THE EFFECT OF WORT OXYGENATION ON BEER ESTER CONCENTRATION

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ABSTRACT

THE EFFECT OF WORT OXYGENATION ON BEER ESTER CONCENTRATION

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Oxygenation of wort during the beer brewing process is a common practice. It is most commonly used to increase the effectiveness of yeast during fermentation. However, in this experimentation the effect of wort oxygenation is examined through the production of esters and fusel alcohols.

Esters and fusel alcohols are a little known chemical element in beer production, but are the cause of many beer aromas and flavors. These flavors are used to enhance and control the style and flavor characteristics of beer. Many of the esters found in beer represent flavors found in nature, but not the actual ingredients used for making the beer. One such ester, phenyl ethyl acetate, provides flavors such as honey and rose. Other esters provide a range in flavor from solvent-like to banana.

This research looks past theoretical assumptions of the chemical and biological process of ester formation and looks to predict ester formation based on wort oxygenation levels. Samples of beer were oxygenated throughout the ranges of 0-25ppm and the subsequent ester and fusel alcohol concentrations were measured.

CHAPTER I: INTRODUCTION

The flavor of beer is a topic of much dispute from both connoisseurs and brewmasters alike. Beer is one of the more complex drinks in terms of the components involved in creation and their multisided relationships. There are many different approaches to achieve a similar product, but even small changes can develop a drastically different end result. The processes used are just as important as the components used. Using wort oxygenation to alter the beer's final flavor by affecting ester production is the focus of this research. Oxygenating wort seems like a simple concept at first glance, but has large consequences on flavor and quality. Brewing processes have advanced and so have brewer's ability to control and observe microscopic changes. This paper is a logical step in developing and improving the body of knowledge of beer manufacturing and flavor control. Reducing the guesswork and helping future brewers develop a higher quality product is the aim of this research. Over time the brewing industry has expanded beyond creative interpretation and guesswork to allow for an increasingly controlled approach, though many refrain from new technology due to increased costs, complexity, and personal philosophy. The market for these advancements can be limited, but the effort is worthwhile as it has the potential for ubiquitous use. (Renger, 1992)

Esters are small chemical compounds that show up in the form of various tastes and smells that account for a large portion of the characteristics that consumers note when tasting and smelling beer. When taking into account esters in the brewing process there is no right or wrong way to approach the subject, as there are styles that both encourage and frown upon the inclusion of esters in their character. Since there is no right

way in brewing methodology this has encouraged the experimentation and incorporation of new technologies into the brewing process. The growing microbrew industry in the United States best exemplifies this trend. The Brewer's Association reports that "Growth of the craft brewing industry in 2012 was 15% by volume and 17% by dollars compared to growth in 2011 of 13% by volume and 15% by dollars." This growth is spurred by increasing public interest and has spurred scientific research. Western North Carolina, in particular, is not immune to this trend and has kept the craft brew industry in the headlines. The brewing industry in WNC is a potential factor in the tourism and the manufacturing industry with the opening of several new breweries in the past years and many more planned. Despite the recent trend of microbrewery growth, it's important to remember that research into the production of beer is nothing new.

The research performed in this thesis experiment aims to benefit both the growing micro/home brewery industry, as well as established large-scale brewing facilities by looking at an accessible form of brewing process control. One of the most common forms of brewing process control is wort oxygenation prior to the fermentation process. Wort oxygenation is used on scales ranging from small home brewers, to large production facilities. Oxygenation is used for several reasons, the most common being the encouragement of successful and timely fermentations. Less widely known is the usage of oxygen enriched wort to reduce ester development and prevent unwanted flavors. Application and understanding of this concept is the primary focus of this study.

Background and Need for Study

Ester control and oxygenation have been subjects linked together since the 1960s. Early knowledge on the subject was limited. Most brewing facilities used wort

oxygenation to ensure successful fermentations, but had little knowledge of the effect that aeration had on the quality of beer (Maule, 1966). Over time knowledge of yeast and its usage of oxygen developed further. This led to a growing realization of the correlation between ester levels and initial wort aeration. Technology and scale drove the desire for understanding as brewers were beginning to experiment with more beer styles as well as high gravity fermentations. As production began to focus on large-scale, high-gravity fermentations, issues with excessive ester build up began to push research towards ester control and fermentation quality. High gravity brewing was the major culprit in pushing research in this direction, as the concept allowed breweries to increase production capacity by brewing high gravity wort in a smaller batch, then dilute with water as needed (Palmer, 1974). Issues with increased gravity in brewing arose due to the change in brewing conditions; as with most processes, if one aspect is scaled, then other aspects have to be modified to successfully utilize the changes. Yeast has the same difficulties with increased-gravity production environments. The increased sugar content and wort density puts more strain on the yeast causing increased ester production and untimely fermentations (Jones, 2007; Verstrepen & Derdelinckx, 2003; Lima, 2011).

As time progressed, understanding of wort aeration and its importance in ester control and fermentation quality increased. However, current research does not pinpoint the exact effect that aeration has on individual ester production, or if the effect can be predicted through regression analysis. Discovering these pieces to the fermentation puzzle is particularly beneficial to brewers wishing to control certain esters and develop a prediction equation for their own production facilities. It should be noted that wort oxygenation is not the most important aspect of ester control, yeast properties weigh

much more heavily on the actual ester profile. Yeast variants can produce ester levels of diverse types at a wide range of levels. This means that in order for wort oxygenation to be a successful factor in flavor control, a brewer must first know the style of yeast that is desired. Oxygenation is then used to fine tune the fermentation and flavor intensity of the beer.

Goals for the Study

The main goal of this study is to further understand the effect wort oxygenation has on the development and production of esters in a quantitative manner. The following research questions will be answered:

- What major esters are the most affected by wort oxygenation?
- What is the optimal range for maximum ester concentration, while still performing a timely fermentation?
- Can ester concentration be predicted through regression analysis and batch comparison?

Objectives of the Study

To successfully perform ester analysis the following objectives were used as guidelines:

- Develop a production procedure using all a grain brewing process to develop a single, large volume, malt batch.
- Perform 30 standardized fermentations using researched oxygenation levels as the only modified independent variable.
- Develop a uniform testing procedure using Gas Chromatography Mass Spectrometry analysis.

- Analyze results using regression analysis to represent correlation between oxygenation and ester concentration.

Significance of Study

While the background understanding of ester concentration levels and wort aeration has already been performed, specific research into the topic of individual ester influence and prediction is relatively unknown. It is theorized that some esters have increased response to initial oxygenation over their peers. The experimentation performed here shows the correlation between oxygenation and individual ester levels.

Brewing operations that wish to fine tune their wort oxygenation levels to maximize ester production will find this information significant as well, as the prediction capability of esters in relation to initial oxygenation is unavailable at the current time. This information is key to finding the balance between maximum ester production and fermentation success.

Definitions and Key Terms

Acetyl-Coenzyme A (Acetyl-CoA)- molecule produced during yeast's respiratory phase. It is used as a molecule in the yeast's fatty acid metabolism for the development of esters within the fermentation process.

Acyl-Coenzyme A (Acyl-CoA)- a coenzyme involved in the metabolism of fatty acids. This coenzyme is formed when a fatty acid attaches to a coenzyme and in turn aids in the production of Acetyl-CoA.

Alcohol Acetyl Transferase I & II- an enzyme that catalyzes the reaction between Acetyl CoA and Alcohol to form esters (see Enzyme below).

Enzyme- large biological molecules that are often proteins. They catalyze many biological functions performing specific internal conversions necessary for life.

Ester- a chemical compound defined by having a carbonyl (double bonded carbon and oxygen atom) adjacent to bonded ether (oxygen atom bonded to either an alkyl or aryl group).

Ester Synthase Gene- enzyme that catalyzes the reaction within fermentation that forms esters.

Enzyme-Catalyzed Condensation Reaction- a reaction that combines two smaller molecules into one larger one while leaving a new smaller molecule behind. This reaction is catalyzed by enzymes during the fermentation process.

Fatty Acid- a carboxylic acid that is often made up of a long chain of carbon atoms. Fatty acids are an important source of fuel when metabolized.

Fermentation- the general term for the yeasts processing of sugars within the wort to produce alcohol, carbon dioxide, and flavor compounds.

Fusel Alcohol- group of alcohols created as a byproduct of a yeast's fermentation process. These alcohols are similar to esters in their effect on beer flavor.

Gas Chromatography-Mass Spectrometry- a form of molecule separation that uses heat and an inert carrier gas to move molecules through a column. These molecules separate out by their volatility before being applied a charge and sent through a sensor that counts the number of particles in relation to the time of measurement to determine the particle type and amount.

Lipid- a group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, and phospholipids among others.

Mashing- brewing process where the germinated malt is soaked at high temperatures to convert the starches contained within into usable sugars for the yeast to process during fermentation.

Saccharomyces Cerevisiae- strain of yeast used to make a beer style called ale. S. cerevisiae is different from the lager strain saccharomyces carlsbergensis, in that it requires a higher temperature for fermentation. The flavor characteristics and alcohol content are subsequently different.

Sonication- a physical process that removes gases from liquids by introducing vibration through controlled-frequency sound waves. Sonication was used in this instance to remove latent carbon dioxide in the post fermentation beer and increase the clarity of gas chromatography readings.

Specific Gravity- the density of the wort in comparison to the density of water. Measurements come out as a direct multiplicative comparison between water and the substance, wort usually is rated at about 1.040, or more specifically, 1.040 times the density of water.

Threshold Value- the concentration value at which the human tongue can detect the presence of esters. Threshold value varies from ester to ester making some esters more influential than other on beer flavor.

Wort- a sweet substance developed through the mashing process. Wort has most of the necessary ingredients for the yeast to perform fermentation.

Yeast- a microorganism used in the beer development phase to convert sugars, carbohydrates, and water into alcohol, carbon dioxide, and flavor components. Yeast operates anaerobically, but has some aerobic-like functions. There are two main types of yeast used in beer production: saccharomyces cerevisiae and saccharomyces carlsbergensis. Each operates under different conditions and produces various flavor components.

Table 1: Esters and alcohols measured in analysis

Component	Threshold Level (mg/L)	Flavor Description
Acetaldehyde	10	Acidic, pungent
1-Propanol	2.6-40	Fusel, sweet solvent-like
Ethyl acetate	25-30	Solvent-like
Isoamyl alcohol	30-70	Alcohol, banana
2-methanol 1-butanol	10-65	Alcohol, solvent-like
Isoamyl acetate	1-1.6	Fruity, banana
Ethyl hexanoate	0.001	Aniseed, apple-like
Phenyl ethanol	28-135	Rose or rose oil
Ethyl octanoate	0.0035	Sour apple
Ethyl decanoate	0.002	Floral

Delimitations of the Study

This study was constrained by the following main criteria:

- Ester Production- The main outcome of this research would only be the ester concentrations in parts per million (ppm) no other data from this sample is used

for data analysis. Other data, such as specific gravity and in-process brewing information is discussed, but only in painting a larger picture of the process.

- Wort Oxygenation- Only oxygenation between the samples is changed. All fermentation samples were set as equal with no variations in atmospheric condition.
- Brew Process- The methodology of this batch of beer was developed with uniformity in mind. The recipe used was a predetermined all grain recipe from the Tuckaseegee Brewing Cooperative. All fermentations were based off of the same brew batch, with as little variation between each setup as possible.
- Equipment- Ester measurement was limited to the available tools in the Chemistry and Physics Department at Western Carolina University as well as the brewing equipment of Tuckaseegee Brewing Cooperative.
    - Brewing Equipment
        - Fermentation
        - Mashing
        - Boiling
        - Specific Gravity
        - A General DOM 22 Dissolved Oxygen Meter was used to measure both temperature and the levels of dissolved oxygen in the various fermenters prior to airlock application.
    - Testing Equipment

- An Agilent G1888 Headspace Analyzer was used to heat and analyze the components prior to separation from the gas chromatography.
- An Agilent 7890A Gas Chromatograph was used to separate volatile molecules for detection by the mass spectrometer.
- An Agilent 5975C Mass Spectrometer was used to detect the individual particles after being separated by the gas chromatograph

- Data Analysis
  - Microsoft Excel was used for basic data analysis and chart creation
  - The Minitab software suite was used to process the data collected by the GC-MS system and perform regression analysis

CHAPTER II: LITERATURE REVIEW

The majority of ester formation in the brewing process takes place during fermentation (Engan, 1974). Fermentation takes place in a sealed container with limited exposure to the environment. An isolated environment is needed to prevent interference in the fermentation process which can negatively impact beer flavor due to wild bacteria contaminating the beer (Lewis, 2004).

During the fermentation process, yeast is added to the finished wort in a process called “pitching” to convert the sugars developed through mashing and brewing into alcohol, flavor components, and carbon dioxide. There are two basic types of yeast: saccharomyces cerevisiae (ale yeast) and saccharomyces carlsbergensis (lager yeast). These two basic types operate in a similar manner in the fermentation process, but produce different results and operate under different conditions. Lager yeast prefers colder fermentation temperatures and produces a beer different form its ale yeast cousin (Lewis, 2004).

Yeast is a microorganism that requires nitrogen, carbon, vitamins, water, oxygen and metal ions to properly ferment (Rees, 1999). While oxygen is important for successful fermentations, yeast can operate anaerobically, or in the absence of air. Anaerobic operation is of high importance as it contributes to the creation of alcohols and the flavor compounds found in beer. It has been proven that most creation of flavor and alcohols happens during the anaerobic phase of fermentation. This aspect of production is a key component of the research, as most flavor compounds are formed when the oxygen levels of the wort have been depleted. These flavor components are primarily found in the form

of esters (Verstrepen, K. J., Derdelinckx, G., et al., 2003). Other flavor components consist of:

- Alcohols
- Carbonyls
- Acids
- Sulfur Compounds
- Amines
- Phenols, and
- Other (Engan)

Yeast reproduces asexually in a process called budding. As each yeast cell grows and splits, they share DNA amongst each other (Lewis, 2004).

Brewing Process Overview

Beer creation follows five basic steps:

1. Malting
2. Brewing
3. Fermenting
4. Finishing
5. Packaging

The malting and brewing steps are performed to create a more fermentable substance; finishing and packaging exist to ensure the beer matures and maintains its quality after storage (Goldhammer, 2008).

Malting

Malting begins after the grain producer decides to sell the barley to a malt house. Malting processes the grain for proper levels of cleanliness and to break the hard barley shell for easier processing in the brewing stage.

The malt producer first soaks the barley. After soaking, it is turned and aerated to allow for slight germination. Germination causes the shell to break down through slight growth of the barley seed and allows the brewers to quickly and easily process the malt. The processors then take the soaked malt and heat it in a kiln. Kilning dries the malt to provide a darker color and a richer flavor to the final product. It is the final step in the malting process (Lewis, 2004).

Malt Grinding

Malt crushing is needed to crush the barley interior while leaving the exterior husk intact. This process opens up the grain to allow for a more efficient and complete mashing process. A malt grinding station consisting of two adjacent cylindrical pins crush the grain as it falls between. (Goldhammer, 2008).

Brewing

Brewing is the process used to make the malt ready for fermentation. The malt enters this stage with no fermentable sugars available for yeast processing. The malt is a very starchy substance; however, these starches can be easily converted to sugar.

Brewers take the malt and grind it before mixing it. Water is used to soak the malt before heat is applied to create a mash substance. This converts the starches to sugars. After this process the spent grains must be filtered out of the mash.

The remaining substance is called wort. Wort is modified in the fermentation process through yeast consumption. Brewers may incorporate other ingredients into the wort and boil it to bestow alternate flavors (Lewis, 2004; Goldhammer, 2008).

Fermentation

Brewers use fermentation to change the sugars developed through the brewing phase into alcohol and carbon dioxide. The wort is taken directly from the brewing phase and placed in a fermentation vessel.

The strength of the wort determines the strength of the alcohol content and the beer. If the brewer is brewing in high volume they might use much stronger wort, but dilute it to create more beer from a batch (Lewis, 2004).

The fermentation process takes place within a large cylindroconical vessel of 6000hL (150,000gal) or more in industrial operations. Brewmasters initiate the brewing process by pitching to add yeast to the wort. As the yeast begins to grow the process speeds up exponentially, the byproducts of alcohol and carbon dioxide are created as the yeast eats through the sugar. The container vents the carbon dioxide to prevent damage to the vessel. The brewmaster cools down the container to encourage the process to conclude; this causes the yeast to settle. The brewmaster will remove the yeast at the bottom of the vessel to reuse it to begin the post processing and packaging (Lewis, 2004).

Yeast

Yeast is a bacteria implemented in the fermentation stage. Yeast consumes the sugars developed within the brewing phase and converts them into the previously mentioned alcohol, carbon dioxide, and flavors. Micro-bacteria in yeast use budding as their form of reproduction. Yeast consumes these sugars without the need for oxygen using an anaerobic metabolism. Despite yeast's nonessential need for oxygen, the efficiency of the process will increase if oxygen is added into the mix. However, brewers desire this inefficiency due to the formation of flavor compounds.

Brewers use yeast to not only process sugars for beer modification, but also to aid in the quality control and consistency of batch production. Because of its importance in the fermentation process, brewers reuse yeast from previous batches, or keep spare cultures to ensure that yeast characteristics are consistent in all batches. Ease of recovery is a necessary quality in yeast and influences yeast selection.

There are two basic types of brewing yeast:

Ale Yeast or Saccharomyces cerevisiae

- Floats atop the batch

Lager Yeast or Saccharomyces carlsbergensis

- Settles on the bottom
- Ferments at low temperatures
- Produces higher levels of carbon dioxide

Uncountable differences occur between yeast strains. These differences account for taste variations; determine the bulk of variation between beer types and batches; and are the driving force behind monitoring the consistency in yeast characteristics.

Once fermentation is complete, the substance is now called green beer, and is ready for post processing and packaging (Lewis, 2004).

Post Processing & Packaging

Brewers process the beer using three techniques: aging, krausening, and lagering. Green beer lacks carbonation; and needs to be matured, stabilized, and clarified before consumption. A brief description of each process follows:

Aging

- Beer is chilled to 0°C for a week
- Carbonation is added at any point

Krausening

- Reintroduces yeast into the beer
- Self carbonates
- Reduces undesirable flavor compounds

Lagering

- Slows the end of the fermentation process by cooling
- Self carbonates
- Reduces undesirable flavor compounds
- Speeds the maturation process

Once brewers complete the finishing process, the beer is ready to be packaged. Brewers remove the excess particulates and remaining yeast through either filtration or centrifugation and apply a stabilizer to the mixture, if desired. Stabilizers are designed to remove certain proteins from the mixture and prevent the formation of haze and free oxygen.

Brewers ship the beer to a packaging plant where the beer is bottled for easy transportation and consumption.

A lot of variables affect what is desired from post processing. Post processing is important if the product is consumed. The two basic types of storage containers are the keg and the simple bottle. If the beer is brewed for consumption, then CO_2 is added back into the beer for desired carbonation. When the fermentation process is performed, CO_2 is released back into the atmosphere.

Bottling could also be used in research as an integral part of the lab. Bottling is a necessary part of the production process outside of keg filling. In this lab, however, it is an optional piece of equipment (Lewis, 2004; Goldhammer, 2008).

Esters

Esters are the backbone of beer flavor components. Esters are volatile chemicals within beer that provide a key majority of beer flavor; they are found to have only comparatively small concentrations, and are measured in parts per million. Ester concentration alone is a misleading characteristic, as only small amounts of these esters are actually needed before they impact beer flavor. Ester threshold values are the concentrations needed before an ester's flavor is detectable, these values vary between ester types. Some esters are available in much higher concentrations, but they will never be noticed in the beer due to having a high threshold.

Esters provide a diverse array of possible flavors both good and bad (Engan, 1974; Verstrepen, K. J., Derdelinckx, G., et al., 2003; Lewis, 2004). Below is a list of

common esters and their associated properties taken from Flavor Active Esters: Adding Fruitiness to Beer:

Table 2: List of sample esters and their properties

Component	Concentration Range (mg/L)	Average Concentration (mg/L)	Threshold Level (mg/L)	Flavor Description
Ethyl acetate	8.00-32.00	18.40	21.00-30.00	Fruity, solvent-like
Isoamyl acetate	0.30-3.80	1.72	0.60-1.20	Banana, pear
Ethyl caproate	0.05-0.3	0.14	0.17-0.21	Apple, aniseed
Ethyl caprylate	0.04-0.53	0.17	0.30-0.90	Apple
Phenyl ethyl acetate	0.10-0.73	0.54	3.80	Roses, honey, sweet

This is only a small sample of esters found in beer. Experimentation will look at many other esters through the gas chromatography mass spectrometry analysis. This aspect of experimentation will be discussed later.

Ester Formation

Esters are formed through a reaction called enzyme-catalyzed condensation reaction, which occurs between an enzyme called acyl-coenzyme A (acyl-CoA) and higher alcohols with an ester synthase gene in the cell called the alcohol acetyltransferase gene (AATase). Acyl-CoA is a coenzyme group that is responsible for the metabolism of fatty acids and is affected by their presence (Pratt, 2004). The two most important aspects of ester formation for research are the concentration of acyl-CoA and fusel alcohols, and the activity of the enzymes found in the wort (Verstrepen, K. J., Derdelinckx, G., et al.,

2003). Factors that increase the levels of acyl-CoA and fusel alcohols will invariably affect the amount of esters produced.

The AATase gene is responsible for the formation of esters by processing the fusel alcohol and acyl-CoA. Unsaturated fatty acids formed through wort oxygenation also repress the AATase gene. AATase repression prevents esters from forming while oxygen is present in the wort, making it a common form of ester control. However, it should be noted that there are factors that have no effect on these enzyme levels that also control ester production. Factors such as top pressure, nitrogen, and glucose levels all modify ester production, but have no effect on enzyme levels. Many of these methods are not fully understood and will be discussed further. (Verstrepen, K. J., Van Laere, S., et al., 2003; Verstrepen, K. J., Derdelinckx, G., et al., 2003).

Certain esters flow freely through the cell membrane due to their high lipid solubility; these are called acetate esters. Fatty acid ethyl esters are not quite as soluble as acetate esters. Ethyl ester chains become less soluble as their chain length increases, and therefore cannot leave the yeast cell. Due to this factor, certain esters will naturally have higher concentrations simply through their ability to permeate the cell wall. Lager yeasts keep higher amounts of esters within their walls during fermentation, thus causing them to release fewer esters. The ability of a yeast cell to retain and release esters controls the concentrations of released esters, thus giving different yeast strains high variability in yeast producing characteristics (Engan, 1974).

Ester Control Methods

Ester control in beer is a highly desirable capability. No two beer styles are the same, and no two breweries use the exact method and recipe. Controlling ester production is an important aspect of the brewing process. One reason to control ester production is to hide undesirable flavors that appear in high gravity brewing. Another reason is to increase desirable flavors in their beer to bring about flavor characteristics. There are many factors that control the production of esters in beer. Each one of the topics listed below is merely touched in brief. The majority of the information provided is based on wort oxygenation. (Verstrepen, K. J., Derdelinckx, G., et al., 2003; Lima, 2011)

Yeast.

Yeast strain is the strongest factor controlling ester production (Engan, 1974). Yeast strain controls the basic favor profile of a beer and is the foundational component of beer flavor. Yeasts can change not only the overall concentration of esters produced, but also the concentrations in relation to each other. This means that similar strains can have different flavor characteristics if one ester is more prevalent. Other changes made to beer will only affect the performance of the yeast. This means that despite some changes in beer flavor, most production methods for modifying ester concentrations will not change the basic characteristic of the beer flavor, only the intensity of esters (Palmer, 1974; Verstrepen, K. J., Derdelinckx, G., et al., 2003).

Nitrogen

Nitrogen can also be used to control ester levels, as nitrogen level is increased, ester production increases as well. However, the relationship between nitrogen content and ester production is very complex and not completely understood as the research into

nitrogen injection as an ester managing control is relatively new. The time, place, and level of nitrogen influence on ester production are based around three basic concepts. The first concept says that in fermentations using wort of high carbon to nitrogen ratios (i.e. worts using high levels of adjuncts), nitrogen becomes an important factor in controlling yeast growth. Higher amounts of nitrogen increase yeast growth and therefore ester production. When nitrogen levels drop, ester production drops as well. The second concept says that nitrogen has an influence on ester production because it increases the levels of fusel alcohols produced. These fusel alcohols interact with the ester production gene inside the yeast cell to increase ester production (Verstrepen, K. J., Derdelinckx, G., et al., 2003). The third concept says that nitrogen affects the ATF1 gene. The ATF1 gene is one of the many genes represented by the global term ester synthase gene. Nitrogen causes it to increase its ester production (Verstrepen, K. J., Derdelinckx, G., et al., 2003).

Temperature

Temperature is used to control ester levels as well. Increased fermentation temperatures are shown to cause increased ester production. Not all esters are as affected by increases in temperature, and some yeast strains are less influenced by temperature. The specific cause of temperature's influence on ester levels is still unknown (Verstrepen, K. J., Derdelinckx, G., et al., 2003; Engan, 1974).

Yeast Pitching

Pitching rate has been shown to affect the synthesis of esters in beer. Higher pitching rates are shown to decrease the levels of esters produced in fermentation (Verstrepen, K. J., Derdelinckx, G., et al., 2003).

Drauflassen

Drauflassen has been shown to increase the number of esters produced. Drauflassen is the act of adding low oxygen wort into fermenting yeast. (Verstrepen, K. J., Derdelinckx, G., et al., 2003).

Fermentor Design

It is important to note that fermentor design will affect the production of esters. Larger fermentation containers will lead to less efficient yeast propagation and a lower production of esters. (Verstrepen, K. J., Derdelinckx, G., et al., 2003)

Wort Oxygenation to Control Ester Levels

Since many of these alternate factors are difficult or expensive to control, their methodology is less widely used. This research is focused around a common method of ester control: oxygen injection into wort. This method is widely used by home brewers and large production facilities alike due to simplified and inexpensive implementation. Due to the widespread usage, there is also widespread misinformation found in locations such as informal sources, such as internet forums, to incorrect company researched product manuals. The goal of this experimentation is to take the background knowledge and understanding of wort oxygenation in respect to ester production and quantify the relationship between oxygen and esters. The analysis attempts to show that ester production can be controlled and predicted through regression analysis and controlled production techniques.

