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THE EFFECT OF YEAST STRAINS USED
FOR FERMENTATION ON THE CONGENER
CONCENTRATION IN DISTILLED FRUIT BRANDIES

presented by

Matthew D. Berg

has been accepted towards fulfillment
of the requirements for

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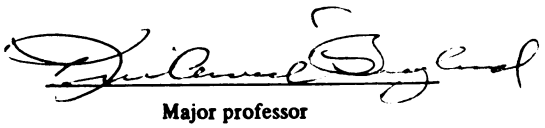

Major professor

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THE EFFECT OF YEAST STRAINS USED FOR FERMENTATION ON THE CONGENER CONCENTRATION IN DISTILLED FRUIT BRANDIES

By

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ABSTRACT

THE EFFECT OF DIFFERENT YEAST STRAINS USED FOR FERMENTATION ON THE CONGENER CONCENTRATION IN DISTILLED FRUIT BRANDIES

By

Matthew D. Berg

The goal of this research was to demonstrate that different yeast strains produce varying amounts of flavor components when fermenting and distilling fruit to produce brandy. Five strains of yeast were used for fermentation of nine different fruits that are commonly grown in Michigan. The resulting distillates were analyzed using gas chromatography to identify ethanol, methanol, fusel alcohols, benzaldehyde, acetone, and acetaldehyde. Based on an objective ranking system, the five yeasts were compared to one another to determine which yeast was best for fermentation by analyzing the resulting distillates. These data will benefit distillers by increasing the predictability and quality of brandy production thus making the process more profitable.

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1. INTRODUCTION

1.1 Michigan Fruit Brandy Industry

Agricultural advances in the United States and particularly in the state of Michigan have increased the number of apples, cherries, grapes, peaches, pears, and plums grown each year. Despite this increased growth, the demand or consumption of these fruit crops has remained relatively stable resulting in large surpluses of fruit crops. These surpluses combined with fruit determined to be unfit for market due to cosmetic defects have created a need for the development of alternative uses for excess fruit crops.

A relatively new use currently being introduced into the United States for excess fruit crops is the production of brandy. This technique has been extensively used in Europe, but is still in its infancy in the United States. The number of stills in the state of Michigan used for the distillation of fruit spirits has grown from zero in 1996 to seven in the year 2002. This growth in the number of distilleries can be accounted for by the need to develop a market for excess fruit crops combined with changes in the state laws of Michigan regarding the licensing of fruit distilleries to facilitate the creation of a distilling industry.

1.2 The History of Distillation

Although the distillation of fruit spirits is a relatively new technology being developed in the United States, it has been utilized for many centuries. Distillation is a very old technique which was used by the Chinese 3000 years BC, the East Indians 2500 years BC, the Egyptians 2000 years BC, the Greeks 1000 years BC, and the Romans 200 B.C.[6] Early stills consisted of a copper boiler heated over an open flame. The alcohol vapors were condensed and collected using wool fibers. Distillation of this type is called

alambic distillation. The Arabs brought the technique of distillation to Europe around the sixth century AD. In 1250, Arnaud de Villenueve was the first to distill wines in France; he called the product that resulted from this process "eau-de-vie" or water of life.[6] Over 750 years later Villenueve's name for distilled wines is still used to identify distilled fruit spirits. The Dutch, French, Irish, Scottish and others started producing distilled spirits around the 15th and 16th century.[6] They created gin (Holland), whiskey (Scotland and Ireland), armanac (France), and cognac (France).

1.3 Different Distillation Approaches

There are two styles of fruit distillation currently in use. The "French" style involves distillation through a simple pot still, which is called alambic. Multiple distillations are required in order to obtain high proof spirits. Multiple distillations cause a loss of ethanol and more importantly a loss of aromatic compounds that lend flavor to the distillate. Spirits from the "French" style of distillation are termed cognac style spirits. The "German" style of distillation involves a single pass through a batch still with a reflux column to obtain high proof spirits. These spirits are usually called eau-de-vie (French) or schnapps (German). They are traditionally stored in glass and served as water clear brandies.[18] The batch column still is designed to trap the fruit essence, which adds to the brandy's aroma and flavor. The additional aroma and flavor of fruit spirits distilled using the "German" style of distillation makes it the preferred method for most fruits except for grapes (cognac and armanac) and apples (calvados).

1.4 Objectives

The objective of this study was to determine the relationship between the strain of yeast used to ferment various fruits and the resulting composition of the distillate. The

five strains of yeast studied were Lalvin Bourgoblanc CY3079, Lalvin ICV 254D, Lalvin Prise de Mousse EC-1118, Lalvin Rhone L2056, and Lalvin S6U. The following chemical compounds in the distillate were analyzed: acetaldehyde, acetone, methanol, 2-propanol, ethanol, 2-methyl-2-butanol, 2-butanol, 1-propanol, 1-butanol, 3-methyl-1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, benzaldehyde, and benzyl alcohol. A comprehensive guide for yeast strain selection for a given variety of fruit was developed.

2. BACKGROUND INFORMATION

2.1 Raw Material

Generally all sugar containing and fermentable materials can be considered as basic materials for the production of brandies.[18] In this study the focus was on the production of brandy from fresh fruit sources grown in the state of Michigan. The domestic fruits studied were Bartlett pears, black plums, Braeburn apples, Gala apples, Granny Smith apples, green grapes (seedless), Montmorency cherries, Newhaven peaches, and red grapes (seedless).

Fruit for fermentation was selected that was at an advanced stage of ripening yet visibly free of mold. With advanced ripening the sugar content increases at the cost of the acid content, a fact usually desired.[18] Higher sugar content leads to the production of greater quantities of ethanol; however, fruit with an acid content too low (high sugar/acid ratio) not only affects the taste in an unsatisfactory way, the mashes obtained from them are more susceptible to the development of undesired microorganisms.[18] Acetic acid, lactic acid, and butanoic acid bacteria are among the most common microorganisms potentially found in overly ripe or moldy fruit. The microorganisms utilize sugars such as glucose and fructose to produce unwanted products other than ethanol. 2-Butanol, 1-propanol, and ethyl acetate are typical compounds formed due to bacterial activity.[2]

The main constituents of fruit include water, carbohydrates, fruit acids, protein like substances, phenolic substances, vitamins, aromatic agents, and mineral agents. The water content of fresh fruit is between 80 and 85%.[18] Water decreases the viscosity of a fruit mash thus assisting fermentation.

The main carbohydrates found in fresh fruit include glucose, fructose, and sucrose. Glucose and fructose are monosaccharides and thus are directly fermentable by yeast. Sucrose is a disaccharide and thus is not directly fermentable by yeast. Bakers yeast typically possesses a potent invertase activity.[10] This enzyme splits sucrose into glucose and fructose.[10] Invertase therefore allows the yeast cells to use sucrose in the production of ethanol.

Citric, malic, and tartaric acid are the main acids found in fresh fruit. These acids maintain a lower mash pH and thus aid in preventing the growth of unwanted microorganisms. Protein like substances comprise 1% of the mass of fresh fruit. They provide amino acids which serve as a breeding ground for the yeast cells and they are affiliated with enzymes. Phenolic substances give fruits their distinctive colors. These molecules can condense to larger molecules that can give distillates a harsh and astringent taste. Vitamin C is present in fruits but does not effect the distillate aroma or flavor. Aromatic agents such as alcohols, volatile acids, esters, aldehydes, acetals, and ketones comprise less than 1% of the mass of fresh fruit. These compounds increase with fruit ripening and give distillates their distinctive aroma and flavor. Fresh fruit contains mineral agents such as calcium, iron, magnesium, phosphorus, and potassium that assist the growth of yeast cells.

2.2 Fruit Mashing

The primary purpose of mashing is to increase the surface area of the fruit. Increased surface area gives yeast cells increased exposure to carbohydrates such as glucose, fructose, and sucrose. The type of utensil or machinery chosen to mash fresh

fruit is dependent on the volume and/or type of fruit. For small volumes of fresh fruit mashing can be accomplished through squeezing, grinding, or mixing. A wooden pestle is often the utensil of choice to aid in the mashing of small volumes of fruit.

Larger volumes of fresh fruit are mashed using a rolling mill or a ratz mill. Rolling mills are used to mash stone and berry fruit. They consist of parallel rollers that rotate in opposite directions. The distance between rollers can be adjusted to allow large stones to pass through the mill. Ratz mills are used to crush seed fruits. The fruit is pressed against a grinding casing by means of a rotor with several blades. This casing has milling blades and slits at the bottom that are arranged axially. The crushed fruit is pressed through the slits to the outside.[18]

2.3 Fermenter

The vessel for fresh fruit mash storage is called a fermenter and is usually constructed of glass, stainless steel, or plastic. These materials are non-reactive with the slightly acidic fruit mash. This non-reactivity prevents the distillates from developing a metallic or other objectionable taste.

The fermentation of fresh fruit by yeast to produce ethanol is an exothermic reaction. In large scale fermentations, even with some cooling, it is almost impossible to prevent a significant temperature rise or peaking because of the large amount of heat evolved during fermentation.[5] To counteract the evolved heat, fermenters are equipped with some type of cooling device. A water jacket or copper piping running through the mash are the typical methods employed to cool the mash. The optimal temperature range of a mash is between 15 and 20°C. There are two competing mechanisms as temperature

increases: on the one hand there is a tendency for the rate of reaction to increase; on the other hand there is a tendency for the rate of inactivation of the yeast to also increase.[10]

Temperatures below 15°C slow down the yeast kinetics and subsequently slow down the production of ethanol. Temperatures above 20°C accelerate the fermentation process but they also favor the growth of undesired microorganisms. High temperatures can lead to increased production of acetic acid, lactic acid, and butanoic acid.

Another concern within the fermenter is a temperature variation within the mash itself. Such inhomogeneity can occur as pockets of mash ferment, with the subsequent release of heat. The fresh fruit mash is agitated to maintain a relatively consistent temperature profile. Agitation is typically accomplished using a motor, shaft, and propeller setup. The final aspect of a quality fermenter involves proper containment of the mash. The fermenter should allow for the release of carbon dioxide and prevent oxygen from contacting the mash. The descriptive equation for alcoholic fermentation by yeast is typically referred to as the Gay-Lussac reaction (Figure 2.1):

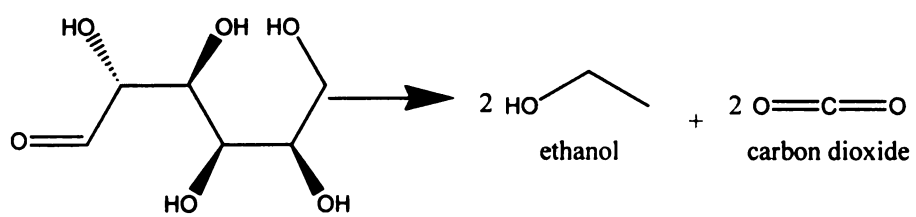


Figure 2.1 Gay-Lussac Reaction: Conversion of Glucose to Ethanol and Carbon Dioxide Using *Saccharomyces Cerevisiae* (Dry Yeast)[10]

To allow for the release of carbon dioxide the fermenter was equipped with an air lock (Figure 2.2) which can be constructed of plastic and is U-shaped. The lower part of the U-shape is filled with water. The production of carbon dioxide within the sealed fermenter causes an increase in pressure above atmospheric levels. This pushes carbon

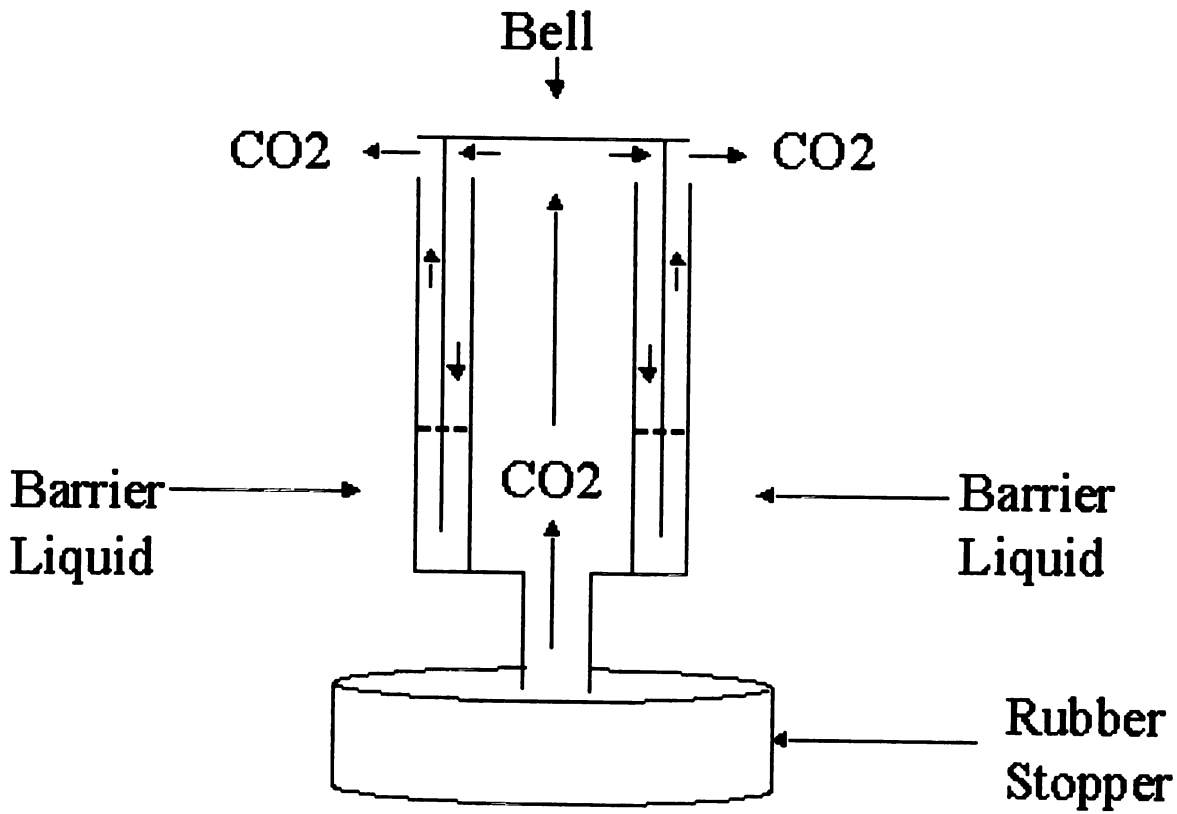


Figure 2.2 Diagram of an Airlock

dioxide bubbles through the water in the bottom of the U-shaped air lock. The water in the bottom of the U-shape prevents the entry of oxygen into the fermenter. Oxygen contact with the mash promotes the formation of undesired bacteria while the yeast used for the fermentation of sugar does not require any oxygen.

2.4 Yeast

The similarity between the aroma compounds formed in nitrogen free sugar fermentation and the aroma fraction of alcoholic beverages indicates that the yeast is responsible for the bulk of the aroma compounds produced.[16] This statement summarizes the current study quite well.

The intensive aroma research work carried out during recent years has shown that the aroma composition of alcoholic beverages consists of several hundred distinct chemical compounds.[17] Amino acids, pectin, and sugars such as fructose, glucose, and broken down sucrose provide the substrate for yeast cells to produce the majority of the chemical compounds that give fruit spirits their distinct aroma and flavor.

