcohol to produce acetic acid in the presence of oxygen. In fact the word vinegar (VINAIGRE in french) comes from the French words for wine (vin) and sour (aigre)

a. The chemistry of vinegar. The reaction that produces vinegar from alcohol is as follows:

(8.1)

The first compound is an alcohol called ethanol. The second is an organic acid called acetic acid or vinegar. What is the difference between the two? The first carbon carries the same number of hydrogen atoms in both compounds. However the second carbon in ethanol carries two hydrogen atoms in ethanol but those are replaced by one oxygen atom in acetic acid. Acetobacter possesses the enzymes that enable it to use ethanol as food and produce acetic acid as a waste product. Because it is a waste it does eventually kill the bacteria producing it and for this reason we use vinegar as a preservative. What else do we use vinegar for? Taste of course. Who likes the taste of vinegar here? Where is it used to change the taste of food? Pickles, ketchup, relish and of course salad dressing. Most of these foods however also have a lot of sugar added and that attenuates the acidic taste of the vinegar. So why do we use vinegar in our foods on the one hand and call its presence in wine spoilage on the other? It may be a question of expectations or possibly of how well or poorly some flavors, such as ethanol and vinegar, mix

b. How vinegar is made. There are two ways of making vinegar: chemically and biologically. In other words we can make vinegar by chemical reactions in a test tube or let Acetobacter make it from alcohol. The latter is extremely easy actually. In fact when making wine precautions must be taken for that not to happen. It is because Acetobacter bacteria are in the air and could easily be deposited on the wine if it were left open. It is therefore not surprising to read that the production of vinegar came at the same time as the production of beer and wine in Antiquity. The second condition to make vinegar is the presence of oxygen. Remember what we said about oxygen and alcoholic fermentation. The absence of oxygen discourages the growth of other microorganisms and prevents oxidation.

Traditional biological production of vinegar is one of the oldest in use. It consists of laying a wood barrel on its side and filling it with diluted wine to the levels of holes drilled at each end so that the barrel is three-quarters full. The holes allow air to enter the barrel but are covered with gauze to prevent insects from entering. After a starter solution of a mixture of Acetobacter strains is added to the beer or diluted wine the barrel is stored at 85 ^{o}F (29 ^{o}C) for several months. After this time 85 % of the solution of vinegar is removed, leaving 15% to inoculate the next batch of diluted wine. This vinegar solution is called wine vinegar and can be red or wine depending on the starting wine used. It is often sold in that form. However the vinegar can be concentrated by distillation and removed from flavor and color compounds of the wine solution. This type of vinegar is called white vinegar and is usually sold in 5 to 7 % acetic acid solutions.

Balsamic vinegar was already made in Italy in the eleventh century. Its name describe the soothing or healing properties this vinegar was thought to possess. It was and still is produced by first simmering grape juice down to 30% of its original volume. The resulting solution is placed in wood barrels where fermentation first produce ethanol and subsequently vinegar. Barrel aging goes on for a minimum of 12 years and up to 25 years. During this time the vinegar concentrates further by evaporation while the barrels give the vinegar flavor and a deep brown color. The balsamic vinegars that are available commercially are made of red wine mixed with white vinegar, caramel and sugar, a far cry from the traditional product.

Acetic acid can also be synthesized by chemical reactions. This is the cheapest way to make vinegar but in this case distillation is required to remove the final product from left over reactants. Distillation can yield almost pure acetic acid, a liquid which is called glacial acetic acid, but it is still sold in grocery stores as 5 to 7 % solutions.

Answers to chapter questions.

p. 4: Can you describe what accelerates the evaporation? If molecules are not held together by strong forces of attraction the will more easily escape the liquid and become a gas.

p. 4: So if I said that ethanol boils at 78 ^{o}C while water boils at 100 ^{o}C what could you conclude from this information? Since the temperature at which ethanol boils is lower than that of water it means that the forces that hold the molecules of ethanol together must be weaker than those present in water. It is actually true.

p. 7: Do you know of other liqueurs other than those named on p. 7? Cointreau and Triple sec (oranges), Frangelico (hazlenut), Sambuca (anise-flavored, derived from the elderberry bush), Bailey's Irish Cream (cream and chocolate), Kahlua(coffee)

EXERCISES.

1. What is the difference, if any, between a liqueur and liquor?

2. Compare the alcohol content of wine and brandy.

3. Is it possible for yeast cells to produce brandy from grape juice? Explain.

4. Is there a fermented drink from which a distilled beverage could not be produced? Explain.

5. Where does the word whiskey come from?

6. Why can you walk through a room but not through a wall?

7. What is the main difference between a gas and a solid?

8. Why are some substances liquids at room temperature while others are gases at room temperature?

9. What is meant by the term :"concentrating the alcohol by distillation of wine"?

10. Why can ethanol mix with water but gasoline cannot mix with water?

11. Explain the principle by which distillation is possible.

12. You decide to spend some time a the bottom of a 12 foot-deep pool. So you strap some weights to yourself and plunge in. After a while at the bottom of the pool you become restless and decide to walk from one end of the pool to the other . Is it easier to walk in water than in air?

13. In question 12, can you think of an explanation for your answer?

14. In question 12, could you still move back and forth in the pool at -20 ^{o}F? WHY?

15. Define vapor pressure.

16. Does vapor pressure increase or decrease when temperature is increased? Explain

17. Ether is a liquid that boils at 40 ^{o}C. If equal volumes of water and ether are left at room temperature which one will evaporate first? Explain.

18. Describe the chemical reaction by which vinegar is produced from alcohol.

19. Vinegar is a solution of acetic acid in water. What does that tell you about the forces of attraction between molecules of water and molecules of acetic acid?

20. Could you separate one liquid from another by distillation of a mixture of the two if they both had the same boiling point? Explain.

21. Why is vinegar a good preservative?

22. How can you separate acetic acid from water if they have similar forces of attraction?

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APPENDIX 1 http://tomsaaristo.com/liqueurs.html

Liqueurs

What exactly is a liqueur?

In simplest terms, a liqueur, sometimes known as a cordial, is a flavored, sweetened spirit. The liqueur category is the most wide-open on the spirits market. Where else do you find drinks colored hot pink or neon yellow and flavored with banana, peppermint, licorice or white chocolate? Where else do you find bottles shaped like giant cones or chocolates, and filled with liquid studded with gold flakes? These drinks are among the most imaginative and, at times, the most bizarre that you will encounter.

How can you make sense of this creative jumble?

By dividing the liqueur category into two parts: old products and new. Most of the old ones have been around for centuries and were typically formulated by monks trying to come up with remedies for the ailments of their congregations. The new liqueurs, on the other hand, have been around for much less time: some for decades, others for just a few years. Some are the work of artisans; another might be the brainchild of committees of slick marketing pros.

The OLD liqueurs that have survived for hundreds of years are elegant tasting, enticing distillations, or macerations, of a variety of flavorings, including herbs, fruits, nuts, seeds, peels, beans, pods, and spices. These potions are wonderful to sip after dinner, though some
people drink them as aperitifs.

Bénédictine, a sweet, aromatic, rich liqueur flavored with more than twenty herbs and plants, was first produced in 1510 at the Benedictine Abbey at Fécamp in the Caux district of Normandy. Often referred to as the world's oldest cordial, dark-amber-colored Bénédictine has never been copied well [as is typical of drinks made from complicated secret formulas], although many have tried.

A second French liqueur claiming ecclesiastical origins is Chartreuse, which was devised by Carthusian monks in the Massif de la Chartreuse near Grenoble more than three hundred years ago. Perhaps the most sophisticated of herbal elixirs, it is blended from 130 different plants. This potion, memorialized in the name of the bright green hue of its liquid, also comes in a mellower yellow version that was created in 1838

Grand Marnier, an exquisite liqueur that is based on Cognac and the peels of Haitian bitter oranges, was the product of a nineteenth-centuryFrench family enterprise. Sip it on the rocks or straight in a snifter.

A popular orange-flavored liqueur from France is bittersweet Cointreau, originally made in the Cointreau family distillery, which was set up after the French Revolution in the historic Loire Valley town of Angers.Theunique taste of small wild bitter oranges sampled by a family member on travels to the Caribbean and South American inspired the Cointreau recipe, eventually formulated with the dried peels of those oranges. Because the drink was three times drier than the liqueurs of the day, imitations proliferated under the name triple sec [triple dry]. Cointreau is delicious in a Margarita, but also wonderful sipped,just like Grand Marnier.

On a different citrus note is Belgian Mandarine Napoléon. This Cognac-based drink perfumed with tangerines was so named because the French emperor was said to have wooed his
favorite actress with a similar tonic.

Another venerable fruit-flavored liqueur is Peter Heering from Denmark, known as Cherry Heering in Europe. Dating back to 1818, this crimson drink with a complex taste is made from the luscious cherries that grow in southern Denmark. Winy and dry for a liqueur, with a distinctive almond-tasting bitterness that comes from the use of the cherry pits, it is heavenly on its own or over ice cream.

Italy's celebrated almond-flavored liqueur, amaretto, is actually made not with almonds but with apricot kernels. It is a perfect romantic drink, as it is said to have been created in Saronno in 1525 as a tribute to artist Bernardino Luini by his model. [Not surprisingly, the original brand, Amaretto di Saronno, remains the standard against which others are measured]. Sweet, syrupy amaretto can be served straight, on the rocks, in mixed drinks,
or in coffee.

Frangelico, a shimmering brown hazlenut-scented liqueur, was first concocted out of woodland nuts and herbs by a reclusive seventeenth-century Italian monk, hence its distinctive bottle, shaped like a robed cleric. It is now produced commercially [and secularly] in the Piedmont region of northwestern Italy. Frangelico is smooth and delicate: it's terrific straight, on the rocks, and in mixed drinks.

Clear, anise-flavored Italian Sambuca is derived from the elderberry bush, which grows all over the hills of Italy and has long been praised for its medicinal virtues. The sweet, potent liqueur produced from the bush's white flowers is often presented con mosche, or "with flies," which means that coffee beans are floated in the liquid. The alcohol is then generally set aflame, a process that roasts the beans, releasing an extra aroma into the drink. [For those who would like to forgo the pyrotechnics, sambuca already infused with coffee is available under the generic name negra. The dark brown liqueur is sumptuous and dense]. Sambuca Romana isprobably the best-known producer of sambuca, but Giovanni Buton, Moninari, and Oblio also make good versions.

Unlike the aforementioned liqueurs, which are based on virtually neutral alcohol, Drambuie and Irish Mist are based on a specific spirit: whisky. Both come from the British Isles, have colorful histories, and are usually drunk straight or on the rocks.

Drambuie was first made on the island of Skye in Scotland by member of the Mackinnon family using a recipe given to hem by Bonnie Prince Charlie in 1746, when the Mackinnons helped theprince escape to France after the Battle of Culloden Moor. The complex combination of Highland malt whisky, heather honey, herbs, and spices lives up to its Gaelic name, an dram buidheach, which means "the drink that satisfies." Irish Mist, produced in the old distilling town of Tullamore, is a blend of Irish whiskey, heather honey and herbs and is said to be based on a secret recipe for heather wine that disappeared in 1691 and resurfaced in 1948, according to a very complicated storythat involves quests, wars, and immigration.

The most dramatic NEW liqueur introduction came in 1979, when International Distillers and Vintners (IDV) pioneered a process thatkept fresh cream and alcohol from separating when they were combined. A rich chocolate-flavored, whiskey-and-heavy-cream liqueur of low
alcohol content known as Baileys Original Irish Cream was born and experienced tremendous success. Needless to say, it spawned many imitators.

One of the most recent cream liqueurs is called Sheridan's. The bottle actually has two sections, one filled with a black liqueur, the other filled with white. The idea is that when you pour the liquids into a glass, you float the white atop the black. More often than not, however, the colors mix together into a milky brown. Nonetheless, the final product is smooth and decadent-tasting.

If you love chocolate, another good choice is Godiva; a satiny liqueur from the candy manufacturer of the same name, this Godiva has been around for five years. About four years ago, IDV and the French Cognac producer Godet began making an opulent liqueur called Godet that is flavored with white chocolate
.

Moving from chocolate to coffee, we come to the other great success story in new liqueurs: Kahlúa, from Mexico, was made prior to WWII but not introduced into the U.S. until 1962. This dark, sweet stimulant is enjoyed straight or on the rocks, but it is also employed in many beloved mixed drinks. Tia Maria, from Jamaica, produced commercially since the forties with famous Blue Mountain java, has an intense flavor, but it is lighter-bodied and drier than Kahlua.

A pair of Asian cordials adds interesting flavors to the liqueur family. Midori, first produced in the early eighties by giant Japanese distiller Suntory, is a bright green drink that has a sweet taste of ripe melons. It works best as an accent in fruity, tropical drinks. The much younger Original Canton liqueur from China, with its pungent ginger and honey flavors, is delightful on the rocks or even teamed with a bit of malt whisky.

Certainly one of the most eye-catching among the newest of the new liqueurs is Goldschlager, an intense cinnamon-flavored concoction that features gold flakes suspended in a clear liquid. Another potent cinnamon drink is After Shock, which as edible crystals lining the inside of the bottle. Both of these spicy potions are in with the "shooter" crowd. On the subtler side is an appealing late-model orange-flavored liqueur called Sublime, which comes in a distinctive pyramid-shaped bottle.

There are dozens more new liqueurs to choose from, including Agavero, a Mexican blend of tequila and damiana flower tea, and Celtic Crossing, an Irish combination of whiskey and Cognac, as well as three of Italian extraction: Blue Caos, a sprightly citrus cordial; Paolina, a cappuccino cream elixir; and Tiramisù, a tonic that tastes like, well, tiramisu.

Whether you try one of these whimsical recent additions to the liqueur category or a classic standby, you are sure to be entertained by these lively drinks, bursting with personality and flavor.

Lesson 9. **Wine as food and medicine**.
2 figures

A. INTRODUCTION
As we have seen earlier wine has been used as food and medicine as well as for religious purposes for as long as it has been made.

Greek records show that Hippocrates, the father of medicine, was one of the first to advocate the use of wine as medicine. Others followed: Erasistrates, Horace, Pliny and Dioscorides in ancient times and Alexander of Trelles, Guy de Chauliac and Pasteur in more recent periods. In the Bible, Paul is quoted to have said: " Use a little wine for thy stomach's sake." This may seem a bit surprising to us but remember that food quality in those days was not up to our standards and stomachs may have been indeed upset a lot more often than today. Pasteur said that wine was the most healthful and the most hygienic of beverages. He may have meant that in two ways. Water was not at all safe to travelers coming to foreign towns and drinking the water there would have meant exposing oneself to a range of bacteria unknown to the traveler's intestinal system. The wine that the innkeeper offered would have been a far safer bet for this traveler. He could have also meant that wine could have relieved some of the milder ailments afflicting the general population.

Today we understand that as a therapeutic agent wine has two types of effects:

- Psychological effects: it makes us feel good

- Physiological effects: it is of actual therapeutic value.