Oxygen in the fermentation process is needed to help synthesize sterols and unsaturated fatty acids for cell membrane biosynthesis. Yeast will not bud when sterol

levels are too low. This means that while yeast operates anaerobically, it still requires oxygen from successful fermentation (Rees, 1999; Briggs, 1999). If oxygen is not added to a fermentation it can slow or stall the process. If a slowed fermentation occurs a known solution is to increase oxygen levels. It is difficult to control oxygen levels during fermentation, so yeast or wort oxygenation is the most common method to avoid this instance and is shown to be more efficient than oxygenation towards the end of fermentations. Reduced sterol levels due to insufficient oxygen levels lower the strength and stability of the yeast's cell wall. Sterols are an important factor in cell wall strength. Oxygen levels as low as 7 mg/l have shown reduced cell wall structures and reduced stability in fermentations. (Jones, 2007; Cowland, 1966; Fornairon-Bonnefond, C., Aguera, E., 2003; Anderson, R., & Kirsop, B. 1975)

Wort oxygenation is a common method for ester control and has been proven to reduce the amount of esters produced in fermentation. However, too little oxygen will stall fermentation by providing insufficient sterol and unsaturated fatty acid creation. This means that brewers desiring a more pronounced ester concentration through oxygenation control must avoid having concentrations below optimal levels. If oxygenation levels are too high, then the perceived quality of the beer can suffer from reduced ester concentrations, decreased alcohol production, and increased fermentation times. However, when the yeast runs out of oxygen, it begins to produce high amounts of esters. In a basic sense, this means that the less oxygen in fermentation, the faster it will reach the point of ester synthesis (Jones, 2007; Cowland, 1966; Anderson, 1974; Engan, 1974; Rees, 1999; Verstrepen, K. J., Derdelinckx, G., et al., 2003).

Oxygenation in the wort reduces the amount of acyl-CoA produced, and therefore, the amount of esters. AATase is responsible for the formation of esters by processing the fusel alcohol and acyl-CoA. Unsaturated fatty acids formed through wort oxygenation are known to repress the AATase gene. This prevents any esters from being formed while oxygen is present in the wort. Ester measurement during oxygenated fermentations has shown that adding oxygen not only reduces ester formulation by 80-90% during application, it also has a lingering effect of reduced ester formulation after oxygenation has occurred (Verstrepen, K. J., Derdelinckx, G., et al., 2003; Anderson, R., & Kirsop, B., 1975).

This picture taken from "Expression levels of the yeast alcohol acetyltransferase genes ATF1, Lg-ATF1, and ATF2 control the formation of a broad range of volatile esters" (Verstrepen, K. J., Van Laere, S., et al., 2003), shows a simple example of the internal processing of esters and alcohols within the yeast:

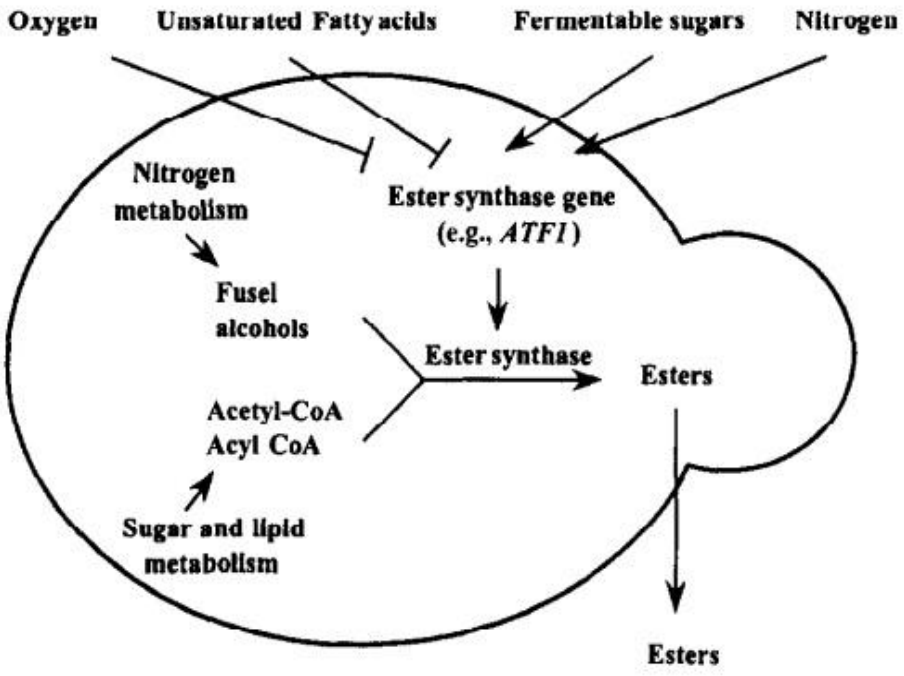

Figure 1: Ester processing within the yeast cell during fermentation

Ester Measurement through Gas Chromatography Mass Spectrometry

Gas chromatography mass spectrometry is a widely accepted form of beer ester analysis. Gas chromatography separates the volatiles, such as esters, and mass spectrometry reads and measures these separated particles. Gas chromatography is split into two major components: the injector system and the column. Within these components are three major steps: ionization, separation, and detection (Huimin, 2012).

Gas Chromatography is a method of separating multiple components called volatiles to easily identify and measure them. Volatiles are particles that vaporize at high temperatures without changing chemical form. To inject the particles and evaporate the volatiles the system uses injection system. The injector vaporizes the particles; this

separates the volatiles from the non-volatiles. To ensure that all particles are evaporated, the injection system typically operates at 50°C higher than the boiling point of the least volatile substance. To transport the volatiles, the system typically uses an inert gas system (Huimin, 2012; Robinson, K. et al., 2005; Miller, 2005).

Once vaporized, the column system separates the molecules out by heating the volatiles along a long tube. This tube represents the column aspect of the chromatography system. There are two phases that the substance is simultaneously converted to. The first phase is the mobile phase. The mobile phase refers to the gaseous volatiles within the system. These volatiles are separated from their original liquid form and moved through the column. They separate out based on their vapor pressures. As the particles travel through the column system they are heated by a programmed oven and will separate out. The stationary phase of the substances remains immobile within the system. The more volatile a substance, the faster it moves through the column. The oven is designed to heat the particles in a particular and often programmable way, to encourage more efficient separation. The column system can be made of many materials. The more similar the polarity of the column is to the volatile being measured, the more effective the system is at separating out the substances for better analysis (Huimin, 2012; Robinson, K. et al., 2005; Miller, 2005).

Once the particles have been separated they travel into the mass spectrometry's ionization system. The goal of ionization is to apply a charge to the particles that an electronic system can measure.

Once a charge is applied, the system separates the ions by weight and records them electronically using a sensor. The sensor can distinguish the molecule based on the time it takes to move through the mass spectrometry machine (Robinson, K. et al., 2005).

Regression Analysis

Statistical regression analysis is the method of variable prediction that was used to predict ester production based on initial oxygenation values. Regression analysis utilizes the relationship between independent and dependent variables. Sir Francis Galton first developed regression analysis in the late 1800s. The two types of relationships found are statistical and functional. (Neter, 2006)

Functional regression is the more simplistic methodology; it uses a direct relationship between two points to predict the exact amount. This is as simple as plotting the line between points and measuring the slope. The formula for this methodology is shown in the form of $y = f(x)$. In this case "y" is the dependent variable and "x" is the independent variable. (Neter, 2006)

Statistical regression is a more complex form of data analysis. Statistical regression utilizes the probability that an outcome will happen based on similar inputs. It then plots a line based on these probabilities to best follow the relationship of the variables. These lines are typically in a linear or quadratic formation, depending on the relationship. This is not as precise as a functional relationship since it shows the relationship based on probability, rather than a functional prediction. (Neter, 2006)

Regardless, both types provide a formula for predicting the outcome based on the determined relationship. This formula can be used to predict future events using a similar

experiment and environment. Regression however, is not a guarantee; it is only an approximation of future events and will not work if some previously unmodified variable is changed. (Neter, 2006)

Regression analysis is important in developing both prediction equations as well as plotting trend lines. MiniTab statistical analysis software is a useful tool for performing this analysis and plotting the data

CHAPTER III: METHODOLOGY

Overview

The basic design of the experimental methodology is an all grain brewing setup with a few changes made to allow for wort oxygenation and uniform fermentation. One batch of beer was brewed using Tuckaseegee Brewing Cooperative's all grain brewing setup and separated into 30 individual fermentations where the initial oxygenation levels for each batch were randomized to concentrations between 0-20ppm (mg/L). These results were recorded and the batch order randomized. Once this was complete, gas-chromatography mass-spectrometry was performed on individual fermentations to assess ester and fusel alcohol concentrations.

Preliminary Procedures

Procedural Equipment

Brewing procedure was established using Tuckaseegee Brewing Cooperative's (TBC) beer methodology for an American Pale Ale. Methodology followed TBC's usage of hops, grain, and brewing procedures to ensure a consistent brew parallel with their past production cycles. The majority of equipment used TBC's own from their predesigned brew methodology.

The basic setup for the TBC brew process involves a few basic components. The first major component is a malt crusher and a scale. The malt crusher is an optional component, and was not used in this setup as all grain purchased was pre-crushed. The scale was used to measure out the correct malt weights, as well as hop amounts for the recipe before their respective processes.

A 55 gallon Blichmann BoilerMaker was needed to perform the mashing operation. This tub was externally insulated to hold heat in and prevent temperature loss during mashing. An instant water heater was used to heat and maintain the water at the desired temperature. All temperatures were measured using a General DOM22 dissolved oxygen meter with an accuracy of ±1.5 °F as well as a Blichmann Weldless Thermometer for verification. The malts used were German Munich, German Pilsner, American Crystal, and American 2-Row Pale.

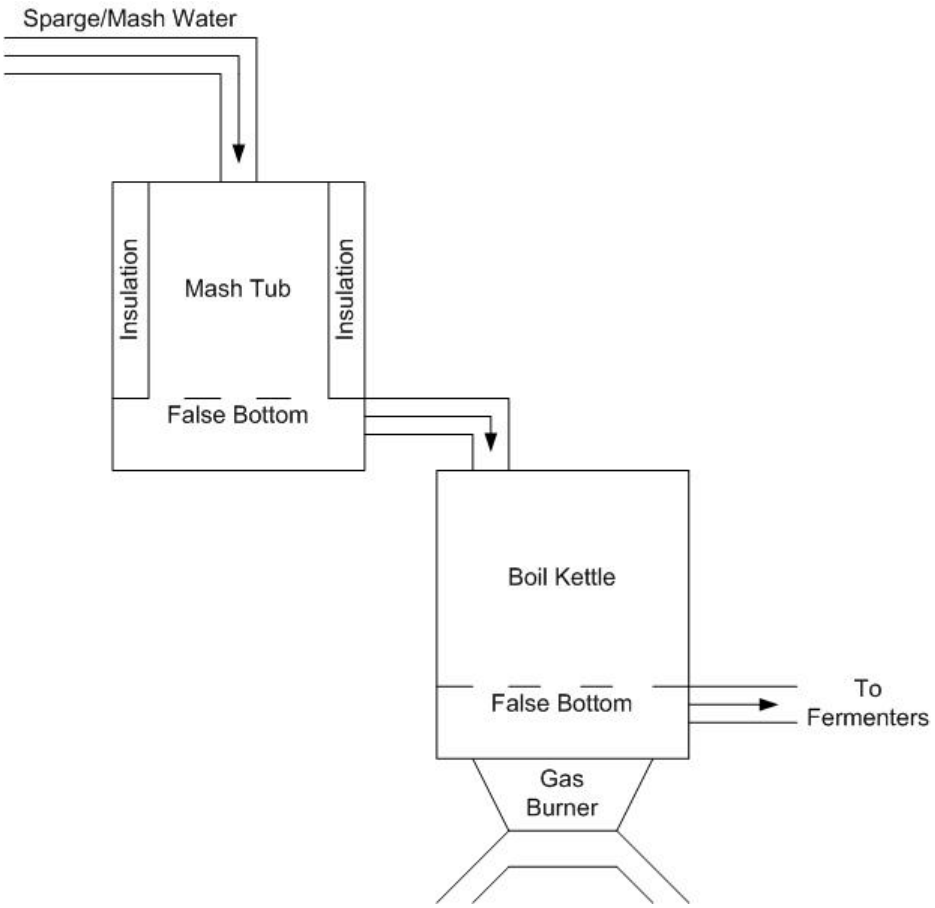

Figure 2: Basic diagram of mash, sparge, and boil setup

Two 55 gallon Blichmann BoilerMaker brew kettles were used for the sparging process. A gravity feed system was used to circulate fresh water. A refractometer was

used to monitor the specific gravity of the sparge. A Blichmann false bottom filter was used to remove the grain during the sparging process.

One 55 gallon Blichmann BoilerMaker was reused during the boiling process with a propane burner. Temperature was monitored as described above. A false bottom was used again to remove the hops. The hops used were Magnum and Cascade.

Transportation to the fermenter was performed using a March Model 809 transfer pump. Dry hopping was performed using a Blichmann HopRocket and wort chilling was performed using a pumped cold water wort chiller.

Oxygenation was performed using an air stone in tandem with a pure oxygen bottle. Dissolved oxygen measurement was taken using the previously mentioned DOM22 meter with an accuracy of ±0.4 mg/L.

Fermentation was performed in 1 gallon collapsible fermenters with iodine mixture filled airlocks. The yeast used was California Ale style yeast from the company White Labs.

Analysis Equipment

For the analysis of the sample, the Department of Chemistry and Physics provided means for preparation and analysis of the samples. For particle analysis and separation an Agilent 7890A Gas Chromatogram was used in tandem with a 5975C Mass Spectrometer and a G1888 Headspace Analyzer.

Procedure

Brewing process involves all steps previously mentioned with the process flow going in the following order:

1. Sanitation prep
2. Malt and hop portioning
3. Mashing
4. Sparging
5. Boiling
6. Oxygenation
7. Fermentation
8. Sample Preparation
9. Ester measurement
10. Data analysis

Sanitation was performed prior to the brew date using iodine and water mixtures in each plastic fermenter. These containers were allowed to soak overnight. The airlocks and caps for each piece were soaked in a similar manner in large containers filled with iodine solution. Most other components do not need to be soaked as they are boiled at high temperatures during various points in production. Sanitation of the oxygenation components was performed using an iodine mixture for sanitation, and sanitized water to rinse the DO meter and injection system. These two mixtures were kept in separate containers. The iodine water mixture was used to sanitize the components, and the clean water was used to rinse the iodine off prior to contact with wort.

Malt and hops were initially weighed before brewing and separated. In some procedures the malt is crushed, but this was not needed as the grain was pre-crushed. 62.7 lbs of grain was added to a preheated Blichmann mash tub. The grain soaks for an hour at a temperature of 170 °F. The mash kettle was wrapped in insulating material to hold the temperature constant.

The sparging process was performed using a second Blichmann tub. The gravity of the beer was reduced to an amount close to the desired original gravity.

After sparging is complete, the wort was boiled in the second brew kettle. During this procedure 10 oz of hops were added during their desired times. Hops were used to fully utilize TBC's standard recipe and encourage predictable results. Boiling lasted for an hour and the specific gravity reached was 1.048.

Fermentation and oxygenation was performed after the wort was boiled. In this scenario, the yeast was added to the boiling container prior to oxygenation in order to thoroughly mix the yeast into the wort and simplify the pitching process. After the yeast is pitched, the mixture was stirred slowly before being pumped into the gallon fermenters. These fermenters were used to separate the large batch into separate fermentations. A specially designed system was developed to oxygenate each fermenter separately and in a relatively random fashion. A simple oxygen tank with a hand nozzle and tube was used to pump into each container. Once the container was pumped with a random amount of oxygen, each container was shaken to encourage thorough mixture of the oxygen within the fermenters. Each mixing was done with the cap closed to prevent extra oxygen from entering the system and prevent spilling. Once shaken, three

measurements were taken using the dissolved oxygen probe. Each measurement was taken while stirring the wort with the probe to ensure that the probe was taking measurements in more than one location, and to prevent the measurement of unmixed pockets of wort. After three measurements within 0.5ppm were taken, the points were recorded, and a sanitized airlock and stopper were placed into position. The fermenters were then placed in an organized manner in one location removed from light and heavy temperature fluctuations. This was performed 30 times using various ranges from 0-20ppm with a few outliers. If a dissolved oxygen measurement was outside of the 0.5ppm range then an extra measurement was taken to reduce potential data collection errors.

Chromatographic Analysis

The Western Carolina University Chemistry Department assisted in developing a method for analyzing the ester concentration in the beer and provided the equipment to perform GCMS. This is a widely used methodology for this process, but each individual experiment must be set up in a specialized manner.

In order to perform gas chromatographic analysis on 30 individual samples many preliminary steps must be taken to make each sample comparable. There are many factors that can contribute to samples lacking consistency, but steps were taken to minimize these potential scenarios. Another factor that must be taken into account when planning a gas chromatographic run is the desired data result. The results presented though GC-MS are not in parts per million, which was the desired data format.

Many factors can contribute to inconsistent data reading in a GC-MS including inconsistent headspace sampling size, inconsistent vial batch size, and potential gas

pressure changes. These errors occur through human and machine error and must be assumed present in all GC-MS analysis. To reduce the effect of human and machine error on data analysis, an internal standard is used amongst all samples batches. In this case, each sample contained 1µL of butyl acetate, as it was not in tested samples prior to experimentation. This chemical compound remained the same amongst all batches and normalized the data.

In order to convert between analyte particles and concentration a calibration curve was developed using 3 random beer batch samples at 5 levels of ester addition using known quantities. Initially, a run was made to determine proper levels of sample that should be added in order to prevent overwhelming the sensor. This initial run was used to pinpoint problems such as flooded sensors, improper measurements, improper mixing, and reduced the potential for human error on the second calibration run. The measurements were adjusted and the following volumes for the calibration samples were devised:

Table 3: Initial unadjusted calibration curve (µL)

Mult	1-propanol	Acetaldehyde	Ethyl acetate	isoamyl alcohol	2 methyl 1 butanol	Isoamyl acetate	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate	Phenethanol
0	0.6386	1.8224	1.0812	1.1567	0.3418	0.0031	0.0005	0.0014	0.0035	0.0386
1.5	0.4967	1.4174	0.8409	0.8996	0.2659	0.0024	0.0004	0.0011	0.0027	0.0300
2.5	0.3548	1.0125	0.6007	0.6426	0.1899	0.0017	0.0003	0.0008	0.0020	0.0215
3.5	0.2129	0.6075	0.3604	0.3856	0.1139	0.0010	0.0002	0.0005	0.0012	0.0129
4.5	0.6386	1.8224	1.0812	1.1567	0.3418	0.0031	0.0005	0.0014	0.0035	0.0386

These standard sizes were determined to adequately produce results that would not overwhelm the system's mass spectrometer sensor and would be easily distinguishable and measureable.

Table 4: Volume of secondary calibration curve compounds and their anticipated concentration multipliers

	Anticipated Concentration Multiplier			
	1.5	2.5	3.5	4.5
calibration standard	Volume of Std. Added			
1-propanol (µL)	0.235673	0.392788	0.549903	0.707018
acetylaldehyde (µL)	0.665428	1.109047	1.552666	1.996285
ethyl acetate (µL)	0.40203	0.670049	0.938069	1.206089
isoamyl alcohol (µL)	0.429756	0.71626	1.002764	1.289267
2-methyl-1-butanol (µL)	0.124768	0.207946	0.291125	0.374303
isoamylacetate (µL)	0.001161	0.001935	0.002709	0.003483
ethyl hexanoate (µL)	0.00029	0.000484	0.000677	0.000871

The secondary calibration curve was performed with success and showed a good correlation between the esters added and the particles measured. The steps for producing this calibration curve can be found in Appendix C.

To perform analysis on all 30 samples each headspace vial was filled with 2mL of beer from each batch. Each sample had 0.025µL of the internal standard added. For batches 8, 18, and 29 the calibration curve was performed. Each calibration batch had one vial without any calibration standards added and contained only the internal standard. The vials were labeled according to the corresponding fermentation container before being placed in the headspace loading system. Each fermentation container was then resealed and stored out of natural light and temperature fluctuation.

The gas chromatogram was initially fine-tuned using multiple third party commercial beer samples to ensure a proper measurement of esters. The parameters used for sampling the data through the gas chromatogram are contained in Appendix B with the initial graphs of ester concentration.

Below is a sample graph of GC-MS spectral analysis taken from sample one. Each peak represents an individual compound:

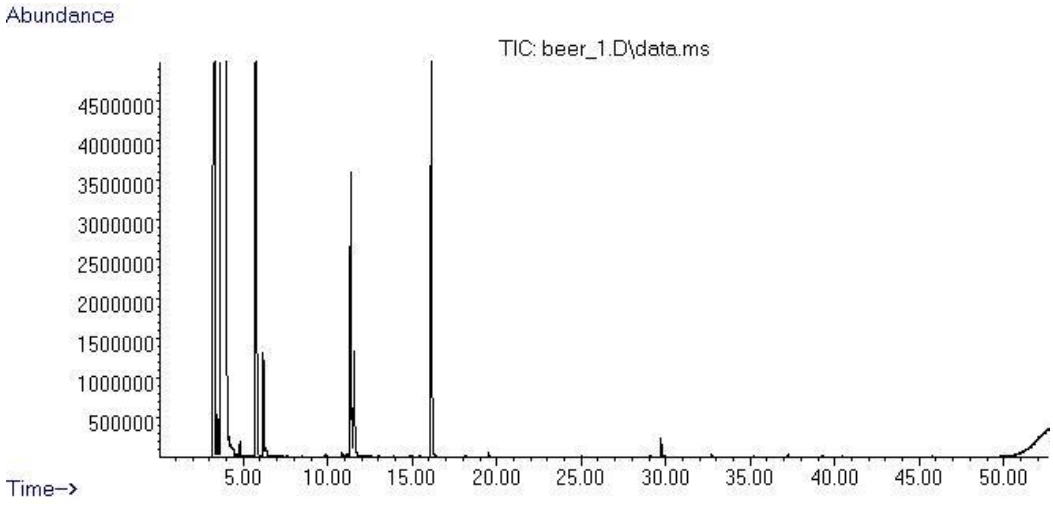

Figure 3: Particle abundance and time comparison in beer 1

Methodology Notes

During the process of adding oxygen to the fermentations there were some incidents, problems, and general notes that became apparent during the process. The first and perhaps most notable is the potential for contamination. In order to perform the oxygenation the oxygen pump and probe must be removes and applied 30 individual times, this increases the opportunities for potential contamination. To reduce the potential for this error's occurrence a soaking solution was created using iodine and hot water.

First iodine, then hot water was applied to sanitize the oxygen meter and pump to avoid iodine contamination.

Another note about the oxygenation process was the sensor itself. The instructions contained within the sensor did not explain the full methodology needed to prepare the sensor for operation. It was only after trial and error, as well as some extra research that it was discovered that the system needed 20 minutes to charge the sensor probe and properly measure oxygen.

Another note on the sensor was that it is highly sensitive and fragile, particularly the film at the end of the sensor probe. This caused issues later on in the process when the sensor was accidentally bumped in the bottom of a fermentation container. Once bumped the sensor produced inconsistent results and had to be recalibrated. This process is time consuming, but the time during the oxygenation procedure was noted in case data irregularities were recorded.

Another issue of note was that the oxygen-injected wort did not initially provide consistent measurement. It was determined that pumping oxygen alone provided uneven mixing. Within three fermentation fillings it was determined that each fermentation container needed proper shaking, and that the probe needed to be stirred within the mixture to ensure even measurement and even oxygenation levels throughout. These instructions were not included with the probe, and initial tap water testing did not have this issue due to an inherently more even oxygen mixture. Only after secondary research did was proper operational procedure discovered and reassessed. The initial oxygenated fermentation containers were oxygenated again and measured using the updated

techniques. This may cause problems in the data, as the initial fermentation containers were given dissimilar conditions due to the delay of proper measurement and oxygenation.

Filling each bucket was a time consuming process that a more automated procedure could vastly assist in. The process of filling and measuring each batch could be fairly easily automated, as well as the oxygenation procedure. Reducing human error and increasing consistency in time and measurement would improve the credibility of this study and reduce potential criticisms.

CHAPTER IV: RESULTS

Data was split into thirty separate sets representing each fermentation. Each result was processed through the following steps before being analyzed:

1. Data conversion for comparison
2. Acetaldehyde removal
3. Statistical outlier removal

Data conversion was necessary to compare between data collected from previous sources. Acetaldehyde removal was necessary after much research was performed on the source of some of the extreme outliers and the fermentation and storage process. Statistical outlier removal was performed to remove bad batches that did not fit the previous criteria, but represented a potentially bad batch. These steps are explained further in this section.

Data Conversion

Processing the results of the analysis required counting each measured ester and alcohol's individual particles in all 30 batches. In order to convert the visual chart reading into quantitative data, each ester and alcohol peak of the report from the gas chromatogram's analysis software was integrated. Doing so converted the physical peaks from the GC report into total particles for each volatile. To determine the physical composition of each particle, the esters were compared using the analysis software's chemical library. This library compares the composition and volatility of each particle with similar results to determine the probability of composition. It then lists potential matches with their probability of similarity listed as a percentage. To ensure that the correct esters were being measured, past data containing known ester additions were

viewed. Once each compound was matched and the total particles calculated, the data was recorded and converted to parts per million. Chemical compound data was compared to the previous runs using added standards as well as the actual density of the compound using this formula:

$$\frac{Norm\ Vol\ of\ Std\ Added * Compound\ Density}{10 * Avg\ Density\ of\ Beer\ Batch}$$

The outcome of this formula is the ppm reading of the individual compound standards added in the preliminary batches. To apply this to the individual batches divide the slope of this figure and the actual area of each particle, once normalized using the internal standard, into the area of particles in each batch. This equation performs the calculation:

$$\frac{Norm\ Area\ of\ Compound}{Slope\ \ ppm\ of\ added\ compound\ and\ Area\ of\ Compound}$$

These results were then compared to the averaged oxygenation levels in ppm of each batch. Since oxygenation and ester concentration is already a known correlation, no other initial analysis beyond regression was needed on the data. Each individual compound was compared to oxygenation and regression analysis was performed using MiniTab.