Baker's yeast or *Saccharomyces cerevisiae* is classified as a fungus. It is capable of reproducing either sexually or asexually. Under the nutrient conditions prevailing in fruit mashes the reproduction takes place through budding, i.e. asexually.[10] After complete development, a daughter cell separates from the parent cell. The doubling period takes between three to six hours depending on the strain of yeast, pH, temperature, and mineral supply.[18]

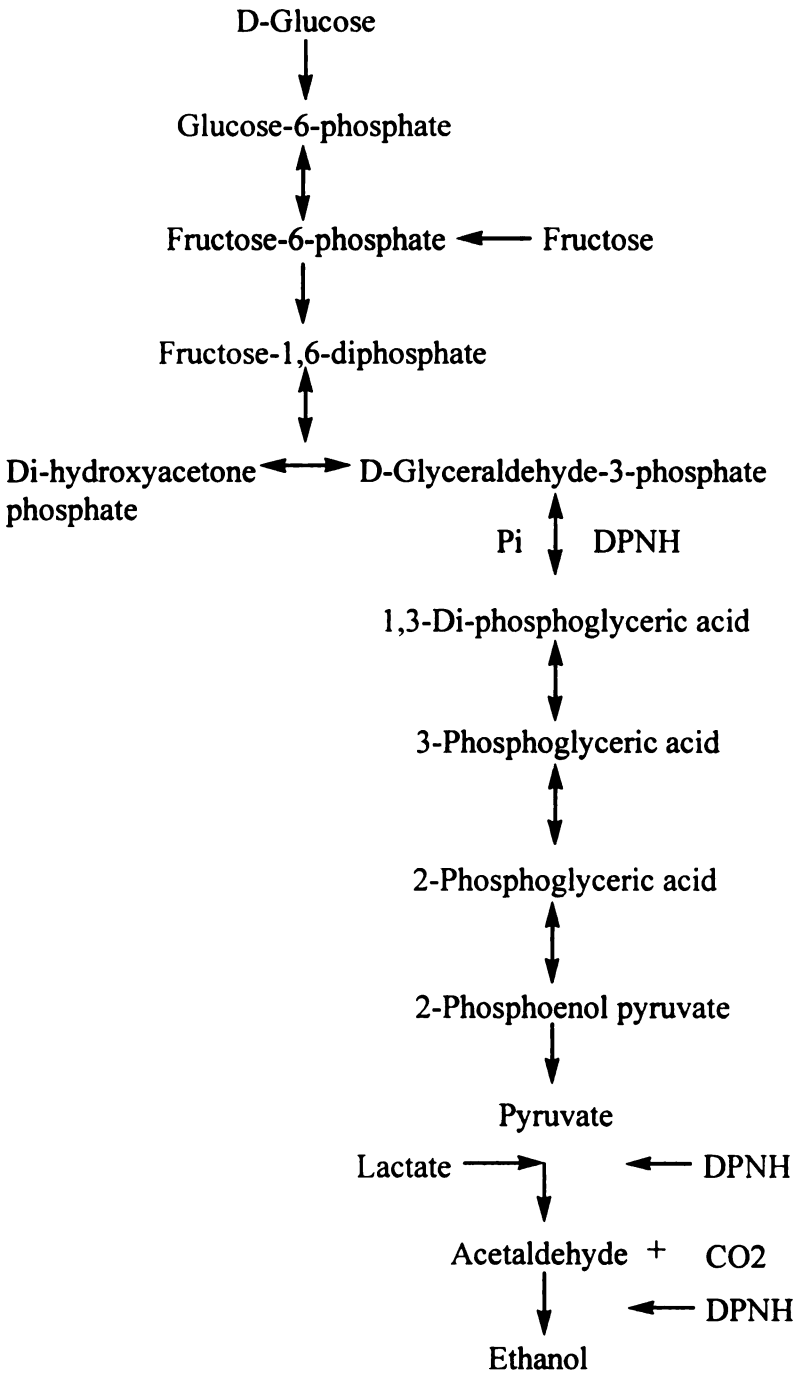
The fermentation process starts when the amount of living yeast cells has increased to 100,000 to 1,000,000 per cubic centimeter. To give *S. cerevisiae* a competitive advantage over wild yeast and bacteria these cellular levels should be

obtained immediately after mashing of the fruit. This is accomplished by starting with a large number of cells. Thick mashes or fruit types rich in tanning agents (danger of fermentation hold up) require an adequately higher dosage, e.g. 40g/hl.[10] In industrial fermentations of complex worts the rate of alcoholic production is restricted by some inhibitors resulting from accumulation of organic or inorganic compounds.[9] These compounds include acetic acid, butanoic acid, and lactic acid all formed due to bacterial activity.

S. cerevisiae is capable of producing ethanol and carbon dioxide from fructose and glucose. The classic reaction describing this process is called the Gay-Lussac reaction as previously described. *S. cerevisiae* is also capable of utilizing sucrose after it is broken down into fructose and glucose by an invertase enzyme. Yeast invertase is located at the cell wall, or perhaps just inside the cell at the cell membrane.[10] It has been shown that very shortly after the yeast comes into contact with sucrose there is no longer any sucrose. The route by which yeast metabolizes common sugars, such as glucose or fructose under anaerobic conditions is referred to as the Embden-Meyerhof-Parnas scheme.[10] This scheme is shown in Figure 2.3. Glucose, fructose, and sucrose are very quickly fermented.[11] Glucose, however, is fermented faster than fructose.

The theoretical yield of such a process is 64.5 L of ethanol produced from 100 kg of sugar.[18] This theoretical yield does not hold up due to incomplete fermentation of the sugars, formation of unwanted side products, and alcohol loss due to distillation. The actual ethanol yield is approximately 56 L from 100 kg of sugar.[18]

Figure 2.3 The Embden-Meyerhof-Parnas fermentation scheme showing the intermediates in the production of ethanol from glucose.[10]



Fructose, glucose, and sucrose must enter a yeast cell through active transport (Figure 2.4). Active transport serves to explain the rapid rate of fermentation of these sugars in yeast, while at the same time permitting accumulation against a concentration gradient.[10] Active transport requires the use of metabolic energy. The binding of sugar to the carrier is visualized as catalyzed by a permease enzyme: a phosphorylation reaction with polyphosphate as a phosphate donor is involved in this binding.[10]

Another aspect of substrate entry into yeast cells involves the penetration of organic acids. Carboxylase is an enzyme located inside the yeast cell. In order for a keto acid molecule to be decarboxylated by yeast carboxylase, it must first penetrate the cell wall and plasma membrane. The plasma membrane tends to be hydrophobic in nature so keto acids with longer carbon chains enter the yeast cell more rapidly. Once inside the yeast cell, keto acids are decarboxylated and reduced into fusel alcohols. Fusel alcohols have a damp cloth odor that has a negative aromatic influence on fruit spirits.

Environmental factors can be extremely influential in the success or lack thereof of yeast fermentation. Nevertheless, it is good operational precaution to maintain pH in the range of 4.0 to 6.0 for best yeast activity.[10] This slightly acidic pH range inhibits the growth of some bacteria. Fruit mashes naturally tend to fall within the suggested pH range. For fruit mashes that do not, lowering the pH is accomplished using sulfuric acid, and the pH is increased using calcium carbonate. The ideal temperature range for yeast fermentations is between 15 and 20°C. This range of temperature balances two competing mechanisms as the temperature rises; the tendency for the reaction rate to increase and the tendency of the rate of inactivation of the yeast to also increase.[10]

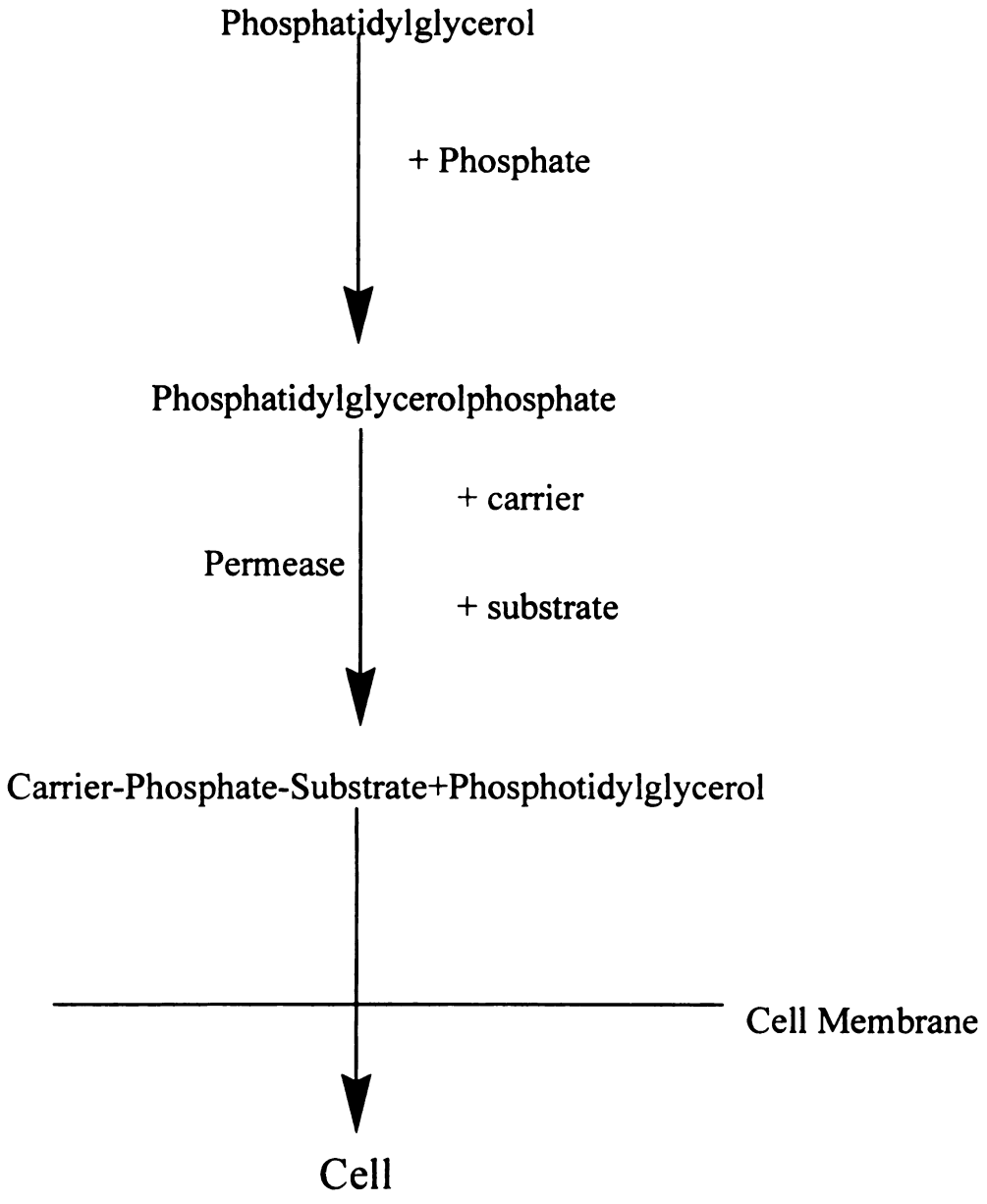


Figure 2.4 Active Transport of Glucose into a Yeast Cell[10]

2.5 Yeast Strains

The Lalvin D254 strain was isolated from Syrah fermentations after screening 3,000 isolates and putting 450 of them through trials for their enological properties.[12] The D254 strain was selected for its ability to ferment in low nitrogen musts, while being a low foamer with an alcohol tolerance up to 16%.[12] The D254 strain has a temperature range of 11-35°C.[12] The sensory profile of the D254 strain is described as showing pronounced butterscotch, creamy, smooth, hazelnut, and almond aromas.[12]

The Lalvin CY3079 strain was selected from fermentations in the Burgundy region, with the objective to isolate a strain that would complement the typical white Burgundy styles of wine making. A slow, steady fermenter even at cooler temperatures, this strain demonstrates a good alcohol tolerance and low production of volatile acids and hydrogen sulfides. It has an alcohol tolerance of 14%.[12] The CY3079 strain releases peptides at the end of fermentations that are believed to enhance many of the aromas such as fresh butter, honey, white flowers, and pineapple.[12] The CY3079 strain has a temperature range of 13-29°C.[12]

The Lalvin S6U strain was isolated and studied by Dr. Cioffi of the Institute Sperimentale per I'Enolagia near Rome. The enological characteristics include the ability to ferment at low temperatures in musts with low levels of suspended solids. The temperature range for S6U is 4-32°C and it has an alcohol tolerance of 15%.[12] Aroma profiles of the wines produced by S6U are described as floral and spicy.[12]

Lalvin EC1118 Prise de Mousse is the original, steady, low foamer, excellent for barrel fermentations. It ferments well at low temperatures with a range of 4-35°C.[12] It

has an alcohol tolerance that is greater than 18%.[12] EC1118 has excellent organoleptic properties.

Lalvin L2056 was isolated from the Cotes du Rhone region because of its ability to maintain a variety of fruit aromas and flavors. The strain demonstrates good alcohol tolerance to 16% and has a temperature range of 15-28°C.[12]

2.6 Fermenter Storage

Fresh fruit fermented between 15-20°C requires a fermentation period of 10-20 days.[18] For consistency in this study, all fermentations were run for 14 days. Fermentations that do not run for ten days run the risk of not utilizing all of the available fructose and glucose. This would mean that the resulting fruit distillate would have a decreased volume of ethanol. Fermented mashes can be stored up to three to four weeks without disadvantageous changes.[18] Beyond four weeks fermentations run the risk of bacterial infection.

2.7 Mash Distillation

The basic concept of distillation involves separating volatile from non-volatile compounds using some type of heat source. Volatility is a physical property of a compound described by its boiling point. Compounds described as highly volatile have low boiling points and compounds described as non-volatile compounds have high boiling points. Heat sources include direct fire, steam, and a water/steam combination. The fermented mash is placed into a still constructed of copper, glass, or high-grade steel. Several reasons favor the use of copper including its ability to conduct heat effectively, it shows optimal resistance to fruit acids, and it effects the quality of the distillate

positively.[18] Copper forms non-volatile products with volatile sulfur compounds, thus improving the fruit distillate's aroma and flavor by removal of sulfur compounds.

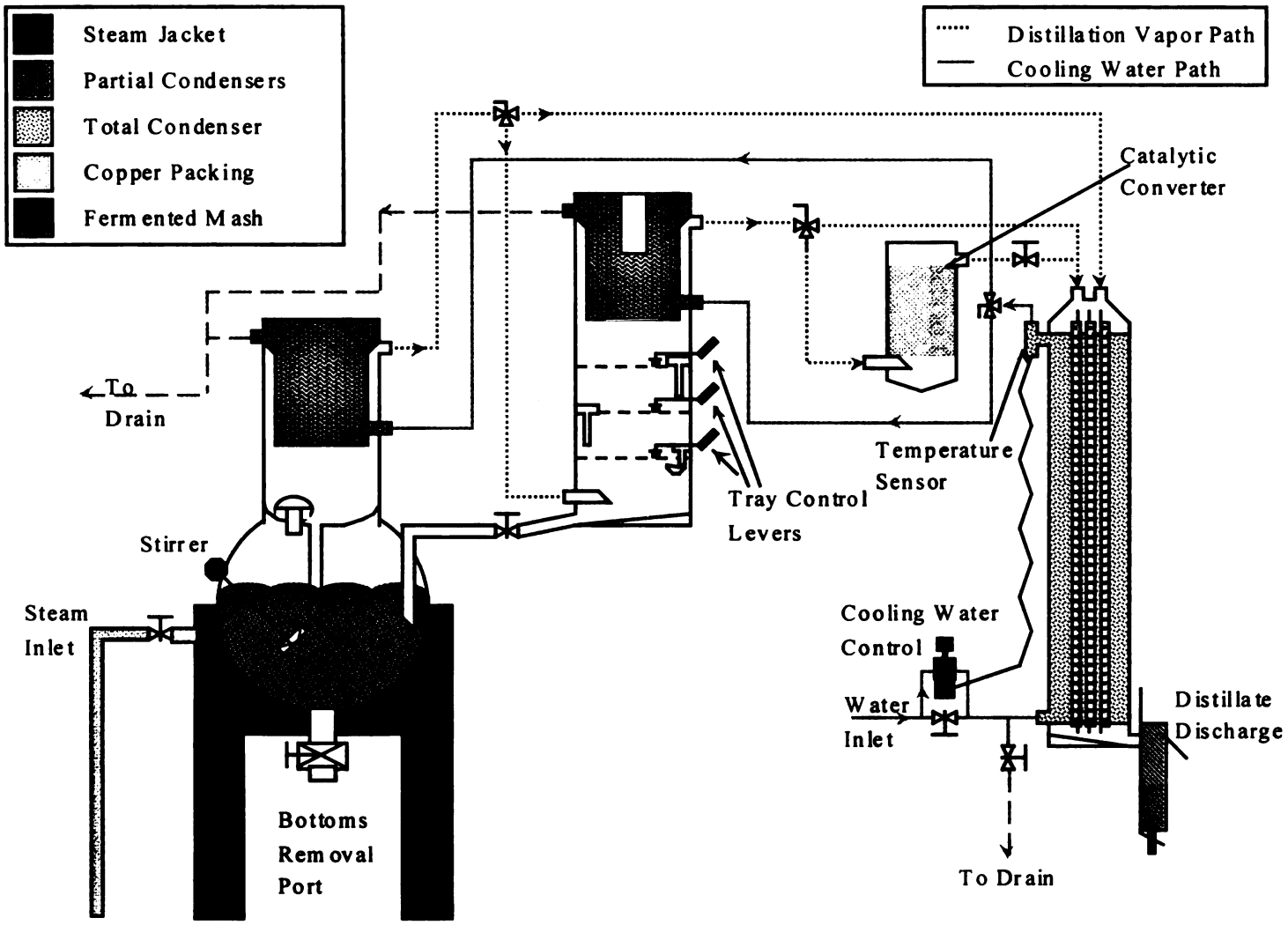
Once vaporized, the volatile mash components travel into the partial condenser. The partial condenser is a cylindrical structure with several trays. The use of a water jacket with cold water flowing through it creates zones of varying temperature. As the vapors travel upward within the partial condenser the temperature gradually falls. With falling temperatures components that are less volatile condense, get trapped on trays, and fall back into the fermentation mash. This process is commonly known as reflux.[18]

Compounds that are more volatile continue to ascend to the top of the condenser and enter a spirit tube. The spirit tube is a pipe that connects the partial condenser to the total condenser. The total condenser also has a water jacket with cold water flowing through it. This condenses the volatile compounds that are collected from a discharge point on the total condenser. The volatile compounds collected from the total condenser comprise the fruit distillate. A schematic of a typical still set-up is shown in Figure 2.5.