Even though both effects are needed in a good therapeutic agent and while few will deny the psychological effects of alcohol in general and wine in particular the latter effects are not clear and have been hotly debated. Claimed effects have ranged from lowering cholesterol in the French (a recent study seems to confirm this claim) to curing bacterial infections. The chemistry of wine is in fact very complex and its effects on our physiology even more difficult to recognize and understand. It is further difficult to differentiate the effects of alcohol from those of the other components of wine. It is nonetheless an area of study that continues to attract the attention of researchers.

B. THE CHEMISTRY OF WINE.

1. Organic chemistry.
Let's first look at the components found in wine and try to understand their chemistry. These compounds all contain atoms of carbon. The chemistry of carbon compounds has been called **organic chemistry**. The reason for this is that carbon atoms have a unique

property, that of being able to attach to each other in a variety of ways. This means that an enormous number of organic compounds are possible and thus organic chemistry encompasses millions of compounds while the chemistry of all other compounds (called inorganic chemistry) looks at only around 1/2 million compounds.

The carbon atom can form bonds with a maximum of four other atoms.

```
  |
- C -
  |
```
(9.1)

Since bonds can also be to other carbon atoms, long chains of carbon atoms can be created:

```
  |
- C - C - C - C - C - C - - -
  |
```
(9.2)

If the other links are to hydrogen, the compounds are called **hydrocarbons**. You use those every day as gasoline in cars, natural gas for cooking, asphalt, lubricating oils etc... Natural gas is called methane and is a single carbon linked to four hydrogen atoms:

```
      H
      |
H  -  C  -H
      |
      H
```
(9.3)

The prefix "met" refers to the fact that there is only one carbon in this molecule. Two carbons carries the prefix of "eth", three carbons is "prop" as in propane etc.

four carbons:	" but"
five carbons:	" pent"
six carbons:	" hex"
seven carbons :	" hept"
eight carbons:	" oct" as in octane.
nine carbons:	" non"
ten carbons :	" dec"

If the carbons are linked to an oxygen and a hydrogen you have an **alcohol**. The same system of prefixes applies here but the ending of the name will be "ol" instead of "ane" for hydrocarbons.

```
      H
      |
H  -  C  -  O  -  H
      |
      H
```
(9.4)

This is called **methanol,** a poison to you and me, because we cannot break it down while rats can. IF you add another carbon to this molecule you actually have **ethanol**, a molecule that our society is very familiar with.

```
            H     H
            |     |
      H  -  C  -  C  -  O  -  H
            |     |
            H     H
```

(9.5)

You notice here that those carbon atoms must always carry four bonds to different or similar atoms. That is one of the rules of organic chemistry.
If the carbons are linked to more than one oxygen-hydrogen combination they are called polyols in chemical terms but sugars in laymen's terms. A molecule of **glucose** is made of six carbons linked to oxygen-hydrogen combinations.

```
           H
           |
           C  =  O
           |
     H  -  C  -  O  -  H
           |
 H  -  O - C  -  H
           |
     H  -  C  -  O  -  H
           |
     H  -  C  -  O  -  H
           |
     H  -  C  -  O  -  H
           |
           H
```

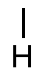
(9.6)

Notice here something interesting: the second carbon has four links but two of them are to oxygen. That is called a double bond and occurs frequently. Triple bonds are also possible. This leads to another class of organic compounds, those where the carbon is linked to oxygen in a double bond. Those are called **aldehydes**, **ketones** and **organic acids**. Let's see if you can recognize the differences between them:

```
           H
           |
           C  =  O
           |
     H  -  C  -  H
           |
           H
```

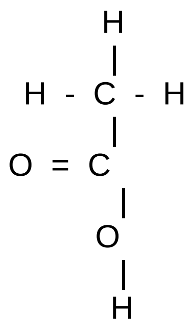
(9.7)

```
           H
           |
     H  -  C  -  H
           |
   O  =    C
           |
     H  -  C  -  H
```



(9.8)

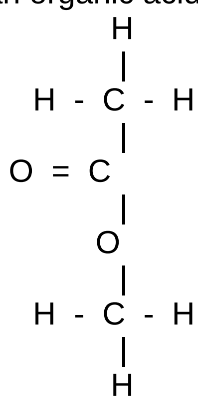
and



Do you see the differences?? The first compound is an aldehyde where the C=O is surrounded by one other carbon atom and a hydrogen atom. The second is a ketone where the C=O is surrounded by two other carbon atoms. Finally the acid has a C=O attached to a oxygen hydrogen combination.

Now tell me what type of compound is the glucose molecule I showed you before. It is an aldehyde.

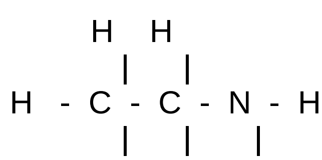
The **ester** is an organic acid where the oxygen-hydrogen combination is replaced by an oxygen attached to another carbon chain. This molecule is formed when an alcohol reacts with an organic acid.



(9.9)

You have to realize of course that I have drawn two carbon chains here each with one carbon but that I could have just easily drawn them with several carbons. Of course the name of these compounds would change with each carbon added to the chain.

Another compound is going to be important in our study of wine chemistry and it is the amine, where the carbon is now linked to another element called the nitrogen.



```
        H    H    H                                  (9.10)
```

Note that while the carbon requires four links, the oxygen two and hydrogen one, the nitrogen will require three links to other atoms (or to itself as in the case of molecular nitrogen (N = N) the most abundant gas in the atmosphere). Amines play an important role in the chemistry of wine as we will see later.

There are other types of compounds such as amides, imines, imidazoles etc. but those are a bit more complicated and we do not need to know what they look like for our study of the chemistry of wine.

These groups we have just seen are called **functional groups** because they determine the function of the molecule or if you will their reactivity. For example you will get drunk if you drink ethanol because of the way ethanol reacts in your body. But you could get drunk if you drank methanol or propanol or isobutyl alcohol (rubbing alcohol) because they are all alcohols and they all react in a similar way in your body. Of course they are not exactly the same and that explains why you would also get dreadfully sick if you drank those alternatives to ethanol.

2. The content of wine.

a. Alcohols. Alcohol is after water the most abundant component in wine. Of course here we are talking about ethyl alcohol or ethanol. That is what yeasts produce mostly when they consume sugars. What would be the name of a three-carbon alcohol???

```
          H    H    H
          |    |    |
    H  -  C -  C -  C  -  O  -  H      propanol
          |    |    |
          H    H    H                            (9.11)
```

In fact yeasts also produce alcohols with 3,4,5,6,7,8 and 9 carbons but in much smaller quantities than ethanol. These alcohols are collectively called "fusel oil" and give a lot of its flavor to wine (and other barrel-aged alcohols as well).

All these alcohols have only one -OH group on the molecule. Other alcohols have two (ethylene glycol or anti-freeze is an example) or three or four or 6 (sugars for example). You can see that adding more -OH groups to the molecule will change its properties drastically. Consider the difference between an alcohol (one -OH group) and a sugar (three -OH groups and up)

A sugar with three carbons and three -OH groups is present in wine. Glycerol, in concentration of 7 to 12 % gives wine a velvety feeling with a sweet flavor. Some unscrupulous wineries have added ethylene glycol to wines in the past to give them a sweet flavor. This compound however is highly toxic and some people have died from consuming this wine.

b. Aldehydes. Do you remember the general structure of aldehydes?

```
        H
        |
H  -  C  -  C  =  O
        |       |
        H      H
```

(9.12)

Compare that to ethanol:

```
          H     H
          |      |
H  -  C  -  C  -  O  -  H
        |       |
        H      H
```

(9.13)

What are the differences? one less hydrogen and a double bond to the oxygen which must then lose a hydrogen.

Aldehydes are often associated with flavor. The flavor of maraschino cherries is benzylaldehyde. In wine the concentration of aldehydes increases when a wine becomes oxidized. The taste is pleasant at low concentrations (the "oak" flavor of red wines that have been aged in barrels) but become unpleasant at higher concentrations.

c. Acids. We have talked about the acids contained in the juice of grapes and therefore contained in wine: tartaric acid, malic acid, citric acid, phosphoric acid, tannic acid. Most carbonated beverages contain phosphoric acid; orange juice has a lot of citric acid etc. All of these give the food they are in a sour taste. That taste is appealing at low concentration but become overwhelming at the higher end of the concentration curve.

The acids named above are present in the wine because they came from the grapes. But the wine making process also creates other acids such as carbonic acid, formic acid, acetic acid, lactic acid and succinic acid. All of these are normally found in wine except for acetic acid; its presence in wine indicates that something has gone wrong with the fermentation and that a bacterium called *Acetobacter* has found a way into the wine.

The total amount of acid in the wine is important for the balance. Usually a young wine will be more acid than an older one. The aging process allows for the crystallization of tartaric acid out of the wine and of course malolactic fermentation reduces the acidity by transforming a strong acid like malic acid into the milder lactic acid.

The wine maker will make the difference here between two types of acids: volatile and non-volatile ones. The former means that the acid can actually evaporate which means that you will smell it in the bouquet of the wine much as you smell the sourness of a lemon. Other acids are non-volatile meaning that you register their presence only when you drink the wine. It makes sense therefore that a balance of these two types of acids would be necessary in a good wine. Usually volatiles are present in a good wine in concentrations of less than 0.11%. That is why the lore said that you had to let the wine breathe after opening the bottle to let some of the volatiles evaporate. It is apparently not true really since the bottle has too small an opening for much volatile acid to evaporate in significant quantities. Acetic acid is one of those volatile acids that will ruin the taste of wine (but not the taste of a good salad dressing). But while only a bit of it may be evaporated off, this compound quickly overwhelms the wine.

d. CO_2. Carbon dioxide is produced during fermentation but most of it disappears, a little bit like a bottle of Coke going flat overnight when left opened. There is however some CO_2 left dissolved in the wine when you drink it. It is like the oxygen dissolved in water. You cannot feel it but the fish actually extract it from the water. The concentration of CO_2 in sparkling wine is higher and there you can feel it. Actually what you feel is the CO_2 coming out of solution because of pressure changes. The opening of the bottle means that the liquid is now at atmospheric pressure instead of under several atmospheres of pressure in the bottle. The carbon dioxide then comes out of the liquid.

e. Nitrogen compounds. Several types of compounds contain nitrogen aside from amines. Amino acids, purines, pyrimidines, amides, nitrosamines etc... Proteins are made of amino acids as we have said before and are an important source of nitrogen for the yeast. On the other hand some nitrosamines are actually cancer causing and are monitored in alcoholic beverages by the government.

f. Coloring. As a wine ages it acquires color. A white wine becomes golden while a red wine will acquire a deeper lightly brownish color. Oxidation of pigments in the grape juice will cause this coloration. Tannins for example give astringency to foods (makes your mouth pucker). These tannins are extracted from the skin and the stems of grapes. Their oxidation will contribute to the darkening of the wine. These tannins also come from the wood of casks in which wine is aged and contribute to flavor and color of the aging wine.

The color of red grapes is due to a molecule called anthocyanin. It is contained in the skin of the grape and its extraction into the juice will color it. This location of the anthocyanin is the reason why a white wine can be made from a red grape. These tannins leach out more or less easily depending on the type of grape you use to make wine and fermentation of skins with juice and hard pressing are sometimes necessary to extract a fair amount of anthocyanins from the grape to make a red wine. A combination of pigments, pH and oxidation of the pigments will give the red wine its characteristic hue.

g. Extract and minerals. We often call the extract the minerals that are present in the wine. The easiest way to find out how much there is to ash a wine i.e. to burn it until all the carbon compounds burn off. What is left then is the minerals like magnesium, calcium etc. Since they do affect taste we do want in there from 1.5 to 3.5 grams per liter; reds contain a little more than whites. The most important minerals in wine are calcium, magnesium, sodium and potassium; iron is also important for taste. Of course yeast also need those minerals for its own nutrition. We find in wine also traces of aluminum, manganese, chlorine and others.

h. Esters. We have seen what esters look like. Can you draw one? There are various ones in wine and they do affect the bouquet of the wine. Esters often have a typical smell and that is why we find them in foods. Some smells are nice like oil of wintergreen (peppermint) methyl anthranilate (grapes), citronella, but then others are not (butyrate gives its smell to rancid butter).
Esters are already present in grape juice (methyl anthranilate for example) and remain in the wine.

i. Vitamins
The vitamins A, B_1, B_2, B_3, B_6 and B_{12} are abundant in grape juice and make it the perfect growth medium for yeast

C. PHYSIOLOGICAL EFFECTS OF WINE.

As wine contains an enormous number of compounds that came from the grape or were formed by yeast during fermentation the study of the effects of these compounds on our physiology will take decades. Of course the presence of alcohol will complicate things enormously because of old beliefs that only alcohol in wine had any effects on our physiology. But as we will see, at least in the few cases studied so far, alcohol is not the only compound affecting our body's chemistry.

1. Alcohol.

a. Ethanol. While other foods are broken down in the stomach and intestine for absorption in the intestine, alcohol is absorbed directly in the mouth, stomach (20%), upper intestine (30%) lower intestine and even the colon and rectum. Absorption occurs quickly because there is no need for any modification or breakdown before absorption. As a result one can feel the effects of alcohol very quickly. In contrast it takes 8 to 12 hours to digest and absorb a steak. Absorption is also faster in the stomach if alcohol is consumed without food. The fats, proteins and acids eaten with food actually coat the stomach walls and slow down alcohol absorption. After absorption alcohol enters the blood stream and is carried to the liver and muscles where it is broken down to carbon dioxide and water faster than fats and proteins because it is a simpler molecule. The chemistry of breakdown is in fact complicated and we do not completely understand how the euphoric effects occur. Lots of research is going on right now to try and understand the psychological effects of alcohol and the chemistry of addiction to alcohol.

Other effects of alcohol include increase in the production of saliva, increase in urine output. Heart rate is not affected but breathing deepens. Alcohol passes through membranes easily so that the fetus receives alcohol from the mother's blood and the milk also increases its alcohol level.

Psychological effects include a sense of euphoria and a lowering of anxieties. Alcohol also slows down reflexes and coordination. Speech becomes slurred and vision is affected. As we said the mechanisms by which alcohol achieves those effects is unclear. We know however that alcohol acts on the pleasure centers of the brain and in effect replaces the levels of pleasure molecules normally produced by the brain in the same way that morphine and heroin act. The dependence on alcohol is therefore similar to a heroin addiction. We will come back to this later.

Reflexes can be simple (as in the arc reflex that makes you remove your hand quickly from a hot burner) or more complicated. The more complicated the reflex the stronger the impairment by alcohol. Furthermore long term use of alcohol may impair these reflexes permanently.

Recent research has also shown that alcohol relieves anxiety in ways that can affect every day lives. In a study of patients recovering from a heart attack, the effects of daily alcohol intake was studied. As shown in the graph below the life span of these patients increased with an increase in daily alcohol intake.