Data Point Removal and Analysis

Initial results in analysis showed little correlation between oxygenation and compound concentrations. Inconsistent results were problematic during data analysis. The initial results resembled charts like this:

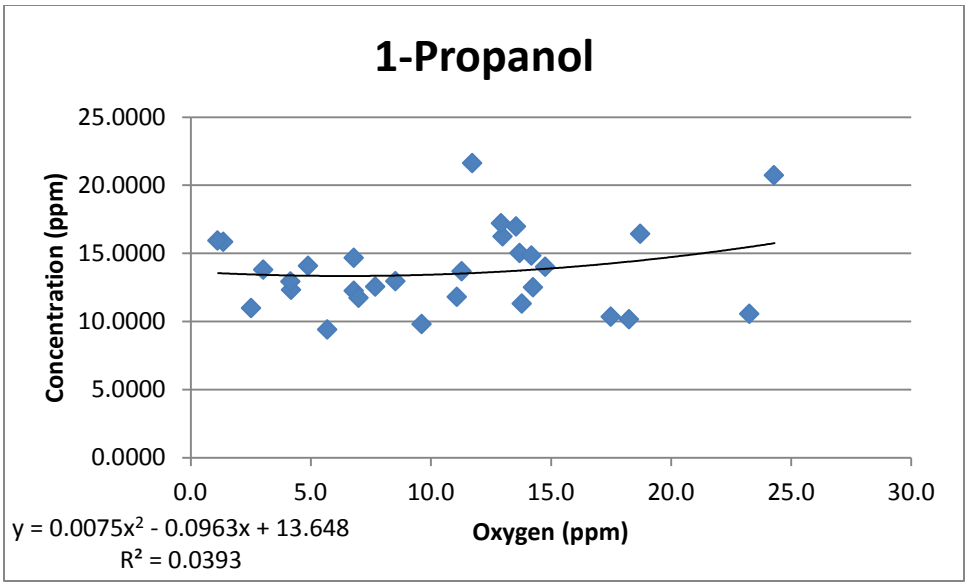

Figure 4: Initial 1-propanol measurements

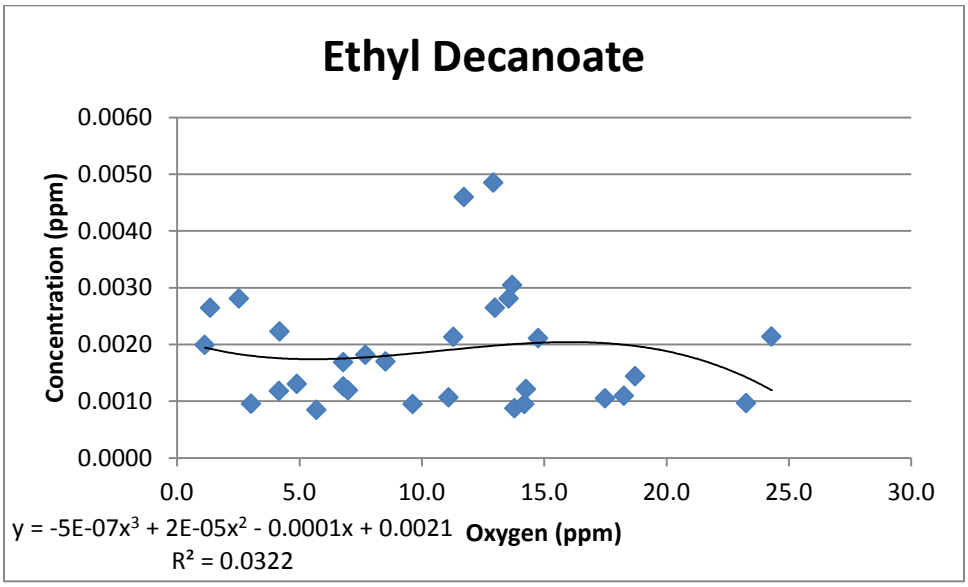

Figure 5: Initial ethyl decanoate concentrations

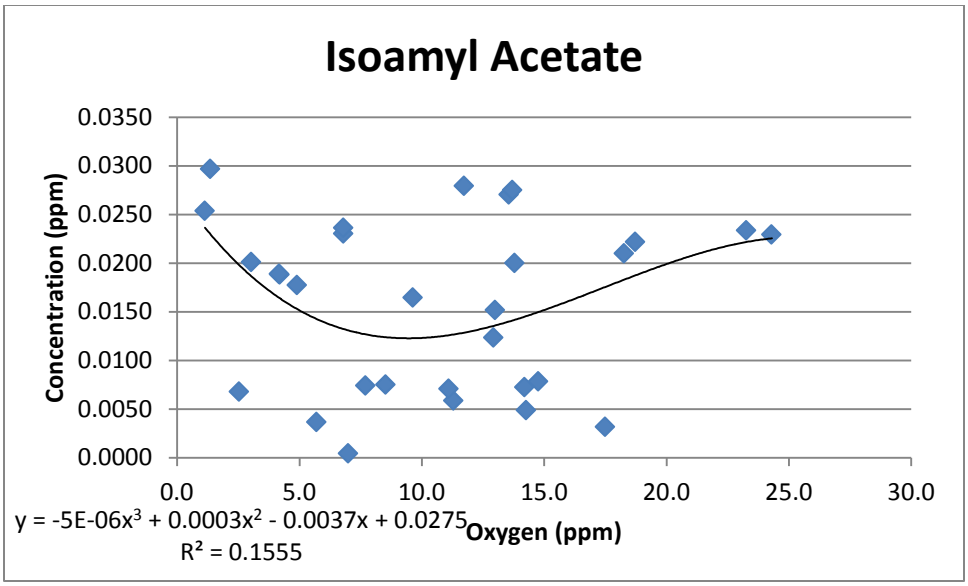

Figure 6: Initial isoamyl acetate concentrations

At this point in analysis outlier removal has not taken place and the points are shown as initially recorded and converted. The data at this point suggests that there is no consistent correlation within the analysis. However, consideration of other factors was important in the data cleanup and outlier removal process.

Acetaldehyde Relationship to Infection and Oxidation

When examining potential outside effects with potential for modifying results and providing incorrect correlations, further research was performed to determine a reasonable approach to assess potential bad batches. The first clue was discovered while examining the Acetaldehyde results:

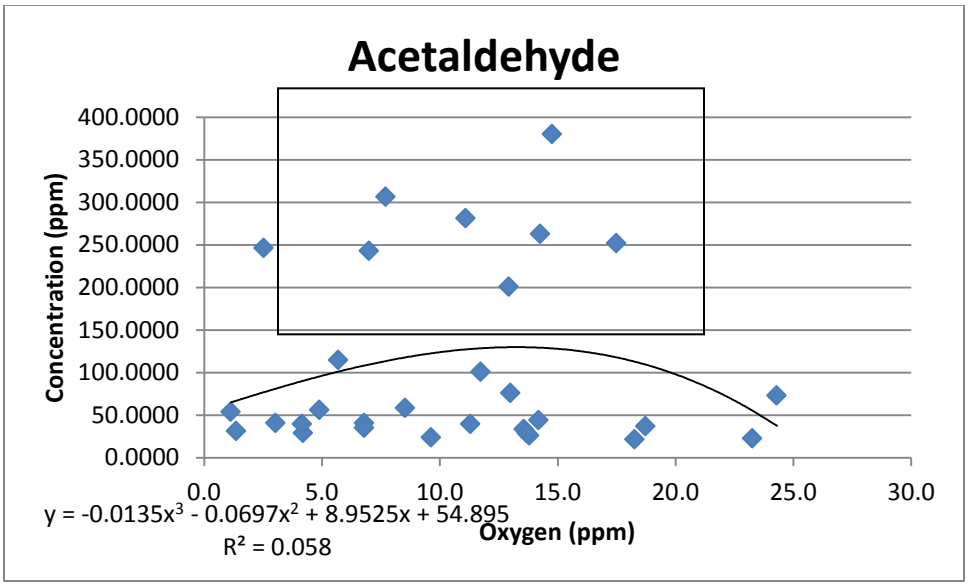

Figure 7: Acetaldehyde and oxygenation comparison

There are two potential separate trend lines in this graph; the secondary correlation is highlighted in the large rectangle. This second trend spurred further analysis into the affect of high acetaldehyde concentrations in beer. Past research has shown that high acetaldehyde concentrations are directly linked to both wild yeast exposure, as well as post fermentation exposure to oxygen. Consequently infected beers, or those stored improperly have potential to develop high levels of acetaldehyde. This information suggests that batches with excessively high levels of acetaldehyde were infected or improperly stored. The container used to store the beer was a simple plastic container with a paper cap seal. Additionally, the potential for human error in correctly applying the lids remains a factor. (Garde-Cerdán, 2006; Otter, 1971; Barker, 1983)

With this information, removal of many data points followed. When removing these points, accepted levels of acetaldehyde, the style of the beer, as well as its threshold levels were all taken into account. With only the acetaldehyde points removed, the data

began to correlate in all samples in a much more expected manner. At this point no other outliers, or inconsistent data points were removed:

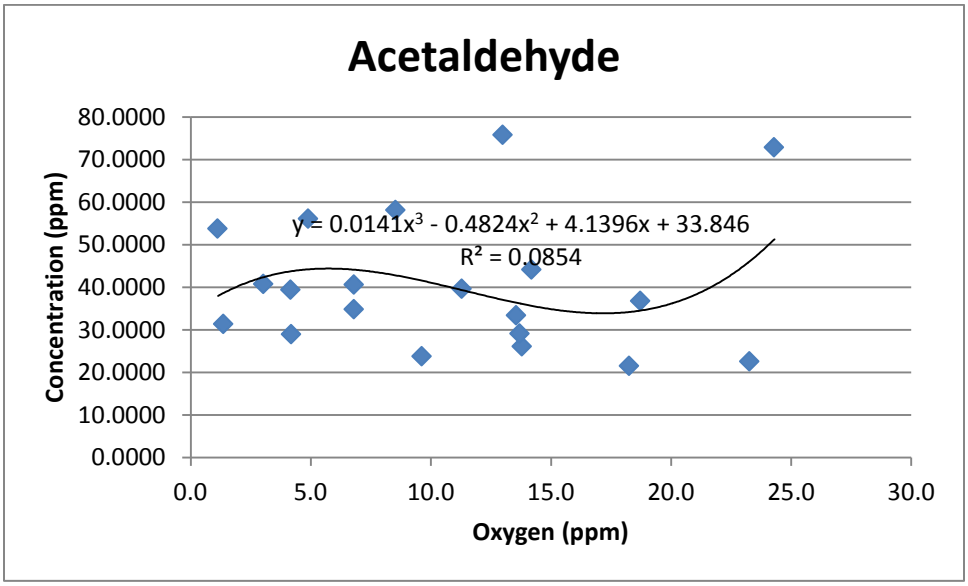

Figure 8: Before and after high acetaldehyde removal.

Alternative Outlier Point Removal

Once all high acetaldehyde points were removed, regression analysis was performed through MiniTab to remove inconsistent outliers. In order to safely remove bad data points, regression was performed multiple times on every analyzed compound while taking into consideration their formation in the yeast's process. Alcohols, in particular, had a much weaker link to wort oxygenation than the esters. In Appendix C, the process and notes on step by step data removal are found.

Once adjusted, the data showed a much stronger correlation than previously seen:

Regression Analysis: 1-Propanol Vs Oxygen (ppm) Before Data Removal

```
The regression equation is
1-Propanol = 14.4 - 0.0870 Oxygen (ppm)


17 cases used, 4 cases contain missing values


Predictor          Coef  SE Coef      T      P
Constant        14.4216   0.9951  14.49  0.000
Oxygen (ppm)  -0.08701  0.08421  -1.03  0.318


S = 2.22270   R-Sq = 6.6%   R-Sq(adj) = 0.4%


Analysis of Variance

Source           DF      SS     MS     F      P
Regression        1   5.274  5.274  1.07  0.318
Residual Error   15  74.106  4.940
Total            16  79.380
```

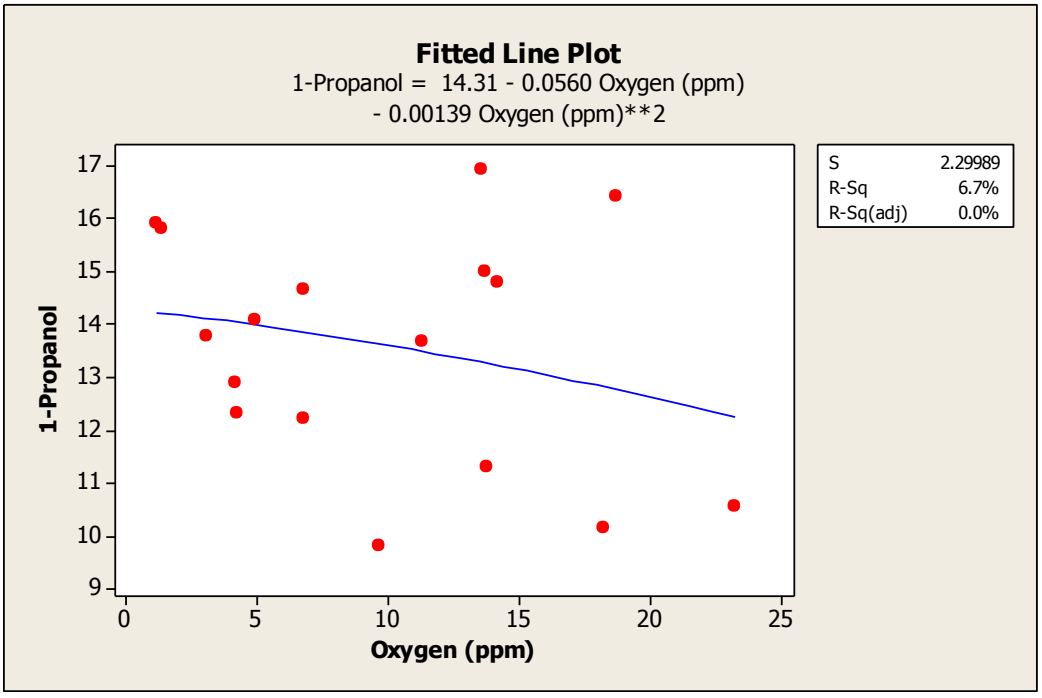

Figure 9: 1-propanol before data removal

Regression Analysis: 1-Propanol Vs Oxygen (ppm) After Data Removal

The regression equation is
1-Propanol = 14.7 - 0.197 Oxygen (ppm)

14 cases used, 16 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	14.7306	0.7299	20.18	0.000
Oxygen (ppm)	-0.19654	0.06720	-2.92	0.013

S = 1.60878 R-Sq = 41.6% R-Sq(adj) = 36.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	22.139	22.139	8.55	0.013
Residual Error	12	31.058	2.588		
Total	13	53.197			

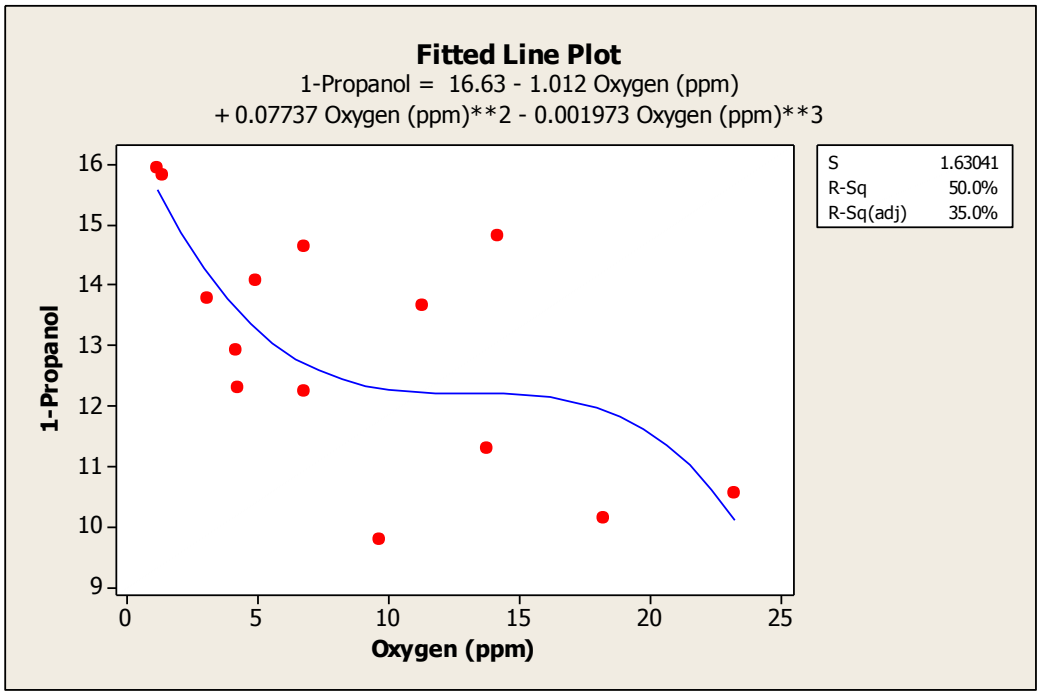

Figure 10: 1-propanol after data removal

Noteworthy Findings of Regression

Regression analysis showed the anticipated correlation between oxygenation and compound concentrations. Chemical compounds correlated to the data at various levels. Previous research suggested this may happen, but the effect was uncertain prior to experimentation. Removal of acetaldehyde as well as researched statistical anomaly removal led to vast improvement of data correlation as well as highlighted the importance of both sanitation as storage containers as important aspects in experimentation.

CHAPTER V: ANALYSIS AND CONCLUSIONS

Restatement of Problem

The beer brewing industry is a growing, ever-evolving market segment that is refining its approach to brewing processes. This segment has facilities ranging from large-scale manufacturing, to small scale professional homebrewers. These facilities all desire one main goal: the improvement of beer flavor. This study approaches the commonly used and commonly misunderstood brewing technique of wort oxygenation. This study lays out a hands-on approach to understanding the effect of wort oxygenation on beer ester and volatile alcohol concentrations to provide useful data on 10 different compounds over a wide variety of wort oxygenations.

Analysis of Findings in Regression Analysis

Using MiniTab statistical analysis software and background research in examining the profile of each ester it was determined that oxygenation did have an effect on the majority of the chemical compounds studied. Compounds with no correlation are discussed with their subsequent results. In this portion of the results analysis, only the fitted line plots are examined. All other information relative to the regression, analysis of variance, and residual plots is found in Appendix C.

Acetaldehyde

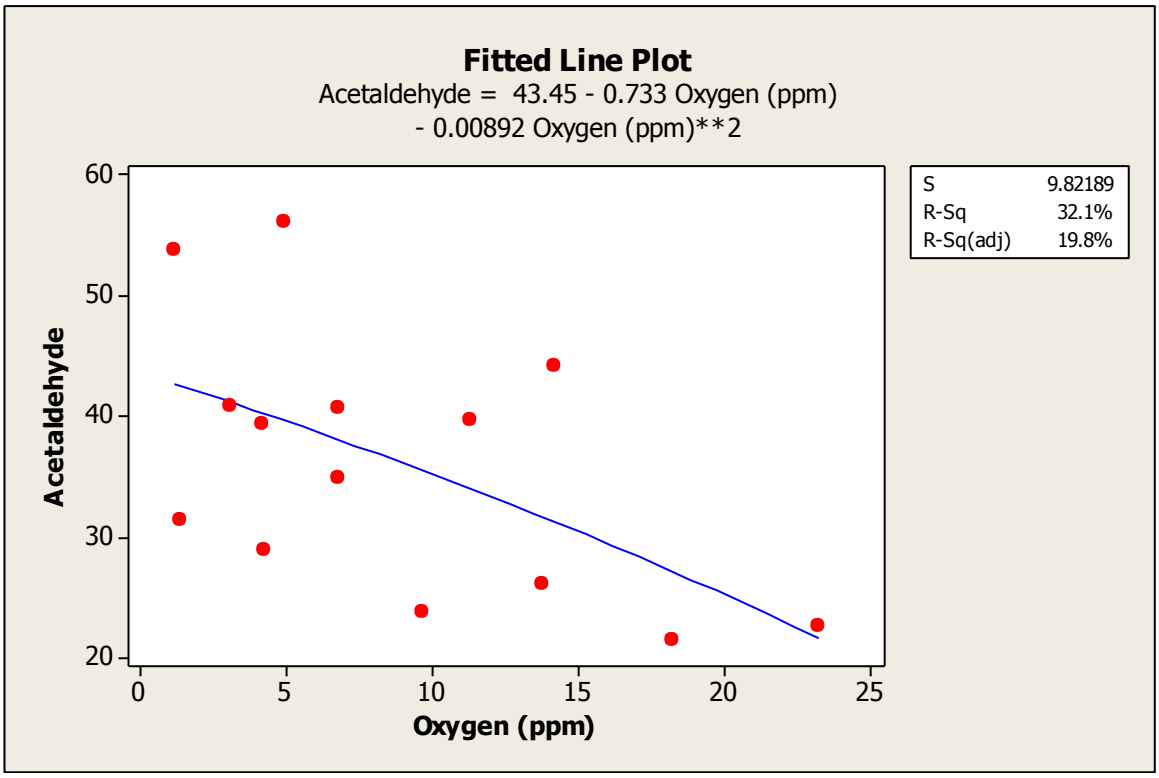

Figure 11: The effect of wort oxygenation on acetaldehyde concentrations

Acetaldehyde shows a general downward trend in respect to wort oxygenation, but considering the previously researched aspects that can also affect acetaldehyde concentrations, it shows an expected lack of correlation. There is still some potential error as latent oxidation and infection can still occur, but the high level batches have been removed at this point, so the potential effect is greatly reduced. This point in analysis is beyond further testing for infected and oxidized scenarios. The p-value recorded was 0.035, suggesting that oxygen was a large factor in acetaldehyde levels.

1-Propanol

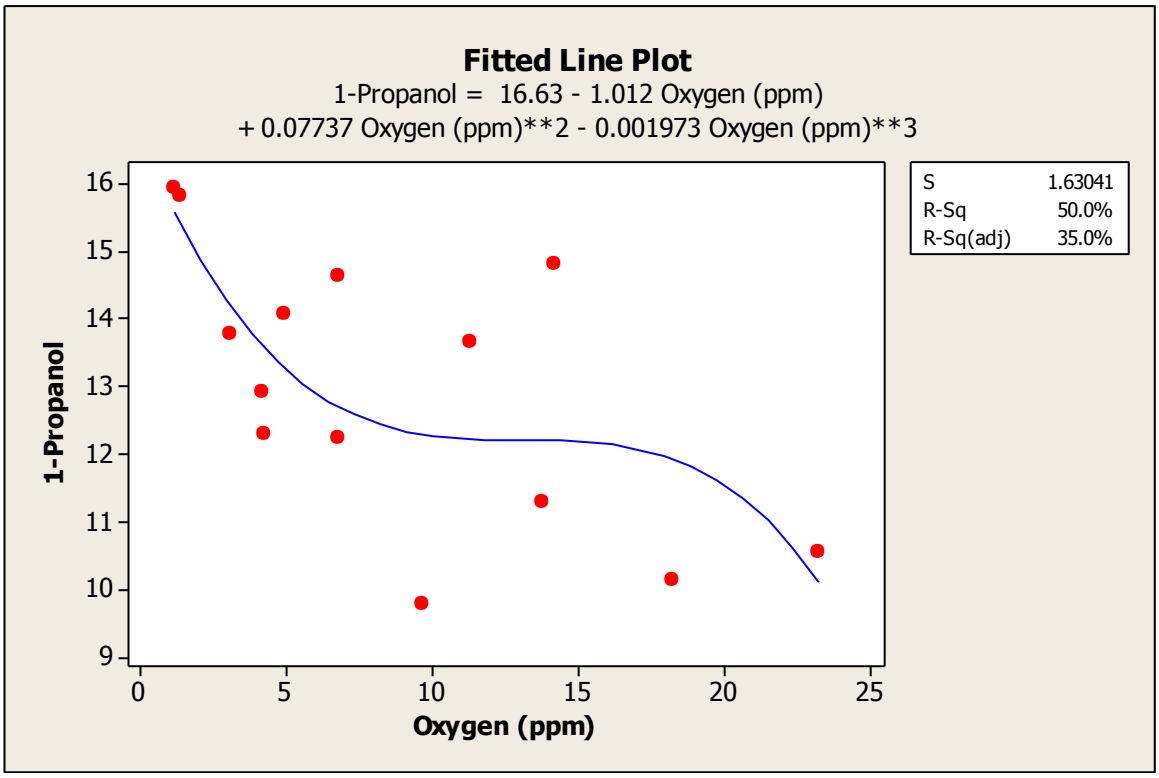

Figure 12: The effect of wort oxygenation on 1-propanol concentration

The fitted line plot for 1-propanol showed a much higher correlation between oxygenation and concentration. The p-value of 0.013 shows that wort oxygenation is a highly likely contributor considering the environment.

Ethyl Acetate

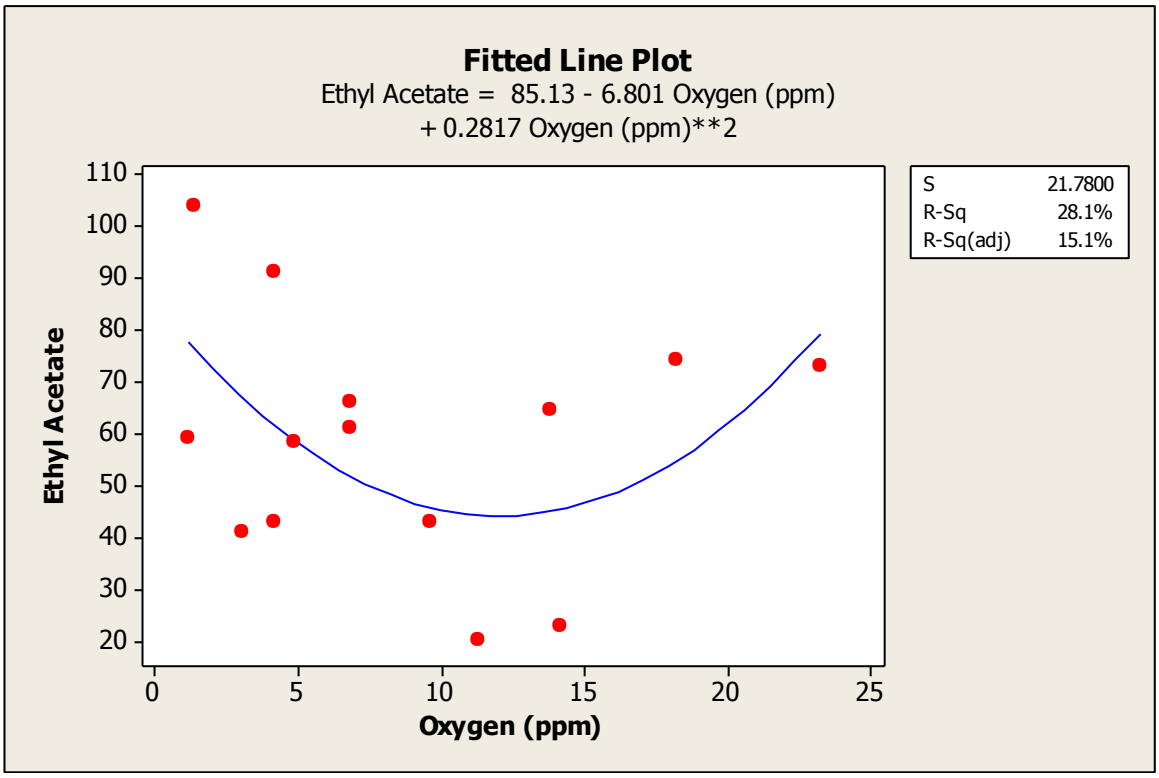

Figure 13: The effect of wort oxygenation on ethyl acetate concentration

In this scenario we find a lower R^2 value which suggests that there is little correlation between ethyl acetate and oxygenation. Ethyl acetate is known to correlate with acetic acid and ethanol in beer, neither of which were tested for in this experiment. A p-value of 0.667 suggests that replication of these results is unlikely.