2.8 Distillate Preparation and Storage

Distillates collected from the total condenser typically are comprised of 60-80 percent ethanol. Distillates are stored at this high concentration of ethanol for a three to sixth month period. Reactions that occur in the aging process include oxidation, esterification, and acetalization. Storage is typically done in an environment that is free of light. It is theorized that the production of ethyl carbamate is a process catalyzed by light. An attempt to minimize the amount of ethyl carbamate found in distillates is made because it is a known carcinogen. Before consumption distillates are diluted to a

Figure 2.5 Schematic of a Christian Carl Still Set-Up[3]


Steam Jacket
Partial Condensers
Total Condenser
Copper Packing
Fermented Mash
Distillation Vapor Path
Cooling Water Path
Catalytic Converter
To Drain
Temperature Sensor
Tray Control Levers
Stirrer
Steam Inlet
Cooling Water Control
Water Inlet
Distillate Discharge
Bottoms Removal Port
To Drain

concentration of ethanol that falls below governmental regulations (40%). This process is termed as cutting the distillate and is done using purified water.

2.9 Gas Chromatographic Analysis

Gas chromatography is a technique used to separate and analyze compounds that are capable of being vaporized in a temperature range of 25 to 250°C. Samples are injected into a flash vaporizer. The flash vaporizer is at least 50°C above the boiling point of the least volatile component of the sample.[13]

A carrier gas such as helium, nitrogen, or hydrogen then carries the sample into the column. The carrier gas acts as the mobile phase. The stationary phase is usually a liquid attached to the walls of an open tubular column.[13] Choice of the stationary phase depends on the polarity of the components that are being separated. Fruit distillates are comprised mostly of alcohols that are polar in nature. Polyethylene glycol therefore is the liquid stationary phase of choice.[13] To improve separation within the column temperature programming is often employed. For samples with a broad boiling range, it is often desirable to employ temperature programming, whereby the column temperature is increased either continuously or in steps as the separation proceeds.[13] The gradual increase in temperature is accomplished by housing the column within an oven.

A flame ionization detector is the most widely used and generally applicable detector for gas chromatography.[13] A hydrogen flame pyrolyzes organic compounds to form ions and electrons that conduct electricity through the flame. A potential of a few hundred volts is passed through the flame and the resulting current is directed into an amplifier for measurement.

2.10 Ethanol

Ethanol is formed by yeast cells in the breakdown of glucose and fructose to form two moles of carbon dioxide and two moles of ethanol. The main goal of the distillation of fruit spirits is to maximize the production of ethanol. Increased ethanol production means that a greater number of bottles of 40% ethanol can be produced. At the end of the day this means that net profits will increase. Making the distillation of fruit spirits more profitable will lead to the continued growth of this industry in the state of Michigan and throughout the United States.

2.11 Methanol

The production of methanol is not quite as straight forward as the production of ethanol. Methanol is a positive aroma and flavor component within fruit spirits yet it is toxic at high levels. The toxicity of methanol has led to its regulation by the United States Environmental Protection Agency and the Food and Drug Administration. The United States Environmental Protection Agency mandates a minimum acute toxicity concentration of methanol in drinking water at 3.9 parts per million.[4] The United States Bureau of Alcohol, Tobacco, and Firearms regulates the amount of methanol to 0.35% volume/volume for both domestic and imported fruit brandy.

Methanol is formed from the breakdown of pectin by pectinesterase. Pectin is a polysaccharide that occupies intercellular spaces in fruit tissue. Pectin contributes to the adhesion between cells and the overall mechanical strength of the cell wall. Fruit cells contain approximately 60% water and 40% biopolymers. Pectin makes up 20-35% of the polymers.[8]

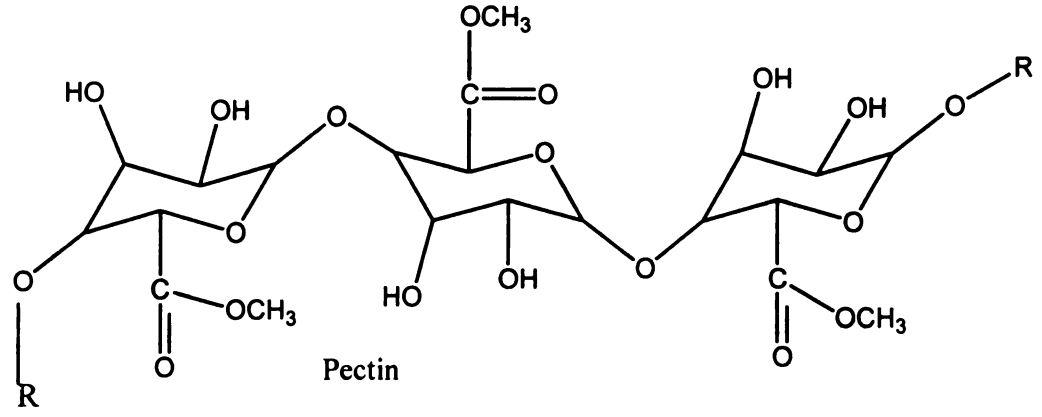
Pectinesterase is an enzyme that catalyzes the hydrolysis of the methyl esters of pectin to generate methanol. Pectinesterase has been found to naturally occur in a wide variety of plants. The structure of pectin and the activity of pectinesterase is shown in Figure 2.6.[2]

2.12 Acetaldehyde

Acetaldehyde has a distinctive aroma characteristic, which can cause an eau-de-vie to have a poor aromatic characteristic at high concentrations.[14] The production of acetaldehyde should therefore be minimized if possible. The reaction mechanism that describes the production of acetaldehyde is shown in Figure 2.3 (The Embden-Meyerhof-Parnas fermentation scheme). In this scheme, acetaldehyde and carbon dioxide are the last intermediates used to produce ethanol from glucose. Conversion of the carbon dioxide and acetaldehyde to ethanol is not 100% efficient. This leaves some acetaldehyde remaining in the mash. Despite the low boiling point of acetaldehyde it is still seen in the distillate. This is due to the fact that acetaldehyde is completely soluble in both water and ethanol, thus making it difficult to separate from the fruit spirits.

Figure 2.6 Pectin and the Activity of Pectinesterase to Produce Methanol

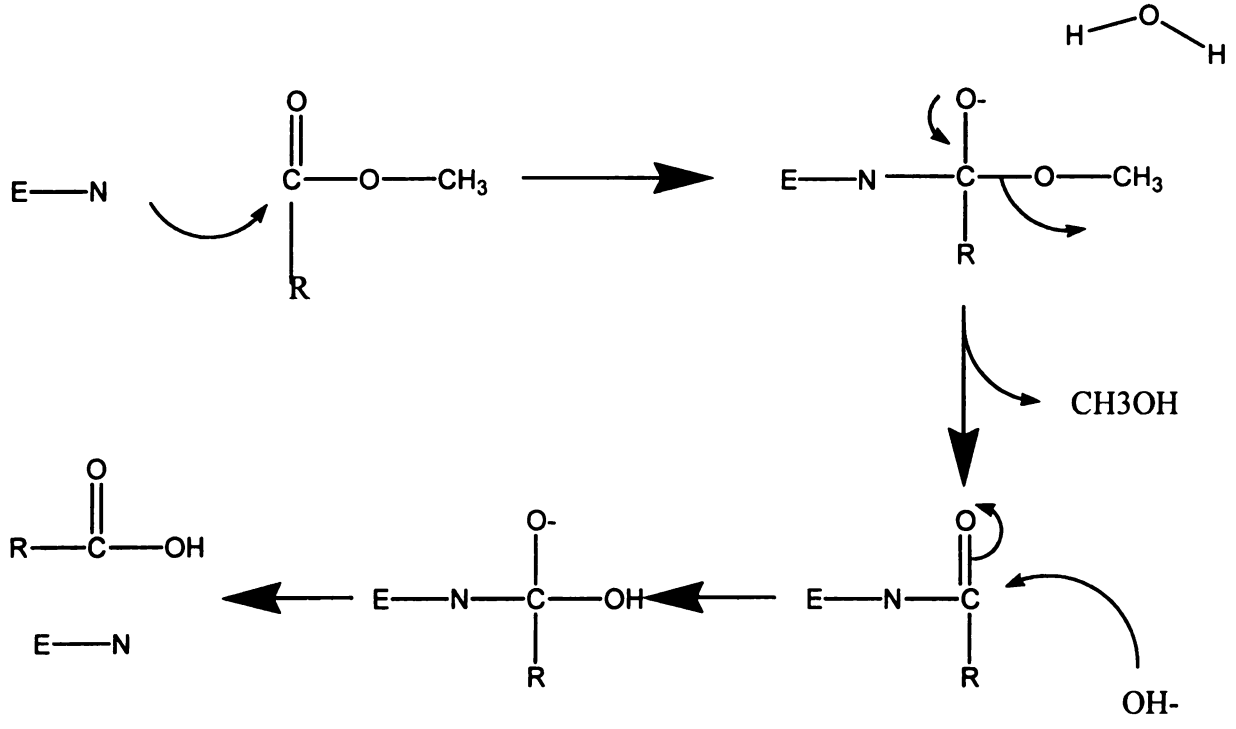
Stucture of Pectin



Pectin

Mode of Action of Pectinesterase

E——N Represents the enzyme



2.13 Ethyl Acetate and Ethyl Formate

Ethyl acetate and ethyl formate are both esters. Esters add fruity aromas to the fruit distillate. If the concentration of the esters is too high, the quality of the fruit spirits will decrease. Esters are typically formed during the aging process. This study does not age the fruit distillate so the ester content should be minimal. The reaction mechanism used in the production of ethyl acetate is shown in Figure 2.7.

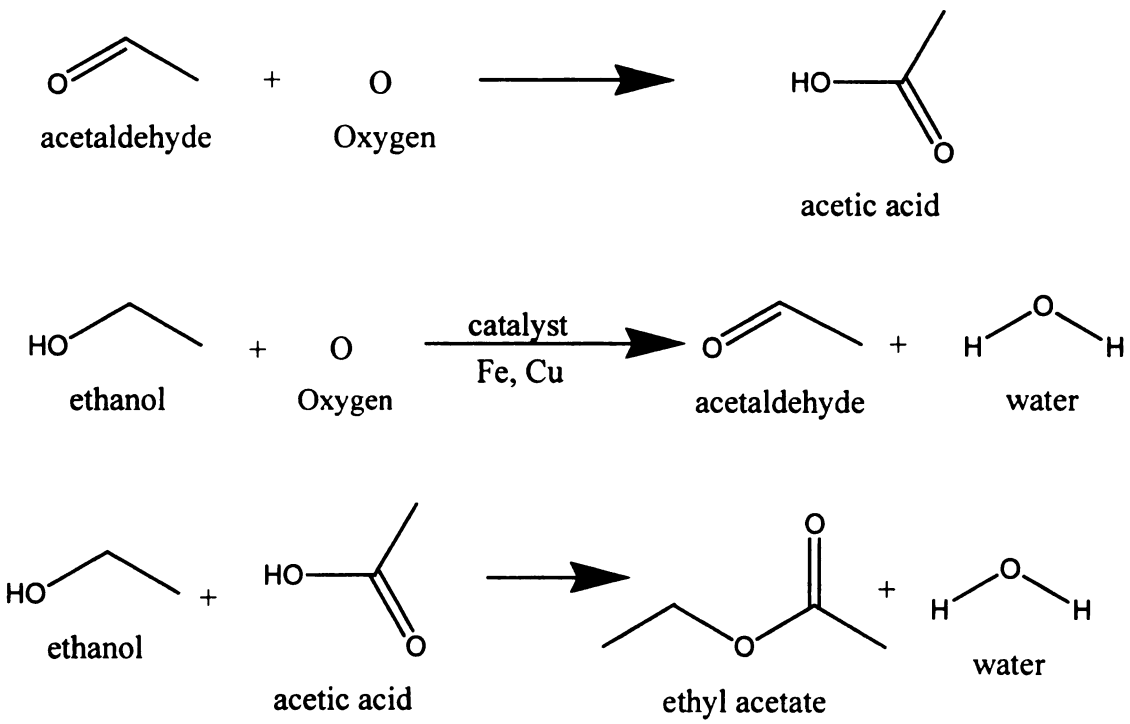


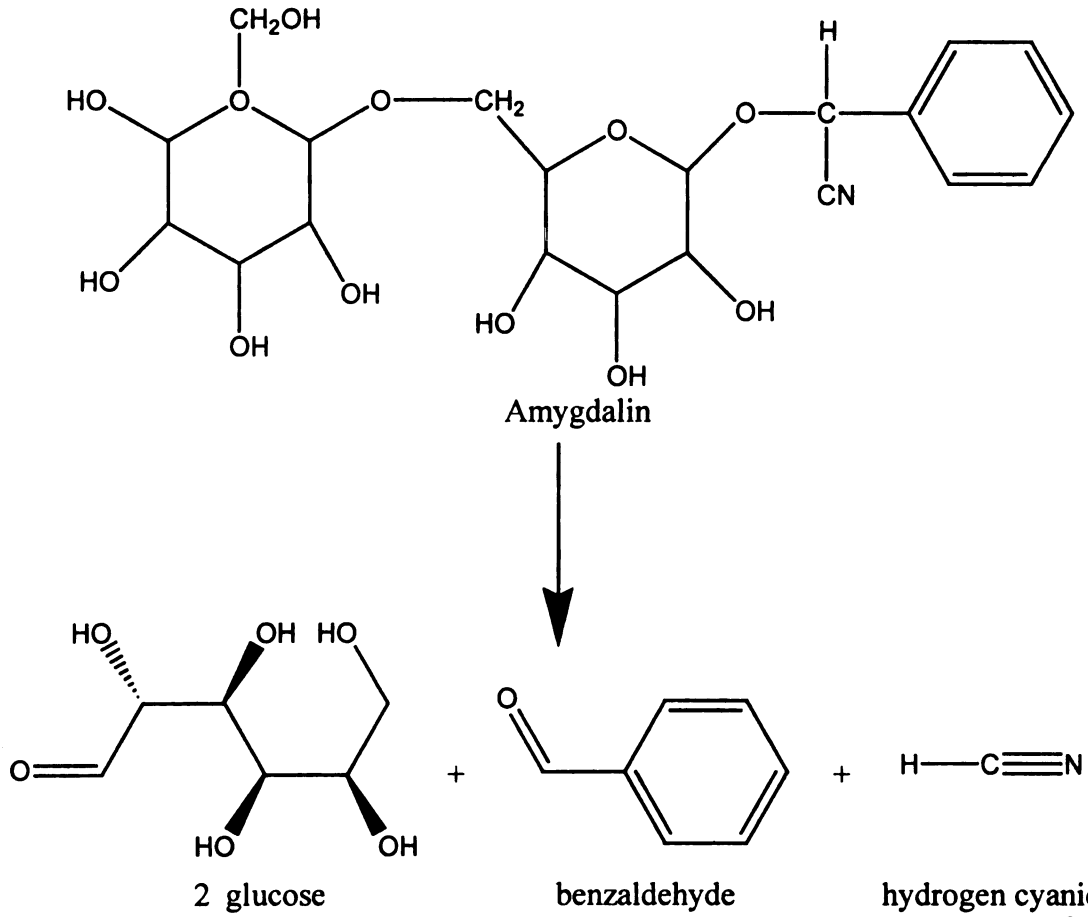
Figure 2.7 The Production of Ethyl Acetate[2]

2.14 2-Furaldehyde

Heating the mash too quickly or using a direct flame can increase the production of 2-furaldehyde. 2-furaldehyde has a burnt-bitter taste. 2-furaldehyde is therefore a compound that has a negative effect on the flavor and aroma of the fruit spirit. Distillation of the mash should be done in a methodical fashion to limit the production of 2-furaldehyde.