Patients (Figure 9.2) who consumed two drinks a day (2 beers or 2 glasses of wine or two mixed drinks all contain the same amount of alcohol) had a significantly longer life span than people who did not drink at all. However life span started declining for patients who consumed more than 2 drinks a day. The tentative explanation was that the low amounts of alcohol relieved anxiety and therefore extended the life span. But higher alcohol consumption brought about side effects of alcohol consumption that affected the life span negatively. Remember that alcohol is a poison and that the liver must detoxify it. That creates a stress on the body that can have negative effects.

b. Other alcohols. We have said that yeast actually produce alcohols other than ethyl alcohol. Methanol is one of them. Produced in trace amounts it is not dangerous for us even though we lack the enzymes needed to break it down. So it accumulates in our system and gets excreted in sweat. Glycerol is another alcohol, made of three carbons and three O-H groups. This compound confers a sweet taste to wine and is not toxic to us at all. Other alcohols, containing more than 3 carbons are toxic however and could cause problems if they were present in more than trace amounts in wine. Of course they do present problems, along with methanol if you consume a lot of wine. The hangover experienced by heavy drinkers is due in part to those alcohols.

2. Acids.

The sharp taste in NY white wines is given by their acid content. It is interesting to note that often red wines have lower acid content because their tannins have helped precipitate the acids. It is also known that the taste of foods is enhanced at higher temperatures. It is not surprising therefore to note that white wines have traditionally been consumed cold while reds are drunk at room temperature. Now you know why.

These acids also have physiological effects on the human body. Lactic acid for example affects the pyloric muscles; these control the opening of the stomach into the intestine. Acids also give **antiseptic** properties to wine. It has been shown that alcohols and acids in wine kill very rapidly a variety of bacteria such as staphylococcus, streptococcus etc. Furthermore Russian scientists have shown that wine has antibacterial properties that cannot be ascribed to its alcohol content. They have isolated from wine a compound with an amine functional group that exhibits very strong antibacterial properties.

Figure 9.2. The effect of moderate drinking on human life span.

3. Carbon dioxide.
Carbon dioxide has several physiological effects on the person consuming it, in champagne for example or in soda pop. Its first effect is that is alters the taste of the solution it is in. It fools our taste buds into thinking that there is more in the drink than is the case. This property is not unique to carbon dioxide. How do we change or alter the taste of our foods?
We add:

a. NaCl (table salt)
b. Sugar
c. Carbon dioxide
d. Acids (such as lactic acid in yogurt and vinegar in relishes and pickles)
e. Bitter flavors (such as quinine in mixed drinks and bitters)
f. Alcohol
g. Others such as sodium glutamate (especially in Chinese foods).

These compounds act on our taste buds to enhance the flavor of our foods.

Look at your tongue (Figure 9.1) The taste buds are distributed throughout the tongue but certain areas of the tongue specialize in recognizing certain flavors such as bitter, sweet, salty and sour. Interestingly these areas correspond to the flavor enhancers that we have just described. By exciting these taste buds others taste buds get excited as well and tell you that there is more taste to your food than there actually is. Carbon dioxide acts in a similar way. Try to drink your favorite beverage (beer or pop) without carbonation and see what the differences are. Let the carbonation run out of your Pepsi overnight and cool it again. Now compare. You will find very little flavor to it compared to the unadulterated one. This explains why some people like to drink carbonated water and find a taste to it.

It has also been known for centuries that the dissolved CO_2 in champagne had euphoric properties on the user. We now know that carbon dioxide increase the rate at which alcohol is absorbed by the body. This explains the increased high reported by some champagne drinkers. Carbon dioxide also increases the volatility of **aromatic** compounds in wine which have **anesthetic** properties (they numb you) that reduce nausea caused by alcohol. The experience of drinking champagne is therefore more pleasant. Carbon dioxide is also a **diuretic** (increases urine flow) and it increases heart rate.

4. Tannins.
Tannins make your mouth pucker. It is the compound that gives **astringency** or **tartness** to foods. The reason for this is that it tightens the membranes in the mouth for that puckering effect. It has the same tightening effects on the stomach.

5. Minerals.
We all need iron in our food for good health and wine is a good provider of iron in the diet.

D. WINE AS FOOD**.**

The early civilizations in Greece considered wine as food. Spartan warrior ate barley meal, figs, cheese, meat, fish and wine as a fighting diet. The Bible expresses the same opinion. To this day Mediterranean cultures consider wine a staple of every day life. It was part of the rations of French soldiers in World War I because it remedied sugar deficiencies. It also made life in the trenches a little more bearable.

As we have mentioned before wine supplies energy because its alcohol and acids are metabolized by the body for energy. Alcohol is completely burned in the body to carbon dioxide and water. This makes for an excellent source of energy and can replace fats and sugars in the diet. In fact when rats are given alcohol they reduce their intake of other foods in proportion to the alcohol ingested. This means that the number of calories ingested remain the same. Of course one could not replace completely those foods with alcohol because of the toxic effects of alcohol.

Wine also provides a different type of food, compounds that maintain the ability of the body to extract energy from foods; these are called minerals and vitamins. Wine contains 13 of the minerals needed by the human body (calcium, phosphorus, magnesium, sodium, potassium, chlorine, sulfur, iron, copper, manganese, zinc, iodide and cobalt). Sodium and potassium are especially important because they maintain the right conditions for metabolic reactions that occur in cells. Iron is a component of hemoglobin, the protein that transports oxygen from the lungs to the muscles. The other minerals are needed by **enzymes** assigned to specific reactions in the body. Vitamins (especially the B complex) are present in wines and are also needed by enzymes in the body to perform specific reactions.

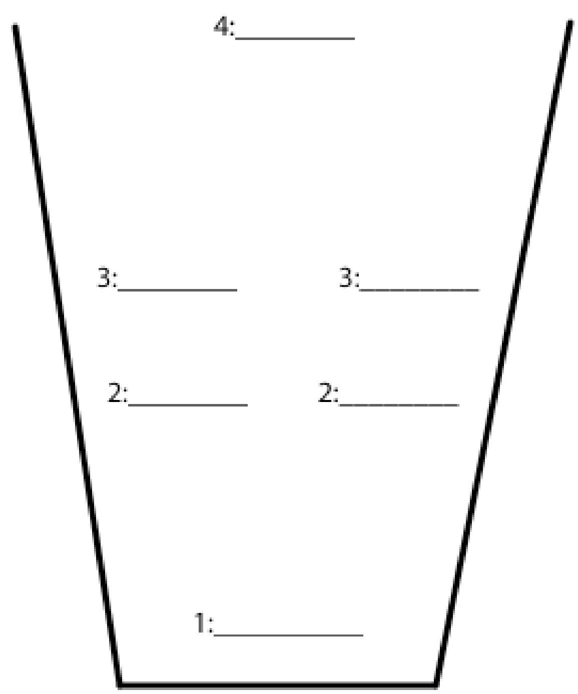


Figure 9.1. Your tongue. Your tongue can taste four basic flavors: sweet, salty, bitter and acid. Can you tell which areas are associated with each of th four basic flavors?

E. WINE AS MEDICINE.

1. Digestive system.

The action of wine on the digestive organs and uses in disease treatment.
We have already said that wine increases the secretions of the stomach and the salivary glands. It also decreases hunger contractions in the stomach and increases **peristaltic** movements. These movements are rhythmic contractions along the intestinal walls that move the food along. These effects are not due to alcohol alone but also to other as yet unknown factors present in wine because corresponding quantities of alcohol do not duplicate the effects of wine. It is also known that wine has bactericidal effects that can help prevent food poisoning. Wine reaches the liver after absorption through the intestinal and stomach walls into the blood stream. It reaches the liver through the portal vein. In the liver alcohol is digested (broken down). Its presence however can stimulate liver function. In fact at low levels alcohol is not toxic to the liver and does produce energy. In large quantities alcohol causes cirrhosis of the liver, a deterioration that is now thought to be more the result of poor nutrition on the part of the alcoholic than a direct effect of alcohol itself. Alcohol also has a mild diuretic effect in that it stimulates the kidney to increase the output of urine.

From these properties one can use wine, in moderation, to alleviate the effects of diseases that cause:

- Deficiencies in liver function
- Deficiencies in stomach secretions
- Spastic constipation (by stimulating peristaltic motion)
- Infections in the gastro-intestinal tract.
- Nausea.
- Disorders of the urogenital tract (diuretic effect)

Since wine is very acid (pH = 3) it should not be ingested by sufferers of hyperacidity or ulcers.

Of course one should never **self-medicate**. If wine is to be used as part of a therapy it should be after proper diagnosis has been made. It is best to have wine before a meal. The use of low alcohol wines is recommended and aged red wines are superior to whites or young wines. Finally drink in moderation; the term moderation cannot be defined with absolutes and does depend on the individual.

2. Cardiovascular and respiratory systems.

a. Respiratory system. Wine does increase the respiratory volume so that you will find yourself taking deeper breaths. Alcohols and esters present in wine do cause this effect. The following table shows that alcohol alone does not have the same effects as wine.

Percent increase in respiratory volume

state	alcohol alone	wine

rested	12 %	20 - 50 %
tired	50 %	70 - 80 %

Therefore the ingestion of wine results in increased oxygen absorption and a feeling of invigoration as if you were breathing pure oxygen. That is part of the feeling of well being experienced after drinking wine or alcohol. The lungs are continually exchanging gases (and more interestingly carbon dioxide and oxygen) between the air and the blood. Since alcohol is volatile it will also be exchanged as a gas and give you the alcohol breath that is always unmistakable. This means that the mild antiseptic properties of alcohol can be beneficial in cases of infectious respiratory disorders such as bronchitis, colds and especially during convalescence. Despite its effects on respiratory volume alcohol does not affect basal metabolic rates.

b. Cardiovascular system.

Alcohol in general and wine in particular have been considered a cardiac tonic for centuries. We now know that they stimulate the pulse and coronary circulation; more blood is pumped out with each stroke. At low levels alcohol causes **vasodilatation** i.e. an opening of the blood vessels. In fact low alcohol concentrations are a better vasodilator than **acetyl choline**, the body's own vasodilator. Hence the old saying that alcohol can warm a person coming out of the cold. While this is true, ironically, if one continues to drink, one will then experience a **vasoconstriction** as alcohol levels rise in the body. Note also here that brandy and wine are far better vasodilators than pure alcohol. Thus here again other components of wine and its distilled product, brandy, appear to affect dilation and constriction of blood vessels. Russian scientists have found in wine an amine that was absent from grape juice and that doubled the heart's output in dilutions of 1:500,000. These effects are way beyond those of alcohol. Since low alcohol concentrations cause vasodilatation venous blood pressure drops after alcohol intake but this is counterbalanced by increased cardiac output. This means that at low concentrations alcohol has very little effect on blood pressure. **Atherosclerosis** is a condition caused by blockage of arteries by cholesterol deposits (**arteriosclerosis** by contrast is the buildup of calcium inside the walls of the arteries making them brittle and hard). Since cholesterol in more soluble in alcohol solutions than in aqueous solutions it is not surprising to find in alcohol drinkers only half the incidence of atherosclerosis than in non-drinkers.

Finally wine also has been found to relieve the pains of angina and hypertension. However since those pains are at least in part caused by the patient's apprehensions it is not clear whether alcohol actually reduce the pains experienced and reduces anxieties that cause these pains.

In summary, wine and brandy

- Have stronger physiological effects than alcohol alone
- Increases coronary circulation
- Relieve pain of angina and vascular blockages.

- Increase volume output of each stroke.
- Alleviate the effects of hypertension.

3. Neuromuscular system.

Alcohol is not a stimulant. This fact is confirmed by the fact that it reduces sleep movement if taken just before bedtime. Alcohol is classified as a narcotic with sedative effects. The sense of exhilaration and vigor that one feels after drinking are due to another effect of alcohol, the release of inhibitions and loss of restraints. Because the energy contained in alcohol can be made available very quickly to muscles muscular energy increases. However a loss of coordination occurs simultaneously. Typing errors increase 80% after the ingestion of one half glass of wine on an empty stomach; the increase is 160% after one glass on wine. The same results are obtained using target practice as the test tool. The number of errors is directly proportional to the alcohol ingested and the complexity of the task used in the test.

Wine and alcohol do however reduce tension and anxiety at the end of the day for example. and slightly the power of voluntary movement. Thus one will experience the feeling of being re energized. However this increase in power of voluntary movement is brief and is followed by a decrease. That is why wine and alcohol should be taken with food so that the digestion of the food will provide energy to alleviate the secondary feeling of lethargy.

Alcohol has very few bad effects on the brain per se but little therapeutic effects as well. It is a mild sedative that helps relaxation, has no effects on manual labor (in small amounts) but decreases neuromuscular coordination. We will come back to that point shortly. Because it is a sedative and not a stimulant it is not recommended for people suffering from depression.

4. Miscellaneous.

We have already mentioned that alcohol has a mild diuretic effect on the kidneys.

5. Conclusion.

Alcohol has always been prescribed as a treatment and a preventative for infectious diseases. For example Frenchmen were less likely to contract malaria (because they drank wine) than groups who did not drink wine or drank only beer or alcohol. In World War I, the French regiments had a lower incidence of infectious diseases and infections due to cuts and abrasions than any other regiments. Remember that the French had their army drink red wine as a part of their daily diet. Today of course wine is not considered a cure for anything but is still prescribed as a supplement to medical treatment and as a preventative. However It has been shown that moderate alcohol intakes can alleviate the effects of stress and increase lifespan (Figure 9.2).

F. ALCOHOLISM.

1. Genetic and social alcoholism.

So far we have looked at the effects of moderate intakes of alcohol. We can now look at what happens when larger doses are absorbed. As we have said alcohol is absorbed very rapidly by the body. In the body alcohol distributes itself throughout the body fluids. So if we say that **blood alcohol levels (BAC) or Blood alcohol level (BAL)** are 0.2% it means that all fluids in your body contain the same level of alcohol. The following levels of blood alcohol correspond to specific states of drunkenness:

a. 0.1%. The legal limit in most states. The person is considered intoxicated; reflexes are slightly affected; response time is down.

b. 0.2%: Speech is slurred at this point. Walking is difficult and walking a straight line is impossible. Driving impairment is obvious to an observer. A person would fail the coordination test that police administer in the field.

c. 0.3%. The person is in a stupor. They would have a hard time standing up from their chair. They cannot follow a conversation anymore. Hopefully the person will fall asleep before they have a chance to drink any more.

d. 0.4%. The person will fall into a **coma**. This usually occurs only if alcohol was ingested very quickly and created the condition before the person fell asleep.

e. 0.5%. **DEATH**. This happens only if alcohol was ingested very quickly. It is not impossible however and several cases are reported each year.

These numbers and figures vary from one person to another but are used as guidelines. NY state considers that a person with a BAC of 0.08% and above is **driving while intoxicated (DWI).** However a person with a BAC of 0.05 to 0.07% will be convicted of **driving while ability impaired (DWAI).** The state also has a zero tolerance policy for drivers under 21.