Isoamyl Alcohol

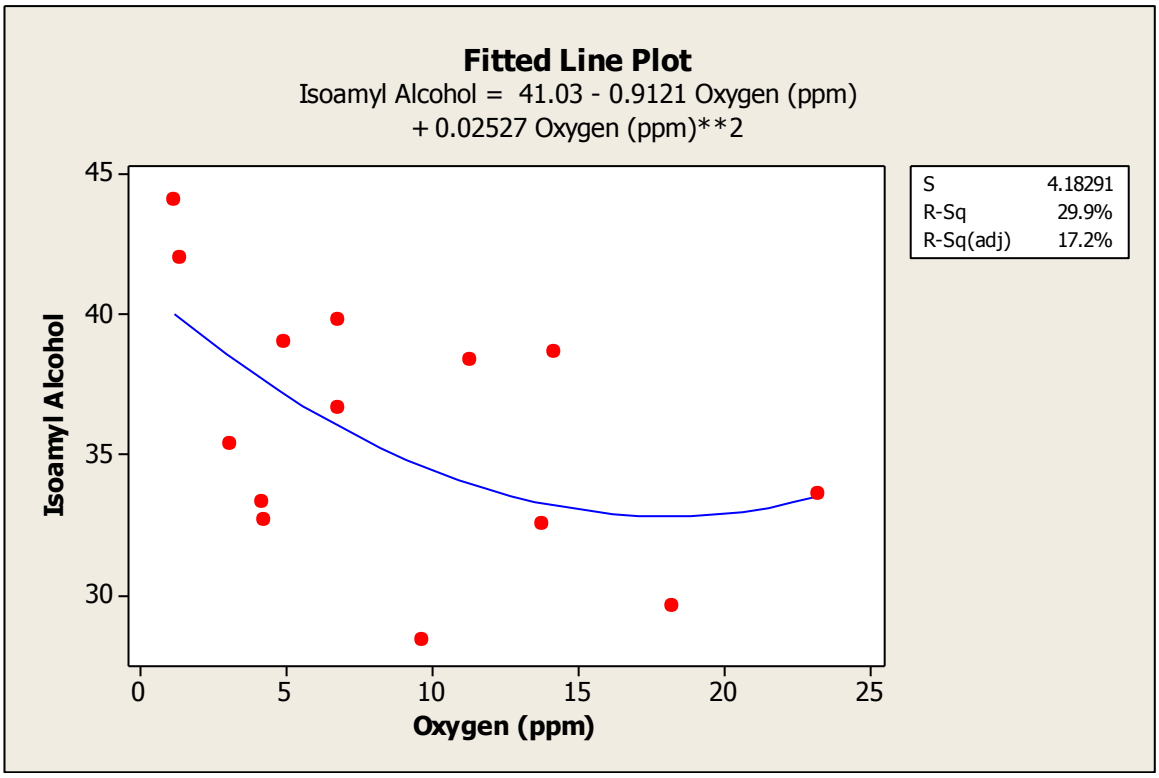

Figure 14: The effect of wort oxygenation on Isoamyl alcohol concentration

The correlation between yeast function and Isoamyl alcohol is much more limited as it is not a yeast byproduct, but is processed within the yeast. The recorded trend line shows some correlation, but the R^2 value suggests that this data does not change enough to show that work oxygenation is a large factor here. The p-value is 0.073, which is not close enough to consider statistically significant.

2-Methyl 1-Butanol

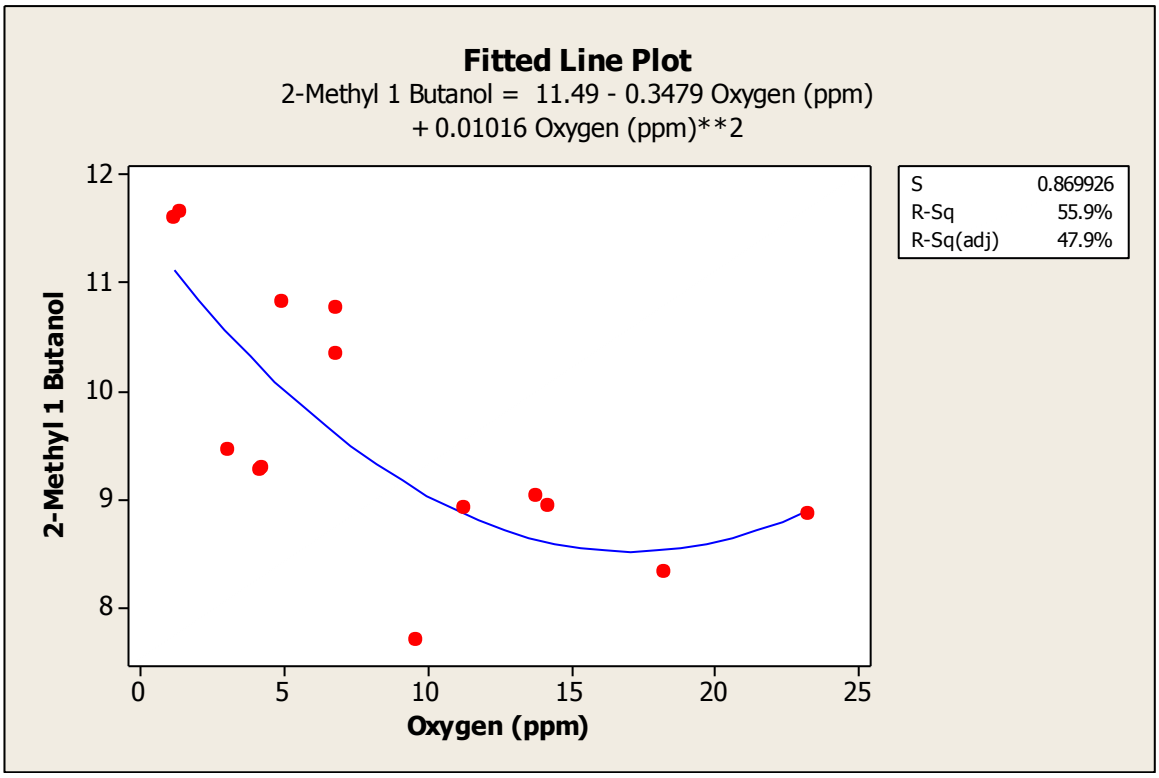

Figure 15: The effect of wort oxygenation on 2-methyl-1-butyl concentration

2-methyl-1-butanol shows a higher correlation between oxygenation and chemical compound levels. A very low p-value of 0.011 shows a high statistical significance.

Isoamyl Acetate

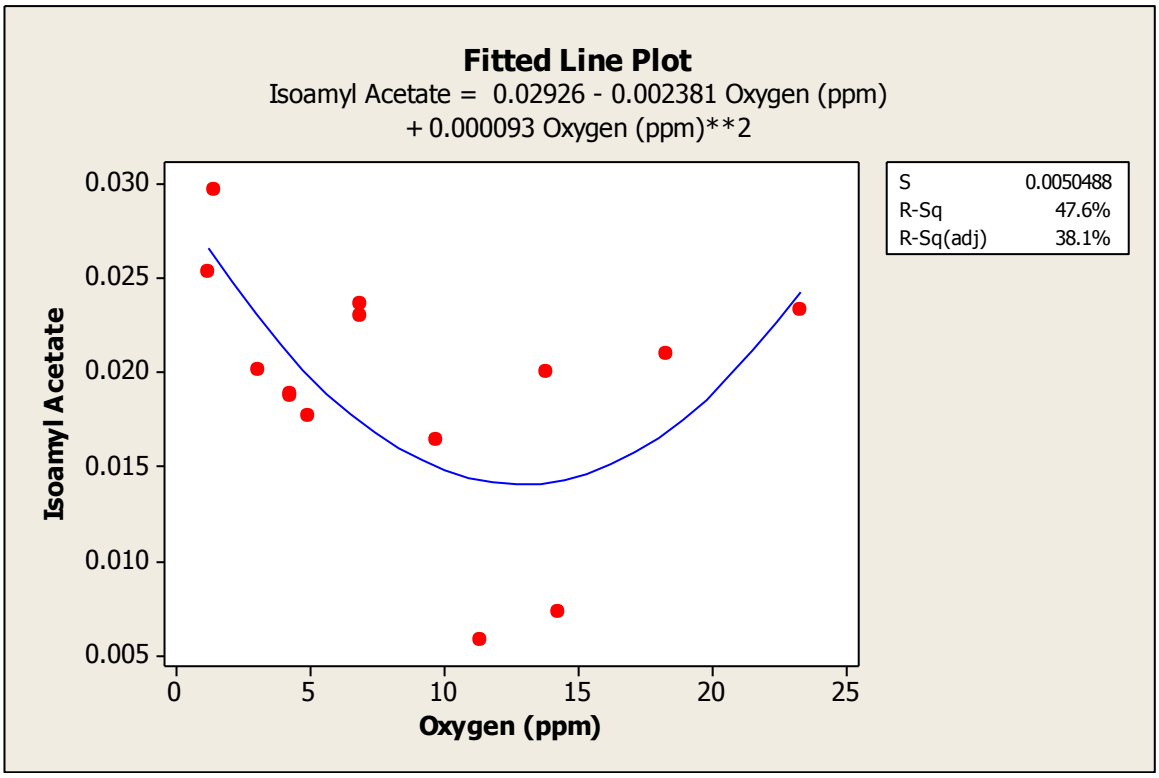

Figure 16: The effect of wort oxygenation on Isoamyl acetate concentration

Isoamyl shows a higher correlation than Isoamyl alcohol, but does not follow in the predicted trend like the other compounds in this study. It decreases in concentration until roughly 13ppm oxygenation levels before steadily increasing as oxygenation. The data shows a higher R^2 value, but has a low p-value of 0.327 rendering this statistically insignificant.

Ethyl Hexanoate

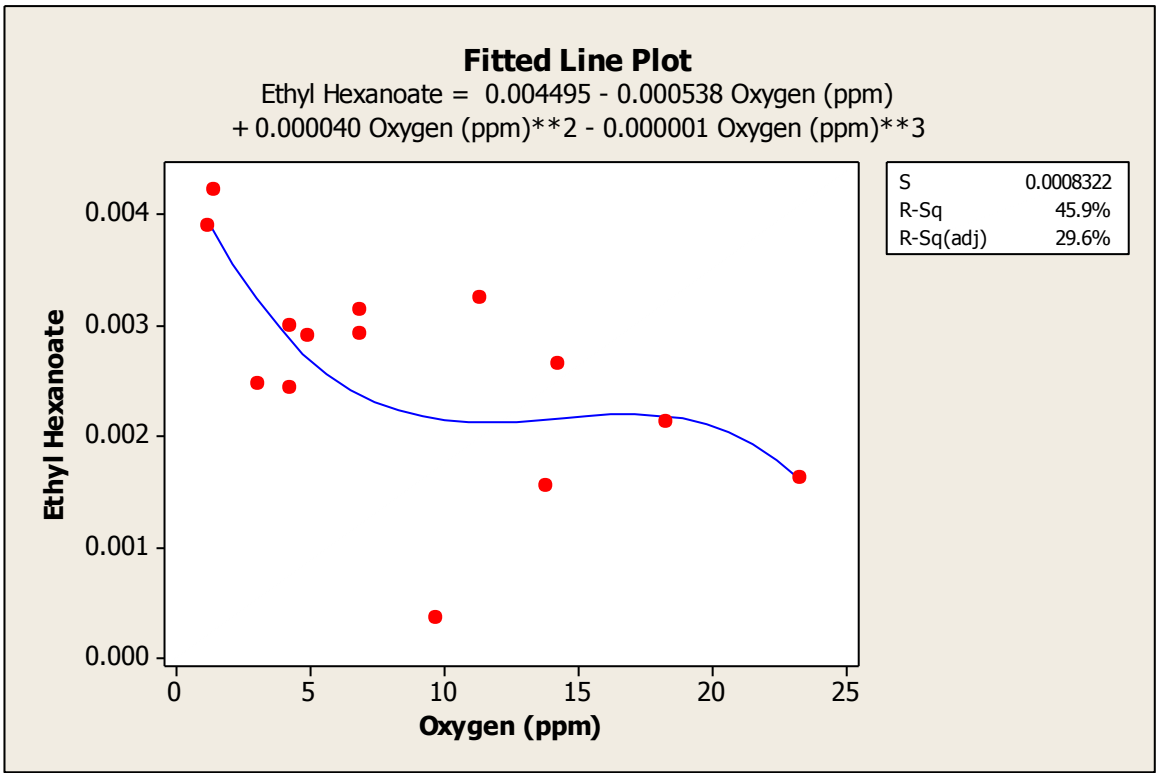

Figure 17: The effect of wort oxygenation on ethyl hexanoate concentration

This graph shows a trend that suggests a decrease in oxygenation reduces ester concentration. A p-value of 0.032 makes this statistically significant. The trend is not linear, but this may account for slight variations found within fermentations, as well as a potential outlier at 9.6 ppm. This point was not removed because it was rarely considered a statistical outlier, and did little to change the data when removed.

Phenyl Ethanol

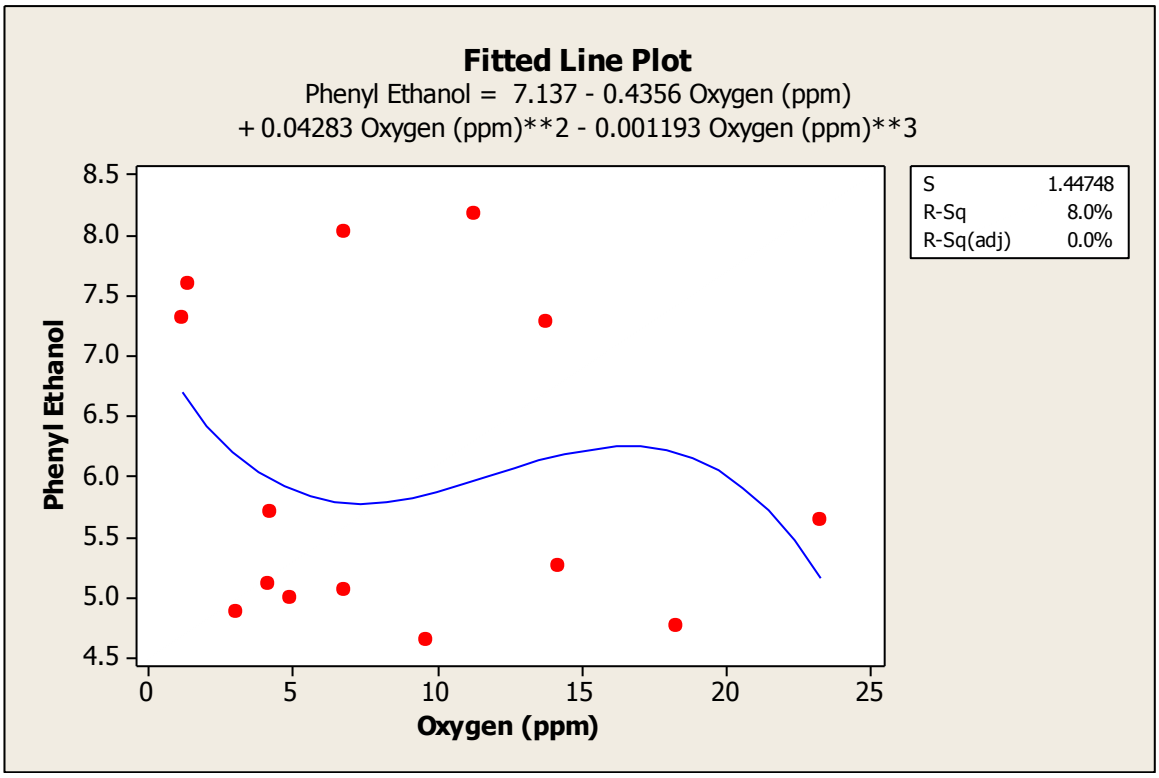

Figure 18: The effect of wort oxygenation on phenyl ethanol concentration

Phenyl ethanol showed very little correlation, if any with wort oxygenation. Research suggests that nitrogen is the largest factor in affecting phenyl ethanol concentrations rather than wort oxygenation. Organic compounds with no oxygenation correlation within the data set help ensure that the data trends found are actually related to wort oxygenation rather than a product of data removal, or coincidence.

Ethyl Octanoate

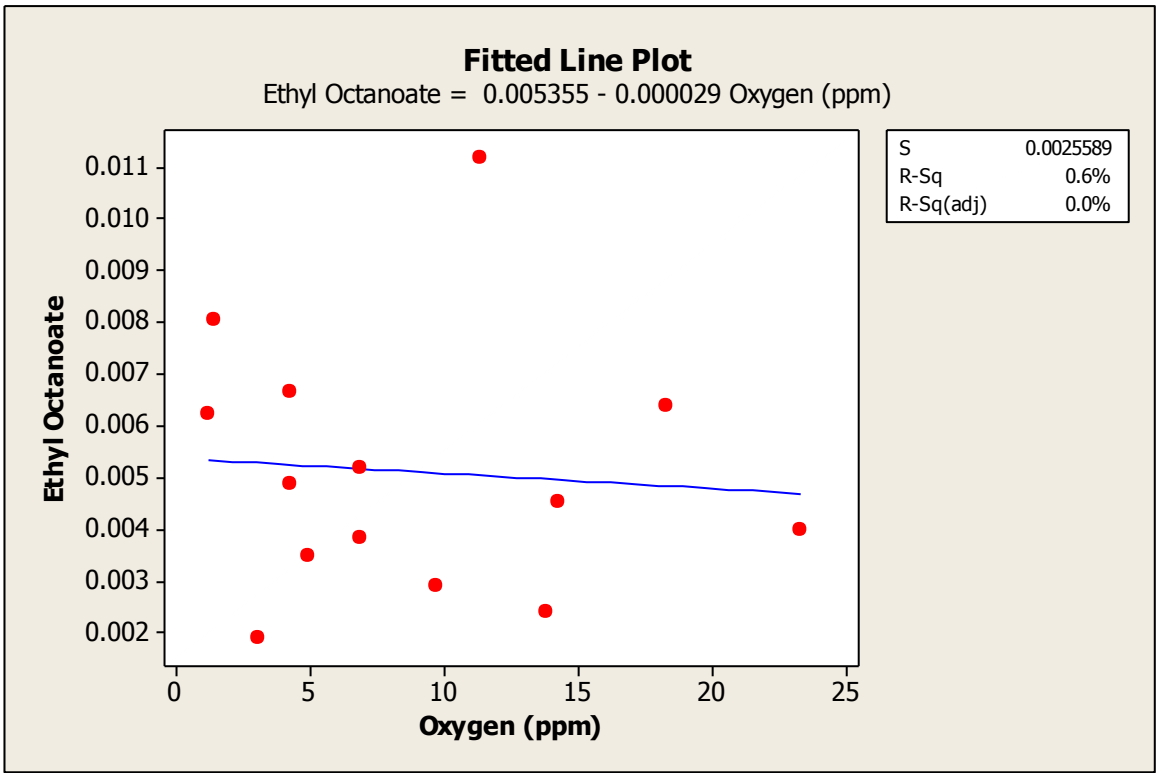

Figure 19: The effect of wort oxygenation on ethyl octanoate levels

Ethyl octanoate also showed little correlation between oxygenation levels and concentrations. This suggests that oxygenation is insignificant in the formation of ethyl octanoate. This ester had the lowest p-value of 0.793 with supplements the low R^2 values. Research suggests that temperature is a larger factor in contributing to the formation of ethyl octanoate.

Ethyl Decanoate

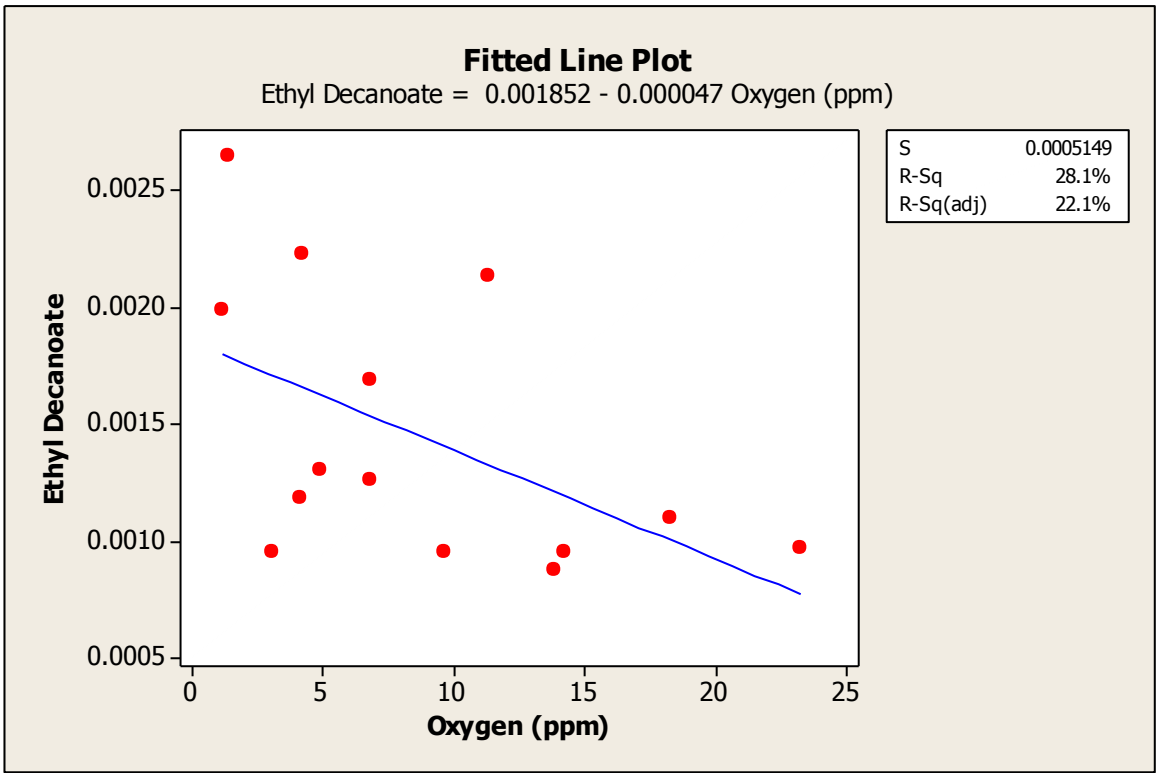

Figure 20: The effect of wort oxygenation on ethyl decanoate concentration

This graph has quite a similar appearance to ethyl octanoate. Research suggests that temperature also heavily contributes to the formation of ethyl decanoate. The correlation is much stronger here than in ethyl octanoate's fitted line graph. This suggests that while they share a similar response in relation to heat, ethyl decanoate levels strongly correlate to oxygenation. Only more data collection could prove or disprove this theory. The p-value is very close at 0.051, suggesting that this isn't statistically significant, but is very close.

Changes and Future Studies

There were many potential improvements noted during experimentation that are useful for streamlining future studies. The most important change is the use of better

sealing fermentation containers. The fermentation container doubles as the storage device in this experimentation for convenience. Modifications to the cap or lid could create a more effective seal. This is necessary to prevent latent oxidation of the samples. Time, being a large factor in this experiment, dictates performing the experiment at the earliest convenience. Cold storage is advised for all timelines of experimentation as it will slow any further yeast development along with alternative bacterial growth. This reduces the potential for ester and alcohol profiles developing that are not representative to the yeast being used. On the experimental side, proper mixing of the oxygen in the wort is necessary. Stirring, shaking and aeration stones all help in this process. Shaking and stirring can modify the current oxygenation level, so performing the sample measurement after every effort to mix oxygen is important. Reducing beer oxygen exposure outside of wort enrichment is also important to consider. Another improvement is the use of gas chromatographic column that is more oriented towards this type of research. The data collected was clear, but there are columns that would enhance the data collection accuracy and timeliness. As with most experiments, there is room for increased data measurement. Alcohol content, fermentation final gravity, and fermentation temperature could provide more detailed analysis of the results.

This research outlines several avenues for future study and continued data collection. The most logical step is to refine the previous experiment by improving the data collection process through revised oxygenation techniques and measurement, timely analysis, sample sonication, and improved GC-MS parameters. Another avenue for future research is to continue the analysis of beer ester flavor concentrations. Wort oxygenation is not the only form of flavor compound control: nitrogen addition, fermentation

temperature, mid-fermentation oxygenation, yeast type and pitching rate, as well as yeast quantity. These are easy transitions for future research as much of the preparation and background information has already been sourced.

Conclusions

Previous studies on individual esters and alcohols on their behavior support the data collected in this study during fermentation, as well as research into the effect of wort oxygenation on beer ester concentrations. The data shows a direct correlation that reduces ester, and for the majority of the results, alcohol concentrations in the beer. The correlation was different in each compound, but generally showed a similar trend. Once a distinction was made between alcohols and esters, a general correlation between the two categories was even stronger. Looking at data points from 0-10 ppm was especially useful as it shows that having low concentrations of oxygen in the wort is the most important factor in brewing beer when controlling esters is desired. After 10 ppm the results became less predictable and the changes were less drastic in nature. Based on the results from this study, prediction of ester and alcohol concentration is possible with various degrees of accuracy. Oxygenation did not affect every compound; this was accurately predicted by prior research and helped confirm that the results accurately represented the yeast formation process.