2.15 Benzaldehyde

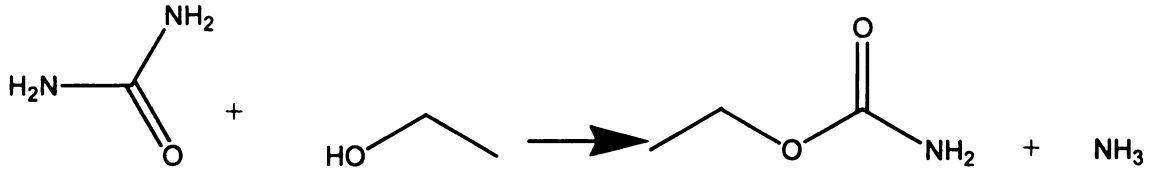
Benzaldehyde has a bitter almond aroma and flavor that has a positive influence on the fruit spirit. Benzaldehyde is formed from amygdalin found in the pits of fruit. Under hot, mineral conditions amygdalin is hydrolyzed as shown below.[2]



2 glucose benzaldehyde hydrogen cyanide

Figure 2.8 The Production of Benzaldehyde by the Hydrolysis of Amygdalin[2]

The production of hydrogen cyanide presents a potential problem. Hydrogen cyanide is apparently converted to urea.[2] Urea then reacts with ethanol to form urethane in the reaction mechanism shown in Figure 2.9. Urethane is a carcinogen; therefore, benzaldehyde production should be carefully controlled.



urea ethanol urethane ammonia

Figure 2.9 The Production of Urethane[2]

2.16 Fusel Alcohols

Fusel alcohols refer to any alcohol that contains more than two carbons. Excess fusel alcohols can contribute an unpleasant, damp cloth aroma to fruit spirits. This unpleasant aroma makes the minimization of the production of fusel alcohols a goal of every fruit spirit fermentation/distillation. Fusel alcohols analyzed in this study were 2-butanol, 2-methyl-2-butanol, 1-propanol, 2-methyl-2-propanol, 1-butanol, 3-methyl-1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, benzyl alcohol, 2-methyl-1-butanol, and 2-methyl-1-propranol. The length of this list demonstrates that fusel alcohols are the largest group of aromatic compounds in alcoholic beverages.[15]

There are two primary pathways in which fusel alcohols are produced. They are catabolically produced from amino acids and anabolically produced from glucose (Figure 2.10). At the beginning of the century, Erlich showed that several higher alcohols can be formed from amino acids: he demonstrates the formation of 3-methyl-1-butanol from leucine, 2-methyl-1-butanol from isoleucine, and isobutanol from valine.[1] Amino acids are found to occur naturally in domestic fruit and are produced due to the anaerobic nitrogen metabolism of yeast cells. The reaction method used to produce 3-methyl-1-butanol is shown in Figure 2.11.

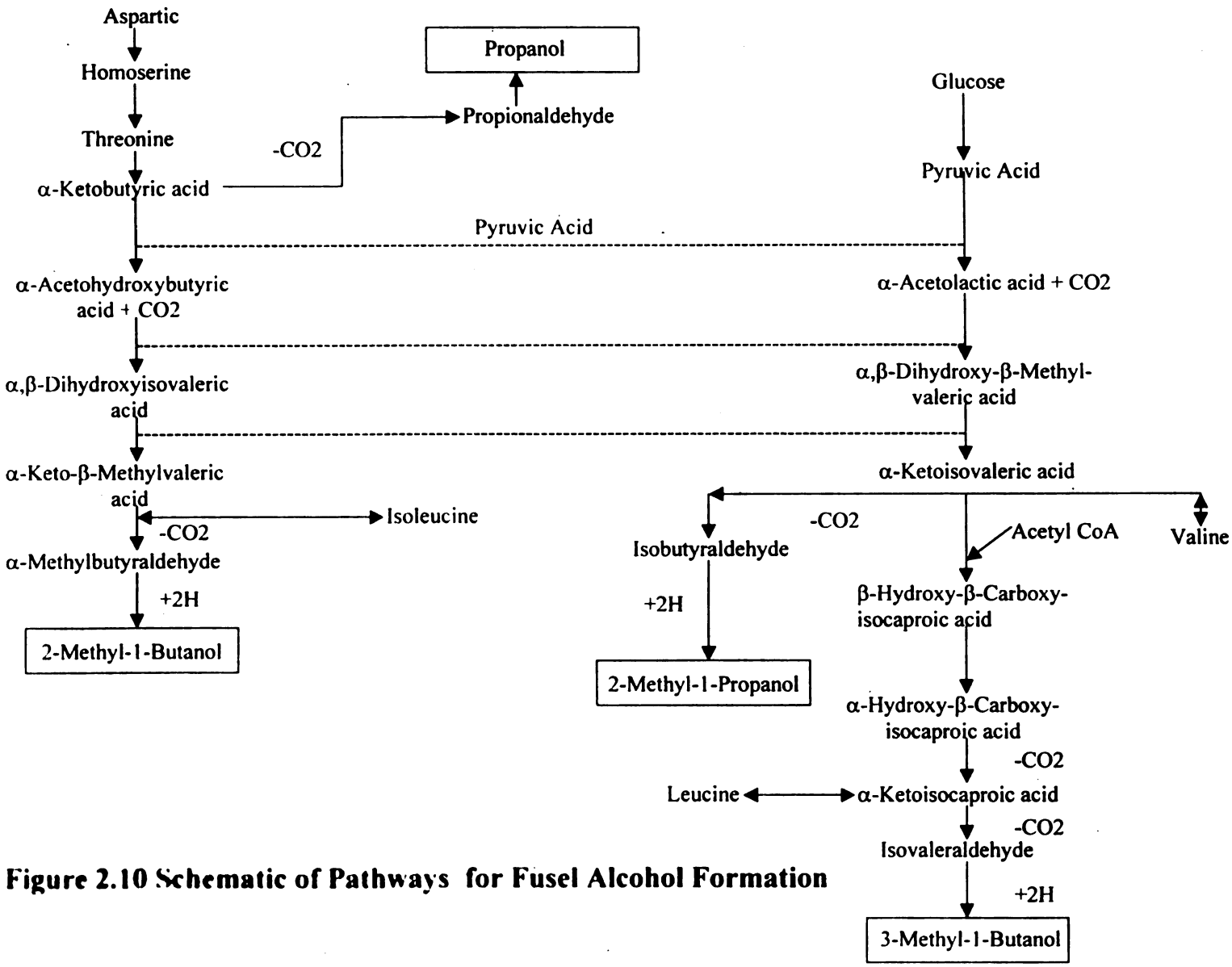


Figure 2.10 Schematic of Pathways for Fusel Alcohol Formation

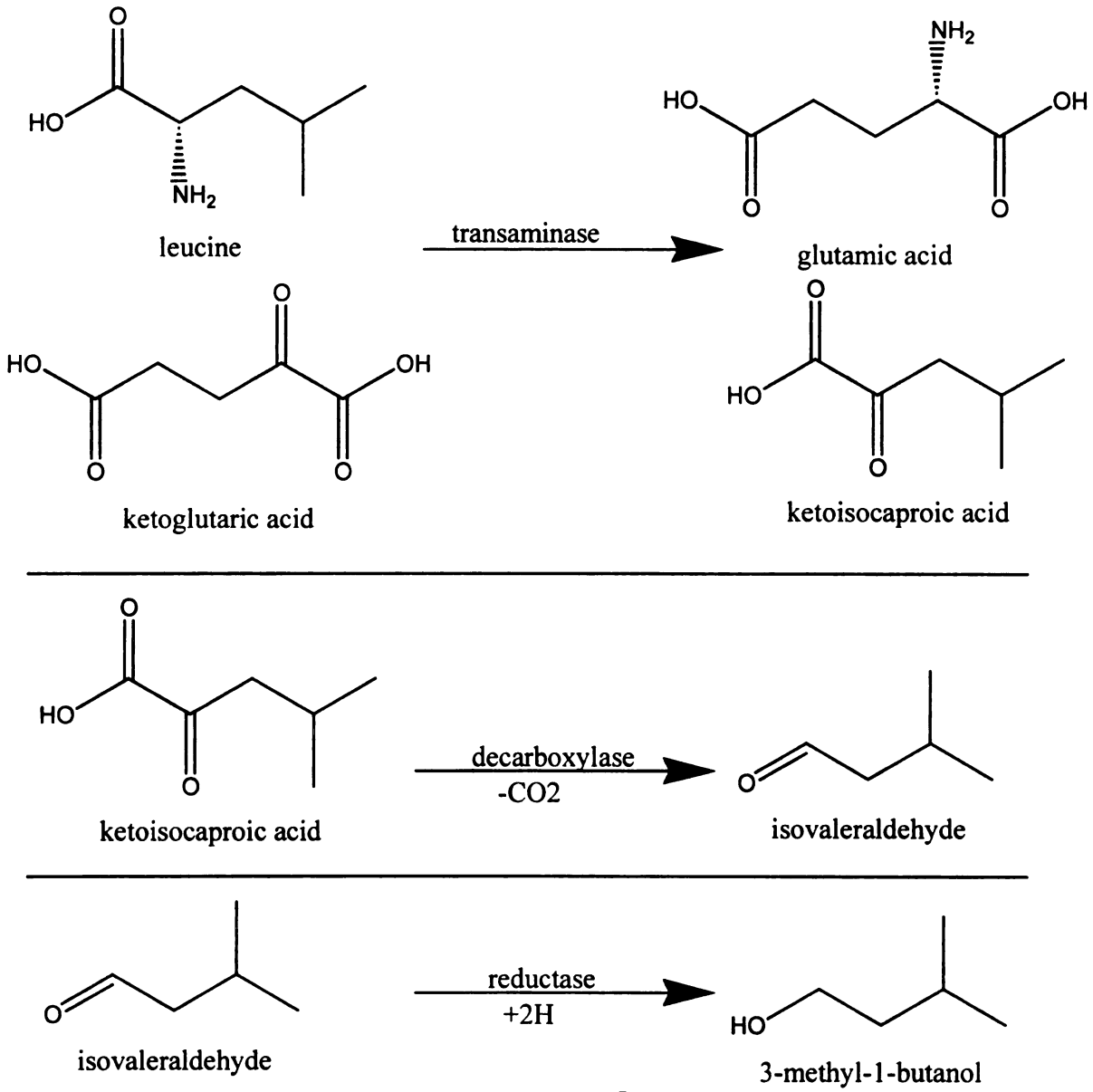


Figure 2.11 The Production of 3-Methyl-1-Butanol[7]

S. cerevisiae has the ability to synthesize all amino acids. Sugars such as glucose and fructose can be used to synthesize leucine.[2] Leucine then enters the reaction mechanism described above to produce 3-methyl-1-butanol. The production of 3-methyl-1-butanol is anaerobic.

3. MATERIALS AND METHODS

3.1 Prefermentation Set-up

A suitable fermenter set-up was designed before fermentation could be initiated. The major difficulty facing the project involved finding a method that would allow for a large number of relatively small volume fermentations. To accomplish this thirty, 300-mL Fleaker Beakers® were purchased. The Fleaker Beakers® came with an air tight rubber cap. The rubber caps assured that the fermentations would be run in an anaerobic environment. To facilitate the insertion of an air lock, one hole was drilled into each rubber beaker cap. Each hole was just small enough to allow for an air lock to pass through the rubber beaker cap. Each air lock was then partially filled with water. This set-up allowed for each fermentation to be run in an anaerobic environment with the release of carbon dioxide.

A chest freezer, thermocouple, and On/Off controller were used for temperature control. Kenmore manufactured the chest freezer and its model name was Galaxy. The model number was 253.19501992 and the serial number was WB10805488. The thermocouple was manufactured by Omega. It was a K-type thermocouple and was constructed of chromium and aluminum. The On/Off controller was manufactured by Omega. It was called a Micromega Series CN7700 Controller.

The On/Off controller received power from a wall-mounted outlet. The On/Off controller was placed outside the chest freezer. The thermocouple was connected to the On/Off controller and the temperature probe was placed in a water bath inside the chest freezer. A water bath was used to minimize the temperature fluctuation due to the opening and closing of the freezer. The chest freezer received power from the On/Off

controller. By connecting the chest freezer and the On/Off controller in series to the wall mounted power supply, the On/Off controller could control the power input to the chest freezer based on temperature. If the temperature within the chest freezer was below the set point, the On/Off controller would not allow power to enter the chest freezer. Without power, the compressor was unable to run and the temperature within the chest freezer rose. If the temperature within the chest freezer was above the set point, the On/Off controller would allow power to enter the chest freezer. With power, the compressor was able to run and the temperature within the chest freezer dropped. This set up allowed for equilibration of the chest temperature control within a 0.1°C (Table 3.1).

3.2 Selection and Preparation of Fresh Fruit

Fruit was selected that was of optimal ripeness yet free of any visible blemishes such as bruising, animal/insect bites, and mold. All stems, leaves, and/or vines were removed from each piece of fruit. The fruit was then rinsed with water to remove any residual soil matter that might have been on the surface of the fruit. The fruit was then dried using toweling and cut into smaller pieces using a pairing knife. The fruit pieces were placed in a five-gallon bucket and then crushed using a wooden pestle. This created a mixture of juice and small pieces of fruit.

A 300-mL Fleaker Beaker® was placed on a mass balance and tared. 200 grams of the previously mashed fruit tissue and juice were placed in a Fleaker Beaker® using a large weigh boat. The weigh boat was used in a shovel like manner to scoop the juice and fruit pieces into each Fleaker Beaker®. The mashed fruit tissue and juice in the five-

Table 3.1 On/Off Micromega CN7700 Controller Settings

1. Set Point 1=15 degrees
2. Set Point 2=15 degrees
3. Input Type= Thermocouple
4. Reading Configuration
   A. Decimal Point=FFF.F
   B. Temperature Unit=degrees Celsius
   C. Filter Constant=0001
5. Alarm 1
   A. Enable
   B. Deviation
   C. Unlatched
   D. Normally Open
   E. High-Low
   F. Alarms Enable/Disable at Power On
   G. Alarm 1 Low=.5
   H. Alarm 2 High=.5
6. Alarm 2
   A. Disable
7. Loop Break Alarm
   A. Disable
8. Output 1
   A. Control Type=On/Off
   B. Action Type=Direct Acting
   C. Dead Band=000.1
9. Output 2
   A. Control Type=On/Off
   B. Action Type=Direct Acting
   C. Dead Band=000.1
10. Ramp and Soak
    A. Disable
11. Angle Out
    A. Not Installed
12. Communication Option
    A. Not Installed
13. Reset Point
    A. Not Installed

gallon bucket was stirred using the weigh boat after removal of each 200 gram sample to assure a consistent mash profile. This process was repeated until a total of fifteen Fleaker Beakers® were each filled with 200 grams of mashed fruit. The Fleaker Beakers® were then sealed with the aforementioned rubber cap/air lock set up. The sealing of the Fleaker Beakers® was done to prevent the invasion of external bacteria while the yeast was prepared.

3.3 Yeast Preparation

Twenty mL of sterile water preheated to 40°C was added to 0.08 grams of yeast. Each yeast/water mixture was placed in a 40°C water bath for fifteen minutes. While in the heated water bath, the yeast/water mixture was stirred. After fifteen minutes the yeast/water mixture was removed from the heated water bath. The yeast/water mixture was allowed to cool at room temperature for fifteen minutes. Cooling of the yeast/water mixture was done to prevent temperature shock of the yeast cells when added to the fruit mash. This process was repeated with each of the four additional strains of yeast. Each of the five yeast strains was done in triplicate for a total of fifteen fermentations per fruit type.

3.4 Fermentation

Fermentation was initiated by adding the cooled yeast/water mixture to the fruit mash contained within each Fleaker Beaker®. Each Fleaker Beaker® received a separate yeast/water sample. The fermentations were done in triplicate to improve the accuracy of the results. The yeast/water mixture was added to the Fleaker Beaker® and stirred to assure that the yeast/water mixture penetrated all areas of the fermentation mash. The rubber cap/air lock was then firmly placed on the Fleaker Beaker® that was then placed

into the chest freezer. The chest freezer was held at 15°C using the On/Off controller and the thermocouple. Each fermentation was allowed to run for a total of 14 days. Each fermentation was determined to have been run to completion by the lack of production of carbon dioxide. Carbon dioxide is a by-product in the conversion of glucose to ethanol. When no carbon dioxide was seen bubbling out of the airlock, the fermentation had reached completion.