DID YOU KNOW:

Ten years ago the U. S. Department of Health and Human Services found that college students used alcohol more often than people who had not gone to or finished high school.

Further the higher education levels were related to higher use of alcohol. In contrast Holder found that the level of abstention from alcohol use was higher in the U.S. than in Canada, Britain, Germany and Italy.

Interestingly Heath found that countries where the population abstains less from alcohol consumption have lower levels of alcoholism.

The U.S. has the highest minimum drinking age in the western world.

Alcohol is metabolized at a rate of 15 ml per hour. That is the time it takes and nothing can speed that up. Coffee or a cold shower will not sober you up. Alcohol brings a lot of calories and alcoholics drinking a bottle of gin per day will ingest 2000 calories, enough to supply their energy needs. That is why they often do not feel hungry. They are deprived however of the vitamins, minerals and essential amino acids their body needs as well. Not surprisingly their health tends to decline while they are drinking. In the US it is estimated that 2-3 million people are heavy drinkers, possibly alcoholics and that 1.5 million people are genetic alcoholics. We will come back to this distinction in a few minutes. The cost of treatment amounts to 2 billion dollars annually. The cost in lost wages is about the same. It is of course difficult to define alcoholism but a strong and frequent compulsion to drink alcohol must be included in this definition. We now recognize two types of alcoholics, social and genetic alcoholics. While the reasons for a social alcoholic to drink are usually the need to conform or the need to escape problems and responsibilities, it is becoming clear that some people (10% of men and 5% of women in this country) drink because of their genes. It is important here to open a parenthesis and ask ourselves why more men than women are genetic alcoholics. Is it possible that genetics could be biased for one gender or the other? Men and women have in fact a pair of chromosomes that is different. This pair is called XX for women and XY for men because men have a chromosome in the pair that has less DNA than its counterpart in women. Remember that this pair will split up at the time of sperm and egg formation to produce two alternatives for men and women: X or X for women and X or Y for men. Punnett squares can be used to see what the progeny will look like when the egg and the sperm combine:

	X	X
X	XX	XX
Y	XY	XY

Any progeny that receives a combination XY of chromosomes will be male. The XX progeny will be female. If a trait is present on the X chromosome, it is said to be sex-linked and its inheritance will not be the same for male and female progeny. Let’s see how this happens. Imagine a trait X that is dominant and its recessive counterpart, x. This trait could be hemophilia where the blood fails to clot after a cut and the person can easily bleed to death from a paper cut. The person must be homozygous recessive to be affected medically. A person who is heterozygous is not affected but is said to be a carrier. You can see that women could be affected or just a carrier but that men can only be affected; they cannot be a carrier because they have only one copy of the gene(s) and it is working or not. What has to be the genotype of a woman who is a hemophiliac?

Now what about the children a couple might have? The woman could be XX, Xx or xx. She could then marry a man who is XY or xY. The distribution of genotypes and phenotypes in the progeny will be different in each case.
For example if the mother is Xx and the man is XY the progeny will then be:

	X	x
X	XX	Xx
Y	XY	xY

This means that none of the daughters will be hemophiliac and only 50% will be a carrier. However 50% of the boys will be hemophiliacs and the other 50% not only be normal but not even be a carrier. You can see that sex-linked genes are not affecting the phenotype of sons the same way they affect the phenotype of daughters. Can you tell me what the genotypes and the phenotypes of the progeny would be if an Xx woman married a hemophiliac man? What is the genotype of a hemophiliac man? Fifty percent of the daughters would be hemophiliacs and the other 50% would be carriers. Fifty percent of the sons would be hemophiliacs.

Now let us go back to genetic alcoholics. Can you imagine a scenario that would explain the fact that twice as many men as women are genetic alcoholics? In fact this situation is not possible if we assume that one sex-linked recessive gene is responsible for genetic alcoholism. We then have to assume that one recessive autosomal (not sex-linked) and one recessive sex-linked genes are responsible for the genetic disease. For example a woman would have to be aa xx in order to have the disease and a man would have to be aa xY. The combinations in the couples are numerous (9 different genotypes for women and six different genotypes for men). Can you name a possible genotype in women? Men? aaXx, Aa xX, AA XX for women and aa xY, Aa XY, Aa xY for men are possible genotypes. Which of these produce the phenotype of a genetic alcoholic? Can you marry a couple and see what the distribution of phenotypes is for their progeny?
If we uses all possible combinations of genotypes in couples we come up with 54 couples. Counting 16 different possible genotypes for the progeny we arrive at 864 different genotypes. Half of these will be boys for a total of 432. Of these 54 show the genotype of a genetic alcoholic or 12.5% of men. For women we find 28 genotypes of alcoholics or 6.5%. The percentages obtained by Punnett squares are very close to the percentages given above and indeed, for men, double those for women. This suggests that it is possible that genetic alcoholism is the result of carrying one autosomal and one sex-linked recessive gene.

The genetic link has been established by a series of disturbing observations:

a. Families of alcoholics are more likely to have alcoholic children (4x more likely) than families of non-alcoholics, even when children are separated from their families at birth (to eliminate the effects of family influences).

b. Twins separated at birth and put in alcoholic and non-alcoholic families are likely to become alcoholics depending on their biological parents and not on their adoptive parents.

c. Genetic alcoholics report a strong urge to drink very early in their lives. This urge manifested itself even before their first drink.

These genetic alcoholics are also different from the general population in that:

a. They are less intoxicated by modest amounts of alcohol than the general population.

b. They are less able to determine how much they have had and how it affected them because they feel less intoxicated.

c. Their blood level rises to the same level as the general population for a given amount of alcohol ingested.

d. Psychomotor tests show that their bodies are more tolerant of alcohol.

e. Because they metabolize alcohol faster, genetic alcoholics experience a greater high than you and me.

f. They come out of a drunk faster and with stronger withdrawal symptoms than the average person.

g. Electroencephalograms (EEG) show that the sons of alcoholics cannot focus as well on their surroundings even without alcohol.

h. Sons of alcoholics show slower alpha wave patterns in their brains than the average person.

i. Sons of alcoholics are more likely to be impulsive, risk taking and hyperactive than the general population. They also score lower on intelligence tests in early life. There is however no difference at the college level.

From these data and what we know of the effects of alcohol in the brain a theory of genetic alcoholism has been formulated. The first part of it involves the sense of euphoria that we experience when we drink alcohol. We feel better because as alcohol breaks down it gets to interact somehow when the pleasure centers of our brains. These centers are present in order to mask the pain that would be associated with our everyday activities. Imagine the two bones of our legs, the tibia and the femur standing on top of one another with all 150 or 200 pounds of you grinding the ends of these two bones together. That is actually very painful and a runner will tell you that. Our brain makes chemicals made of only a few amino acids (5) called **endorphins** or **opiates** that are produced to bind these centers and make us feel, literally, no pain. The same runner will tell you that after a long run they do not feel the pain but in fact feel almost a high because their brains responded to the pounding they were imposing on their bodies by synthesizing more opiates. Some chemicals such as heroin, morphine, and, alcohol interact with these opiate receptors to make us feel this euphoria. These chemicals are far more potent (or present in greater concentrations) than opiates and the euphoria we experience is much greater than that produced by our own opiates. The problem is that when the brain senses this abundance of opiates (whether they are made in the brain or coming from the outside) it shuts down its own production of opiates. Therefore when the effect of the opiates wears down the brain starts to experience this pain that was hidden from it by the opiates. We call that a hangover in the case of alcohol and **withdrawal** in the case of more potent drugs. The pain can be so great that the person will react by wanting to increase the opiate concentration in their brains at all cost. That is the cause of the addiction that opiates induce in animals.

The second part of the theory of genetic alcoholism lies in the rapidity of metabolism of alcohol in these people. This high rate of conversion increases the sense of euphoria experienced, the level of shut down of the brain in its own production of opiates and the sense of " withdrawal' experienced after the drunk.

Several questions remain however on the mechanism by which alcohol causes this effect on opiates receptors. It appears that alcohol increases the synthesis of opiates by the brain which later shuts down this production when alcohol disappears from the system. The "why" and "how:" are still unclear however.

2. American Indians and alcohol.
It is important to study the effects of alcohol on cultures other than our own in order to eliminate cultural bias. In the American Indian population in the US for example the rates of alcoholism are as high as 30%. Ninety percent of Indian arrests involve alcohol, the highest rate in the US.

This relationship with alcohol dates back to the colony days when missionaries assumed that Indians were in great need of the morals needed to change their depraved ways. The rest of the population assumed that Indians were trying to relive their glory days through the use of alcohol. Even today people in the West refer to Indians as drunks. The problem with these theories is that Indian populations were extremely diverse. New York state Indians were **horticulturists** (relying on gardens for food) while in the West they used irrigation and agriculture. Indians were semi-nomadic in New York, nomadic in the West and sedentary in the South West. In the Plains and New York they were egalitarians but in the West lineage was important, the chief lineage outranking any other. These vast differences make it unlikely that all of these cultures would have reacted similarly to their encounter with alcohol.

When Europeans came to America they started negotiating with Indians as equals and served alcohol as a civilized way to interact with equals. Indians however were unfamiliar with the beverage and warmed to it very quickly without the time to develop they social inhibitions and restraints common to the European societies. Europeans eventually viewed Indians as inferior and displaced them to reservations under the pretext of keeping frictions and wars between the two cultures to a minimum. Resettling on reservations was extremely difficult for nomadic people and indeed we find that alcoholism was higher in uprooted reservations than on reservations created on ancestral land. Deprived of their formal means of support (hunting and gathering on limited space is not possible) they became dependent financially on the government. To people stripped of their sense of independence, culture, dignity alcohol became a part of Indian culture.

New religions were formed among Indians to combat alcoholism (Sundance for example). These religions were relatively effective in their goal to reduce alcoholism on reservations but several other factors counterbalance this effect:

a. The Indian Prohibition Act that forbade the sale of alcohol to Indians actually exacerbated the problem.

b. Drinking on reservations is a public act. There is thus a lot of pressure to drink with your buddies. Closet drinkers do not exist on reservations.

c. In 1853 the Indian Prohibition Act was repealed and it was left to individual reservations to decide whether to keep it or not. Today we find that the reservations that kept it have the highest alcoholism rates.

d. The stigmas associated with alcoholism in our society do not exist on reservations. It is a big thing for us to be thrown in jail for drunkenness but not on Indian reservations especially if it is a "white" jail.

e. Indian society is a "level" society meaning that it is egalitarian. Since a high value is placed on equality an Indian will distribute his winnings among their peers. And alcohol is a great equalizer.

f. Indians do not interfere with someone else's life and thus will not preach about the evils of alcohol and take someone's keys away from them if they are drunk.

g. Alcohol created a sense of unity and solidarity in a society strongly divided over political and social values.

Non-alcoholics are divided into two camps in their views on alcohol. Traditionalists view alcoholics as the least successful of their peers. Non-traditionalists among Indians are more educated and view alcoholism as a problem stemming from a need to succeed in a world of very limited opportunities. Alcohol is then abused as a reason or a scapegoat for one's failure to succeed.

It has been suggested that the oppression that whites have imposed on Indians may be the cause of this high incidence of alcoholism; the reaction of people not in control of their lives may be alcoholism. But this would assume that they cannot adjust to those changes in their lives and cultures. And yet indications are that they have adapted to these changes in their cultures. Further the US government has advocated for year the right to self-determination in Indian reservations and provided these reservations with incentives that are not available to non-Indians such as special programs, no federal taxes, free college for any American Indian etc....

The other argument is that the stripping of their culture created this need for alcohol. However Indians do have a culture even though it was changed considerably over the last two centuries.
It could also be argued that alcohol became a part of their culture at a time when they had not had a chance to adapt to these changes in their way of life.

The very last possibility is that the genetic component is stronger in Indians than in other cultures and that this creates a strong physiological dependence rather than the psychological dependence that has been argued thus far.

From lack of morality to genetic dependence we have not yet come full circle in our long list of assumptions on the cause of the high incidence of alcoholism in Indian populations. The effects of alcoholism however are clear in any population. Alcohol causes a host of health problems from liver problems (the prime center of detoxification of alcohol) to cancer, primarily because alcohol weakens the immune system by imposing a stress on the body as it becomes heavily involved in detoxification. Colon cancer rates in heavy beer drinkers are three times higher than in the general population. We also see increases in the incidence of esophagus, throat and stomach cancers in these same beer drinkers.

Whatever the beneficial effects of alcohol and wine on individuals what a society might gain by the use of alcohol must be measured by what it loses.

G. OTHER USES OF FERMENTATION.

It is interesting to note that fermentation has evolved over the ages. Some uses of fermentation have fallen by the way side because cheaper or more efficient ways have been found or because there is no more demand for this particular application. For example we do not pickle vegetables by fermentation any more but simply put vinegar in our pickle in order to acidify them. Very few companies use fermentation to treat cocoa beans before making chocolate. Sparkling wines are often made today by injecting the carbonation (CO_2) directly into the wine.   However while these processes can be accomplished chemically without fermentation the end product is not always of the same quality. The best chocolates in the world are obtained by the traditional fermentation method. And most experts agree that the methode champenoise produces the best sparkling wines. As for our initial example, I pickle my hot peppers with vinegar and so I have never had the opportunity to compare this method to fermentation.

While fermentation appears less useful in some applications others are appearing every day. It has recently been possible to isolate genes that code for proteins that can be used as cures for diabetes (insulin) for example or other disorders. These proteins are very expensive to isolate from cows or other humans so we have turned to putting these genes into microorganisms and allowing them to grow in large tanks. Since these microorganisms have no use for the new protein they are now synthesizing they excrete it outside into the growth medium. It is then easy to isolate this protein from very few contaminants and provide the medical community with abundant supplies of these cures. Examples include insulin, interferon, clotting factors, hormones etc...

Another recent advance in fermentation is the clean up of oil spills with microorganisms. As you know crude oil often leaks from tankers. It is very viscous and is not degraded very easily in the environment. It has been shown however that some bacteria can feed on this oil and therefore dissolve the pollutants more effectively than the detergents that are now in use. The reason is that the detergents are not penetrating the top layer of soil to reach the more deeply buried deposits of oil. Bacteria can travel more easily down to the lower layers where oil seeps.

And finally some applications of fermentation remain as popular today as they have been throughout history. Bread making, wine making, beer making and cheese making have always been with us and do not give any sign of falling from grace with the human race any time soon.

DID YOU KNOW:

During NATO countries' intervention in Bosnia only soldiers from the US were prevented from drinking alcohol by their commanders.

In Europe many high school cafeteria serve alcohol.

In Europe many McDonald restaurants serve alcohol.

CLASS DISCUSSION.

Should parents allow their children to occasionally have a glass of wine with their meals at home?

Would people be more likely to abuse alcohol after they leave their parents'home if they were allowed to drink at home?