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APPENDIX A

Integration of gas chromatographic data points and normalization adjustments

Run	Acetal dehyde	ADJ	1-Propanol	ADJ	Ethyl Acetate	ADJ	Isoamyl Alcohol	ADJ
1	2.36E+07	2.36E+07	5.80E+06	5.80E+06	4.88E+08	4.88E+08	2.10E+08	2.10E+08
2	2.96E+07	3.68E+07	5.69E+06	7.07E+06	2.49E+08	3.10E+08	1.84E+08	2.29E+08
3	1.86E+07	3.01E+07	5.35E+06	8.69E+06	4.03E+08	6.55E+08	1.76E+08	2.85E+08
4	2.19E+08	3.43E+08	4.57E+06	7.17E+06	1.09E+08	1.70E+08	1.60E+08	2.51E+08
5	2.39E+07	3.67E+07	4.89E+06	7.51E+06	3.26E+08	5.00E+08	1.68E+08	2.58E+08
6	1.94E+07	2.84E+07	5.56E+06	8.11E+06	5.41E+08	7.90E+08	1.87E+08	2.72E+08
7	2.27E+07	3.58E+07	4.44E+06	7.00E+06	9.66E+07	1.53E+08	1.57E+08	2.49E+08
8	5.75E+07	6.58E+07	9.27E+06	1.06E+07	6.31E+08	7.22E+08	2.59E+08	2.97E+08
9	1.90E+08	2.23E+08	4.79E+06	5.62E+06	1.29E+08	1.51E+08	1.72E+08	2.02E+08
10	3.50E+07	5.07E+07	4.98E+06	7.22E+06	3.05E+08	4.42E+08	1.74E+08	2.53E+08
11	3.06E+07	4.86E+07	5.15E+06	8.16E+06	2.83E+08	4.49E+08	1.80E+08	2.85E+08
12	2.13E+07	3.32E+07	5.39E+06	8.42E+06	4.47E+08	6.98E+08	1.77E+08	2.76E+08
13	1.62E+07	2.04E+07	4.28E+06	5.40E+06	4.40E+08	5.55E+08	1.72E+08	2.17E+08
14	1.14E+08	1.04E+08	5.29E+06	4.82E+06	1.05E+08	9.60E+07	1.72E+08	1.57E+08
15	2.56E+07	3.14E+07	5.11E+06	6.27E+06	3.78E+08	4.63E+08	1.94E+08	2.37E+08
16	2.15E+08	2.77E+08	4.99E+06	6.43E+06	1.12E+08	1.44E+08	1.62E+08	2.08E+08
17	1.70E+07	2.63E+07	4.96E+06	7.69E+06	7.16E+08	1.11E+09	1.75E+08	2.71E+08
18	4.85E+07	9.12E+07	5.89E+06	1.11E+07	2.96E+07	5.56E+07	1.83E+08	3.44E+08
19	1.57E+08	2.19E+08	4.30E+06	6.01E+06	9.71E+07	1.36E+08	1.67E+08	2.33E+08
20	5.13E+07	6.85E+07	6.23E+06	8.32E+06	1.21E+08	1.61E+08	1.78E+08	2.38E+08
21	2.02E+07	2.15E+07	4.72E+06	5.02E+06	3.06E+08	3.26E+08	1.73E+08	1.84E+08
22	2.15E+07	2.61E+07	5.19E+06	6.31E+06	5.70E+08	6.93E+08	1.74E+08	2.11E+08
23	2.05E+08	2.28E+08	4.78E+06	5.30E+06	1.08E+08	1.20E+08	1.68E+08	1.86E+08
24	1.91E+08	2.54E+08	4.53E+06	6.05E+06	1.05E+08	1.40E+08	1.63E+08	2.17E+08
25	3.02E+07	3.56E+07	5.61E+06	6.62E+06	2.75E+08	3.25E+08	1.83E+08	2.16E+08
26	1.79E+08	2.37E+08	4.82E+06	6.40E+06	1.34E+08	1.79E+08	1.59E+08	2.12E+08
27	1.70E+07	1.95E+07	4.55E+06	5.20E+06	4.94E+08	5.64E+08	1.68E+08	1.92E+08
28	2.27E+07	3.99E+07	4.31E+06	7.59E+06	9.85E+07	1.73E+08	1.42E+08	2.50E+08
29	1.03E+08	1.81E+08	4.98E+06	8.82E+06	7.03E+08	1.24E+09	1.67E+08	2.95E+08
30	4.53E+07	5.25E+07	5.72E+06	6.63E+06	2.82E+07	3.27E+07	1.59E+08	1.85E+08

Batch	ɒutanol	ADJ	Isoamyl Acetate	ADJ	Ethyl Hexanoate	ADJ	Phenyl Ethanol	ADJ
1	6.75E+07	6.75E+07	2.52E+06	2.52E+06	6.23E+05	6.23E+05	9.58E+06	9.58E+06
2	5.69E+07	7.07E+07	2.04E+06	2.53E+06	8.01E+05	9.95E+05	5.17E+06	6.43E+06
3	4.82E+07	7.82E+07	2.10E+06	3.41E+06	1.00E+06	1.63E+06	7.03E+06	1.14E+07
4	4.24E+07	6.66E+07	6.29E+05	9.88E+05	1.10E+06	1.73E+06	6.61E+06	1.04E+07
5	5.25E+07	8.06E+07	1.89E+06	2.90E+06	8.22E+05	1.26E+06	6.89E+06	1.06E+07
6	5.98E+07	8.72E+07	2.56E+06	3.74E+06	1.16E+06	1.70E+06	6.85E+06	1.00E+07
7	4.23E+07	6.68E+07	4.66E+05	7.36E+05	8.27E+05	1.31E+06	6.82E+06	1.08E+07
8	7.59E+07	8.69E+07	2.52E+06	2.89E+06	9.22E+05	1.06E+06	1.03E+07	1.17E+07
9	4.65E+07	5.46E+07	7.28E+05	8.55E+05	1.38E+06	1.62E+06	6.75E+06	7.92E+06
10	5.58E+07	8.09E+07	1.54E+06	2.23E+06	8.06E+05	1.17E+06	4.54E+06	6.58E+06
11	5.47E+07	8.67E+07	2.01E+06	3.19E+06	9.88E+05	1.57E+06	6.07E+06	9.63E+06
12	5.60E+07	8.74E+07	1.79E+06	2.79E+06	6.59E+05	1.03E+06	7.81E+06	1.22E+07
13	5.26E+07	6.63E+07	2.33E+06	2.94E+06	5.16E+05	6.51E+05	5.89E+06	7.43E+06
14	4.75E+07	4.33E+07	5.07E+05	4.62E+05	7.88E+05	7.17E+05	5.65E+06	5.15E+06
15	6.30E+07	7.73E+07	2.43E+06	2.98E+06	9.56E+05	1.17E+06	5.43E+06	6.66E+06
16	4.57E+07	5.88E+07	7.23E+05	9.31E+05	8.16E+05	1.05E+06	5.68E+06	7.32E+06
17	5.51E+07	8.53E+07	2.24E+06	3.46E+06	7.36E+05	1.14E+06	6.27E+06	9.72E+06
18	5.75E+07	1.08E+08	1.87E+06	3.52E+06	8.72E+05	1.64E+06	7.79E+06	1.47E+07
19	4.84E+07	6.77E+07	3.80E+04	5.31E+04	7.18E+05	1.00E+06	5.13E+06	7.18E+06
20	5.01E+07	6.69E+07	1.43E+06	1.91E+06	1.44E+06	1.92E+06	6.29E+06	8.40E+06
21	5.41E+07	5.76E+07	1.95E+06	2.07E+06	1.34E+05	1.42E+05	5.74E+06	6.12E+06
22	5.73E+07	6.95E+07	1.95E+06	2.37E+06	8.04E+05	9.76E+05	6.19E+06	7.52E+06
23	4.84E+07	5.37E+07	3.57E+05	3.96E+05	9.43E+05	1.05E+06	4.91E+06	5.45E+06
24	4.51E+07	6.01E+07	6.67E+05	8.90E+05	7.05E+05	9.41E+05	6.07E+06	8.10E+06
25	5.88E+07	6.93E+07	2.01E+06	2.38E+06	1.02E+06	1.20E+06	5.70E+06	6.73E+06

26	4.32E+07	5.74E+07	4.62E+05	6.13E+05	8.82E+05	1.17E+06	4.82E+06	6.40E+06
27	5.45E+07	6.22E+07	2.32E+06	2.64E+06	7.49E+05	8.55E+05	5.50E+06	6.28E+06
28	3.80E+07	6.68E+07	5.18E+05	9.11E+05	6.04E+05	1.06E+06	3.94E+06	6.94E+06
29	4.48E+	7.94E+07	8.79E+05	1.55E+06	7.35E+05	1.30E+06	7.58E+06	1.34E+07
30	4.60E+07	5.33E+07	8.15E+05	9.45E+05	2.19E+06	2.54E+06	5.67E+06	6.58E+06

Batch	Ethyl Octanoate	ADJ	Ethyl Decanoate	ADJ
1	7.52E+05	7.52E+05	5.59E+05	5.59E+05
2	4.84E+05	6.02E+05	4.91E+05	6.10E+05
3	1.50E+06	2.43E+06	1.11E+06	1.80E+06
4	2.24E+06	3.51E+06	8.59E+05	1.35E+06
5	7.91E+05	1.21E+06	5.23E+05	8.03E+05
6	1.74E+06	2.54E+06	1.16E+06	1.69E+06
7	2.24E+06	3.53E+06	8.64E+05	1.36E+06
8	1.19E+06	1.36E+06	1.19E+06	1.37E+06
9	3.49E+06	4.09E+06	1.53E+06	1.80E+06
10	7.55E+05	1.09E+06	5.74E+05	8.32E+05
11	1.24E+06	1.97E+06	8.02E+05	1.27E+06
12	9.59E+05	1.50E+06	5.88E+05	9.18E+05
13	1.00E+06	1.27E+06	4.88E+05	6.16E+05
14	7.98E+05	7.27E+05	5.94E+05	5.41E+05
15	1.34E+06	1.64E+06	8.79E+05	1.08E+06
16	2.18E+06	2.81E+06	9.01E+05	1.16E+06
17	2.18E+06	3.38E+06	1.26E+06	1.95E+06
18	1.50E+06	2.83E+06	1.56E+06	2.94E+06
19	6.10E+05	8.53E+05	5.44E+05	7.61E+05
20	1.77E+06	2.36E+06	1.27E+06	1.69E+06

21	8.58E+05	9.13E+05	5.70E+05	6.07E+05
22	1.73E+06	2.10E+06	1.17E+06	1.43E+06
23	1.93E+06	2.14E+06	6.06E+05	6.72E+05
24	1.32E+06	1.76E+06	5.12E+05	6.83E+05
25	1.31E+06	1.54E+06	6.38E+05	7.53E+05
26	1.58E+06	2.11E+06	5.85E+05	7.77E+05
27	1.77E+06	2.02E+06	6.14E+05	7.01E+05
28	8.14E+05	1.43E+06	3.45E+05	6.07E+05
29	1.82E+06	3.21E+06	1.75E+06	3.10E+06
30	3.67E+06	4.25E+06	9.36E+05	1.09E+06

Measured oxygenation levels per batch in PPM

Batch	Oxygenation AVG	Oxygenation Measure 1	Oxygenation Measure 2	Oxygenation Measure 3
1	13.80	13.6	13.8	14.0
2	3.03	3.0	3.1	3.0
3	13.57	13.8	13.7	13.2
4	14.77	14.8	14.7	14.8
5	6.80	6.7	6.9	6.8
6	1.37	1.4	1.3	1.4
7	11.30	11.3	11.3	11.3
8	24.30	24.4	24.3	24.2
9	2.53	2.6	2.5	2.5
10	4.90	4.9	4.9	4.9
11	1.13	1.2	1.1	1.1
12	18.73	18.8	18.7	18.7
13	23.27	23.3	23.2	23.3
14	5.70	5.7	5.7	5.7
15	6.80	6.8	6.8	6.8
16	7.70	7.7	7.7	7.7

17	13.70	13.3	13.9	13.9
18	11.73	12.0	11.7	11.5
19	7.00	6.8	7.0	7.2
20	13.00	13.0	13.0	13.0
21	9.63	9.6	9.6	9.7
22	4.20	4.0	4.1	4.5
23	17.50	17.4	17.5	17.6
24	11.10	11.0	11.1	11.2
25	4.17	4.3	4.1	4.1
26	14.27	15.1	13.7	14.0
27	18.27	17.9	18.3	18.6
28	14.20	14.0	14.2	14.4
29	12.93	13.0	12.9	12.9
30	8.53	8.4	8.4	8.8

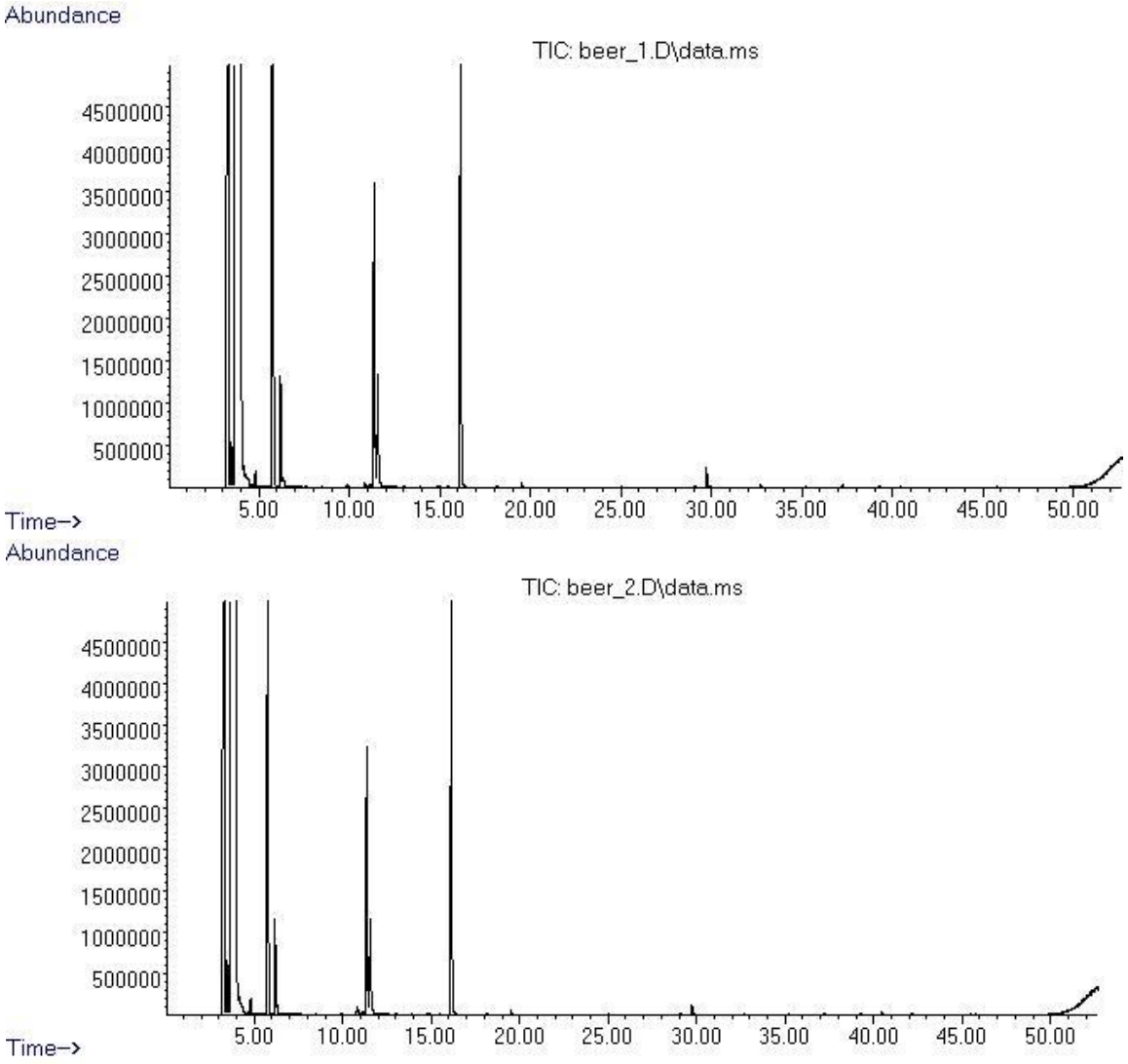
Measured weight of beer samples in grams

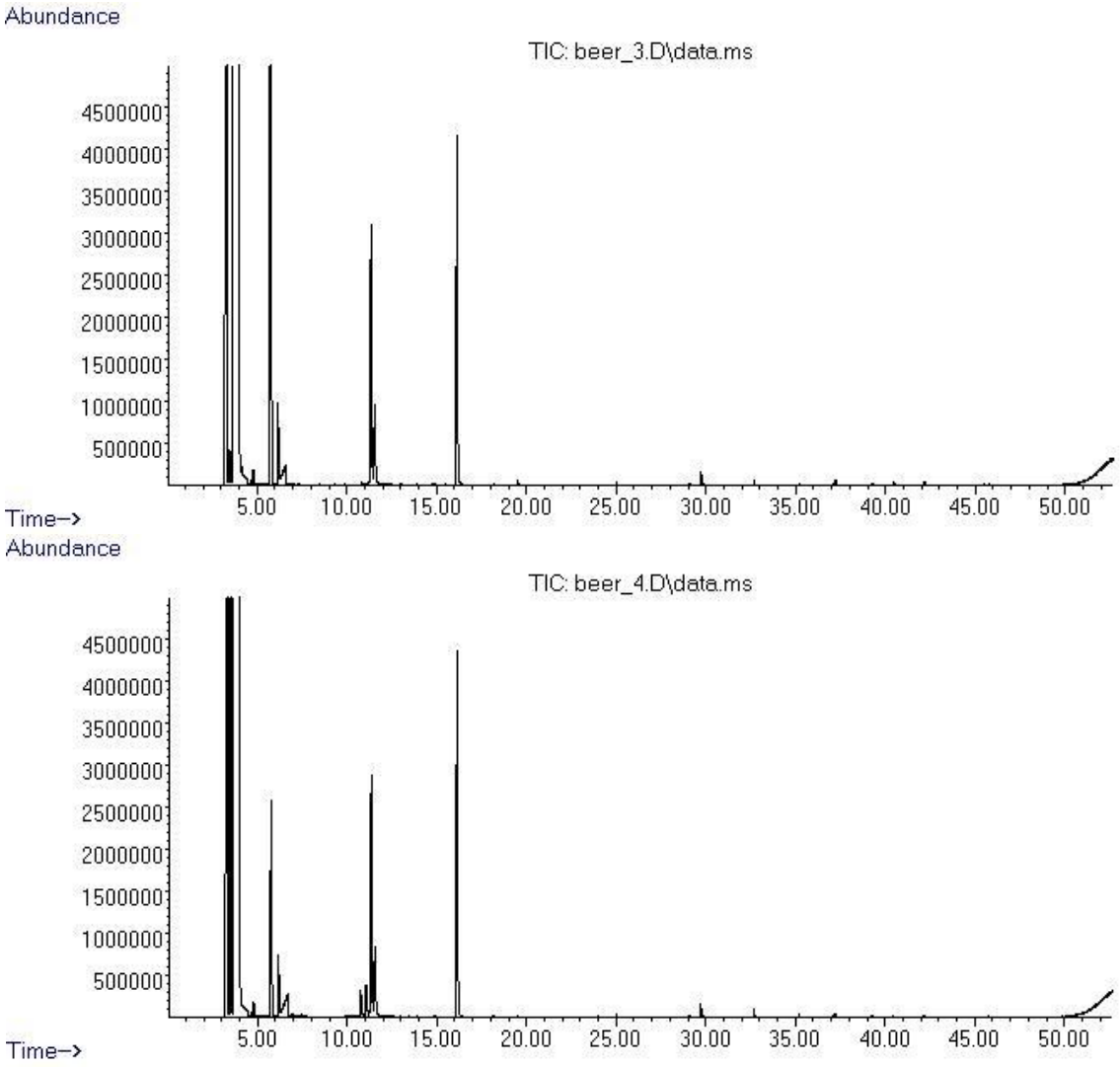
Batch	AVG Weight per mL	Weight 1	Weight 2
1	0.992	0.993	0.991
2	0.987	0.985	0.988
3	0.986	0.986	0.986
4	0.981	0.982	0.980
5	0.979	0.980	0.978
6	0.988	0.988	0.987
7	0.986	0.983	0.989
8	0.985	0.985	0.984
9	0.983	0.984	0.981
10	0.980	0.980	0.979
11	0.982	0.983	0.980
12	0.985	0.983	0.987
13	0.981	0.982	0.979
14	0.992	0.995	0.988
15	0.980	0.978	0.981

16	0.986	0.988	0.984
17	0.979	0.983	0.975
18	0.985	0.980	0.989
19	0.979	0.975	0.982
20	0.982	0.981	0.982
21	0.981	0.982	0.980
22	0.979	0.976	0.981
23	0.975	0.974	0.975
24	0.987	0.997	0.977
25	0.979	0.980	0.977
26	0.980	0.982	0.977
27	0.977	0.977	0.976
28	0.983	0.981	0.984
29	0.982	0.981	0.983
30	0.984	0.986	0.981