3.5 Distillation

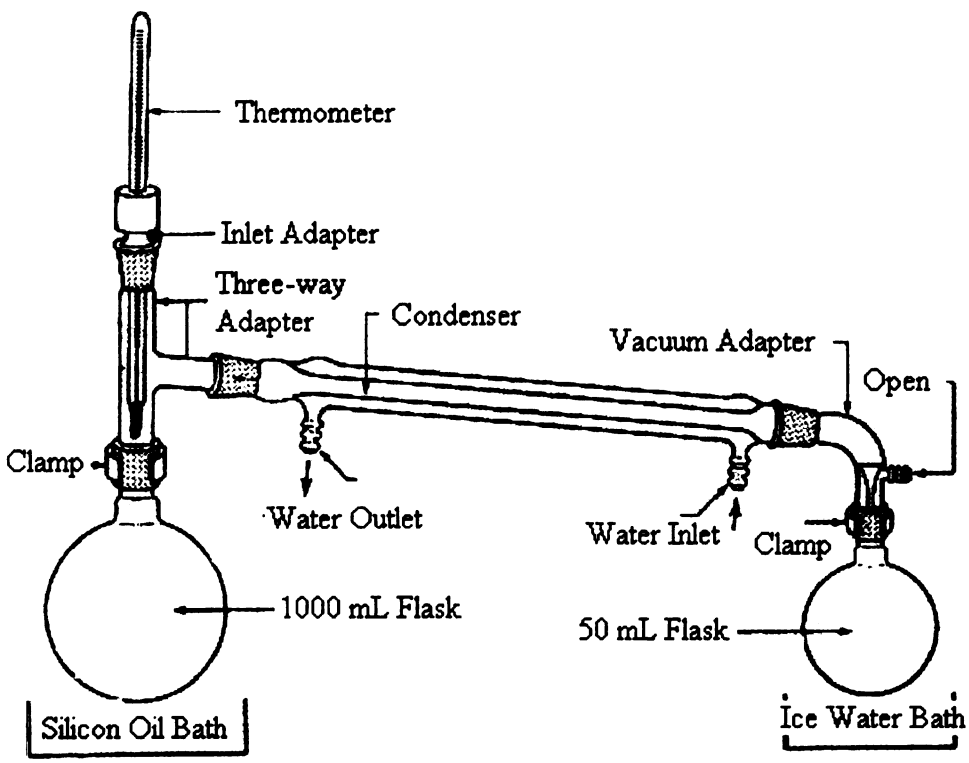
The entire fermented fruit mash was removed from the Fleaker Beaker® and placed into a 1000 mL round bottom flask. The flask was fitted into the simple distillation apparatus shown in Figure 3.1. The fermented mash was heated using a hot plate/stirrer apparatus (Corning Model PC-420) set on a heat setting of four in combination with a silicon oil bath which typically reached temperatures of around 115°C. This temperature was needed so that the vapor temperature in the simple distillation column reached 99°C. At 99°C the distillation was stopped and the products were collected from the 50-mL round bottom flask and placed in a 20-mL storage vile.

3.6 Distillate Analysis

All distillates were analyzed using gas chromatography. The analysis of the distillates using gas chromatography was done within 48 hours of distillation to prevent highly volatile compounds from evaporating before analysis. The run conditions for the gas chromatograph are described in Table 3.2 and were used for all trials throughout the study.

Standard retention times were generated for the following compounds: acetaldehyde, acetone, ethyl formate, ethyl acetate, 2-methyl-2-propanol, methanol,

Figure 3.1 Simple Distillation Set-Up



Column:	30 meters, 0.32 μm Stabilwax®
	0.5 μL split injection
Oven temperature:	40°C (hold for one minute); to 190° @ 2.5°C/min.
	190°C (hold for five minutes)
Injector temperature:	240°C
Detector temperature:	255°C
Carrier gas:	Helium @ 30 cm/second
Split ratio:	65:1
Total GC rum time:	65 minutes

Table 3.2 Shimadzu GC-17A Run Conditions

ethanol, 2-methyl-2-butanol, 2-butanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, furaldehyde, benzaldehyde, 1-octanol, and benzyl alcohol. The retention times in triplicate were then averaged to increase the accuracy of the analysis. Compounds that have low boiling points will have relatively short retention times while compounds that have high boiling points will have relatively long retention times.

The next phase of the experiment involved generating a calibration curve for ethanol. The following concentrations of ethanol were prepared using 100% ethanol and HPLC water: 25%, 27%, 30%, 33%, 35%, 38%, 40%, 43%, 45%, 47%, 50%, 53%, 55%, 57%, and 60%. Each of these concentrations was run five times using the aforementioned gas chromatographic conditions. From this, the peak areas were integrated and averaged. A calibration curve was generated by graphing the percent ethanol versus the peak area. This curve allowed for the determination of the concentration of ethanol and all other compounds within the distillate. Ethanol therefore served as the internal standard.

4. RESULTS AND CONCLUSIONS

4.1 Qualitative Identification of the Compounds within Each Distillate

The first step in determining the influence that a particular yeast strain might have on the taste of a fruit spirit is to determine which compounds make up the spirit. Compounds that have low boiling points have shorter retention times in gas chromatography than do compounds that have higher boiling points because compounds with low boiling points vaporize at lower temperatures. Solubility can also impact the retention time of a compound. Compounds that are soluble in ethanol will have shorter retention times because they are vaporized along with ethanol. Ethanol has a relatively low boiling point (78.3° C) compared to water (100° C). Compounds that are more soluble in water than in ethanol will remain in a liquid state until the temperature within the column reaches the boiling point of water. Compounds that are more soluble in water than in ethanol therefore have increased retention times. Once vaporized, compounds are swept through the column by the carrier gas to the detector.

Table 4.1 lists the compounds of interest that were identified within each fruit spirit distillate along with their respective boiling points. This table illustrates that the boiling point of a compound is not a precise measure of retention order. Compounds with higher boiling points in some instances do have shorter retention times. This supports the fact that compounds that are more soluble in ethanol than in water can have shorter retention times than would be expected based on boiling point alone.

Compound	Boiling Point (deg. C)	Retention Time (min)
Acetaldehyde	21.0	1.6
Acetone	56.5	2.0
Ethyl Formate	54.3	2.0
Ethyl Acetate	77.0	2.5
2-Methyl-2-Propanol	82.0	2.7
Methanol	64.7	2.8
Ethanol	78.3	3.2
2-Methyl-2-Butanol	101.9	4.5
2-Butanol	94.0	4.8
1-Propanol	97.0	5.1
2-Methyl-1-Propanol	107.7	7.4
1-Butanol	118.0	8.6
3-Methyl-1-Butanol	132.0	11.0
2-Methyl-1-Butanol	128.0	11.6
1-Pentanol	138.0	12.8
1-Hexanol	156.5	17.7
1-Heptanol	176.0	22.6
Furaldehyde	161.7	23.1
Benzaldehyde	179.0	25.6
1-Octanol	194.5	27.6
Benzyl Alcohol	205.0	41.7

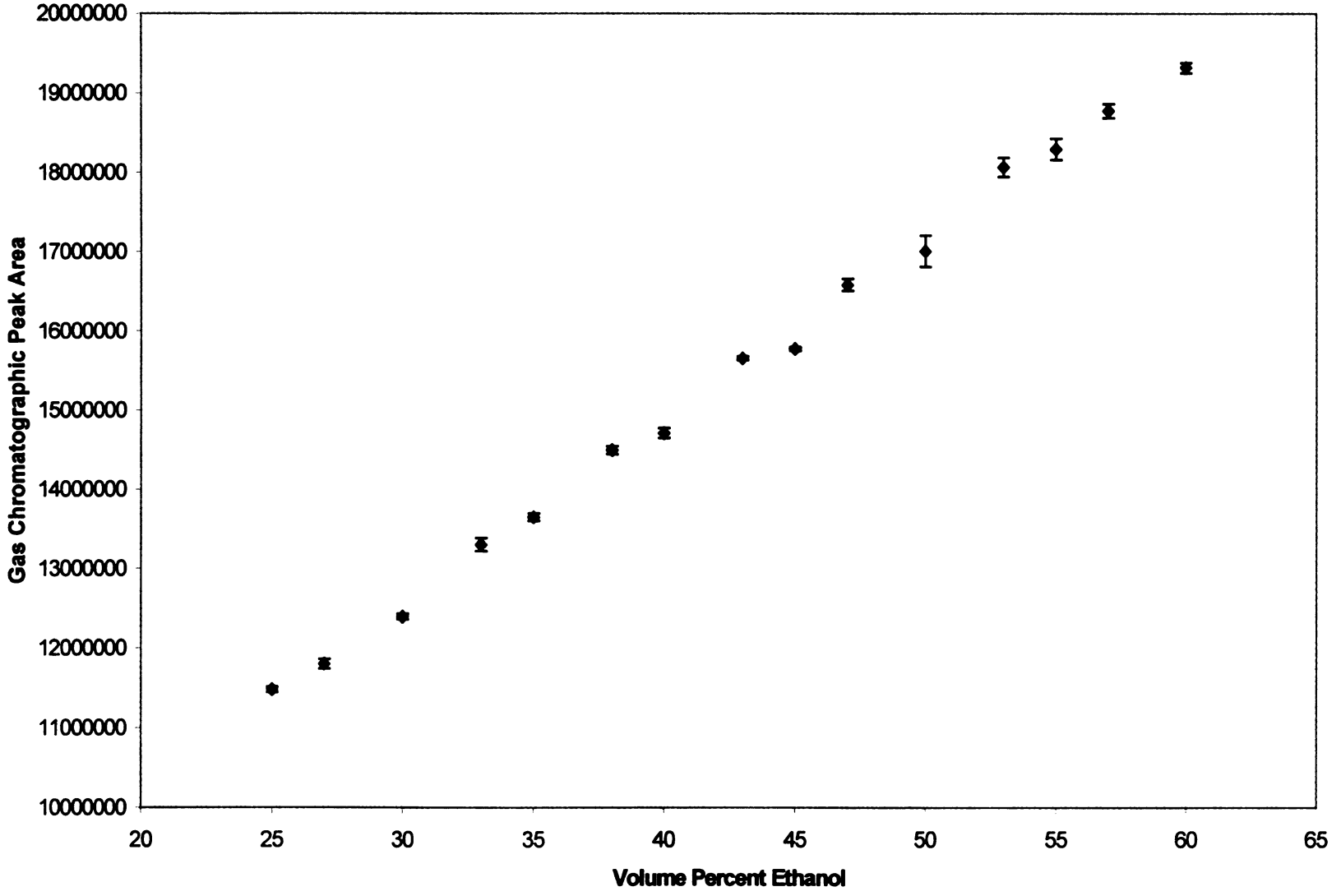
Table 4.1 Typical Fruit Distillate Components, Boiling Points, and Retention Times Seen Experimentally Using a Shimadzu Gas Chromatograph and FID Detector

4.2 Ethanol Calibration

The main goal of every fermentation/distillation is to produce ethanol. Increased ethanol production while maintaining product quality means an increased yield of fruit distillate thus leading to increased income. It is therefore necessary to precisely quantify the amount of ethanol in each distillation. Gas chromatography lends itself well to the precise quantification of ethanol. The quantification of ethanol is directly related to the integrated ethanol peak area in a gas chromatogram.

Fruit distillates can have a broad range of ethanol concentrations depending on such parameters as the sugar content of the fruit, the length of fermentation, the quality of the yeast, and the end temperature of a distillation. The potential range of ethanol concentrations of fruit distillates makes it necessary to use a wide range of ethanol concentrations when producing a calibration curve. Various ethanol concentrations were made by diluting absolute (100%) ethanol with HPLC water. HPLC water was used as the agent to dilute absolute ethanol to other concentrations because it has relatively few impurities. The lack of carbon in HPLC water was important because the flame ionization detector used in this study responds only to substances that contain a carbon source. The HPLC water passes through the gas chromatography detector without registering a peak. The ethanol calibration curve is shown in Figure 4.2. The range of the ethanol calibration curve is between 25% and 60% ethanol. This range encompassed all ethanol concentrations seen in the results that follow.

Figure 4.1 Ethanol Calibration For Gas Chromatography



4.3 Ethanol as an Internal Standard

Ethanol was used as the internal standard in the determination of the concentration of all other flavor components in the fruit spirit distillates. Ethanol was chosen as the internal standard because it is present in the highest concentration next to water and all brandies must be normalized to ethanol content.

The integrated area of all peaks within a single chromatogram produced from a single fruit distillate were integrated and added. Individual peak areas were then divided by the sum of all peak areas to give an area percent for each peak. The volume percent composition of ethanol was determined using the ethanol calibration curve shown in Figure 4.2. By comparing ratios of the unknown peak volume percent composition divided by the unknown peak area percent to the ethanol volume percent composition divided by the ethanol peak area percent the unknown volume percent composition was calculated for each peak. The final calculation involved converting each volume percent composition to a 40% ethanol solution by multiplying by forty divided by the actual ethanol volume percent composition found from the calibration curve. The end result is that each chromatographic peak represents a volume percent composition of a given flavor component in a 40% ethanol solution. These calculations standardized each distillate to a 40% ethanol solution and allowed for the comparison of the production of flavor components between all five varieties of yeast used in this study.

4.4 Ranking of the Yeasts

An objective method of determining which yeast was the best for a given variety of fruit was developed. Each yeast was compared to all other yeasts and assigned an alphabetical ranking using the Duncan Range Test Method for each congener category

identified using gas chromatography. The letter "a" represented the best possible performance in a specific congener category. The letter "e" represented the worst possible performance in a specific congener category. Compounds could have the same rank if they were determined to be statistically similar by the Duncan Range Test Method. The alphabetical ranks were then converted to numerical ranks (a=1, ab=1.5, b=2, bc=2.5, c=3, cd=3.5, d=4, de=4.5, and e=5). The production of ethanol is the primary goal of a fruit fermentation/distillation. The yeast that produced the most amount of ethanol was given an "a" ranking. Yeasts that produced less ethanol were given lower rankings. Methanol is a positive flavor component. The yeast that produced the most methanol was given an "a" ranking. Yeasts that produced less methanol were given lower rankings. Benzaldehyde imparts a positive bitter almond aroma and taste into a fruit distillate. The yeast that produced the most benzaldehyde was given a ranking of "a" and yeasts that produced less benzaldehyde were given lower rankings. Acetone, acetaldehyde, and fusel alcohols all impart negative aroma and flavor characteristics into a fruit distillate. In each of these categories the yeast that produced the smallest amount of each of these congeners was given a ranking of "a" with lower rankings being assigned to yeasts that produced more of each of these congeners. After rankings were assigned for each congener category, an overall average ranking was generated for each yeast. The yeast with the lowest average ranking was determined to be the yeast that performed best for a specific variety of fruit.

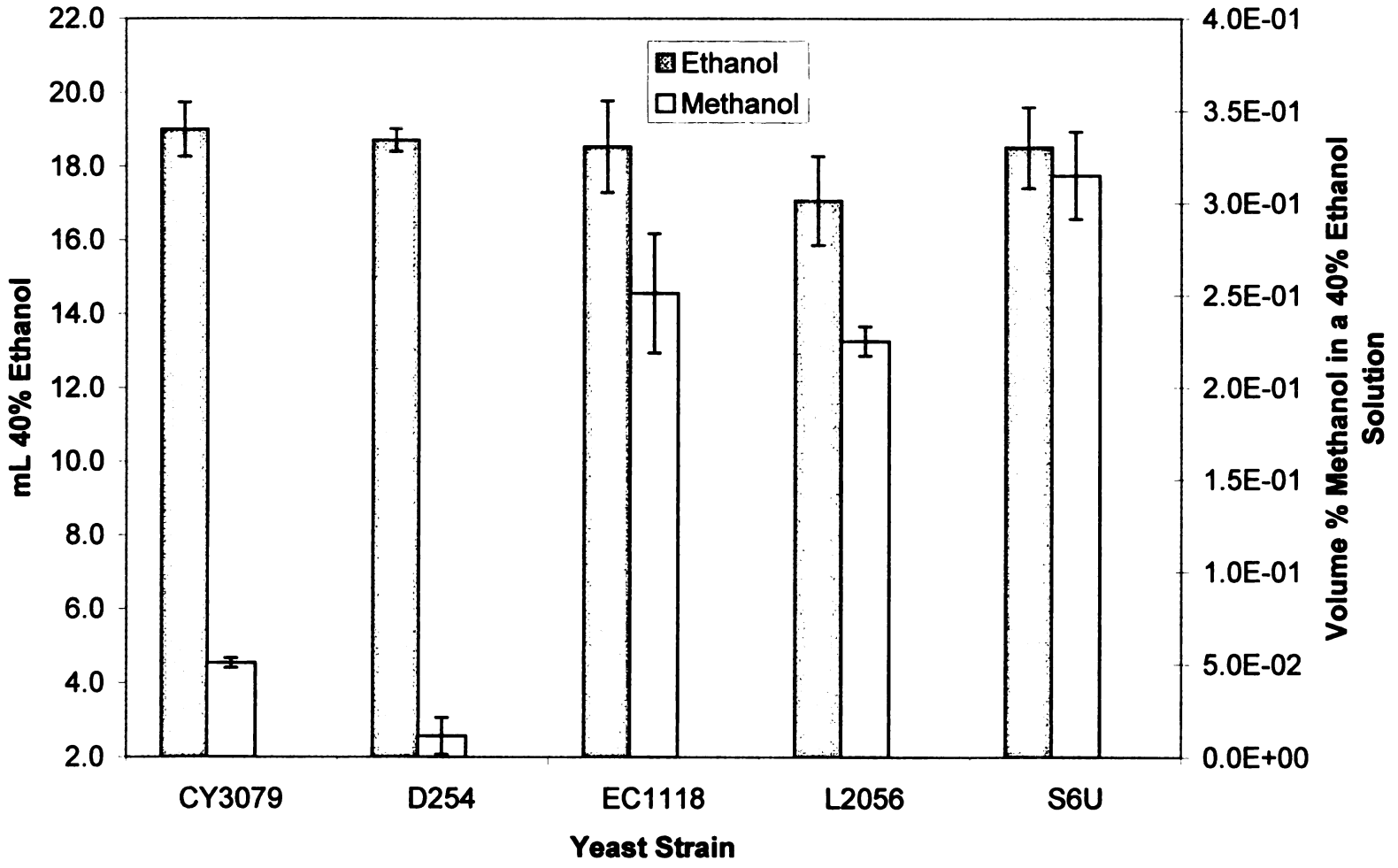
4.5 Bartlett Pears

Table 4.2 shows the results of fermenting and distilling bartlett pears. Figure 4.2 shows the production of ethanol and methanol along with the corresponding standard deviation in the three trials used to generate averages. Ethanol, methanol, acetaldehyde, acetone and a variety of fusel alcohols were identified in the bartlett pear distillates using gas chromatography. The fusel alcohols found in bartlett pear distillates included 2-methyl-2-propanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol, 1-hexanol, and 1-hetanaol. These fusel alcohols were summed together to generate a single fusel alcohol category.