Answers to chapter questions

p. 6: What would be the name of a three-carbon alcohol? The prefix for “3” is “pro” and the suffix for an alcohol iis “ol”. So the name of a three-carbon alcohol is propanol (also propyl alcohol).

p. 6: Do you remember the general structure of aldehydes?

```
   O
  //
-  C
    \
   H
```

p. 9: Can you draw an ester? An ester is very similar to an organic acid but with a group substituting for the hydrogen atom on one of the oxygen atoms:

```
 O
  //
-  C
    \
   O – R
```

p. 21: What has to be the genotype of a woman who is a hemophiliac? Remember that a hemophiliac has no working copy of that gene or set of genes. Since a woman has two copies of all these genes, she has to be xx in order to be hemophiliac.

p. 22: Of aaXx, Aa xX, AA XX for women and aa xY, Aa XY, Aa xY for men , which of these genotypes produce the phenotype of a genetic alcoholic? Only aa xY has the phenotype of a genetic alcoholic. Remember that a person must be recessive for both genes or carry only one recessive gene if the other allele is Y.

p. 22: Can you marry a couple and see what the distribution of phenotypes is for their progeny? A possible example would be:

aaXx with aaxY. Note here that the man is a genetic alcoholic

Separating the genes among haploid sperm and egg we obtain four different possibilities for both the man and the woman:

	aX	ax	aX	ax
ax	aaXx	aaxx	aaXx	aaxx
aY	aaXY	aaxY	aaXY	aaxY
ax	aaXx	aaxx	aaXx	aaxx
aY	aaXY	aaxY	aaXY	aaxY

We find here 8 boys and 8 girls. Of the 8 boys 4 are genetic alcoholic and of the eight girls 4 also are genetic alcoholics. Statistically half the boys and half the girls would be genetic alcoholics. In this marriage we do not see the difference in the percentage of genetic alcoholic between men and women.

But what if we married aaxx with aaXY. In this case the woman is a genetic alcoholic.

	ax	ax	ax	ax
aX	aaXx	aaXx	aaXx	aaXx

aY	aaxY	aaxY	aaxY	aaxY
aX	aaXx	aaXx	aaXx	aaXx
aY	aaxY	aaxY	aaxY	aaxY

Here we see that none of the girls are genetic alcoholics while every single boy is a genetic alcoholic.

EXERCISES.

1. To how many other atoms will carbon bond?
2. Draw an alcohol, an organic acid, an aldehyde, a ketone, an ester, an amine.
3. What are the tastes your tongue can sense?
4. How can you sense other tastes than the ones your tongue senses?
5. Is alcohol absorbed only by your stomach? Explain.
6. Can the foetus become drunk when its pregnant mother drinks? Explain.
7. If alcohol is a poison how can it help you live longer when consumed in moderation?
8. Name six compounds that enhance the taste of foods?
9. Other than the enhancement of taste name another effect of carbon dioxide.
10. Explain how wine is considered a food.
11. Name five effects of wine on human physiology.
12. What is vasoconstriction?
13. If the consumption of low quantities of alcohol can warm you up, would you suggest to a friend planning a long walk on a winter day to drink heavily before leaving?

14. Why do you fall asleep if you consume large quantities of alcohol?
15. Can you die of alcohol consumption? Explain.
16. Why is it a bad idea to drive after drinking alcohol? Give and explain at least two reasons.
17. Explain the differences between a genetic alcoholic and a social alcoholic.
18. Can you establish a genetic link to alcoholism in a person who has never consumed alcohol before? Explain.
19. Explain the biochemistry behind the feeling of euphoria that alcohol gives humans.
20. Explain the term "level" society. How can such a society have potential problems with alcoholism?
21. Consider the graph showing the relationship between consumption of alcohol and longevity. What would the graph look like if you plotted it with longevity on the X axis and # of drinks/day on the Y axis?
22. What do you think is meant by the expression: "alcohol is a great equalizer"?
23. Explain the term vasodilation.
24. How does a hangover occur?
25. How does your brain compensate for the pains of every day life?
26. What is organic chemistry?
27. When a solution has a pH of 4.2 are there more H^+ or OH^- in the solution?
28. When a wine contains 0.08% volatile acids what can be expected when opening the bottle and tasting the wine?
29. What effects do the bubbles in champagne have on the body?
30. Why does alcohol comsumed in moderation increase life span?
31. Why does immoderate alcohol consumption shortens life span?
32. Since wine is a food could you nourish yourself exclusively with it?
33. Before the discovery of aspirin therapy for heart attack patients people suffering a heart attack or chest pains were give a glass of brandy or other alcohol. How could this help their situation?
34. Based on your answer to question 33 what would you suggest is one of the effects of aspirin on the body?
35. Describe two ways in which moderate alcohol consumption can help a person suffering from atherosclerosis.
36. What type of drug is alcohol? Explain.
37. What is the BAC for DWI and DWAI in NY state?
38. What observations led scientists to believe that alcoholism had a genetic connection?
39. What is the biochemical reason for withdrawal?
40. List two physiological and psychological reasons for the high alcoholism rate seen within some American Indian cultures. Do you consider one more valid than the other? Explain.
41. What compound makes it possible for rats to digest methanol but not humans?
42. Under what conditions is alcohol a poison?
43. Organize the following according to who would get drunk faster:

    i. Man after drinking 6 beers
    ii. Man after eating pasta dinner and drinking 6 beers
    iii. Man after eating 2 candy bars and drinking six beers
    iv. Woman after eating 2 candy bars and drinking six beers
    v. Woman after drinking 6 beers
    vi. Man after eating a steak and drinking 6 beers

44. The DWI law states that if your blood alcohol content (BAC) exceeds 0.08 you are arrested for driving while intoxicated. For Bob who weighs 180 lbs, this means that if he drinks more than 4 beers in 3 hours, he can not drive. The beer he drinks comes in 12 oz cans and each can contains 3.5% alcohol.

    i. What does BAC of 0.08 really mean? **8%**
    ii. How many ml of beer can Bob drink in 3 hrs before he can not drive (1 ml = 29.6 ml)? **1420.8 ml**
    iii. What % of alcohol is present in 1 ml of beer? **3.5%**

iv. Calculate how fast Bob metabolizes alcohol in ml alcohol per lb body weight per hour. **2.63 ml/ lb*hr**

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LESSON 10. MAKING CHEESE AND FERMENTED MILK

3 tables
No figures
16 illustrations
1 appendix

A. INTRODUCTION.

Most authorities consider that cheese was first made in the Middle East. The earliest type was a form of sour milk which came into being when it was discovered that domesticated animals could be milked. The first fermentation of milk into cheese or other products was probably an accident. The product however had a pleasant smell and did not taste spoiled. A variety of microorganisms floating in the environment, a Lactobacillus for example, had landed in the milk rather than a *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes* , *Yersinia enterocolitica*, *Salmonella* spp., *Campylobacter jejuni* and several strains of *Clostridium*, *Cornebacterium*, *Arthrobacter*, *Microbacterium*, *Micrococcus* , and *Streptococcus*. As you can see many bacteria can grow in milk using the nutrients available but the Lactobacillus would not produce compounds that make humans sick while the others would. A legendary story has it that cheese was 'discovered' by an unknown Arab nomad. He is said to have filled a saddlebag with milk to sustain him on a journey across the desert by horse. After several hours riding he stopped to quench his thirst, only to find that the milk had separated into a pale watery liquid and solid white lumps. Because the saddlebag, which was made from the stomach of a young animal, contained a coagulating enzyme known as rennin, the milk had been effectively separated into curds and whey by the combination of the rennin, the hot sun and the galloping motions of the horse. The nomad, unconcerned with technical details, found the whey drinkable and the curds edible. These two different theories point to two of the methods used today to make cheese. The milk can be coagulated into curds by means of bacteria which produce an acid that coagulate the curds or an enzyme called rennin that accomplishes the same goal.

B. A BRIEF HISTORY OF CHEESE MAKING.

Cheese was known to the ancient Sumerians four thousand years before the birth of Christ. The ancient Greeks credited Aristaeus, a son of Apollo and Cyrene, with its discovery. While it is believed that milk fermentation originated in the Fertile Crescent between the rivers Euphrates and Tigris 6-7,000 years ago (Iraq today) the fact that it was probably an accident would indicate that milk fermentation originated in several countries simultaneously rather than being spread from a common point of origin.

Sumerian carvings, stoneware and writings show the making of cheese as far back as 3000 years ago. Scientists analyzed material found in tombs of that era to determine that it had once been cheese. Carvings from 100 B.C. show the storage of excess milk in skin bags where this milk would have curdled during the nomads' travels. The acid produced by bacteria would not only curdle the milk but also preserve the curds from spoiling microorganisms. Salt was also added to the newly formed curds to further prevent them from spoiling. It is probably at that time that the separation occurred between fermented milk and cheese. If the curd was solid enough and whey could be separated from it by using the perforated bowls found in several locations in Europe and Asia the final product would be a cheese. If the whey was not separated fermented milks such as yogurt or Kephir or Koumis would be the result.

The bible mentions the consumption of cheese. In the first book of Samuel (c. 1000 B.C.) David's father tells him to:" carry these ten cheeses unto the captain of their thousand". This quote not only shows that cheese was consumed in biblical times but also that it was considered a precious gift.
Throughout Greece cheeses were named after the town from which they originated. From the writings of Aristotle and Herodotus among others we know of the sheep's milk cheeses of Cythnos as well as the Scythian and Phrygian cheeses made from mare's milk. The curds for these cheeses were produced with rennin from hare and kid but vinegar and the milk of the fig tree were also used. Several authors also mentioned the need for cleanliness in cheesemaking and the scalding of the curds before the cheese was pressed. It is also clear from these accounts that cheeses varied in quality because of climate and animal feed used.

In the Roman era cheese really came into its own. Cheesemaking was done with skill and knowledge and reached a high standard. By this time the ripening process had been developed and it was known that various treatments and conditions under storage resulted in different flavors and characteristics.

The larger Roman houses had a separate cheese kitchen, the caseale, and storage areas where cheese could be matured. In large towns home made cheese could be taken to a special centre to be smoked. Cheese was served on the tables of the nobility and traveled to the far corners of the Roman Empire as a regular part of the rations of the legions.

The fermented milk was also found to keep far longer than the original milk it came from and thus could be stockpiled and stored until needed. Later humans probably discovered that this fermented product could be used to inoculate fresh milk and that inoculation would confer to the fresh milk the same properties found in the fermented milk.

Trade of cheeses grew very rapidly in the Roman empire and prices had to be fixed by the emperor Diocletian (AD 284-305) to avoid the overpricing of product which would have hurt the trade. A hard dry cheese called Lunar was very popular in the trade business then because it could keep over long periods of time; this cheese was the ancestor of a cheese we now know as Parmesan.

During the Middle Ages constant hostilities and invasion caused a large decline in cheesemaking throughout Europe. The craft was kept alive in monasteries and regions that were isolated enough to be by-passed by conflicts. During the Middle Ages, monks became innovators and developers and it is to them we owe many of the classic varieties of cheese marketed today. While the monasteries kept written records and recipes to ensure the continuation of the art, recipes and techniques were often passed on from generation to generation by word of mouth in villages. We must deduce from this that recipes were lost and others were generated by this more haphazard method of transmission. Other cheeses simply disappeared because consumers did not like them; some disappeared because of changes in agricultural practices, food habits or sociological conditions.

During the Renaissance period cheese suffered a drop in popularity, being considered unhealthy (maybe because of poor quality). It regained favor by the nineteenth century, the period that saw the start of the move from farm to factory production. In the mid 1800s we see again the appearance of industrial cheese making in eastern Europe (Slovakia, Bohemia)

where cooperative organizations were formed to collect and process milk. Up to that time however producers were still puzzled by the same questions that the Romans had pondered. Why did the milk from different regions give different cheeses even when treated in exactly the same manner? Why did gas holes appear in the curd? Why was the cheese sometimes hard and leathery?
In the late nineteenth century scientists started exploring cheese making and established links between agricultural practices, location of the soils, rainfall, farm management, cheese making techniques and cheese quality.

With the development of microbiology in the late 1800s and the advent of refrigeration the production and trade of cheeses grew rapidly. Pure cultures of Lactobacillus or Leuconostoc could be isolated for inoculation into fresh milk. Spoiling bacteria could be eliminated from the milk by pasteurization; this would reduce the chances of spoiling fermentations during the making of cheese and make possible the testing of cheese for the presence of microorganisms. **How would you develop such a test? Your employer, the Cheesy cheesemaking factory wants to insure that the proper type of bacteria is growing in the cheddar it is making. The company asks you to design the test. What will you do?**

Finally refrigeration allowed for long-term storage. This of course would expand the trade in cheese to the international level.

Some cheeses that were locally produced a few hundred years ago became internationally famous and are now made and sold in many counties. The Roquefort has been made for more than 900 years, the Camembert for more than 300 years. Their popularity meant that these products were eventually made in several countries. The name of the cheese thus has come to describe a type of cheese rather than the region it comes from. Although some cheeses cannot carry a name unless they are made in a specific region most cheeses are named for a specific appearance, texture, taste and aroma. **Compare this system of nomenclature to that used in the naming of French and American wines.** Today our world processes probably more than fifty million tons of milk annually into twelve to fifteen million tons of cheese and fermented milk products. Most cultures ferment milk and their members consume these products on a daily basis. The milk of ruminants (cow, goat, sheep, yak, buffalo, ewe, reindeer, llama and zebu) and non-ruminants (camel, mare and ass) is used in different parts of the world. Students at Cornell University also used the milk of a variety of mammals including rats and mice and concluded that cheese could be made from a variety of milk sources.

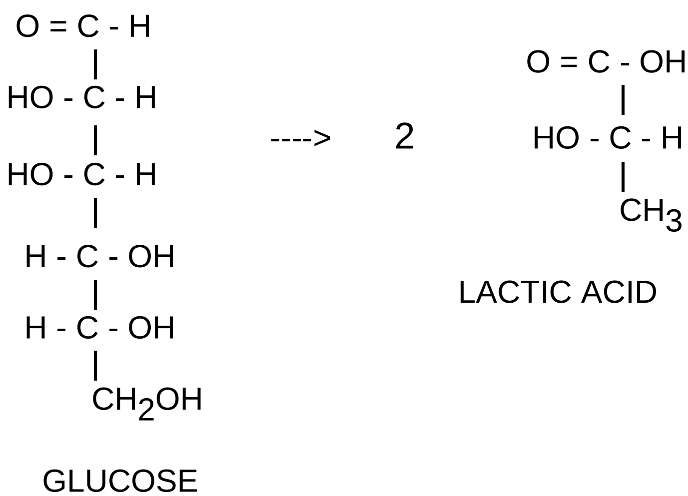
C. TYPES OF FERMENTATION IN MILK.