APPENDIX B

Gas chromatographic graphic results

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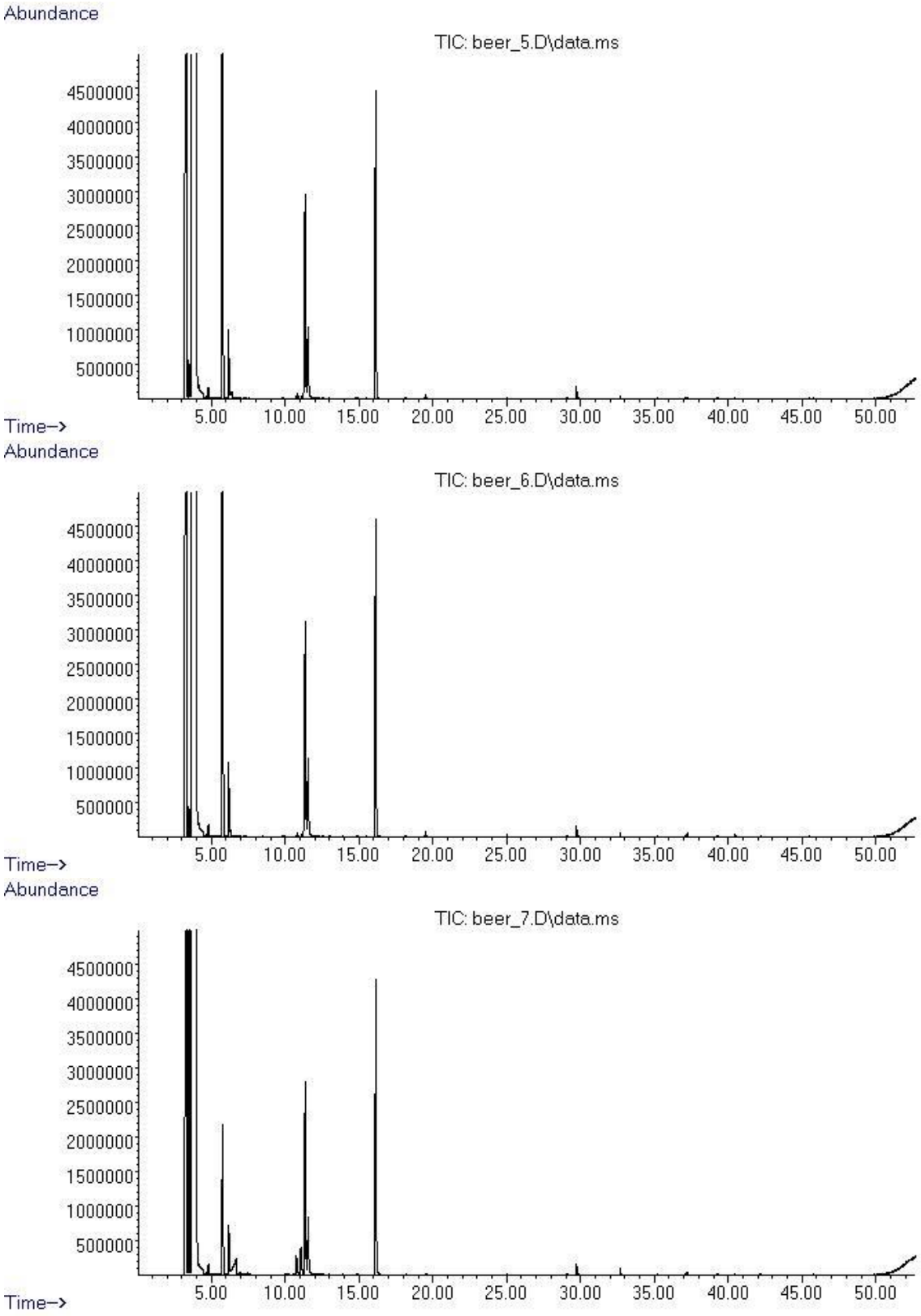
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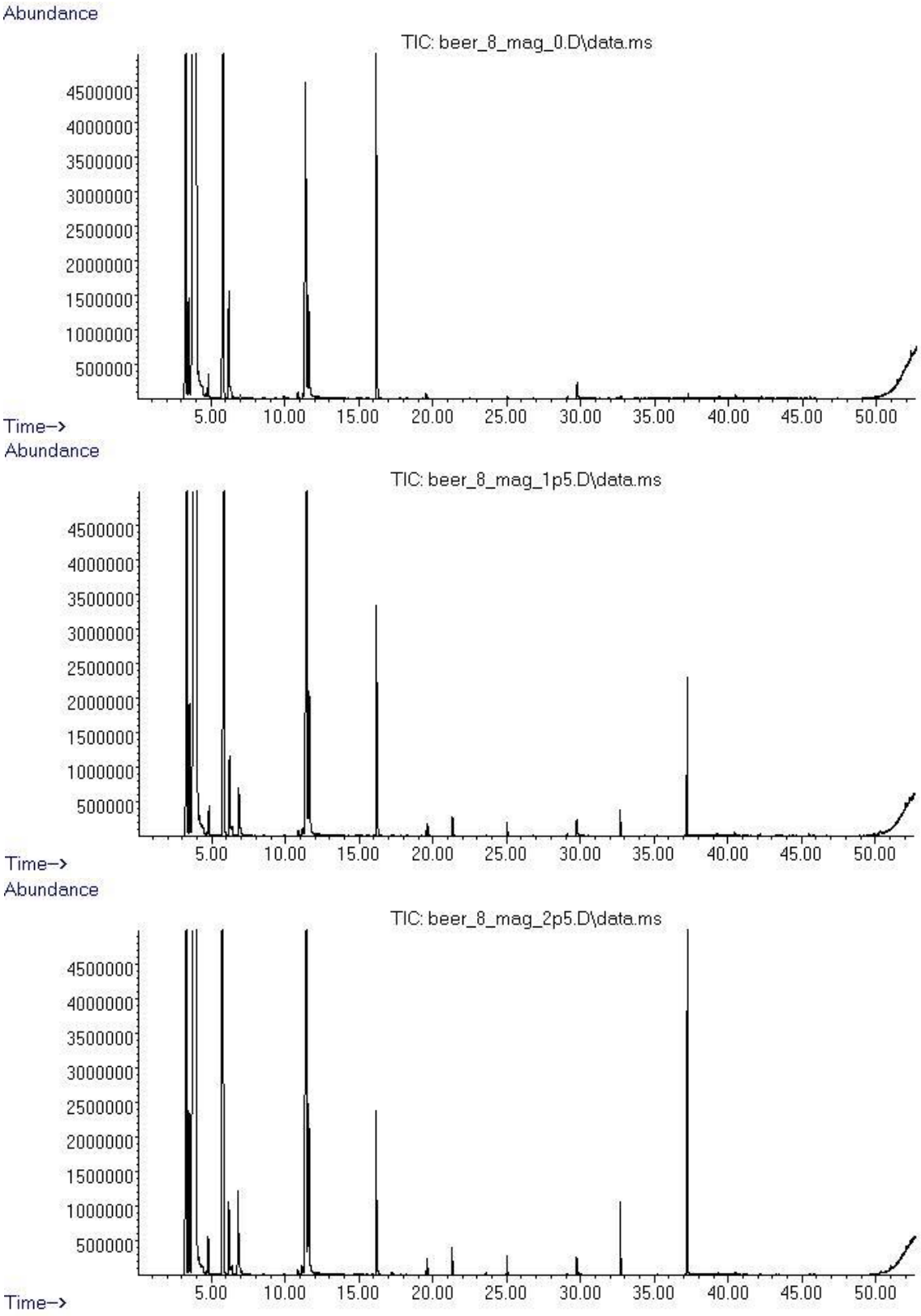
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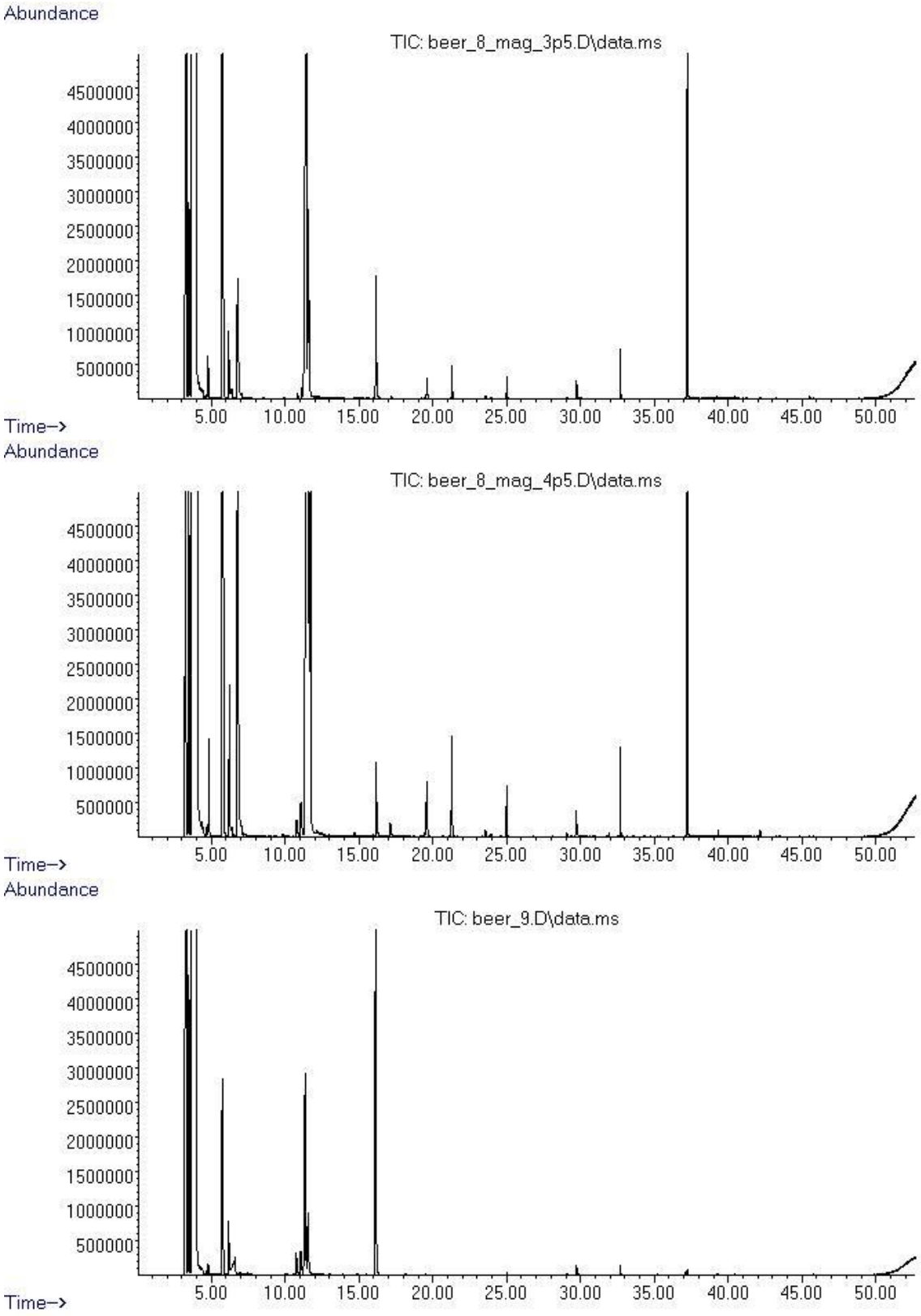
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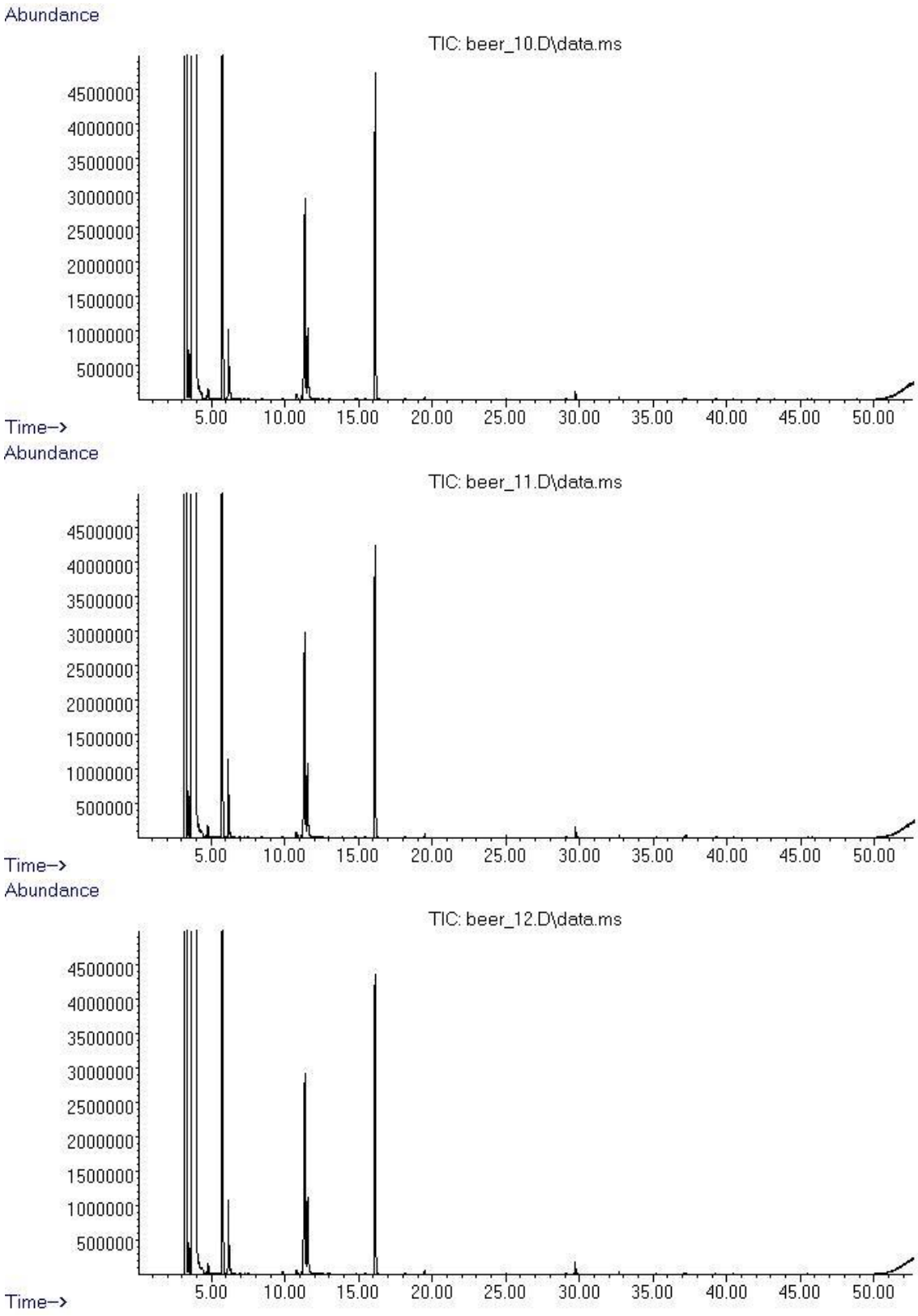
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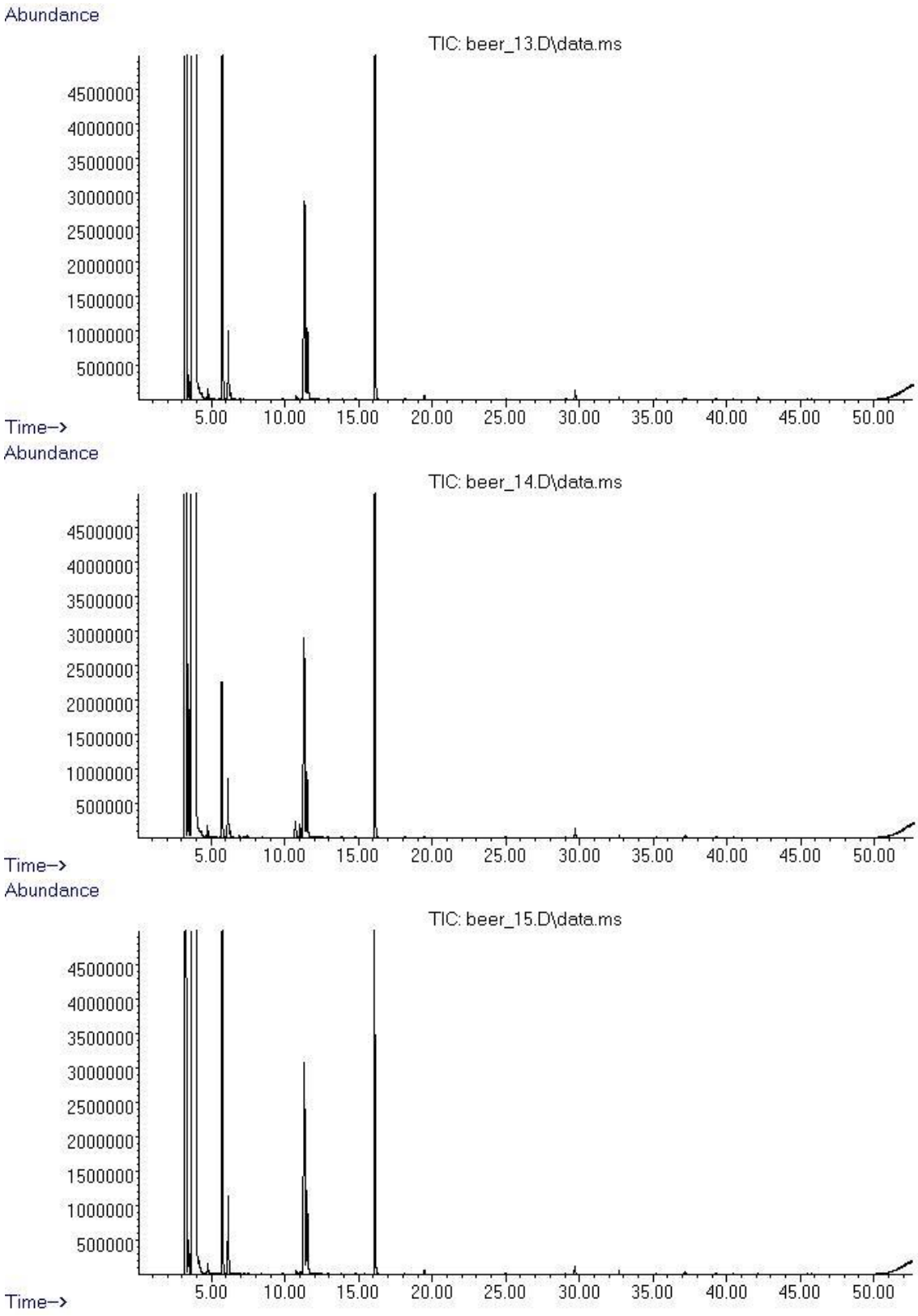
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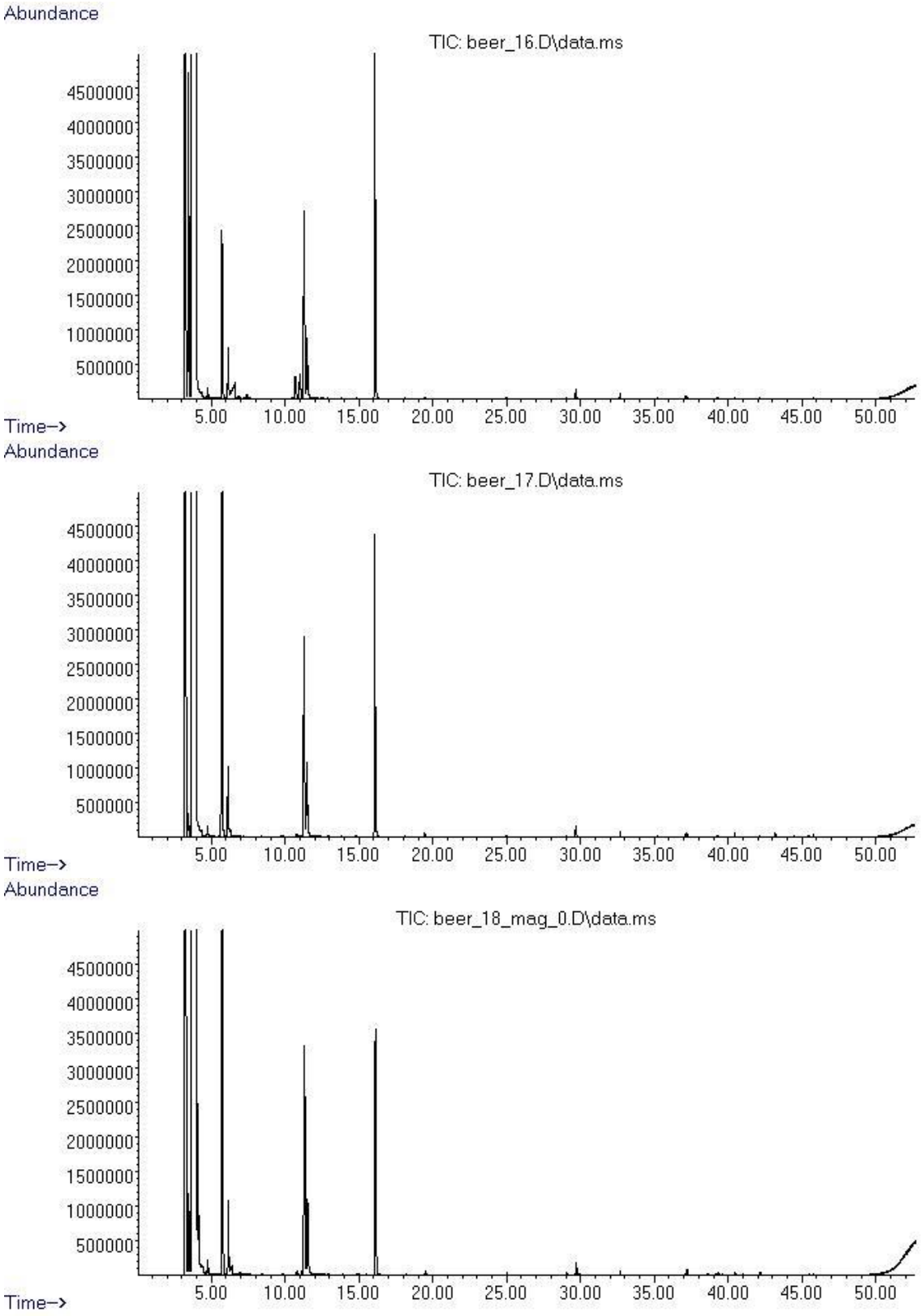
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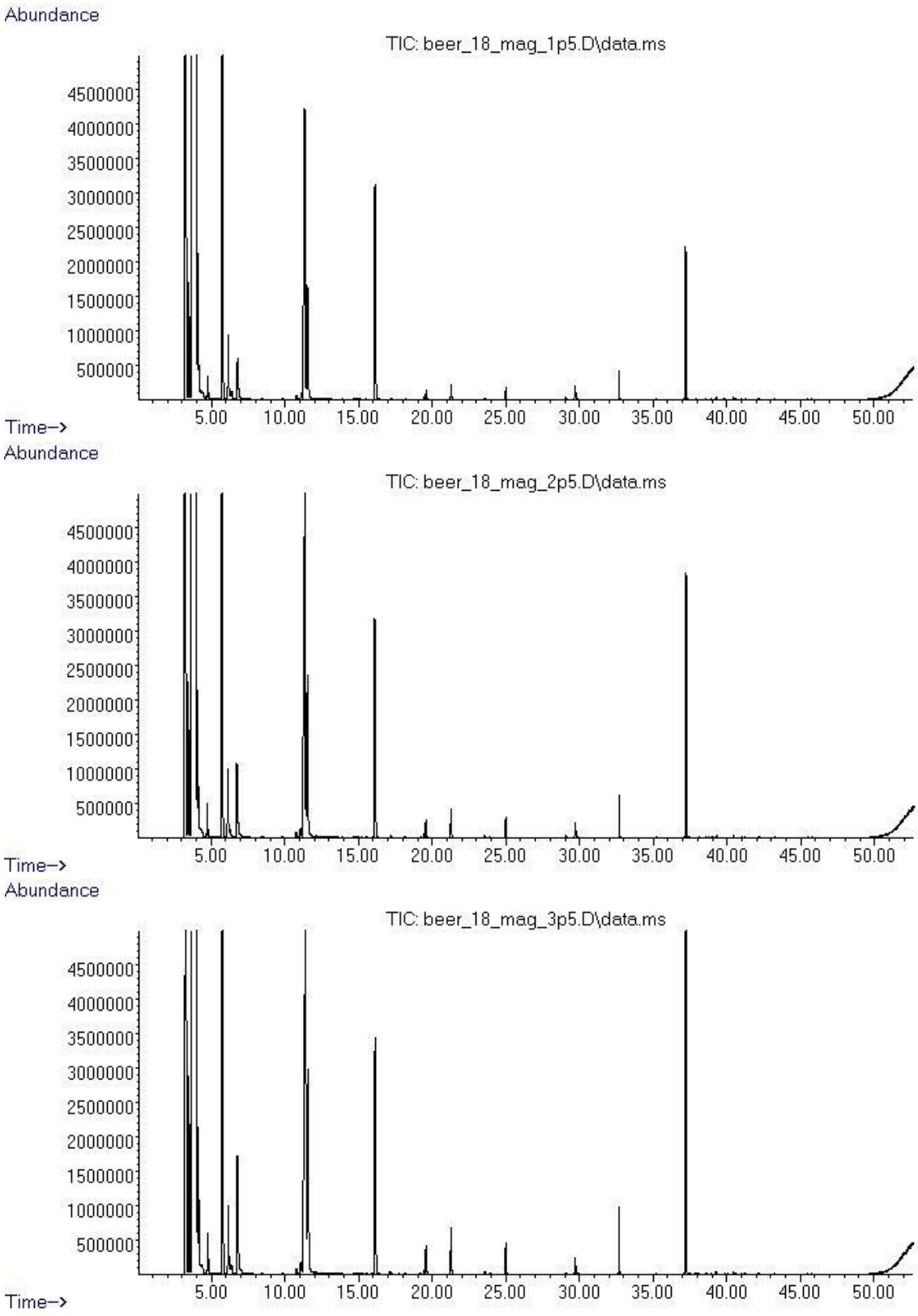
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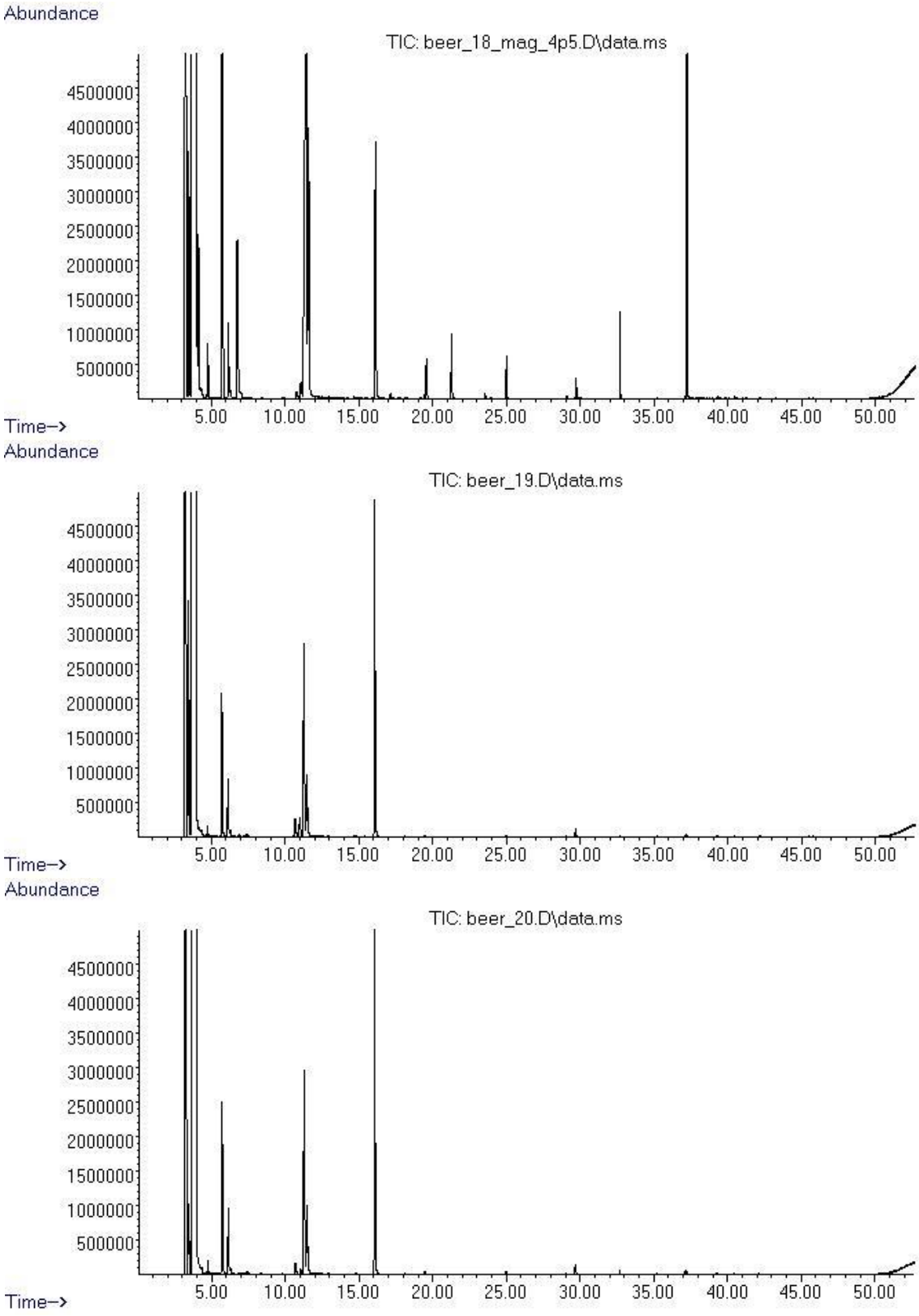
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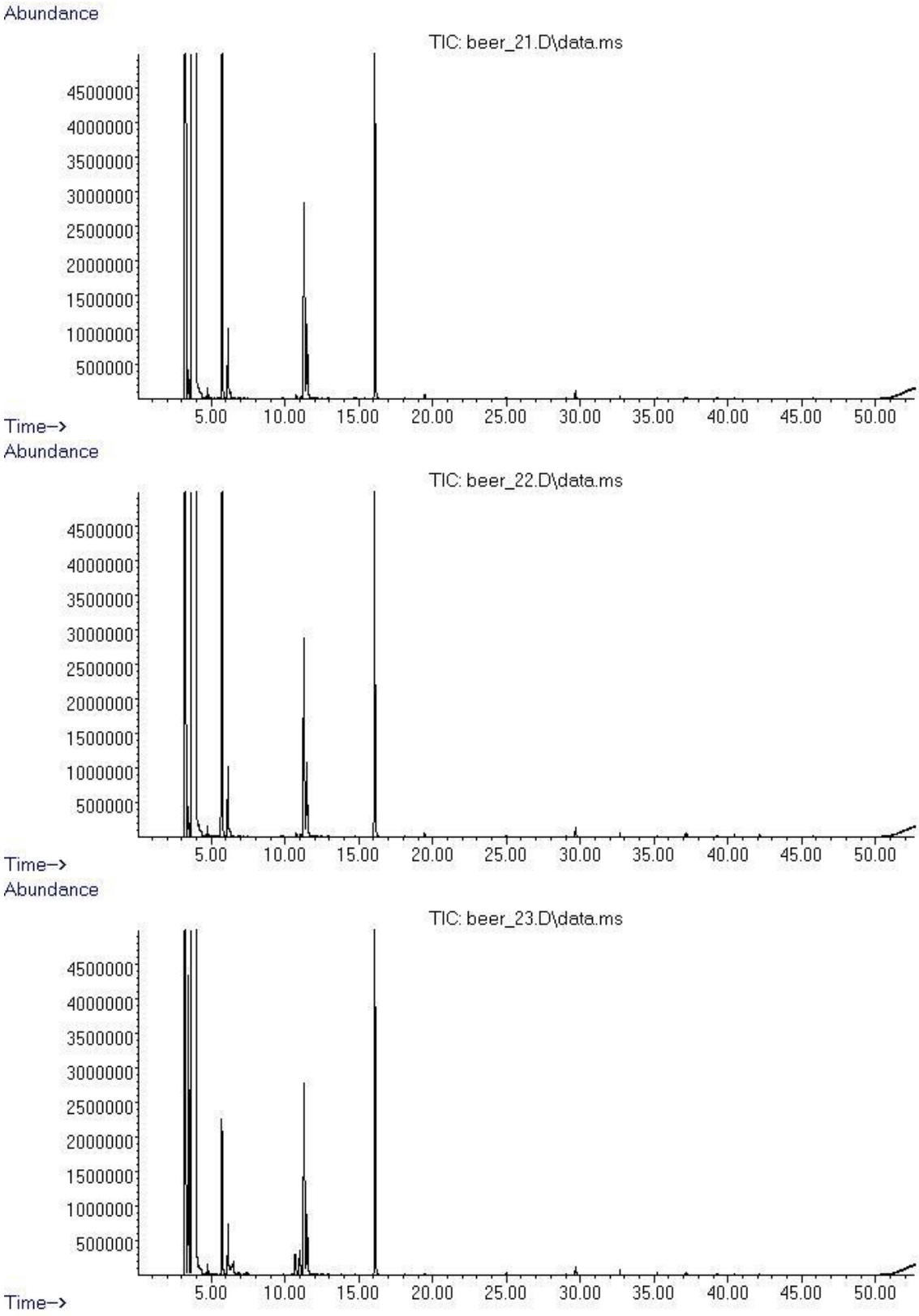
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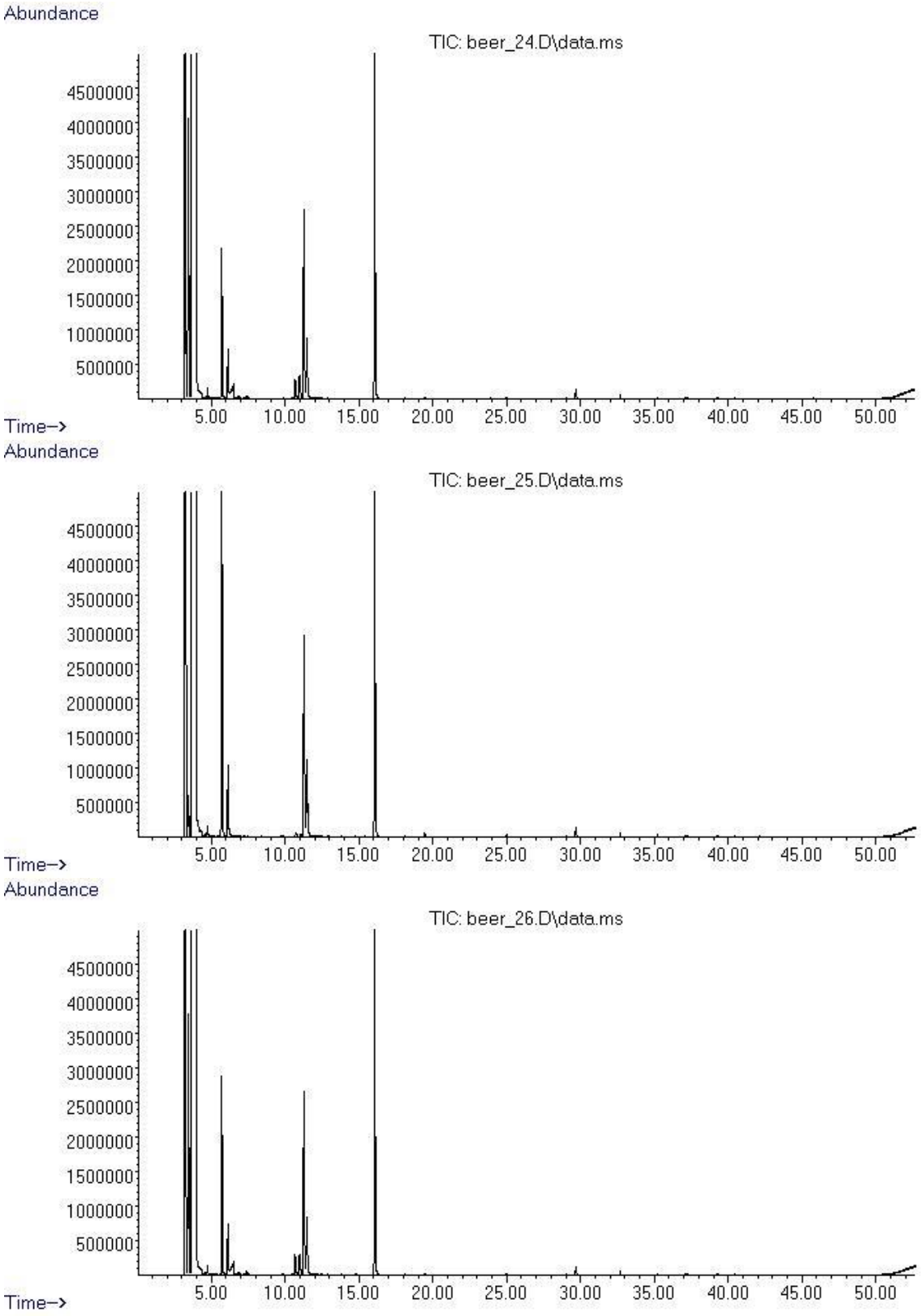
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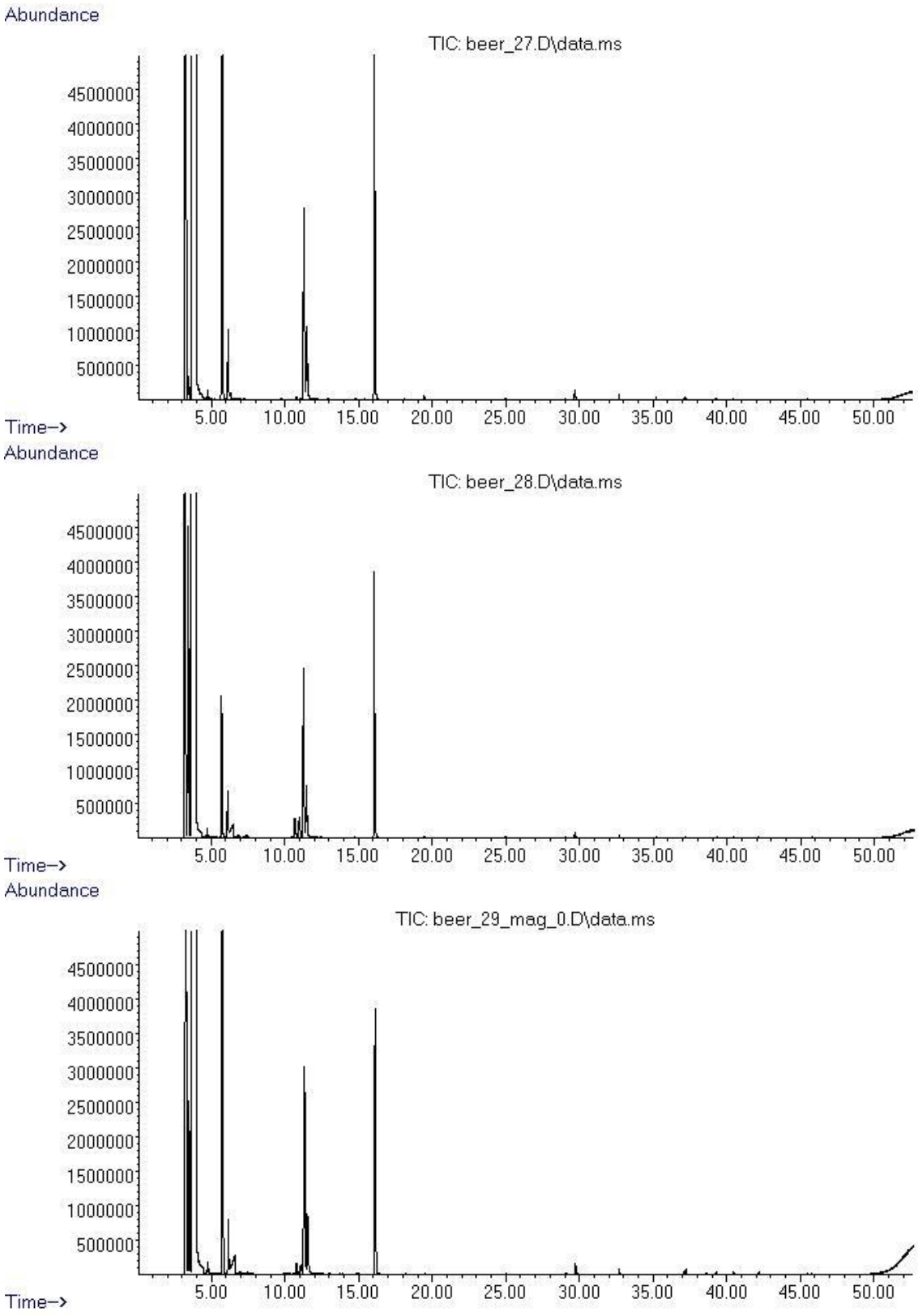
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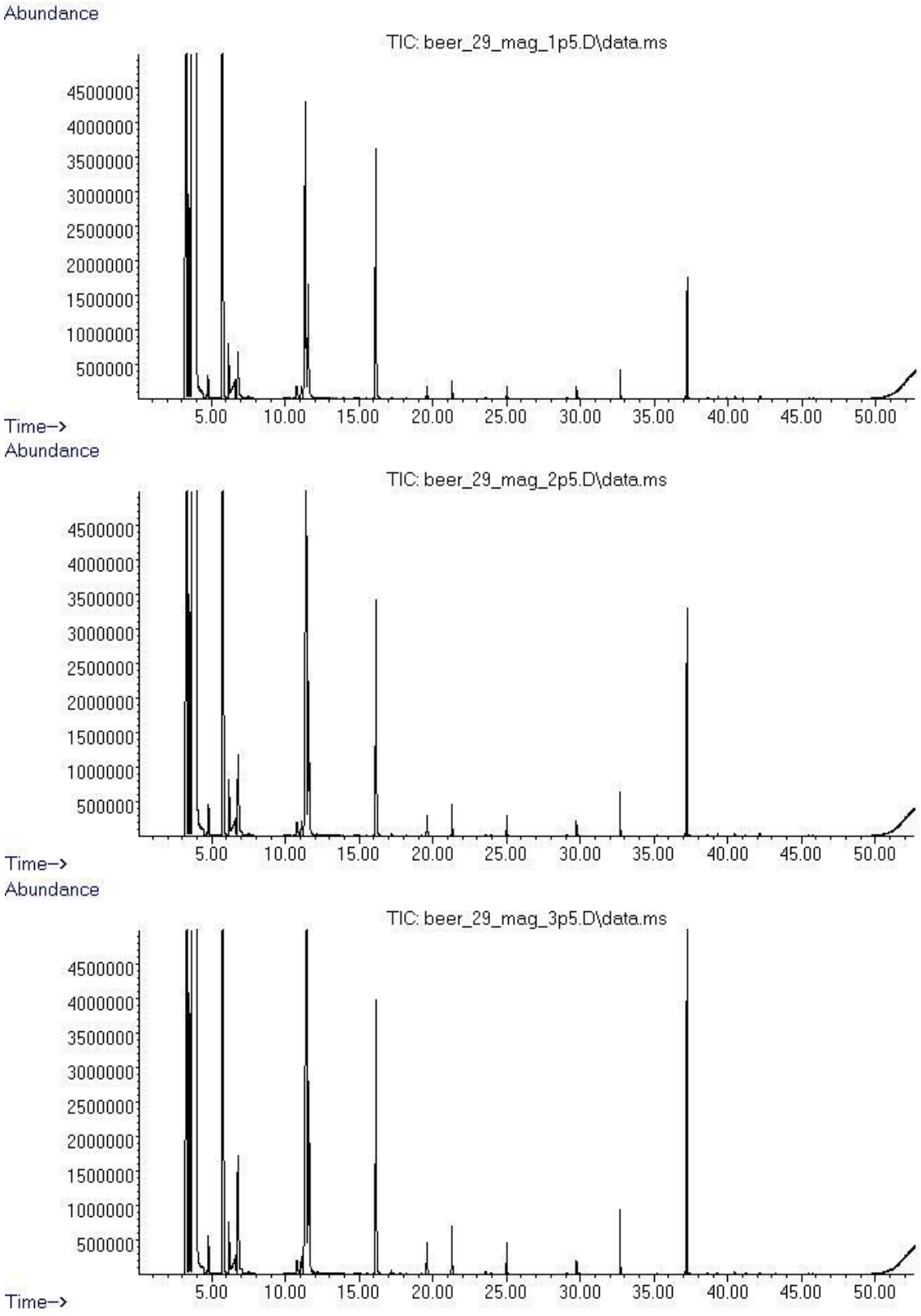
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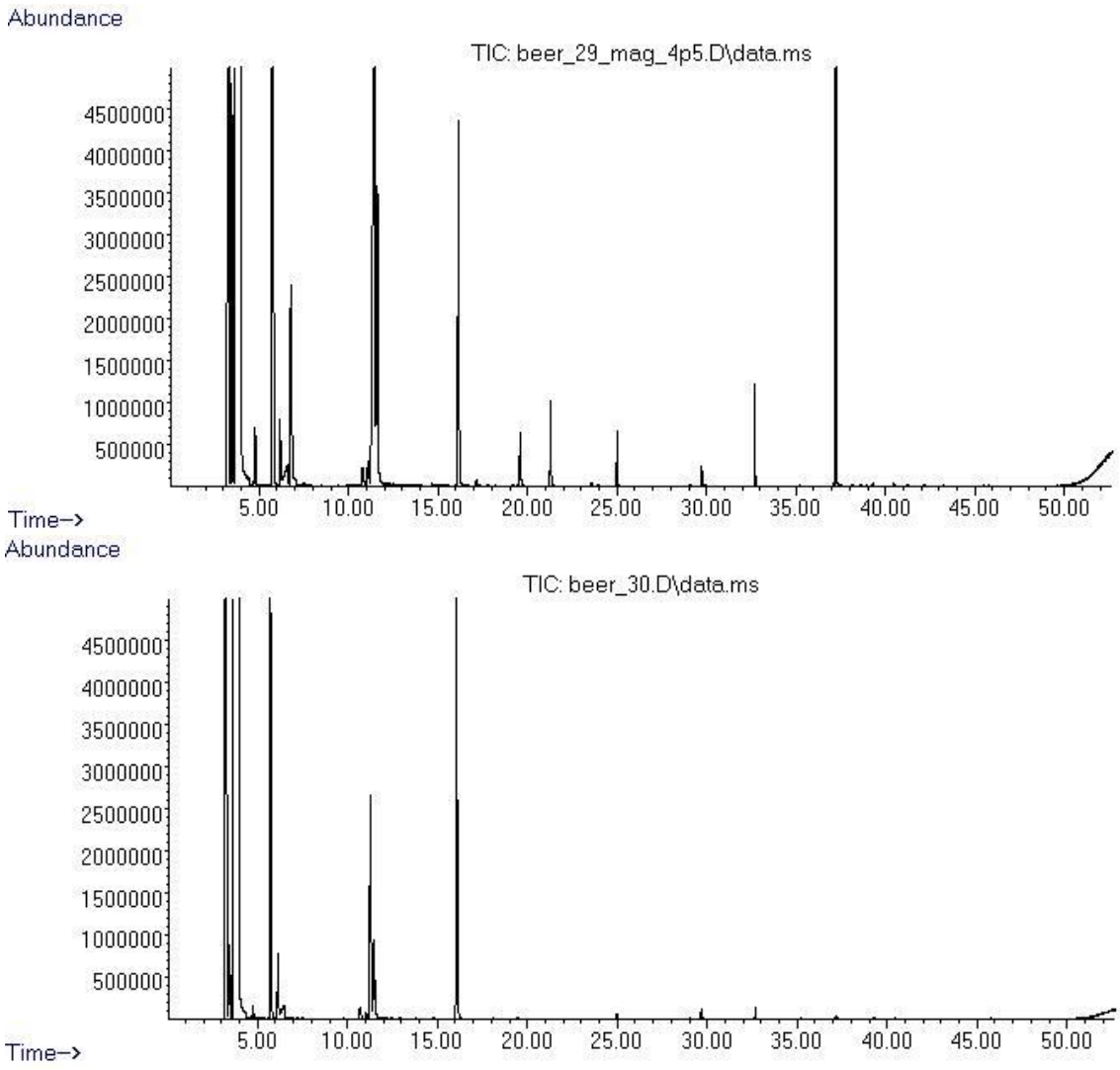
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Gas chromatographic parameters