Table 4.2 Bartlett Pear Distillate Composition and Rank

Yeast Strain	CY3079	D254	EC1118	L2056	S6U
40% Ethanol mL	19.0±.737	18.7±.306	18.5±1.25	17.1±1.21	18.5±1.10
Duncan Test	a	ab	ab	b	ab
Ethanol Rank	1	1.5	1.5	2	1.5
Methanol %V/V	.0506±.00261	.0113±.00984	.251±.0324	.225±.00799	.315±.0237
Duncan Test	c	c	ab	b	a
Methanol Rank	3	3	1.5	2	1
Fusel Alcohol %V/V	.644±.117	.602±.0127	.499±.0541	.507±.0244	.590±.0110
Duncan Test	c	b	a	a	b
Fusel Alcohol Rank	3	2	1	1	2
Acetaldehyde %V/V	.0171±.00347	.0142±.000960	.0155±.00226	.0131±.00171	.0230±.00340
Duncan Test	d	b	c	a	e
Acetaldehyde Rank	4	2	3	1	5
Acetone %V/V	.0000800± .000140	.0000300± .0000600	.00111± .00026	.000980± .0000500	.000520± .000310
Duncan Test	b	a	e	d	c
Acetone Rank	2	1	5	4	3
Average Rank	2.60	1.90	2.40	2.00	2.50
Overall Rank	5	1	3	2	4

Figure 4.2 Bartlett Pear Distillate Ethanol and Methanol Concentrations



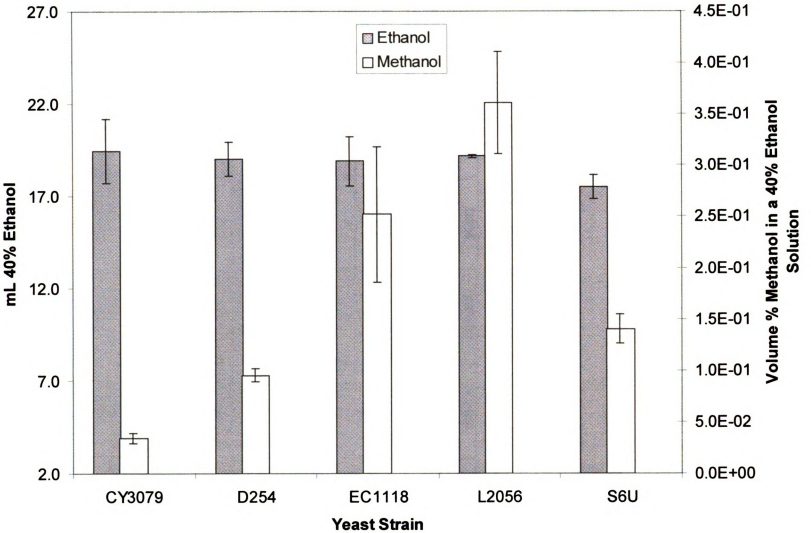
4.6 Black Plums

Table 4.3 shows the results of fermenting and distilling black plums. Figure 4.3 shows the production of ethanol and methanol along with the corresponding standard deviation in the three trials used to generate averages. Ethanol, methanol, acetaldehyde, and a variety of fusel alcohols were identified in the black plum distillates using gas chromatography. The fusel alcohols found in black plum distillates included 2-methyl-2-propanol, 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, and 1-hexanol. These fusel alcohols were summed together to generate a single fusel alcohol category.

Table 4.3 Black Plum Distillate Composition and Rank

Yeast Strain	CY3079	D254	EC1118	L2056	S6U
40% Ethanol mL	19.4±1.73	19.0±.916	18.9±1.34	19.2±.0823	17.5±.638
Duncan Test	a	ab	ab	ab	b
Ethanol Rank	1.0	1.5	1.5	1.5	2.0
Methanol %V/V	.0346±.00488	.0950±.00635	.252±.0661	.361±.0500	.140±.0143
Duncan Test	d	c	b	a	c
Methanol Rank	4	3	2	1	3
Fusel Alcohol %V/V	.648±.0737	.595±.0435	.627±.0562	.508±.254	.482±.0308
Duncan Test	e	c	d	b	a
Fusel Alcohol Rank	5	3	4	2	1
Acetaldehyde %V/V	.00699± .00201	.00517± .00131	.00698± .000610	.0113± .000610	.0336± .00128
Duncan Test	b	a	b	c	d
Acetaldehyde Rank	2	1	2	3	4
Average Rank	3.00	2.13	2.38	1.88	2.50
Overall Rank	5	2	3	1	4

Figure 4.3 Black Plum Distillate Ethanol and Methanol Concentrations



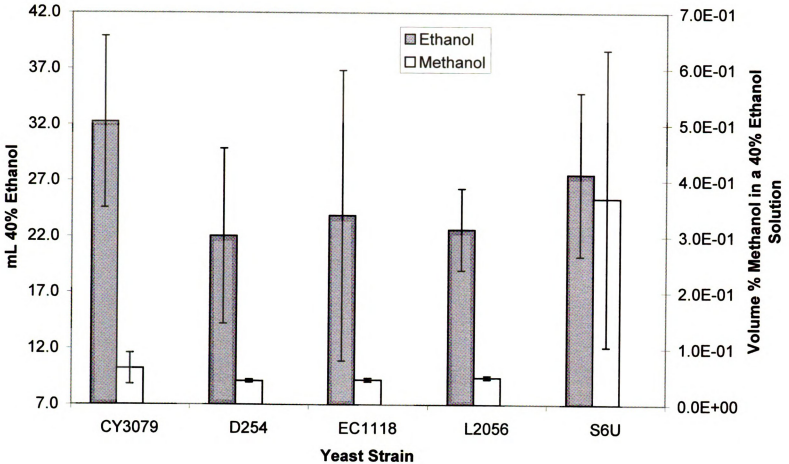
4.7 Braeburn Apples

Table 4.4 shows the results of fermenting and distilling braeburn apples. Figure 4.4 shows the production of ethanol and methanol along with the corresponding standard deviation in the three trials used to generate averages. Ethanol, methanol, acetaldehyde, acetone and a variety of fusel alcohols were identified in the braeburn apple distillates using gas chromatography. The fusel alcohols found in braeburn apple distillates included 2-methyl-2-propanol, 1-propanol, 1-butanol, 3-methyl-1-butanol, and 1-hexanol. These fusel alcohols were summed together to generate a single fusel alcohol category.

Table 4.4 Braeburn Apple Distillate Composition and Rank

Yeast Strain	CY3079	D254	EC1118	L2056	S6U
40% Ethanol mL	32.2±7.67	22.0±7.83	23.9±13.0	22.6±3.65	27.5±7.32
Duncan Test	a	b	b	b	ab
Ethanol Rank	1	2	2	2	1.5
Methanol %V/V	.0640±.0277	.0420±.00270	.0440±.00322	.0480±.00321	.368±.265
Duncan Test	b	b	b	b	a
Methanol Rank	2	2	2	2	1
Fusel Alcohol %V/V	.807±.0687	.635±.0404	.744±.154	.615±.0234	.874±.164
Duncan Test	c	a	b	a	d
Fusel Alcohol Rank	3	1	2	1	4
Acetaldehyde %V/V	.0210±.0127	.00600±.00278	.0130±.00135	.00500±.00338	.0190±.00554
Duncan Test	e	b	c	a	d
Acetaldehyde Rank	5	2	3	1	4
Acetone %V/V	1.59±.470	.513±.286	.000±.000	.0110±.00698	.321±.556
Duncan Test	b	ab	a	a	ab
Acetone Rank	2	1.5	1	1	1.5
Average Rank	2.60	1.70	2.00	1.40	2.40
Overall Rank	5	2	3	1	4

Figure 4.4 Braeburn Apple Distillate Ethanol and Methanol Concentrations



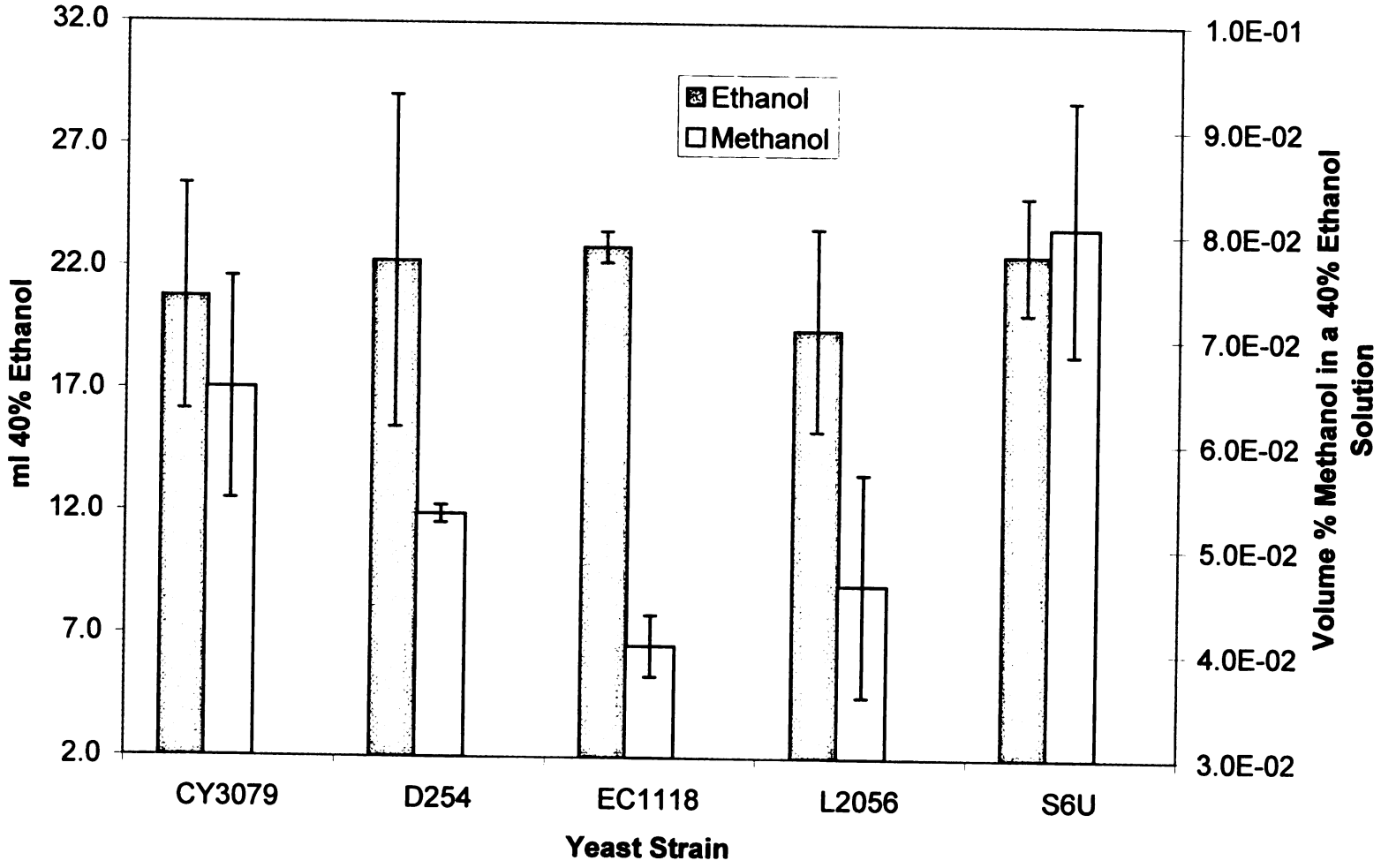
4.8 Gala Apples

Table 4.5 shows the results of fermenting and distilling gala apples. Figure 4.5 shows the production of ethanol and methanol along with the corresponding standard deviation in the three trials used to generate averages. Ethanol, methanol, acetaldehyde, acetone and a variety of fusel alcohols were identified in the gala apple distillates using gas chromatography. The fusel alcohols found in gala apple distillates included 2-methyl-2-propanol, 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, and 1-hexanol. These fusel alcohols were summed together to generate a single fusel alcohol category.

Table 4.5 Gala Apple Distillate Composition and Rank

Yeast Strain	CY3079	D254	EC1118	L2056	S6U
40% Ethanol mL	20.8±4.63	22.3±6.81	22.8±.634	19.5±4.15	22.6±2.39
Duncan Test	a	a	a	a	a
Ethanol Rank	1	1	1	1	1
Methanol %V/V	.0651±.0106	.0531±.000830	.0406±.00292	.0464±.0106	.0806±.0121
Duncan Test	b	c	e	d	a
Methanol Rank	2	3	5	4	1
Fusel Alcohol %V/V	.839±.160	1.06±.157	.656±.0644	.979±.201	.686±.139
Duncan Test	b	c	a	c	a
Fusel Alcohol Rank	2	3	1	3	1
Acetaldehyde %V/V	.00891± .000400	.00881± .00104	.00764± .00136	.0104± .00274	.00762± .000650
Duncan Test	d	c	b	e	a
Acetaldehyde Rank	4	3	2	5	1
Acetone %V/V	.00165± .000190	.00121± .000630	.000370± .000330	.000530± .000200	.000610± .000220
Duncan Test	e	d	a	b	c
Acetone Rank	5	4	1	2	3
Average Rank	2.80	2.80	2.00	3.00	1.40
Overall Rank	3	3	2	5	1

Figure 4.5 Gala Apple Distillate Ethanol and Methanol Concentrations



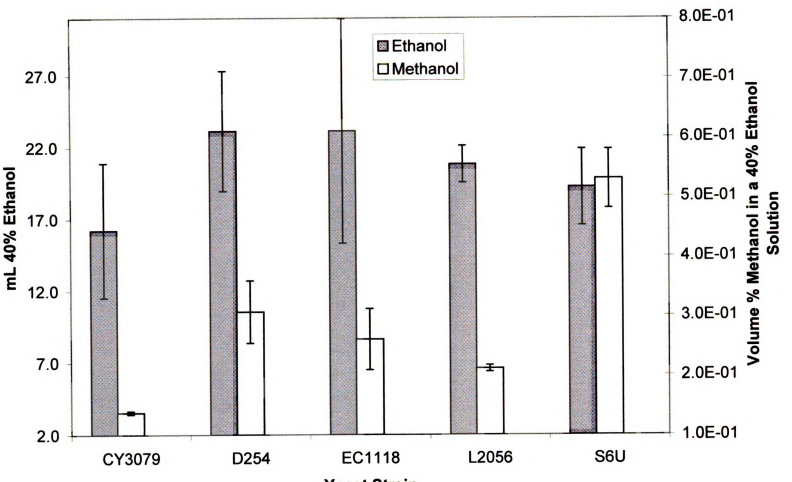
4.9 Granny Smith Apples

Table 4.6 shows the results of fermenting and distilling granny smith apples. Figure 4.6 shows the production of ethanol and methanol along with the corresponding standard deviation in the three trials used to generate averages. Ethanol, methanol, acetaldehyde, acetone, benzaldehyde, and a variety of fusel alcohols were identified in the granny smith apple distillates using gas chromatography. The fusel alcohols found in granny smith apple distillates included 2-methyl-2-propanol, 1-propanol, 3-methyl-1-butanol, 1-hexanol, and 2-butanol. These fusel alcohols were summed together to generate a single fusel alcohol category.