While several types of bacteria can grow in milk, all of them use the sugar present in milk to draw energy from and grow. The most abundant sugar in milk is lactose, a disaccharide made of glucose and galactose. Bacteria can use these simple sugars and release the energy stored in their bonds. This energy is then stored in the bacteria as ATP for building molecules, growth, reproduction, locomotion, transport of molecules across membranes etc...
In the process of using milk sugars to extract energy bacteria produce waste products. These waste products vary with the microorganism and will give characteristic flavors to the fermented product. High concentrations of these wastes are toxic to the organism that produced it but can be used by other organisms as food; an example of this phenomenon is a plant waste compound called oxygen which is necessary to the survival of mammals. Not all milk fermentation products are edible however. We shall see that each type of milk fermenting

bacteria generates specific waste products that will either produce pleasant aromas and flavors or vile and spoiled ones. Fortunately our own senses can detect the difference.

Of the six types of fermentations discussed below only the first four lead to desirable products.

1-. Lactic acid formation. Bacteria of the *Streptococcus* and *Lactobacillus* families are capable of converting glucose into lactic acid. The conversion actually involves a series of eleven reactions, all catalyzed by enzymes.

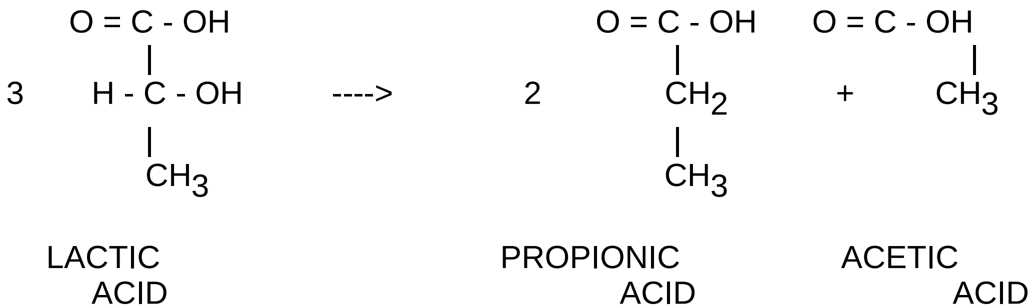


(10.1)

The molecule on the left is glucose and the two molecules of lactic acid produced are depicted on the right. Note that glucose is a six carbon molecule and lactic acid is a three carbon molecule. Eleven steps were necessary to arrive at the product. Note here that the reaction as written must account for the whereabouts of all the atoms in the molecules. Since glucose has six carbons for example the products must show six carbons. This way we can study the fate of the carbons atoms during these reactions. Since these eleven reactions always occur when glucose is broken down to produce energy these are grouped in a pathway called glycolysis. In biological systems these pathways are facilitated by enzymes that speed up the reactions (enzymes are also called catalysts). Enzymes are very large proteins that take reactants into pockets called active sites and orient those reactants in such a way that they touch and react quickly; thus the reaction can proceed at accelerated rates.

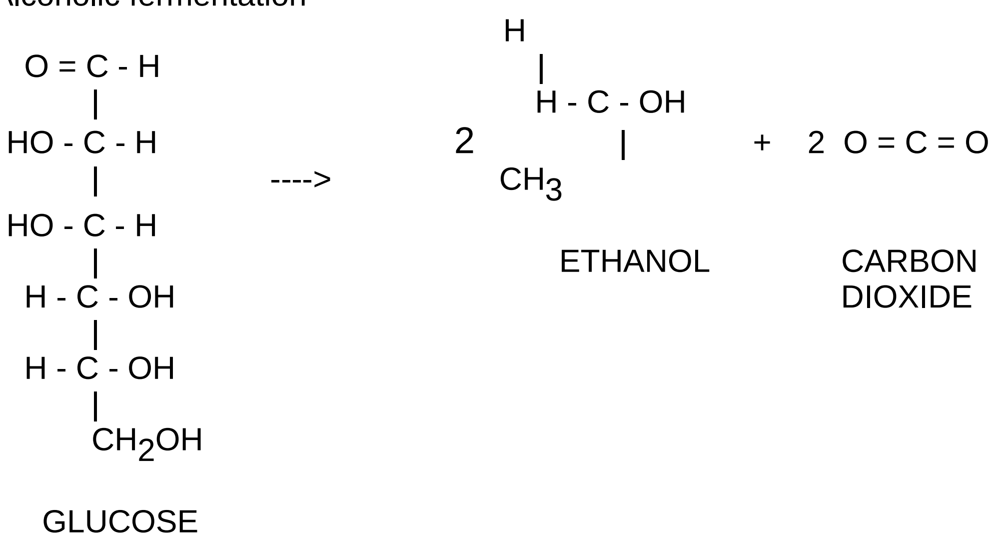
Lactic acid has a sharp biting taste that is characteristic of yogurt for example. When produced in the milk lactic acid precipitates the milk out of solution. They start to aggregate with molecules of fat and with themselves to form **curds** leaving behind a clear liquid called **whey**. As we will see later the acid coagulation of milk can be accomplished with a variety of acids and they do not have to be produced by bacteria; lemon juice is added to milk to make Queso Blanco in Latin America. Similarly other precipitation methods are available to form curds such as the use of salts and enzymes (rennin). Lactic acid fermentation however impart into the cheese a unique flavor that has come to characterize a variety of cheeses.

2. Propionic acid fermentation. Produced by *Propionibacterium shermanii* the acid here is not used to precipitate the curd but rather for flavor and the creation of the eyes that are characteristic of **Gruyere** and **Emmentaler** cheeses.



(10.2)

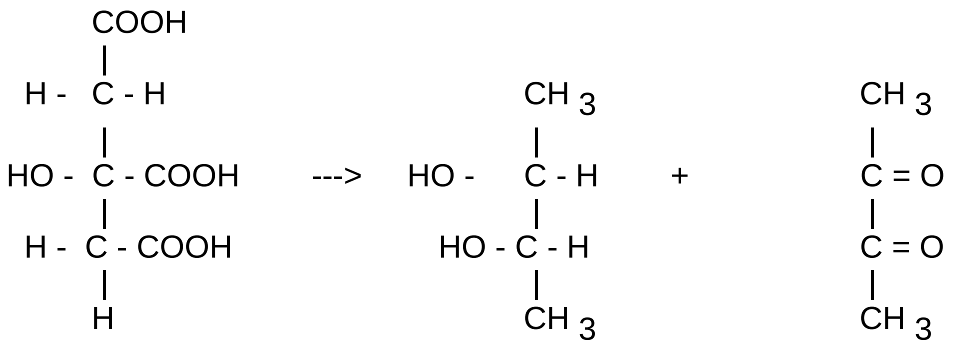
3. Alcoholic fermentation



(10.3)

While the consumption of fermented milk is not popular in this country it is a staple in other parts of the world. These drinks contain 1 to 3% alcohol, about 0.8% lactic acid and are consumed as Kefir(sheep or goat's milk fermented with streptococcus and yeast) and Koumiss (mare's milk fermented with Lactobacillus bulgaricus and **Torula** yeast) in the Caucasus mountains and Russia.

4. Citric acid fermentation

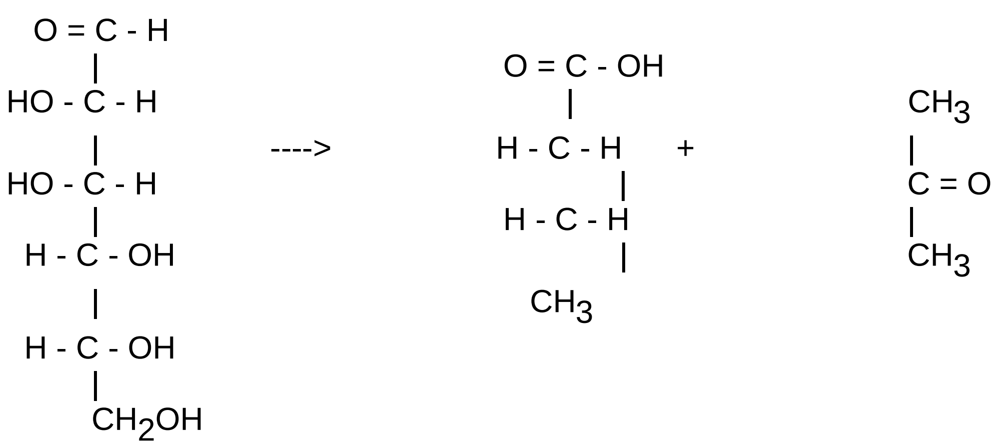


(10.4)

CITRIC ACID — BUTYLENE GLYCOL — DIACETYL

Here the citric acid present in the milk is fermented by organisms such as *Leuconostoc citrovorum* and *L. diacetilactis* to produce aromatic compounds that give its flavor to sour cream and buttermilk.

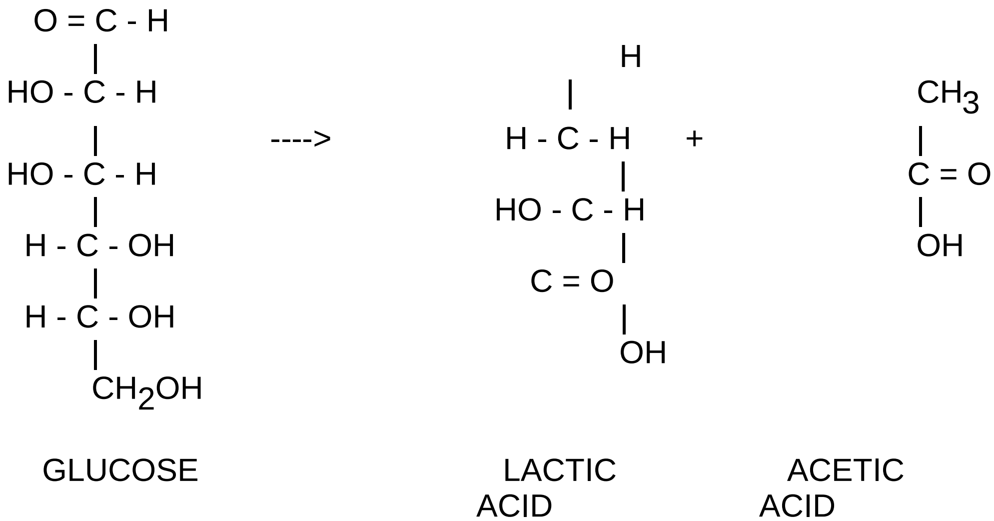
5. Butyric acid fermentation. This type of undesirable fermentation often occurs after the cheese is stored to ripen. *Klebsiella aerogenes* and *Clostridium sporogenes* can resist normal pasteurization and thus can start developing during curing. They use glucose in the cheese to produce butyric acid, carbon dioxide and acetone. The former gives the cheese a rancid flavor while the large carbon dioxide production produces large holes in the cheese.



(10.5)

GLUCOSE — BUTYRIC ACID — ACETONE

6. Vinegar-carbon dioxide fermentation. This fermentation comes from poor quality cleaning practices or contaminated milk. *Escherichia coli* and *Aerobacter aerogenes* use glucose to produce vinegar (acetic acid) and lactic acid.



(10.6)

Ethyl alcohol (CAN YOU DRAW ITS STRUCTURE?) and carbon dioxide are also produced. It is important to point out here that these products were all found in desirable fermentations: lactic acid in the production of yogurt, acetic acid in the production of vinegar and ethanol in the production of wine and beer. Why then is the E. coli fermentation undesirable? There are two reasons to this. The first is that the flavor of a cheese is obtained with a delicate balance of flavor compounds. It one of the components is present is larger quantities the cheese will have a bad aroma and flavor. Thus while several types of bacteria may be able to produce flavor compounds only a few can produce the proper balance or those compounds. Second the rapid production of carbon dioxide can produce holes that deform the curd.

D. THE COMPOSITION OF MILK.

1. Evolution of mammals.

Mammals developed in Triassic (and flourished in the early Jurassic period) from the reptilian order Therapsida, terrestrial herbivores and carnivores. These early mammals had a brain three to four times larger than their reptilian ancestors; this presumably a reflection of greater neuromuscular coordination and improved auditory and olfactory acuity. They were also much smaller animals than their reptilian ancestors. While larger mammals would appear later these early mammals were very small indeed. Their heads could rotate more freely. Their skeleton was simplified. Fewer bones were present in the skull for example and the limbs and limb girdle were simplified.

2. Characteristics of mammals.

While the fossil record clearly shows the evolution in skeletal features it is harder to determine whether these early mammals carried soft tissue characteristics of modern mammals. It is fair to say however that even if these characteristics were not present in early mammals they developed soon after. These characteristics include endothermy, the ability to regulate their own internal temperature. Since there is now evidence to indicate that some reptiles of the period were capable of high energy production it is possible to speculate that these early mammals could as well. Mammals alone have external structures to intercept sound waves, the pinna of the ear structure. The reproductive system in mammals is also unique. Both ovaries are functional and the ovum is fertilized in the oviducts. The embryo develops in the uterus within the confines of a fluid-filled amniotic sac, the placenta. The mother and the fetus are linked by blood vessels that transport food to the fetus and carries wastes out.

Skin glands also appeared in mammals. They cannot be found in other species of vertebrates. Sweat glands provide evaporative cooling. Hair follicles are supplied with sebaceous glands that produce an oil secretion that lubricate the hair and skin. Hair incidentally is a feature unique to mammals. The coat of hair is an insulation for the mammals. It slows down the dissipation of heat to the environment as well as the absorption of heat form the environment. Finally the mammary glands help mammals feed their young for a period of weeks to years after birth with milk, a rich solution of protein, fats, lactose, vitamins and minerals. Since the young's immune system is still not completely developed the mother's milk also carry antibodies (also called gamma-globulins). Mammary glands consist of a system of ducts which reach the surface of the skin through the nipple. Hormonal signals late in pregnancy stimulate the production of secretory alveoli around these ducts that will produce milk. Milk production itself is stimulated by another hormone called prolactin. Nursing and the emptying of the milk stimulate further production of prolactin and milk.

3. Composition of milk.

The components of milk are produced directly or indirectly from blood. Although the osmotic pressure of milk and blood are the same the composition of the two fluids is very different. Milk contains much more sugar, lipids, calcium, phosphorus and potassium than blood. It has less protein, sodium and chlorine than blood. The protein in milk is mostly casein (also some albumins and globulins) while the albumins and globulins are the main proteins in blood plasma. Also quantitatively most of the lipid in milk is in the form of **triglycerides** while **phospholipids** and **cholesterol** are the major blood lipids.

The composition of milk varies widely in mammals. Table 10.1 shows a comparison of milk composition for a few mammals. The major components are fats, proteins and sugars (lactose). Other components include salts (0.9%), pigments (< 0.1%), gases (<0.1%), vitamins (< 0.1%) and volatiles (< 0.1%). The percent water of course varies tremendously from one species to another.

Table 10.1. Composition of Milk (%).