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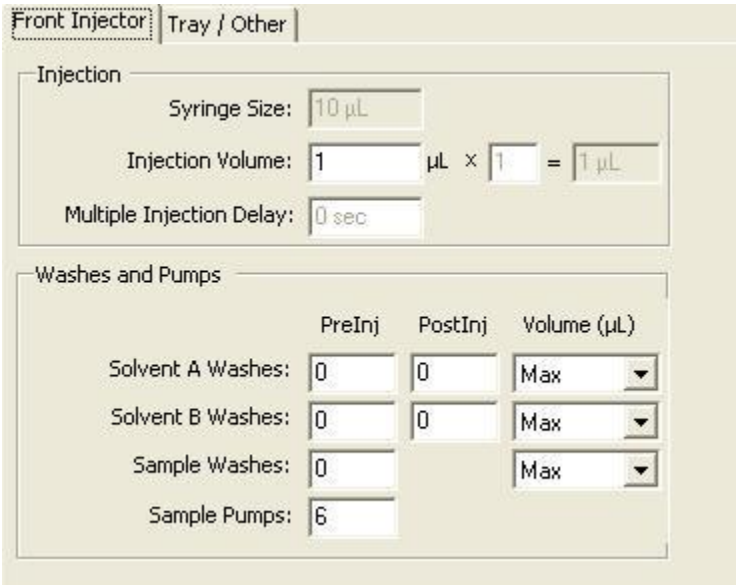

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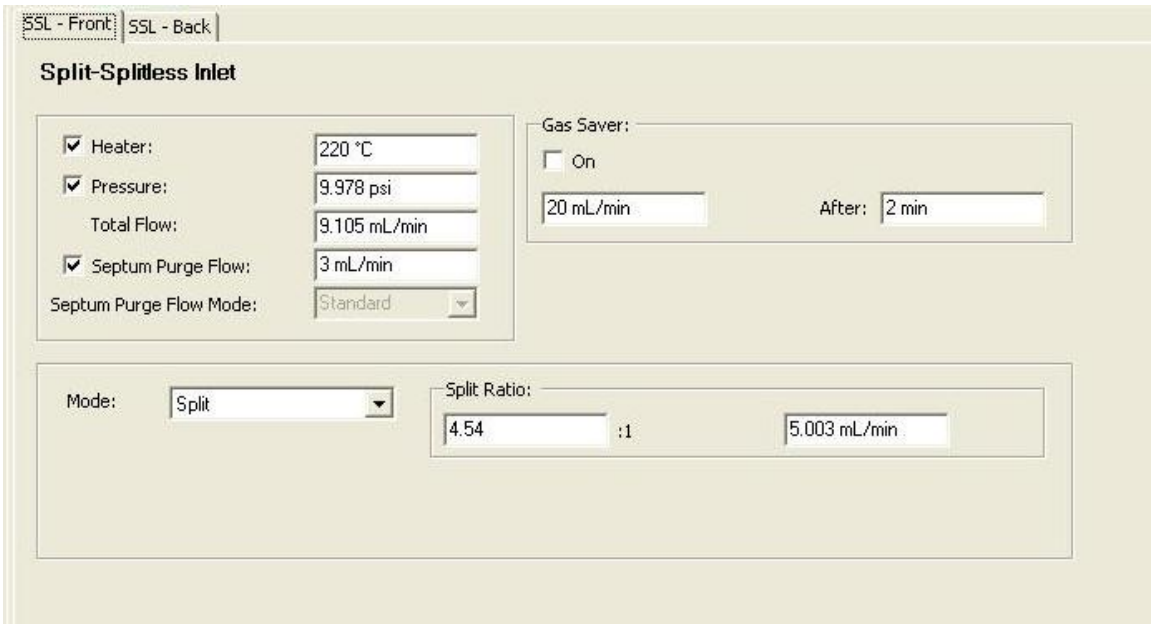

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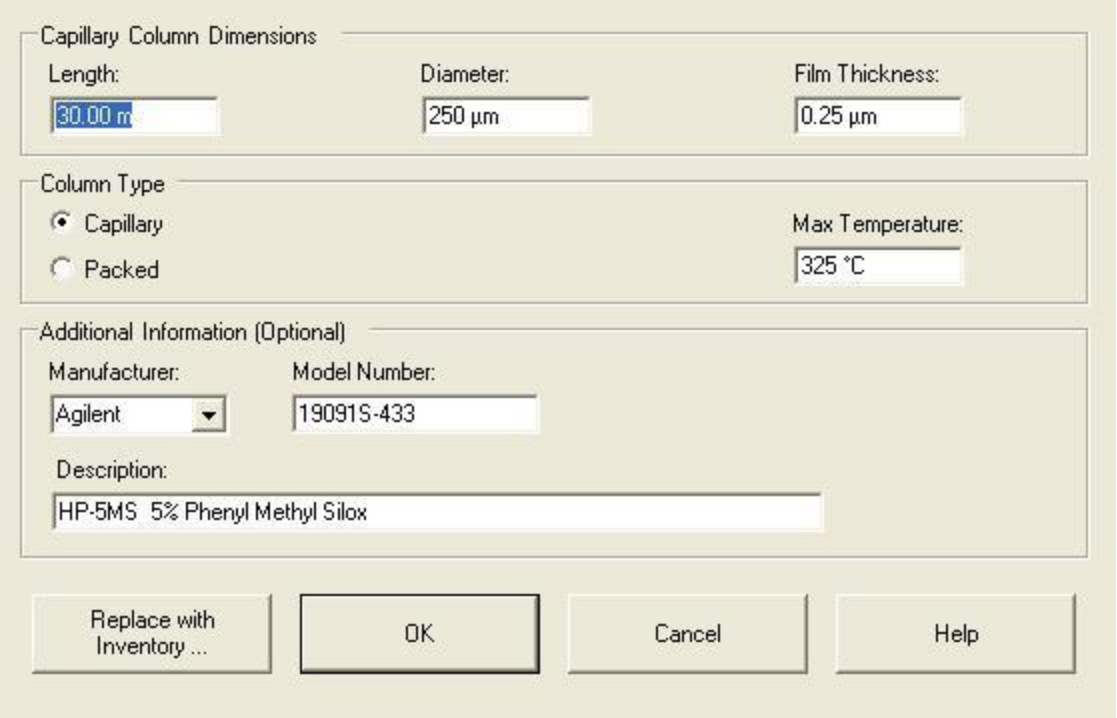

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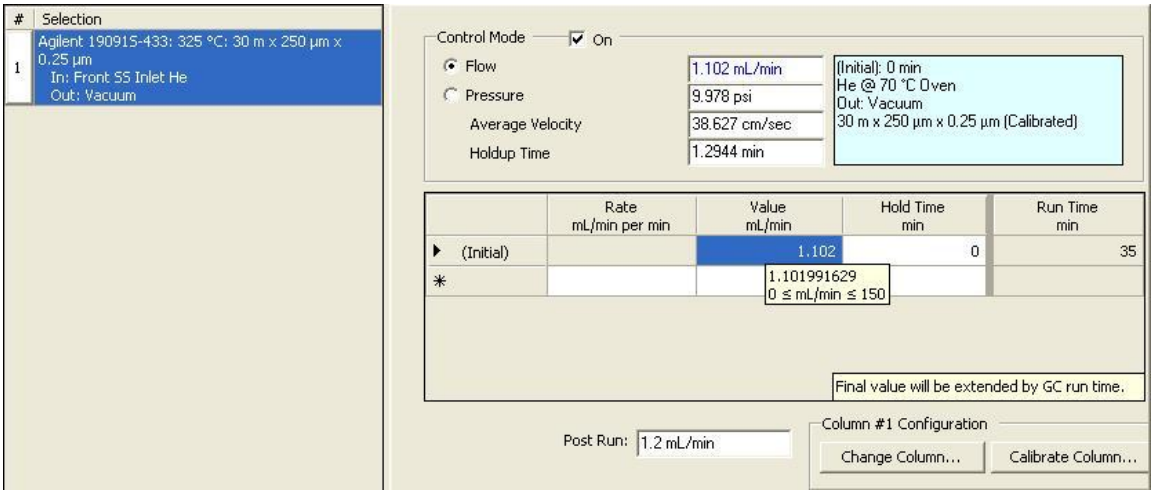
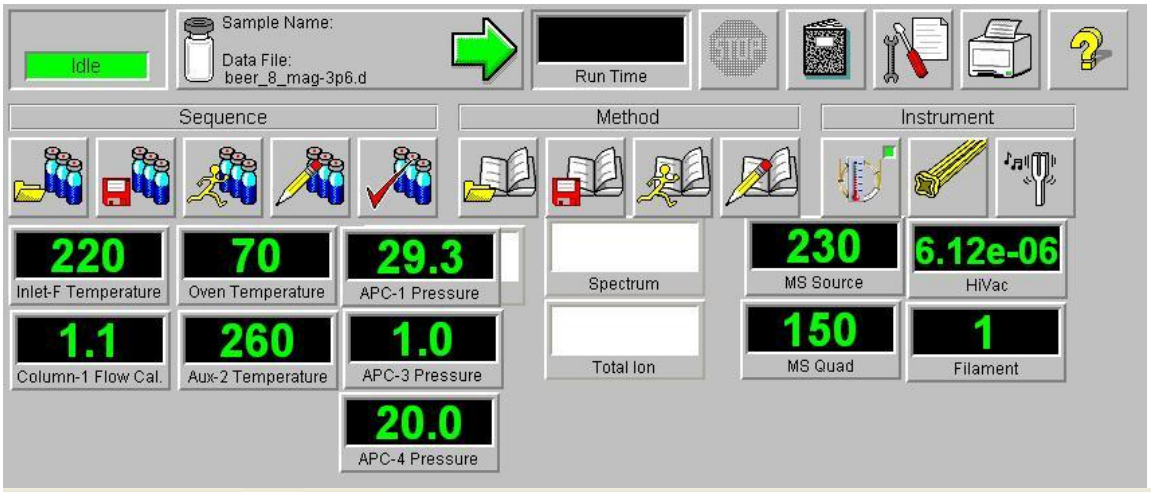
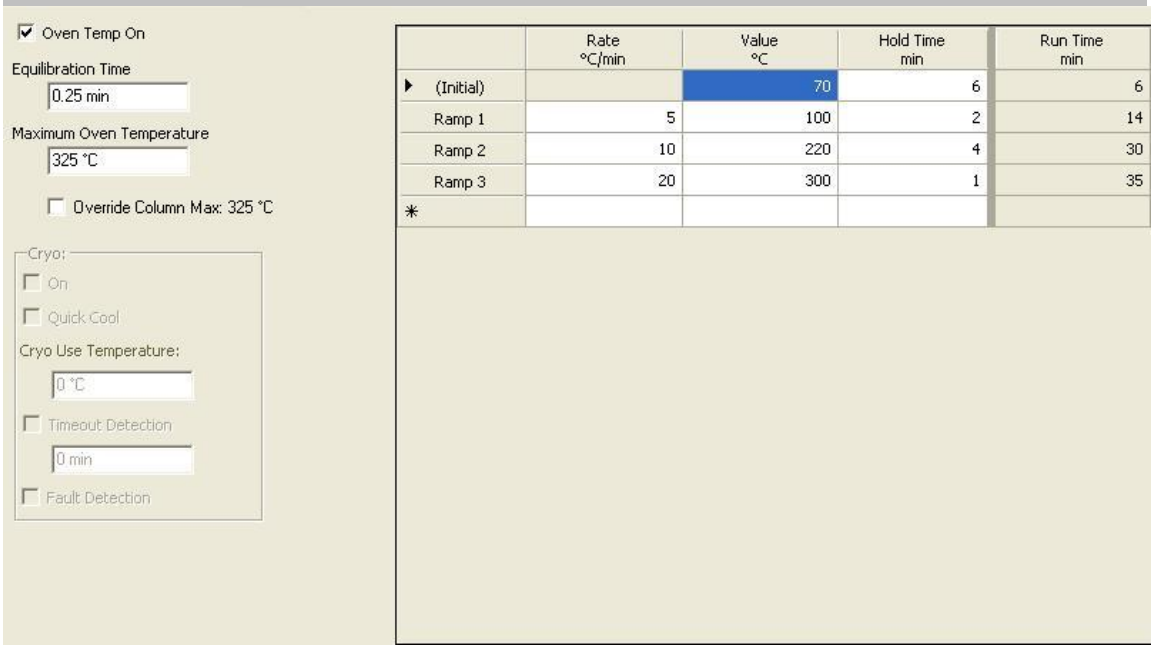