Table 4.6 Granny Smith Distillate Composition and Rank

Yeast Strain	CY3079	D254	EC1118	L2056	S6U
40% Ethanol mL	16.2±4.69	23.1±4.19	23.1±7.82	20.8±1.28	19.2±2.66
Duncan Test	a	a	a	a	a
Ethanol Rank	1	1	1	1	1
Methanol %V/V	.137±.00278	.306±.0524	.260±.0513	.211±.00536	.530±.0495
Duncan Test	d	b	bc	c	a
Methanol Rank	4	2	2.5	3	1
Fusel Alcohol %V/V	.983±.140	.857±.168	.887±.299	2.22±2.15	.867±.163
Duncan Test	a	a	a	b	a
Fusel Alcohol Rank	1	1	1	2	1
Benzaldehyde %V/V	.00170±.00248	.000±.000	.00204±.000	.000±.000	.000±.000
Duncan Test	a	a	a	a	a
Benzaldehyde Rank	1	1	1	1	1
Acetaldehyde %V/V	.0420±.0526	.0148±.00556	.0187±.00382	.0329±.0213	.0200±.00420
Duncan Test	e	a	b	d	c
Acetaldehyde Rank	5	1	2	4	3
Acetone %V/V	.000580± .000180	.000120± .00021	.000180± .000210	.000280± .000130	.000300± .0000500
Duncan Test	e	a	b	c	d
Acetone Rank	5	1	2	3	4
Average Rank	2.83	1.17	1.58	2.33	1.83
Overall Rank	5	1	2	4	3

Figure 4.6 Granny Smith Distillate Ethanol and Methanol Concentration



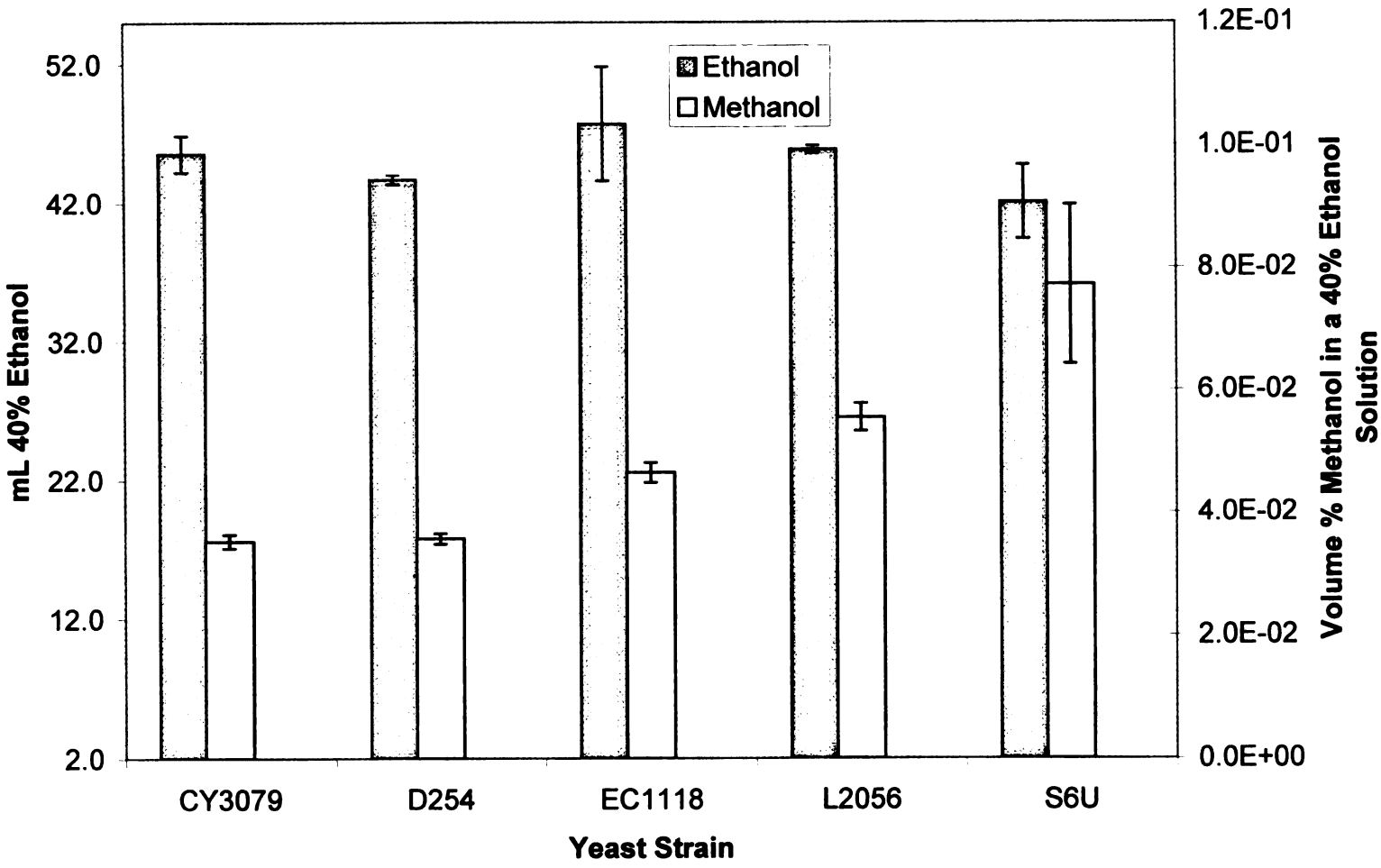
4.10 Green Seedless Grapes

Table 4.7 shows the results of fermenting and distilling green seedless grapes. Figure 4.7 shows the production of ethanol and methanol along with the corresponding standard deviation in the three trials used to generate averages. Ethanol, methanol, acetaldehyde, acetone and a variety of fusel alcohols were identified in the green seedless grape distillates using gas chromatography. The fusel alcohols found in green seedless grape distillates included 2-methyl-2-propanol, 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, and 1-hexanol. These fusel alcohols were summed together to generate a single fusel alcohol category.

Table 4.7 Green Seedless Grape Distillate Composition and Rank

Yeast Strain	CY3079	D254	EC1118	L2056	S6U
40% Ethanol mL	45.5±1.31	43.7±.327	47.7±4.12	45.8±.262	42.0±2.67
Duncan Test	a	a	a	a	a
Ethanol Rank	1	1	1	1	1
Methanol %V/V	.0353±.00112	.0357±.000860	.0465±.00163	.0555±.00225	.0772±.0130
Duncan Test	d	d	c	b	a
Methanol Rank	4	4	3	2	1
Fusel Alcohol %V/V	.419±.0145	.416±.0129	.562±.0582	.506±.0245	.421±.0165
Duncan Test	a	a	c	b	a
Fusel Alcohol Rank	1	1	3	2	1
Acetaldehyde %V/V	.0120±.00123	.00755±.00035	.0417±.0250	.0280±.00151	.0365±.00127
Duncan Test	b	a	e	c	d
Acetaldehyde Rank	2	1	5	3	4
Acetone %V/V	.000±.000	.000320± .000320	.000±.000	.000±.000	.000±.000
Duncan Test	a	b	a	a	a
Acetone Rank	1	2	1	1	1
Average Rank	1.80	1.80	2.60	1.80	1.60
Overall Rank	2	2	5	2	1

Figure 4.7 Green Grape Distillate Ethanol and Methanol Concentration



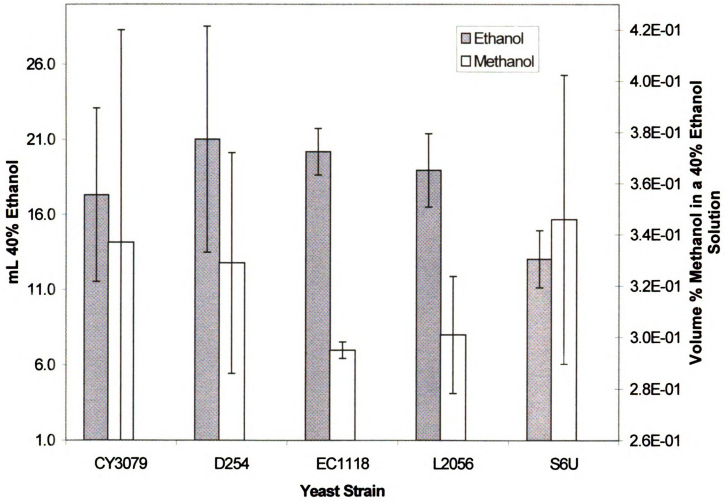
4.11 Montmorency Cherries

Table 4.8 shows the results of fermenting and distilling montmorency cherries. Figure 4.8 shows the production of ethanol and methanol along with the corresponding standard deviation in the three trials used to generate averages. Ethanol, methanol, acetaldehyde, acetone, benzaldehyde, and a variety of fusel alcohols were identified in the montmorency cherry distillates using gas chromatography. The fusel alcohols found in montmorency cherry distillates included 2-methyl-2-propanol, 1-propanol, 3-methyl-1-butanol, 1-hexanol, benzyl alcohol, and 2-butanol. These fusel alcohols were summed together to generate a single fusel alcohol category.

Table 4.8 Montmorency Cherry Distillate Composition and Rank

Yeast Strain	CY3079	D254	EC1118	L2056	S6U
40% Ethanol mL	17.3±5.78	21.0±7.54	20.2±1.56	18.9±2.44	13.0±1.91
Duncan Test	a	a	a	a	a
Ethanol Rank	1	1	1	1	1
Methanol %V/V	.337±.0830	.329±.0430	.295±.00319	.301±.0227	.346±.0563
Duncan Test	b	c	e	d	a
Methanol Rank	2	3	5	4	1
Fusel Alcohol %V/V	.810±.157	.657±.112	.660±.133	.529±.0960	.726±.00319
Duncan Test	d	b	b	a	c
Fusel Alcohol Rank	4	2	2	1	3
Benzaldehyde %V/V	.0495±.00832	.0350±.00431	.0389±.00519	.0198±.0177	.0548±.00525
Duncan Test	b	d	c	e	a
Benzaldehyde Rank	2	4	3	5	1
Acetaldehyde %V/V	.00706±.00407	.00792±.00123	.00701±.00128	.0198±.00549	.0279±.00893
Duncan Test	a	b	a	c	d
Acetaldehyde Rank	1	2	1	3	4
Acetone %V/V	.418±.409	3.82±3.68	.900±1.05	.411±.712	.652.440±
Duncan Test	a	a	a	a	a
Acetone Rank	1	1	1	1	1
Average Rank	1.83	2.17	2.17	2.50	1.83
Overall Rank	1	3	3	5	1

Figure 4.8 Montmorency Cherry Distillate Ethanol and Methanol Concentration



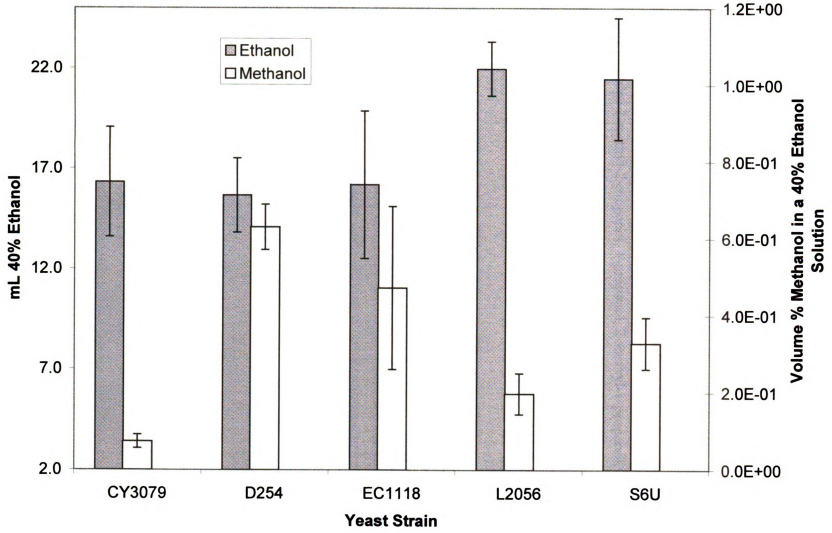
4.12 New Haven Peaches

Table 4.9 shows the results of fermenting and distilling new haven peaches. Figure 4.9 shows the production of ethanol and methanol along with the corresponding standard deviation in the three trials used to generate averages. Ethanol, methanol, acetaldehyde, acetone and a variety of fusel alcohols were identified in the new haven peach distillates using gas chromatography. The fusel alcohols found in new haven peach distillates included 2-methyl-2-propanol, 1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, benzyl alcohol, and 1-hexanol. These fusel alcohols were summed together to generate a single fusel alcohol category.

Table 4.9 New Haven Peach Distillate Composition and Rank

Yeast Strain	CY3079	D254	EC1118	L2056	S6U
40% Ethanol mL	16.3±2.73	15.7±1.85	16.2±3.68	22.0±1.35	21.5±3.04
Duncan Test	a	a	a	a	a
Ethanol Rank	1	1	1	1	1
Methanol %V/V	.0737±.0176	.630±.0591	.472±.212	.197±.0532	.328±.0673
Duncan Test	d	a	b	cd	bc
Methanol Rank	4	1	2	3.5	2.5
Fusel Alcohol %V/V	.596±.165	.391±.0600	.691±.124	.437±.0433	.508±.192
Duncan Test	c	a	d	a	b
Fusel Alcohol Rank	3	1	4	1	2
Acetaldehyde %V/V	.0338±.00513	.0369±.00628	.0566±.0304	.0463±.00666	.0458±.0149
Duncan Test	a	b	e	d	c
Acetaldehyde Rank	1	2	5	4	3
Acetone %V/V	.590±.747	.000±.000	2.100±3.63	.000±.000	.0841±.145
Duncan Test	a	a	a	a	a
Acetone Rank	1	1	1	1	1
Average Rank	2.00	1.20	2.60	2.10	1.90
Overall Rank	3	1	5	4	2

Figure 4.9 New Haven Peach Distillate Ethanol and Methanol Concentration



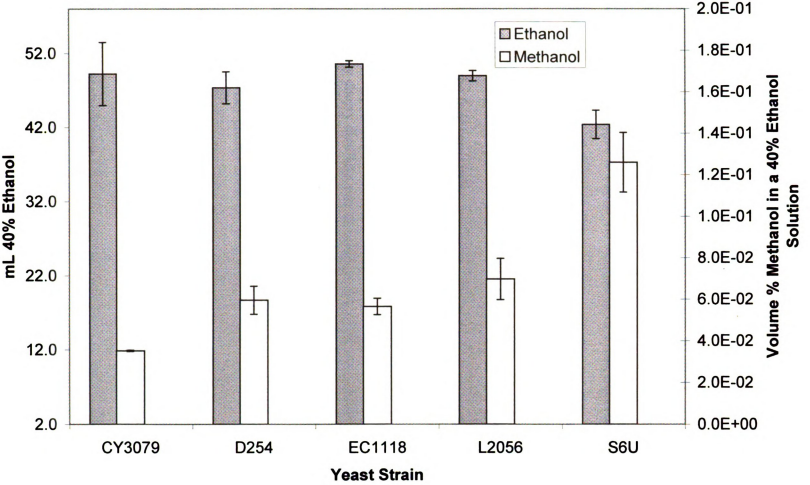
4.13 Red Seedless Grapes

Table 4.10 shows the results of fermenting and distilling red seedless grapes. Figure 4.10 shows the production of ethanol and methanol along with the corresponding standard deviation in the three trials used to generate averages. Ethanol, methanol, acetaldehyde, acetone and a variety of fusel alcohols were identified in the red seedless grape distillates using gas chromatography. The fusel alcohols found in red seedless grape distillates included 2-methyl-2-propanol, 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, and 1-heptanol. These fusel alcohols were summed together to generate a single fusel alcohol category.

Table 4.10 Red Seedless Grape Distillate Composition and Rank

Yeast Strain	CY3079	D254	EC1118	L2056	S6U
40% Ethanol mL	49.2±4.27	47.3±2.16	50.6±.432	49.0±.707	42.4±1.92
Duncan Test	a	a	a	a	a
Ethanol Rank	1	1	1	1	1
Methanol %V/V	.0352±.00028	.0595±.00675	.0565±.00394	.0697±.00997	.126±.0144
Duncan Test	d	c	c	b	a
Methanol Rank	4	3	3	2	1
Fusel Alcohol %V/V	.457±.0338	.471±.0160	.405±.00776	.556±.0261	.474±.0551
Duncan Test	b	c	a	d	c
Fusel Alcohol Rank	2	3	1	4	3
Acetaldehyde %V/V	.0139± .0107	.00913± .00621	.0139± .000280	.0000300± .000055	.0818± .00500
Duncan Test	c	b	c	a	d
Acetaldehyde Rank	3	2	3	1	4
Acetone %V/V	.0000600± .000110	.0000700± .000110	.0000600± .000100	.000150± .000142	.000160± .000216
Duncan Test	a	b	a	c	d
Acetone Rank	1	2	1	3	4
Average Rank	2.20	2.20	1.80	2.20	2.60
Overall Rank	2	2	1	2	5

Figure 4.10 Red Grape Distillate Ethanol and Methanol Concentration



5. COMPUTER SIMULATION

5.1 ChemCAD Batch

ChemCAD Batch® is a type of batch distillation software marketed by Chemstations. Common applications of this software include modeling existing batch column equipment, exploring alternative processes for existing products, design of new equipment, and validation of thermodynamics using batch column equipment. In this study ChemCAD Batch® was used to model the distillation of a fruit mash using the experimentally determined congener concentrations. By simulating a fruit mash distillation using ChemCAD Batch® it was possible to produce the profile of each congener as the fruit distillate was collected. Congener profiles provide a quantitative guide to the quality of the fruit spirit being produced. These profiles are useful in predicting when to make volumetric cuts of a fruit distillation.