Species	Fat	Protein	Lactose
Ass	1.3	1.8	6.2
Bear (Polar)	33.1	10.9	0.3
Cat	7.1	10.1	4.2
Chimpanzee	3.7	1.2	7.0
Cow (Holstein)	3.5	3.1	4.9
Dog	9.5	9.3	3.1
Goat	3.5	3.1	4.6
Horse	1.6	2.4	6.1
Human	4.3	1.4	6.9

Pig	7.9	5.9	4.9
Seal (Gray)	53.2	11.2	2.6
Sheep	10.4	6.8	3.7
Whale	33.2	12.2	1.4
Yak	7.0	5.2	4.6
Zebu	4.9	3.9	5.1

Note here that mammals that live in cold climates or whose young grow unusually rapidly have a very high percentage of fat and protein in their milk. **What would be the percent water in polar bear milk compared to that of a cow?**

Usually low levels of carbohydrates will accompany high levels of fat. Conversely high sugar contents are usually associated with low fat contents. Note also that the chimpanzee and human milks have similar values for the fat, protein and lactose content. This is not surprising since these two species are genetically very similar. Other data have also shown that in cattle for example there are differences among breeds in the composition of milk. This indicates that genetic selection (by Nature or by humans) can lead to significant differences.

Mammals produce youngs that are not completely ready to be on their own when they are born. This means that the mother must provide special nutrition to the infant for a period of time in order to insure full development.

a. Proteins. Proteins are large molecules made of **amino acids**. An amino acid has the following general structure:

```
          COOH
 +         |
  H3N - C - H                                  (10.7)
           |
           R
```

There is a variety of possibilities for what the R group can be. If"R" is simply a hydrogen atom the amino acid is called glycine. If this "R" group is a methyl group ($-CH_3$) the amino acid is alanine: the "R" group $-CH_2$ - SH defines the amino acid cysteine. While these are simple R groups some amino acids carry more complex ones. In all most mammals carry about 19 amino acids and the proteins in their tissues are made of such amino acids. Proteins are put together by stringing these amino acids together with a special bond called the peptide bond:

```
      R1                    R2
      |                     |                    (10.8)
H3N - CH - C - OH   +   H3N - CH - C – OH   -->
           \\                    \\
```

```
              O                      O
Amino acid 1            amino acid 2
                                      R1        H    R2
                                      |         |    |
                              H3N - CH - C  -  N - CH - C - OH        +  H2O
                                          \\              \\
                                           O              O
                                        dipeptide
```

Can you see what has happened in the reaction? Can you describe it?

The large number of different R groups with different properties gives the proteins a variety of properties for the various functions they can have. The number of amino acids also varies in proteins from only a few (5 in opiates) to thousands. The **immunoglobulins** we mentioned above and make up the **antibodies** have 1200 amino acid in their **sequence**. Consider that each antibody is specific to a given **antigen**. Thus each antibody must have a different arrangement of amino acids to bind specifically to that antigen. The possibilities for variety in proteins are astronomical. If each position in a protein can have any of19 amino acids, the possibilities for different sequences would be 361 for a dipeptide (19 x19), 6859 for a tripeptide (19 x19 x19), 130,321 for a tetrapeptide (19 x19 x19 x19), and 2,476,099 for the **opiate** pentapeptide (19 x19 x 19 x19 x19). Imagine the possibilities for an average size protein made of 500 amino acids.

The **primary structure** of proteins consists of a polypeptide chain of amino acids residues joined together by peptide linkages, which may also be cross-linked by disulphide bridges. **Amino acids** contain both a weakly basic amino group, and a weakly acid carboxyl group both connected to a hydrocarbon chain, which is unique to different amino acids. The three-dimensional organization of proteins, or **conformation**, also involves secondary, tertiary, and quaternary structures. The **secondary structure** refers to the spatial arrangement of amino acid residues that are near one another in the linear sequence. The alpha-helix and ß -pleated sheet are examples of secondary structures arising from regular and periodic steric relationships. The **tertiary structure** refers to the spatial arrangement of amino acid residues that are far apart in the linear sequence, giving rise to further coiling and folding. If the protein is tightly coiled and folded into a somewhat spherical shape, it is called a **globular** protein. If the protein consists of long intermolecularly linked polypeptide chains they are called **fibrous** proteins. **Quaternary structure** occurs when proteins with two or more polypeptide chain subunits are associated

Proteins can actually serve in a variety of roles.

- **Enzymes** are proteins that speed up reactions in cells. Without Them life would be impossible since virtually all reactions of organic biomolecules are catalyzed by enzymes.
- **Transport proteins** bind and transport specific molecules from cell to cell, organ to organ. The prime example of a transport protein is hemoglobin which transports oxygen from the lungs to various tissues and carbon dioxide from these tissues back to the lungs.
- **Storage proteins** are proteins that will be used as a reserve of

energy and amino acids for the future growth of an organism. Examples include the ovalbumin of egg white that is broken down by the embryo to provide the amino acids it needs to build its own proteins. Another example is casein in milk that the young mammal ingest for the same reason.

- **Contractile proteins** such as **actin** and **myosin** are present in muscles to help use move around.

- **Structural proteins** support body structures and help keep things in place. Think of **tendons** and **ligaments** for example that keep bones in their proper alignment and connect bones to muscles. Furthermore your skin, hair, and fingernails are made of pure structural protein.

- **Regulatory proteins** such as **hormones** control cellular and physiological functions. Insulin for example control the entry of sugar inside the cells; growth hormone of course control when you grow and how tall you become. These hormones also start controlling our behavior during adolescence.

- **Defense proteins** such as antibodies are made by specialized cells of the immune systems to neutralize invading foreign proteins of bacteria or viruses. The immune system defines you as a unique individual or self and protects you against any invading foreign particles. Think of graft rejection for example where the immune system would fight a kidney that your sister gave you when yours failed. Even though you and your sister are very close genetically she is still a different self than you and your immune system would recognize that kidney of hers as an invader in your body.
  In this category of defense proteins we must also include venom and toxic plant proteins. They protect the organisms from predators. Blood clotting proteins could also be considered defense proteins since they clot the blood when you cut yourself so that you do not bleed to death. The milk of mammals does contain antibodies that are passed on to their young to protect them as their own immune system develops.

b. Fats. We have all heard of fats and our culture has been obsessed with them. These compounds are in fact a reserve of energy that our bodies store for times when foods might be less plentiful. They then supply our energy needs until we find more food. This ability to store energy was a very important feature of our bodies when, thousands and millions of years ago, humans lived a nomad’s life, traveling constantly to find new sources of food and sometimes having to spend many days without eating. Special cells in the body called **adipose cells**, are responsible for storing these fats. The degree to which they are filled can control our appetite. In one theory currently being developed about obesity people are born with different numbers of adipose cells; some people have a lot more than others. These cells require to be filled with fats and the body complies by increasing food intake. This means that a people with more adipose cells put their bodies into a state of starvation in order to comply with our society's norms of thinness.

There are several categories of fats in the bodies of mammals. They serve as energy storage, structural functions and insulation. However all fats have structural characteristics that are similar. They all carry a long chain of carbon and hydrogen atoms. The number of carbons varies from only a few to twenty and thirty carbon atoms. The most common chains in mammals however are 16 and 18 carbons in length. When the last carbon atom on the chain is a carboxyl group (refer to chapter 8 for a brief listing of functional groups):

```
        O
   //
    - C                                   (10.9)
   \
      O – H
```

The fat is now called a fatty acid. This one of sixteen carbon in length is called palmitic acid.

```
   H   H  H   H  H  H   H   H H   H  H   H H   H   H     O      (10.10)
   |   |  |   |  |  |   |   |  |   |  |   |  |   |   |    //
H – C – C- C – C – C – C – C – C – C – C- C – C – C – C – C – C .
   |   |  |   |  |  |   |   |  |   |  |   |  |   |   |    \
   H   H  H   H  H  H   H   H  H   H  H   H  H   H   H     O – H
```

What is interesting about this molecule is that part of it repels water and the other part attracts it. DO YOU KNOW WHICH PART ATTRACTS WATER MOLECULES?

```
        O                    (10.11)
      /   \
    H       H
```

A water molecule contains an oxygen atom. This atom in a molecule means often the presence of a property called hydrophilicity. It means that the molecule will be attracted to other molecules carrying a similar atom and to water. These molecules will mix in water. Think of sugars for example. On the other hand a molecule that does not contain oxygens will likely not be able to mix in with water. Think of gasoline for example which would float on top of the water instead of mixing with it:

```
    H   H  H   H  H  H  H   H
    |   |  |   |  |   |  |   |                              (10.12)
 H – C – C- C – C – C – C – C – C - H.
    |   |  |   |  |   |  |   |
```

```
  H   H  H   H  H  H  H   H
```

This molecule is called octane and is one of the molecules that make up gasoline, a mixture of 5 or 6 molecules similar to octane.

If you look at a molecule of octane and a molecule of palmitic acid you notice that they have a very similar structure: a long chain of **hydrocarbon**, a mixture of hydrogen and carbon atoms. That segment of the molecule does not mix with water. On the other hand the carboxyl group at the end of the molecule of palmitic acid does interact with water. This gives palmitic acid a **hydrophobic** character (repels water) and a **hydrophilic** character (attracts water). For this reason we call the molecules of fatty acid **amphoteric**. This gives them very interesting properties. Soap for example is made of molecules of fatty acids that have been given a permanent charge by taking away the hydrogen atom on the carboxylic group to make the carboxylic group even more attractive to water:

```
    H   H  H   H  H  H   H   H  H  H  H   H  H  H   H      O                (10.13)
    |   |  |   |  |   |   |   |  |   |  |   |  |   |   |     //
H – C – C- C – C – C – C – C – C – C – C- C – C – C – C – C – C .
    |   |  |   |  |   |   |   |  |   |  |   |  |   |   |     \ _
    H   H  H   H  H  H   H   H  H  H  H   H  H  H   H     O
```

When many fatty acid molecules are present in water the hydrophobic segment of these molecules cannot interact with water and thus interacts with the hydrophobic segment of other molecules of fatty acid. They form a ball where the hydrophilic head on the outside of this ball to interact with water. This formation keeps the molecules of fatty acid in suspension in water. This is how a molecule that is mostly hydrophobic can stay in solution in water. This structure is called a **micelle** and they are so large that you can actually see them in water because it gives soapy water a **turbid** or murky look. The particle of dirt on the clothes that you wash will have more affinity for the hydrophobic inside of the micelles and will be carried away from the clothes. Next time your mother washes your clothes you may want to entertain her with the reason soaps and detergents work.

Fats also play a crucial role in the building and maintenance of the cell membrane. A special category of fats (**phospholipids)** is used for this purpose. The molecule is made of two molecules of fatty acid linked to a molecule of sugar (**glycerol**); this sugar is also linked to a variety of large hydrophilic molecules. This gives the phospholipid a larger hydrophilic head than the fatty acids we have seen before. These phospholipids line themselves in rows so that their hydrophobic tails are interacting together. These tails must hide from the water environment they are in and so they will interact with the tails of another row of phospholipids to form a double layer. This double layer will be the basis for a layer called a **membrane** that surrounds the contents of the cell (see chapter 4 for a description of the cell).

```
  O  O  O  O  O  O  O  O  O  O  O  O  ←-hydrophilic head          (10.14)
||  || ||  || ||  || ||  || ||  || ||  ||  ←-hydrophobic tail
```

|| || || || || || || || || || || || ←-hydrophobic tail
O O O O O O O O O O O O ←-hydrophilic head

This membrane is semipermeable which means that it allows only certain compounds to come in and to leave. These compounds that are allowed in the cell will serve as nutrients and building blocks while the compounds coming out are the waste products. Inside the cell the nucleus and the organelles are also surrounded and protected by membranes made in the same way. .

So far we have talked about only a few of the different types of lipids present in nature. Others include waxes and lipids that make up a good part of your brain, called sphingophospholipids. But neither of these are present in the milk that we will use to make cheese and fermented milk products.

E. FERMENTED MILK PRODUCTS: YOGURT

Yogurt is similar to kefir, koumiss, acidophilus milk, buttermilk and kir among others. Traditionally yogurt is made with condensed milk i.e. milk which has lost 30% of its water. But whole milk can be used today to make yogurt although milk solids, gelatin or gums are often added to thicken the final product i.e. hold water better within their structure.
Several bacterial cultures are used to make yogurt but all of them use the sugar lactose for their energy source and convert it to lactic acid. *Lactobacillus bulgaricus , Streptococcus thermophilus*, *Lactobacillus lactis* and *Lactobacillus helveticus* are all bacterial cultures used to make yogurt. These cultures usually grow in milk at higher temperatures (40 ^{o}C and above) are called thermophilic.
Other species in this group of lactic acid bacteria are also able to ferment lactose to lactic acid. They are normally present in the milk and are also used as starter cultures in the production of cultured dairy products such as yogurt. Some examples in milk are:

1. lactococci

L. delbrueckii subsp. *lactis* (*Streptococcus lactis*)
Lactococcus lactis subsp. *cremoris* (*Streptococcus cremoris*)

2. lactobacilli

Lactobacillus casei
Leuconostoc

The major lactic starter organisms and their physiological characteristics are shown in Table 10.2. Generally, *Streptococcus cremoris, Streptococcus lactis. Streptococcus thermophilus* and *lactobacillus acidophilus* are used for acid production. *Streptococcus diacetylactis* and *Lactobacillus bulgaricus* are used for acid and flavor production. *Leuconostoc cremoris* and *Leuconostoc dextranicum* are used for flavor production. The combination of different species and strains of these cultures allows for diversity in acid and flavor production.Yogurt is commonly made with a mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*,

Lactic acid cultures are often called starters. They are **micro-aerophilic** which means they tolerate only low oxygen concentrations. They are not **psychrotrophic** which means that cold storage rapidly depletes their numbers and encourages the growth of spoilage microorganisms. They can be. cocci (spherical cells) 1 µm in diameter OR rods (rod shaped cells) 1 µm wide and 2 to 3 µm long

The lactic acid produced by the bacteria produces a **gel** rather than a curd in which the coagulated milk proteins trap the water. Milk contains small **micelles** (globules) of the protein **casein**. Heating the milk above 85 ^{o}C causes the casein micelles to aggregate with another protein called **lactoglobulin**. The resulting complexes do not aggregate as much under conditions of low pH as in cheese where the action of rennin on casein prevents lactoglobulin interaction and allows for larger aggregate formation. This is the reason why yogurt is a gel that retains all the water present in the milk while the **curd** formed in cheesemaking extrudes the whey.