The experimentally determined percent volume/volume composition of the CY3079 Montmorency cherry distillate was as follows: acetaldehyde (.00706), acetone (.418), 2-methyl-2-propanol (.00773), methanol (.337), ethanol (40.0), 1-propanol (.369), 3-methyl-1-butanol (.432), 1-hexanol (.000770), and benzaldehyde (.0495). It was then assumed that a Montmorency cherry mash has an ethanol percent volume/volume composition of roughly eight percent. Based on this estimate, the other congener percent volume/volume compositions were calculated for the Montmorency cherry mash. The estimated congener percent volume/volume compositions in the Montmorency cherry mash were as follows: acetaldehyde (.00141), acetone (.0837), 2-methyl-2-propanol (.00155), methanol (.0830), 1-propanol (.0738), 3-methyl-1-butanol (.0864), 1-hexanol

(.000154), and benzaldehyde (.00989). The remainder of the mash composition was assumed to be water and had a percent volume/volume composition of 91.7.

The estimated mash composition values were used in the ChemCAD Batch® software as the pot charge. The mash was assumed to be at standard temperature and pressure (25°C and 1 atm). The column specifications included selecting a four stage column with a partial condenser followed by a complete condenser at a pressure of one atmosphere. The operational specifications included selecting a reflux ratio of 1.5, a distillate rate of .333 L/min, and a stopping criterion when the ethanol liquid volume fraction reached 0.50. The display specifications included selecting units of liquid volume fraction versus time in hours.

Table 5.1 outlines the % volume/volume of acetaldehyde, acetone, 2-methyl-2-propanol, methanol, ethanol, 1-propanol, 3-methyl-1-butanol, 1-hexanol, and benzaldehyde in each cut. Acetaldehyde, acetone, and 2-methyl-2-propanol are seen in the highest percent volume/volume concentrations in the initial distillate or heads cuts because of their relatively low boiling points. The first three cuts are termed the head cuts and are not usually used for consumption. Methanol initially has its highest percent volume/volume concentration and then decreases rapidly. It then increases somewhat towards the middle or heart distillate cuts and then once again decreases. Cuts number four through fourteen are designated as hearts cuts and are used for consumption. The methanol percent volume/volume concentration versus the distillate volume is shown in Figure 5.1. Methanol's profile can be attributed to its complete solubility in both ethanol and water. Ethanol (Figure 5.2), 1-propanol, and 3-methyl-1-butanol have a large initial increase and then gradually decrease in percent volume/volume. This decline can be

attributed to the increased concentration of water in the distillate as the temperature within the partial condenser rises closer to the boiling point of water. Finally, 1-hexanol and benzaldehyde have a gradual increase in percent volume/volume as the temperature inside the partial condenser rises closer to their respective boiling points.

Cut Number	Cut Volume Liters	Additive Volume Liters	Ethanol %vol/vol	Methanol %vol/vol	Acetone %vol/vol	3-Methyl 1-Butanol %vol/vol	2-Methyl 2-Propanol %vol/vol	Benz-aldehyde %vol/vol	Hexanol %vol/vol	Acet-aldehyde %vol/vol	Propanol %vol/vol
1	0.5	0.500	65.9	4.12E-01	1.49E+01	3.95E-01	4.22E-02	1.14E-05	7.00E-05	6.43E-02	8.72E-01
2	0.5	1.00	70.3	3.87E-01	2.25E+00	7.83E-01	3.14E-02	2.60E-05	1.46E-04	4.79E-02	1.23E+00
3	0.5	1.50	69.9	3.89E-01	2.09E+00	7.95E-01	2.96E-02	3.35E-05	1.57E-04	4.31E-02	1.19E+00
4	1	2.50	69.2	3.91E-01	1.84E+00	8.08E-01	2.73E-02	5.10E-05	1.74E-04	3.68E-02	1.13E+00
5	1	3.50	68.0	3.93E-01	1.50E+00	8.18E-01	2.47E-02	6.93E-05	1.99E-04	2.93E-02	1.05E+00
6	1	4.50	66.7	3.95E-01	1.23E+00	8.19E-01	2.20E-02	9.27E-05	2.23E-04	2.30E-02	9.69E-01
7	1	5.50	65.1	3.96E-01	1.02E+00	8.13E-01	1.94E-02	1.19E-04	2.47E-04	1.75E-02	8.84E-01
8	1	6.50	63.4	3.96E-01	8.34E-01	7.99E-01	1.69E-02	1.46E-04	2.71E-04	1.33E-02	8.06E-01
9	1	7.50	61.8	3.95E-01	6.67E-01	7.78E-01	1.47E-02	1.74E-04	2.96E-04	9.75E-03	7.25E-01
10	1	8.50	59.8	3.93E-01	5.25E-01	7.53E-01	1.27E-02	2.09E-04	3.20E-04	7.25E-03	6.44E-01
11	1	9.50	57.7	3.91E-01	4.00E-01	7.23E-01	1.09E-02	2.45E-04	3.44E-04	5.50E-03	5.69E-01
12	1	10.5	55.3	3.88E-01	2.92E-01	6.85E-01	9.00E-03	2.86E-04	3.70E-04	3.75E-03	4.94E-01
13	1	11.5	52.7	3.84E-01	2.09E-01	6.44E-01	7.34E-03	3.32E-04	3.94E-04	2.65E-03	4.25E-01
14	1	12.5	50.1	3.78E-01	1.25E-01	5.99E-01	6.00E-03	3.77E-04	4.17E-04	1.90E-03	3.59E-01

Table 5.1 The variation in Montmorency cherry distillate congener concentration estimated by using ChemCAD Batch simulation software. The Montmorency cherries were fermented using the CY3079 strain of yeast.

Figure 5.1 CY3079 Montmorency Cherry Distillate Methanol Profile Using ChemCAD Batch Simulation

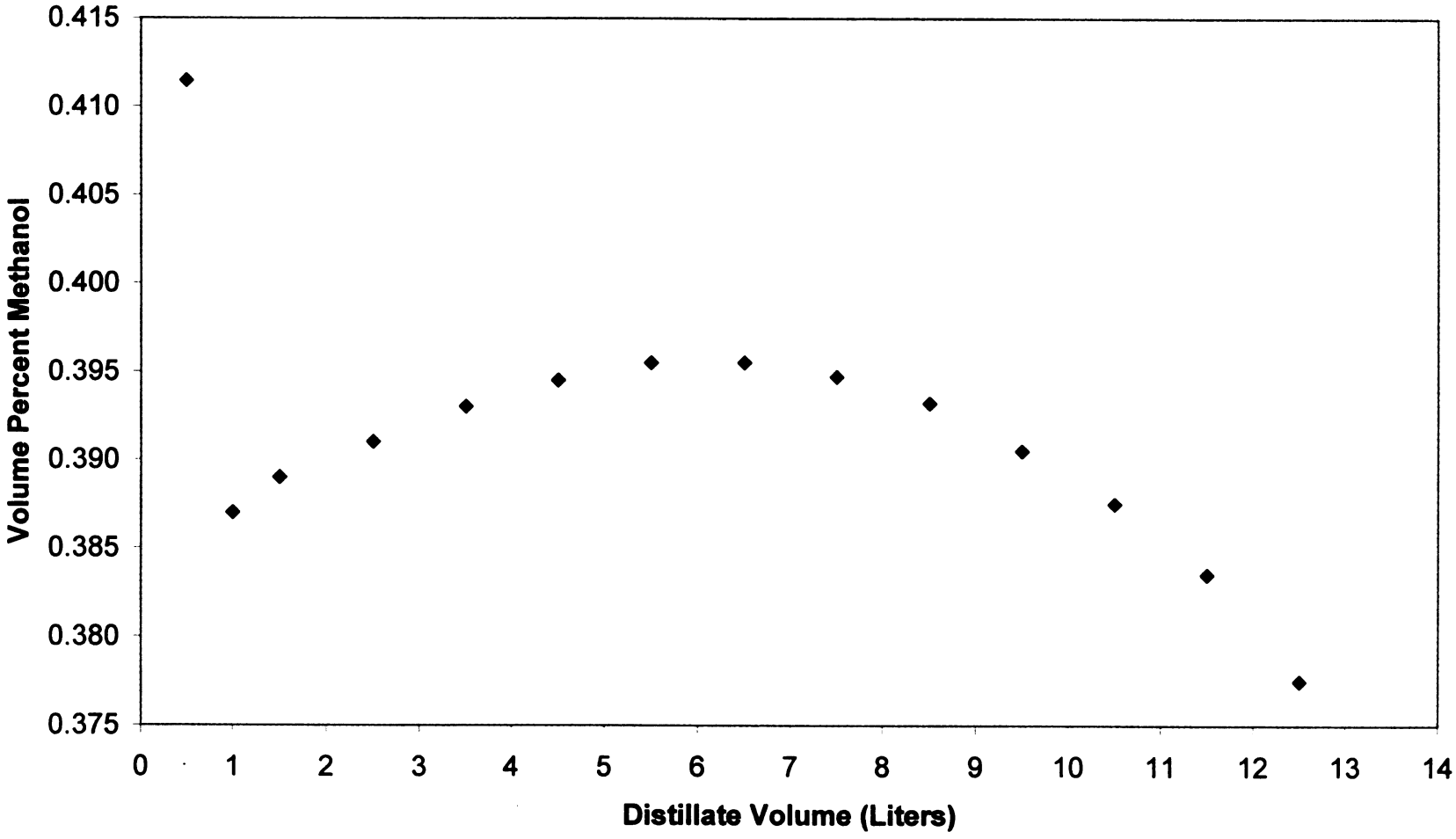
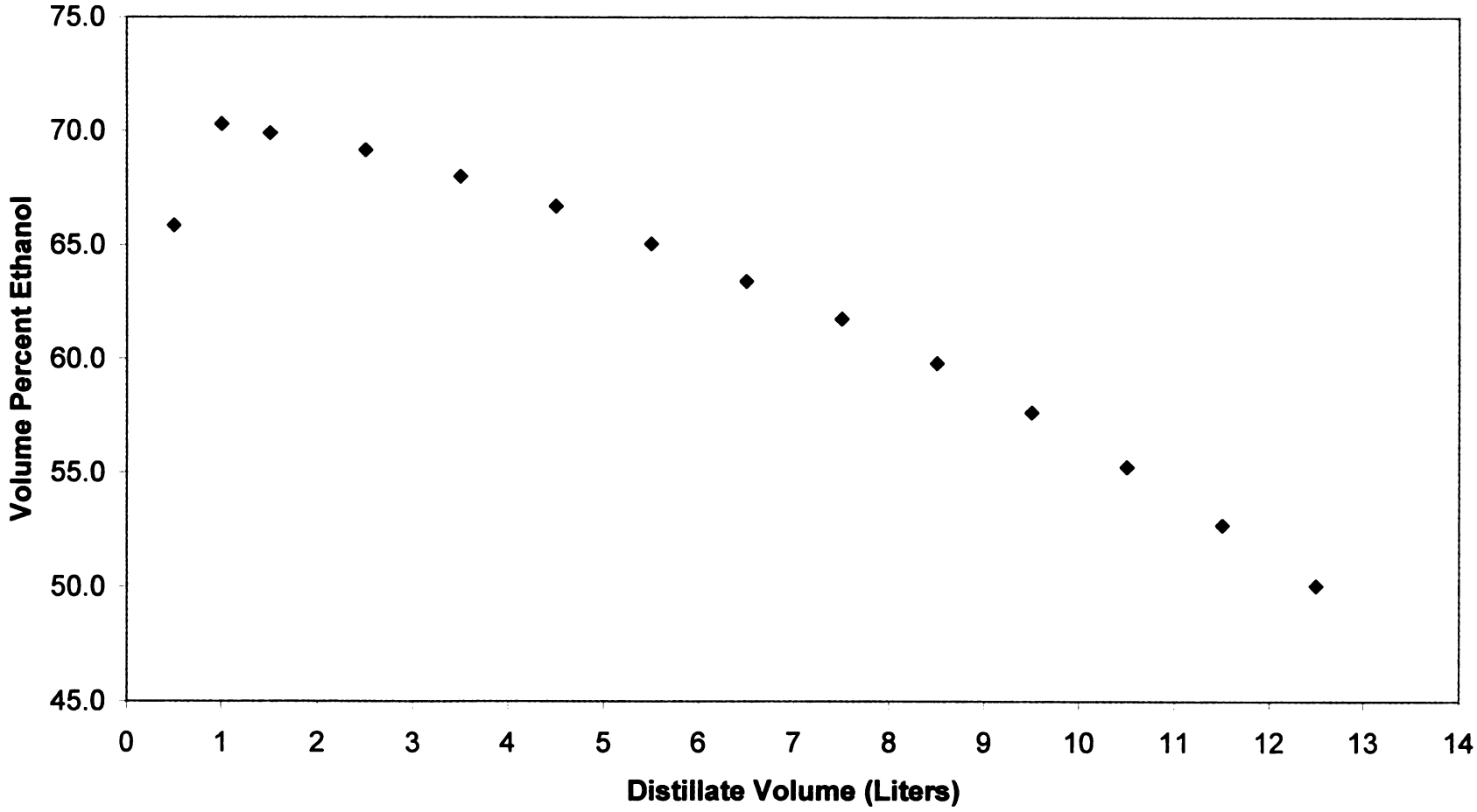


Figure 5.2 CY3079 Montmorency Cherry Distillate Ethanol Profile Using ChemCAD Batch Simulation



6. SUMMARY AND CONCLUSIONS

The fermentation and distillation of fruit beverages has been in practice since at least 3000 B.C. Despite this extensive history, the process of fermenting and distilling and its effect on the resulting product has not been fully understood. It was the goal of this research to develop some insight into the effects of altering the strain of yeast used to ferment and distill fresh fruit to produce brandy. By determining the effects of altering yeast strains, these data could then be used to improve the process and profitability of the Michigan fruit brandy industry.

This research has proven that yeast strains directly influence the congener concentration of a fruit brandy. The congeners identified using gas chromatography were ethanol, methanol, fusel alcohols, benzaldehyde, acetaldehyde, and acetone. When comparing the performance of different yeast strains for the same variety of fruit, it can be seen that different congener concentrations were produced. These fluctuations in congener concentration were used to compare the performance of five different strains of yeasts for the same fruit. The method of ranking the yeasts was done in an objective manner. Yeasts were ranked using the Duncan Range Test Method with one being the best ranking and five being the worst ranking. An overall average was then generated to compare the performance of different strains of yeast for a single variety of fruit. The nine varieties of fruit used in this research included Bartlett Pears, Black Plums, Braeburn Apples, Gala Apples, Granny Smith Apples, Green Seedless Grapes, Montmorency Cherries, New Haven Peaches, and Red Seedless Grapes.

Table 6.1 shows the yeast that was determined to be the best to ferment and distill a specific variety of fruit. This figure shows that of the five yeast strains studied all five

were determined to be the best performing yeast for at least one variety of fruit. This indicates the importance of the yeast strain selected to be used to ferment and distill fruit brandy. The intent of this research was accomplished by providing distillers in the state of Michigan a guide to yeast selection that will allow them to increase the amount and quality of fruit brandy that they produce.

Variety of Fruit	Best Performing Yeast
Bartlett Pears	D254
Black Plums	L2056
Braeburn Apples	L2056
Gala Apples	S6U
Granny Smith Apples	D254
Green Seedless Grapes	S6U
Montmorency Cherries	CY3079 & S6U
New Haven Peaches	D254
Red Seedless Grapes	EC1118

Table 6.1 The Best Performing Yeast Determined by Ranking the Congener Composition of Each Fruit Distillate.

7. FUTURE WORK

The research in this study focused on the fermentation of nine varieties of fruit with five different commercial strains of yeast. All fermentations within this study were done at 15°C. Future work could be done at different temperatures to see if the yeast performance is altered by temperature changes. Work of this nature would provide an optimal yeast and temperature combination to ferment and distill particular fruit varieties. Additional work could also be done on other varieties of fruit and yeast that were not studied in this research.

Other future work could involve using alternative analytical methods when determining the production of congener compounds. The research in this study focused on the major congener categories (ethanol methanol, fusel alcohol, benzaldehyde, acetone, and acetaldehyde). By using analytical techniques such as gas chromatography-mass spectroscopy instead of gas chromatography, congeners in smaller concentrations could be detected and quantified.

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