F. CHEESE.

1. The curdling process

Coagulating or curdling the milk until it turns into curds and whey is the first step taken when making cheese. Today, cheese is curdled with a bacterial culture (Table10.2) and a coagulating enzyme called **rennin**, both of which help to speed the separation of liquids and solids. The curdling process begins by warming the milk until it reaches a bacteria-free temperature. During the warming period, a coloring dye is sometimes added to produce a particular color in the finished product. Once the milk has reached a consistent temperature, the starter culture is added and the milk begins

Table 10.2 Characteristics of starter organisms used in manufacturing fermented diary products

Culture	Incubation temperature (Centrigrades)	growth 10 oC	growth 45 oC	acid production % of milk	diacetyl production	pH drop	salt tolerance
Streptococcus cremoris	22-30	+	-	0.8-1.0	-	++	4.0
Streptococcus lactis	21-30	+	-	0.8-1.0	-	++	4.0-6.5
Streptococcus thermophilus	40-45	-	+	0.8-1.0	+	+	2.0
Streptococcus diacetylactis	22-28	+	-	0.8-1.0	+	++	4.0-6.5
Lactobacillus acidophilus	37	-	+	0.3-2.0	-	+,-	6.5
Lactobaciltus	42	-	+	1.5-4.0	-	++	2.0

bulgaricus							
Leuconoctoc cremoris	20-25	+	-	0.1-0.3	+	+,-	6.5.0
Leuconoctoc dextranicum	20-25	+	-	0.1-0.3	+	+,-	4.0-6.5

to coagulate into one large curd. Starter cultures are those microorganisms that are used in the production of cultured dairy products such as yogurt and cheese. The natural microflora of the milk is not efficient or predictable enough and is destroyed by pasteurization. A starter culture provides particular characteristics in a more controlled and predictable fermentation. They produce lactic acid but also flavor and aroma compounds as well as prevent the growth of other organisms.

There are two groups of lactic starter cultures:

a. Single strain
b. Mixed or compound: with more than one strain, each providing its own specific characteristics

Starter cultures may be categorized as mesophilic or thermophilic:

Mesophilic

* *Lactococcus lactis* subsp. *cremoris*
* *L. delbrueckii* subsp. *lactis*
* *L. lactis* subsp. *lactis* biovar *diacetylactis*
* *Leuconostoc mesenteroides* subsp. *cremoris*

Thermophilic

* *Streptococcus salivarius* subsp. *thermophilus* (*S.thermophilus*)
* *Lactobacillus delbrueckii* subsp. *bulgaricus*
* *L. delbrueckii* subsp. *lactis*
* *L. casei*
* *L. helveticus*
* *L. plantarum*

Mixtures of mesophilic and thermophilic microorganisms can also be used as in the production of yogurt.

Raw milk at warm temperature will support a variety of micro-organisms in succession as the pH changes over time. In controlled conversion of milk to fermented dairy products, a primary component of fermentation is development of acidity by lactic acid bacteria. Acid development in cheese making is absolutely essential to cheese flavor, cheese texture and cheese safety. Acid is required to:

a. Assist coagulation. High pH produces soft, soapy, fruity and bitter cheese. Low pH produces cheese with brittle texture and mottled color

b. Help the protein matrix in the curd to contract and squeeze out moisture. That process of contraction is called **syneresis**.

c. Prevent growth of pathogenic and spoilage bacteria. Proper rate and extent of acid development is the most important principle with respect to quality and safety of natural cheese. With the exception of noncultured cheese varieties such as ricotta, proper culture growth and acid development is probably equal in importance to pasteurization with respect to safety.

d. Develop cheese texture, flavor and color. The following general associations are relevant to most cheese varieties. In addition to lactic acid cultures many special or secondary cultures are used to promote specific ripening (both flavor and texture) characteristics that are specific to different types of cheeses..

- Large holes: *Propioni bacterium freudenreichii subsp. shermaniee*
- White molds: *Penicillium camembertii, P. caseiocolum, and P. candidum*
- Blue/green molds: *Penicillium roqueforti, Penicillium glaucum*
- Smears i.e. yeasts and molds
- Various coryneform bacteria including *Brevibacterium linens*, several species of *micrococci*, and several species of *Staphylocci*
- Ripening adjuncts: such as salt.
- Bacterial or yeast culture additions.
- Attenuated cultures which are not intended to grow but only to contribute their enzymes
- Species of Lactobacilli and pediococci which are intended to grow during cheese ripening and contribute enzymes

2. Removing the whey.

As the milk forms into a huge curd, it is stirred and cut, allowing the whey to drain off. The milk is then reheated and pressed to remove as much whey as possible.

3. Molding and shaping.

When the whey removal process is finished, the warm curd is molded or shaped into a cheese. Many cheeses today are shaped by using a cheese wheel or similar mold. The warm curd is poured and pressed into the molding.

4. Salting and shaping.
High amounts of salt are added to cheese during or before the process of molding. Salt plays an important role in the formation of the cheeses rind or outer coating. Heavily salted cheeses will develop thick outer coatings, such as that found on swiss cheese.

5. Ripening.
Once the cheese has been molded and salted, it is allowed to ripen. Some cheeses take only two weeks to mature and others can take as long as 7-years. Temperatures remain exact during this time.

It is during this period that the rind of the cheese is formed. Some form naturally and others, artificially. Many cheese surfaces are treated with bacteria, alcohol, wax, oils, or water during the maturing phase to enhance flavor and coloring. Washed rind varieties are washed and brushed regularly to promote an even bacteria growth across the surface and prevent their insides from drying out. Cheddar cheeses are salted and then wrapped with cotton, after which time they are left untouched until they are mature. In Swiss cheese carbon dioxide production by *Propionibacterium* is encouraged by exposure to 200 ^{o}C for about 3 weeks after brining and drying off in the cold room. For smear ripened cheese, *Brevibacterium linens* , coryneform bacteria, and yeasts are encouraged by high humidity (90-95%) and washing to discourage molds
Other cheeses are colonized by a blue green mold that the crumbly open texture of the cheeses is invaded by the *Penicillium glaucum* (synon. *P. roquefortii*). *Penicillium sp.* for Camembert, Brie and Blue types require 85-90% humidity and air circulation to provide oxygen.

According to the type of surface characteristics, cheese treatments are grouped as follows:

a. Ripened by surface molds as for Camembert, Brie and Blue cheeses.
b. Washed rinds without (or with little) bacterial growth, e.g., St. Paulin types.
c. Washed rinds with smear, e.g., Muenster types and Oka
d. Dry rinds which may be coated with oil or butter to prevent cracking and desiccation, e.g., Edam, Scamorza, and Parmesan.
e. Waxes and resins which may be applied by dipping, brushing or spraying. These provide good protection but are more permeable than plastic films, so it is still desirable to maintain 85% Relative Humidity to prevent drying.
f. Rindless cheese which are cured in moisture and gas impermeable film or in large blocks (eg., 640 lb Cheddar)

G. NUTRITION.

Milk contains 87% water, 5% sugar (although yogurt and cheese are devoid of sugar because bacterial cultures used the sugar to produce lactic acid), 4%fat, 3% protein and 1% vitamins and minerals (Table 10.3). One pint of milk contains 18g of high quality protein, no starch, 28g of carbohydrate as the sugar lactose and 23g of fat. (28g = 1oz) It should be used as part of a protein meal.

Milk is particularly useful as a source of calcium and of riboflavin. Most children can drink and digest milk and it contains a useful amount of most vitamins, except for vitamins C and D. It contains most minerals except for iron. Skimmed milk has less than 1.8% fat and has lost most of the fat soluble vitamins. It still retains the same calcium levels as full milk. Heat treated milk contains fewer vitamins than fresh milk

Cheese is formed from casein and fats, and still contains most of the protein, fat and vitamin A of the milk. It has lost most of the lactose and the B vitamins (Table 10.3).

Yogurt is nutritionally similar to milk, except that the lactose level has been reduced. It still contains excellent levels of protein, calcium and riboflavin. Fat levels can vary from 0.2% to 9% according to the type of milk used (Table 10.3). Yogurts often have added flavorings and sugar, but plain yogurts to which you add your own flavorings give the greatest control of the nutritional content.

Single cream is 21% fat, whipping cream is 21% fat and double cream is 48% fat. It still contains vitamins A, E and D (Table 10.3).

Eggs contain 12% protein and 10% fat. and contribute useful amounts of vitamins D, retinol, riboflavin and the minerals iodine and iron. The iron is best absorbed when eaten with a meal that contains plenty of vitamin C (Table 10.3).

1. Proteins.

Cheese protein belongs to the food proteins with the highest biological valency. Which means that it contains a lot of important amino acids. These special protein components must be taken with the food.

Milk and cheese proteins increase the biological valency of other food proteins, for example the ones from bread or potatoes. In a cheese sandwich or a cheese-potato-baked pudding the essential amino acids optimally complement one another. Also, a snack of cheese and nuts is a high quality protein combination. Cheese protein is especially digestible, as it is further split up during the ripening of the cheese.

Table 10.3. Protein, sugar, fat and calcium content of various foods

Food	protein	starch	sugar	fat	calcium/100g
Whole milk	3.2%	0	4.8%	2.4%	115mg
Yogurt, full	5.7%	0	7.8%	3%	200mg
Skim milk	3.3%	0	5%	0.1%	120mg
Cream, single	2.6%	0	4.1%	19.1%	91mg
Cheddar	25.5%	0	0	34.4%	720mg
Brie	19.3%	0	0	26.9%	540mg
Eggs	12.5%	0	0	10.8%	57mg

Allergies from cows milk depends on the protein structure of the milk. In comparison to cow milk, the goat milk has a little less milk sugar and lower fat portion. These differences are sufficient enough that some cow milk allergies don't flare up when digesting milk from goats or sheep.

2. Fats.
The fat content of milk (and therefore cheese) is of economic importance because milk is sold on the basis of fat. Milk fatty acids originate either from microbial activity in the rumen, and transported to the secretory cells via the blood and lymph, or from synthesis in the secretory cells. The main milk lipids are a class called **triglycerides** which are comprised of a glycerol backbone binding up to three different fatty acids. The fatty acids are composed of a hydrocarbon chain and a carboxyl group. The major fatty acids found in milk are:

a. Long chain
- C14 - myristic 11%
- C16 - palmitic 26%
- C18 - stearic 10%
- C18:1 - oleic 20%

b. Short chain (11%)
- C4 - butyric*
- C6 - caproic
- C8 - caprylic
- C10 - capric

Butyric fatty acid is specific for milk fat of ruminant animals and is responsible for the rancid flavor when it is cleaved from glycerol by lipase action.

Saturated fatty acids (no double bonds), such as myristic, palmitic, and stearic acids make up two thirds of milk fatty acids. Oleic acid is the most abundant **unsaturated fatty acid** in milk with one double bond. While the **cis** form of geometric isomer is the most common found in nature, approximately 5% of all unsaturated bonds are in the **trans** position as a result of rumen hydrogenation.

```
  H   H           H
  |   |           |
- C - C - C = C - C -          Cis form                    (10.15)
  |   |   |   |   |
  H   H   H   H   H
```

```
  H   H       H   H
  |   |       |   |
- C - C - C = C - C -          Trans form                  (10.16)
  |   |   |       |
  H   H   H       H
```

Triglycerides account for 98.3% of milk and cheese fat. The distribution of fatty acids on the triglyceride chain, while there are hundreds of different combinations, is not random. The fatty acid pattern is important when determining the physical properties of the lipids. In general, the SN1 position binds longer carbon length fatty acids, and the SN3 position binds mostly shorter carbon length and unsaturated fatty acids. For example:

- C4 - 97% in SN3
- C6 - 84% in SN3
- C18 - 58% in SN1

The small amounts of mono-, diglycerides, and free fatty acids in fresh milk may be a product of early lipolysis or simply incomplete synthesis. Other classes of lipids include **phospholipids** (0.8%) which are mainly associated with the fat globule membrane, and **cholesterol** (0.3%) which is mostly located in the fat globule core

The taste of a cheese has a lot to do with its fat content. On one hand, much of the taste is derived from the fat soluble milk characteristics of the cheese, on the other hand much of the flavor comes about during the maturing process and from the fatty acids.

The milk fat contained in the cheese is especially easy to digest because of its high amount of short and middle chained fatty acids. As these are components that are easily burned off, they will be immediately processed and not stored as fat. The fine distribution of the smaller fat balls makes it easier to digest milk fat in the intestine. Milk fat is also a carrier of the fat soluble vitamins A, D, E and K.

3. Vitamins and minerals.

In no other food is calcium so prevalent and in such a useful form as it is in milk, milk products and cheese. Calcium is essential for the building and for the preservation of bones and teeth and the prevention of osteoporosis. 100 g sliced or hard cheese per day covers the necessary daily amount of calcium!. Next to calcium, magnesium is also an important mineral in the cheese. It fulfils essential functions for muscles and the co-operation of nerves and muscles.

Cheese also provides Vitamin A, Beta Carotene as well as several B group vitamins.

Vitamin A is essential for the function of the optic cells. It is needed for the mucous membranes of the body and it is an essential vitamin for all growing processes.

Vitamin B group has in total eight vitamins, cheese contains Vitamin B2, B12 and Pantothenic Acid. They are important for the control and the regulation of important metabolic functions. They are primarily found in meat products, however cheese is also rich in these vitamins.

Answers to chapter questions.

p. 3: **How would you develop such a test? Your employer, the Cheesy cheesemaking factory wants to insure that the proper type of bacteria is growing in the cheddar it is making. The company asks you to design the test. What will you do?**
From what you know of microbiology it is possible to recognize certain microscopic and microscopic features of bacteria, yeasts and molds. A cheese culture can be plated on solid medium and grown into isolate colonies. The shape and consistency of these colonies can be indicative of molds present. Microscopic observations will enable you to recognize bacteria, yeasts and molds based on size and shape of the cells. Bacterial types (coccus, bacillus etc) can also be differentiated. One can also identify Gram + and Gram – bacteria.

p. 8: Can you draw the structure of ethyl alcohol ? Remember that the prefix "eth" means 2 carbon atoms and the functional groups of alcohols is –OH. Also remember that each carbon atom must be surrounded by 4 bonds. Putting together we have:

```
          H    H
          |    |
H  -  C  -  C  -  O  -  H
      |    |
      H    H
```

p.10 : **What would be the percent water in polar bear milk compared to that of a cow?** Both animals are of comparable size but the polar bear lives in much colder climates. Fat is very important to this animal as it acts (among other things) as an insulation against the cold. After birth the young animal will have to quickly build up and maintain this fat insulation. Therefore one would expect the milk of its mother to have a very high fat content. Table 10.1 shows that the milk of the polar bear contains 33% fat while the milk of a cow contains 10 times less fat.

p. 11: **Can you see what has happened in the reaction**? **Can you describe it?**

What is known as an amide bond has formed. The COOH of the first amino acid and the NH_3 group of the second amino acid have “fused”. The carbon atom of the carboxyl group is now bonded to the nitrogen atom of the amino group. The resulting reaction has also formed H_2O since the carboxyl group lost an OH group and the amino groups has lost an H.

p. 14: Do you know which part of the fatty acid attracts water? The COOH part of the molecule will be attracted to water because they both contain similar bonds (-OH).

EXERCISES.

1. Why is cheese hard in texture and yogurt soft in texture?

Appendix 1.

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