	Rate °C/min	Value °C	Hold Time min	Run Time min
(Initial)		70	6	6
Ramp 1	5	100	2	14
Ramp 2	10	220	4	30
Ramp 3	20	300	1	35
*				

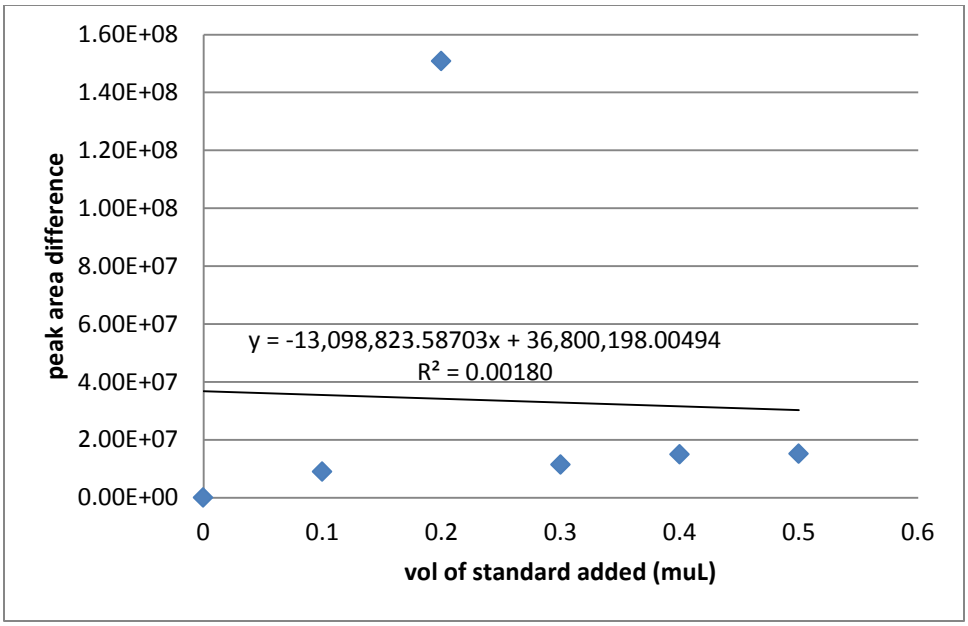
APPENDIX C

First calibration curve with analysis

vol of standard added (muL)	peak area	peak area difference
0	15865619.1	0.00E+00
0.1	24790671.24	8.93E+06
0.2	166675420.2	1.51E+08
0.3	27235955	1.14E+07
0.4	30800658.29	1.49E+07
0.5	30978343.4	1.51E+07

magnitude diff lowest	highest	
1.56254	1.952545514	*lower than 2

Slope	Ord Mag	Vol (muL)	Est Area	
30225449	2	1.049818602	31731238	
	1.75	0.918591277	27764833	
	1.5	0.787363952	23798429	
	1.25	0.656136626	19832024	
	slope	-13098823.6	36800198	intercept
		154134491.2	46666535	
		0.001802277	64479084	
		0.007222124	4	



ı standard	amount required (µL)	amount to be used (µL)	vol. conc. (µL std / µL mix)	amount to be used (µL)	vol. conc. (µL std / µL mix)	dilute 125 muL of standard to 10 mL with beer (vol conc (µL std / µL mix))	1.5	2.5	3.5	4.5	↑amount of standard in each beer
1-propanol (µL)	1.703036	20000	0.985222	170	0.125692	0.001571	0.235673	0.392788	0.549903	0.707018	
acetylaldehyde (µL)	4.859766			480	0.353895	0.004436	0.665428	1.109047	1.552666	1.996285	
ethyl acetate (µL)	2.883158			290	0.214416	0.00268	0.40203	0.670049	0.938069	1.206089	
isoamyl alcohol (µL)	3.084461			310	0.229203	0.002865	0.429756	0.71626	1.002764	1.289267	
2-methyl-1-butanol (µL)	0.911504			90	0.066543	0.000832	0.124768	0.207946	0.291125	0.374303	
isoamylacetate (µL)	0.008164	100	0.004926	0.837438	0.000619	7.74E-06	0.001161	0.001935	0.002709	0.003483	
ethyl hexanoate (µL)	0.001465	25	0.001232	0.20936	0.000155	1.93E-06	0.00029	0.000484	0.000677	0.000871	
ethyl	0.0036	50	0.0024	0.4187	0.00	3.87E-06	0.0005	0.0009	0.0013	0.0017	

octanoate (µL)	23		63	19	031		8	67	54	41	
ethyl decanoate (µL)	0.009389	125	0.006158	1.046798	0.000774	9.67E-06	0.001451	0.002419	0.003386	0.004354	
phenyl ethanol (µL)	0.102985			10	0.007394	9.24E-05	0.013863	0.023105	0.032347	0.041589	

To make the calibration standard solution

step 1: follow the purple column

add the volumes (in microliters) of the four components to 20 mL of 1-propanol

step 2: follow the red column

add the volumes (in microliters) of the five red components to a clean vial

add the volumes (in microliters) of the mixture from step 1, in yellow

step 3: follow the green column

add 125 µL of the step 2 standard solution to 9.875 mL of the appropriate beer

this is your final standard solution

step 4: follow the blue column

for each beer, make 5 standards

for the 0, add none of the final standard solution
for the 1.5, add 150 microliters of the standard solution to 9.850 mL of butyl acetate containing beer

for the 2.5, add 250 microliters, stc.

be sure to also follow yesterday's procedure for adding the correct amount of butyl acetate.

butly acetate solution

5 microliters iof butyl acetate and 4.995 mL of beer

APPENDIX C

Regression analysis

Regression Analysis: Acetaldehyde versus Oxygen (ppm)

The regression equation is
Acetaldehyde = 44.2 - 0.934 Oxygen (ppm)

14 cases used, 16 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	44.163	4.270	10.34	0.000
Oxygen (ppm)	-0.9340	0.3932	-2.38	0.035

S = 9.41232 R-Sq = 32.0% R-Sq(adj) = 26.3%

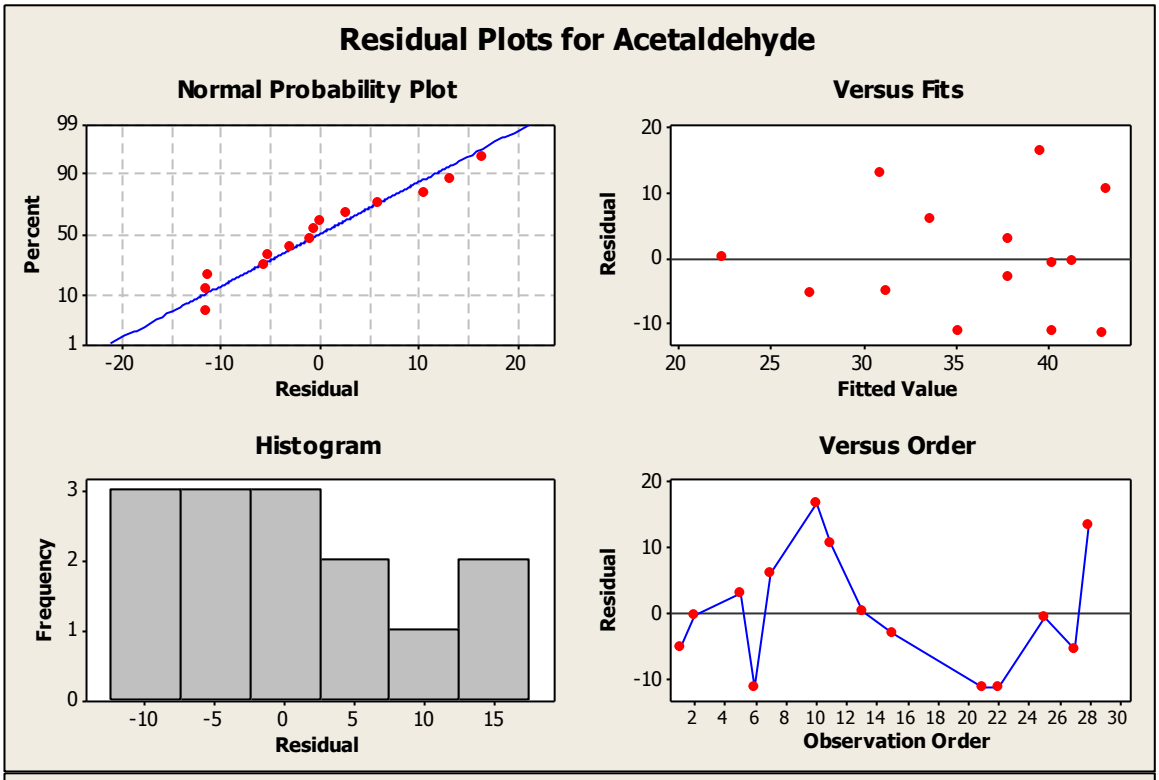
Analysis of Variance

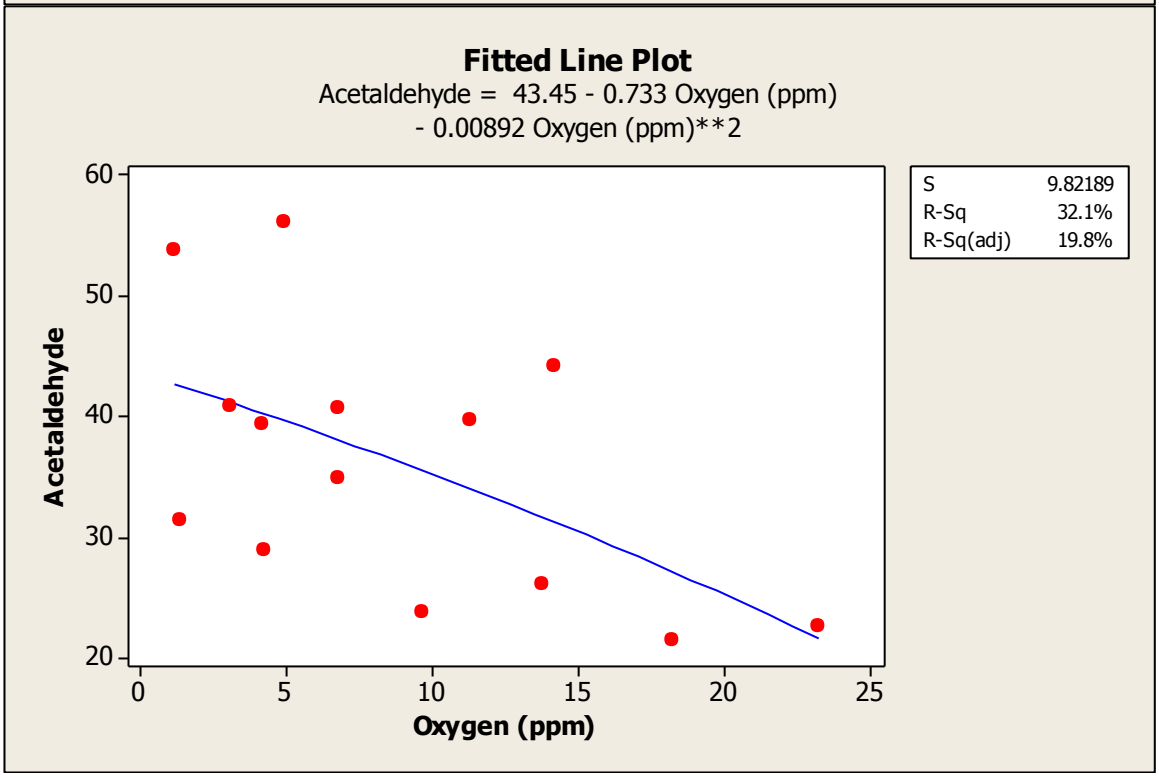
Source	DF	SS	MS	F	P
Regression	1	499.92	499.92	5.64	0.035
Residual Error	12	1063.10	88.59		
Total	13	1563.02			

Unusual Observations

Obs	Oxygen (ppm)	Acetaldehyde	Fit	SE Fit	Residual	St Resid
13	23.3	22.58	22.43	6.23	0.14	0.02 X

X denotes an observation whose X value gives it large leverage.


Residual Plots for Acetaldehyde
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Fitted Line Plot
Acetaldehyde = 43.45 - 0.733 Oxygen (ppm)
- 0.00892 Oxygen (ppm)**2
S 9.82189
R-Sq 32.1%
R-Sq(adj) 19.8%
Acetaldehyde
Oxygen (ppm)

Regression Analysis: 1-Propanol versus Oxygen (ppm)

The regression equation is
1-Propanol = 14.7 - 0.197 Oxygen (ppm)

14 cases used, 16 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	14.7306	0.7299	20.18	0.000
Oxygen (ppm)	-0.19654	0.06720	-2.92	0.013

S = 1.60878 R-Sq = 41.6% R-Sq(adj) = 36.8%

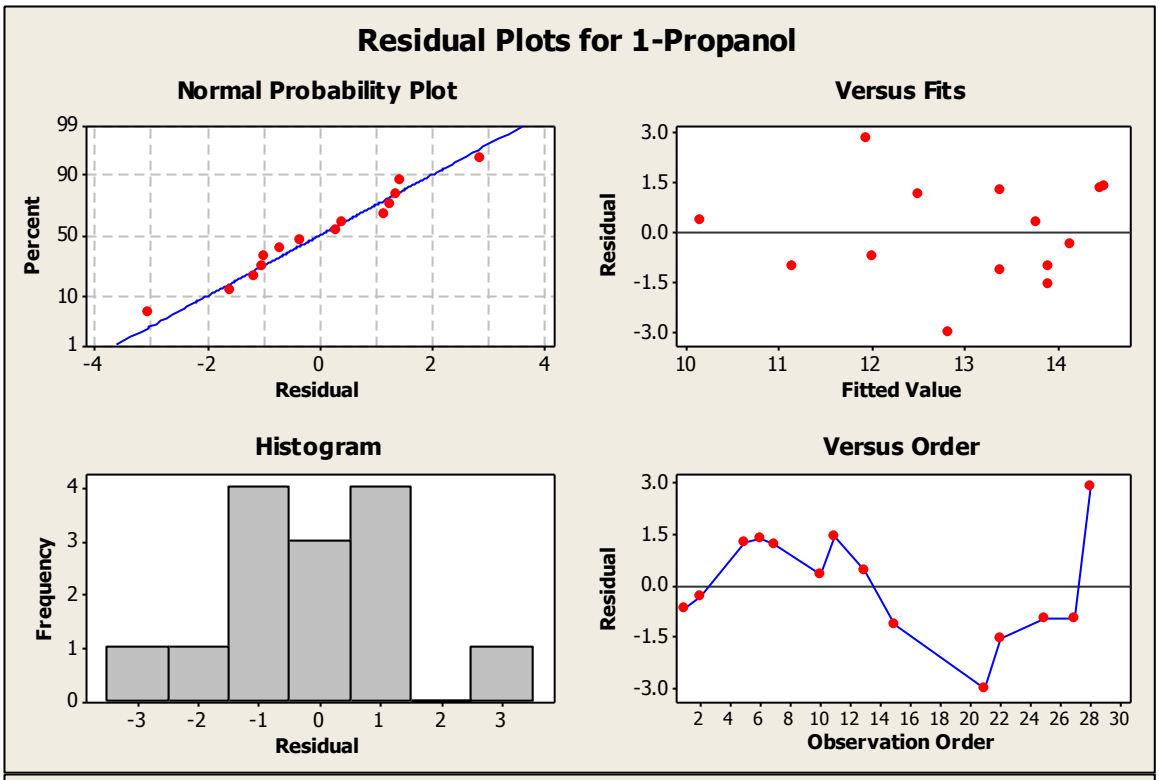
Analysis of Variance

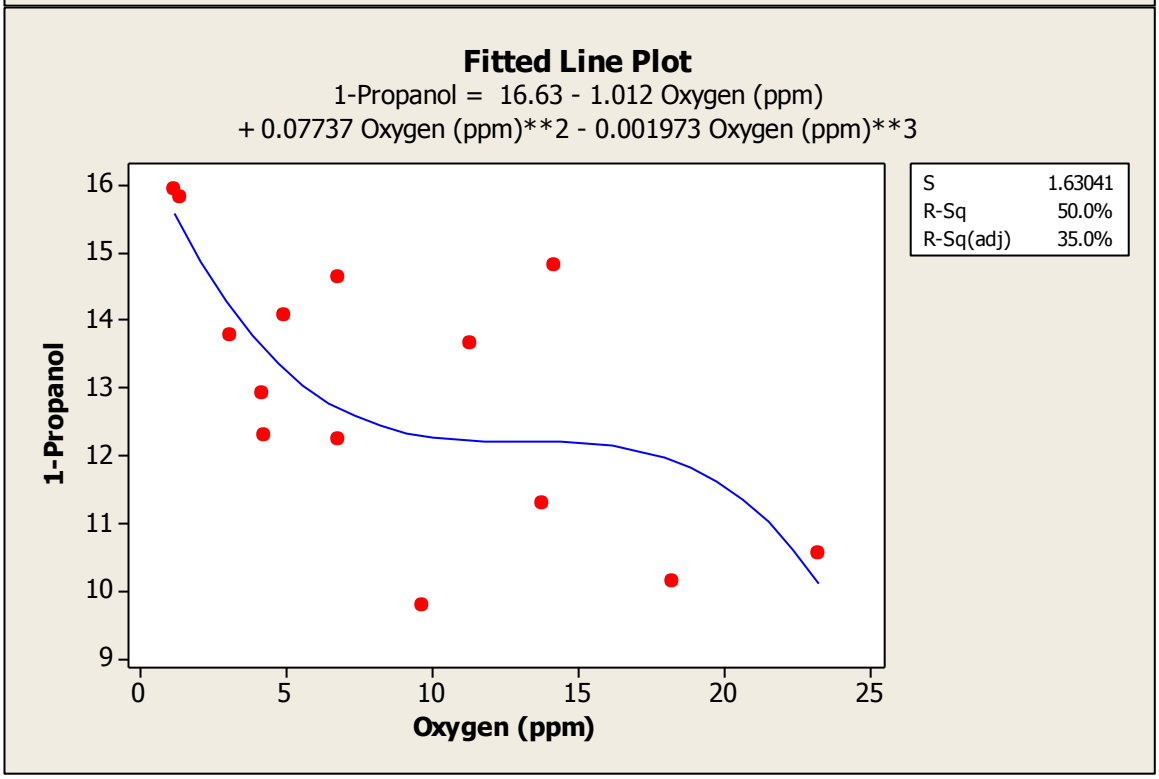
Source	DF	SS	MS	F	P
Regression	1	22.139	22.139	8.55	0.013
Residual Error	12	31.058	2.588		
Total	13	53.197			

Unusual Observations

Obs	Oxygen (ppm)	1-Propanol	Fit	SE Fit	Residual	St Resid
13	23.3	10.550	10.158	1.064	0.393	0.33 X

X denotes an observation whose X value gives it large leverage


Residual Plots for 1-Propanol
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Fitted Line Plot
1-Propanol = 16.63 - 1.012 Oxygen (ppm)
+ 0.07737 Oxygen (ppm)**2 - 0.001973 Oxygen (ppm)**3
S 1.63041
R-Sq 50.0%
R-Sq(adj) 35.0%
1-Propanol
Oxygen (ppm)

Regression Analysis: Ethyl Acetate versus Oxygen (ppm)

The regression equation is
Ethyl Acetate = 62.5 - 0.44 Oxygen (ppm)

14 cases used, 16 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	62.49	11.08	5.64	0.000
Oxygen (ppm)	-0.435	1.020	-0.43	0.677

S = 24.4129 R-Sq = 1.5% R-Sq(adj) = 0.0%

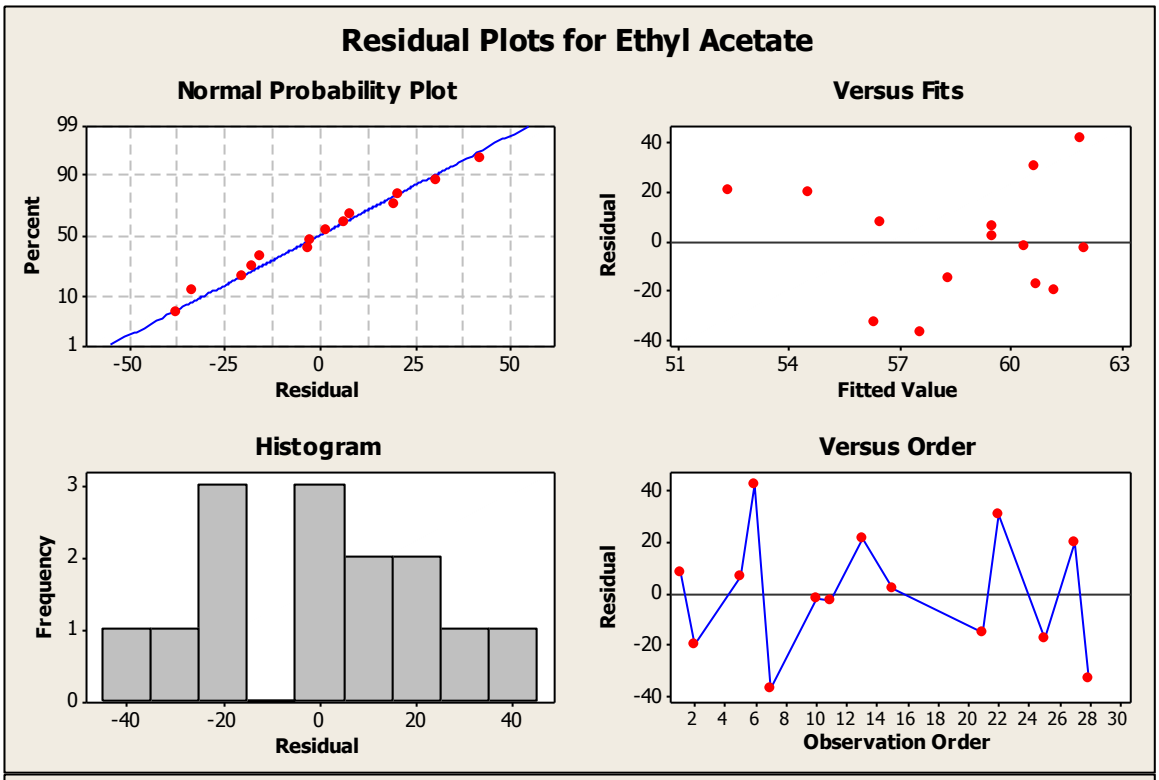
Analysis of Variance

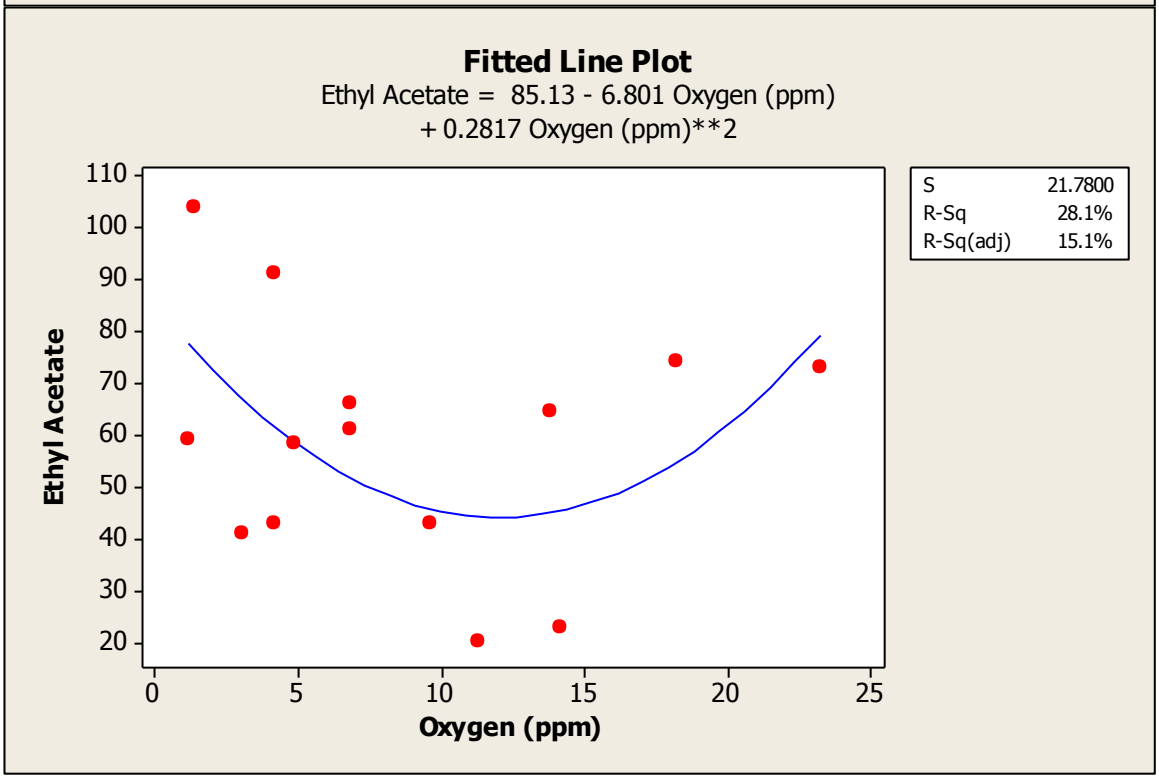
Source	DF	SS	MS	F	P
Regression	1	108.6	108.6	0.18	0.677
Residual Error	12	7151.9	596.0		
Total	13	7260.5			

Unusual Observations

Obs	Oxygen (ppm)	Ethyl Acetate	Fit	SE Fit	Residual	St Resid	
13	23.3	73.21	52.37	16.15	20.84	1.14	X

X denotes an observation whose X value gives it large leverage.


Residual Plots for Ethyl Acetate
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Fitted Line Plot
Ethyl Acetate = 85.13 - 6.801 Oxygen (ppm)
+ 0.2817 Oxygen (ppm)**2
S 21.7800
R-Sq 28.1%
R-Sq(adj) 15.1%
Ethyl Acetate
Oxygen (ppm)

Regression Analysis: Isoamyl Alcohol versus Oxygen (ppm)

The regression equation is
Isoamyl Alcohol = 39.0 - 0.341 Oxygen (ppm)

14 cases used, 16 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	38.999	1.889	20.65	0.000
Oxygen (ppm)	-0.3412	0.1739	-1.96	0.073

S = 4.16352 R-Sq = 24.3% R-Sq(adj) = 18.0%

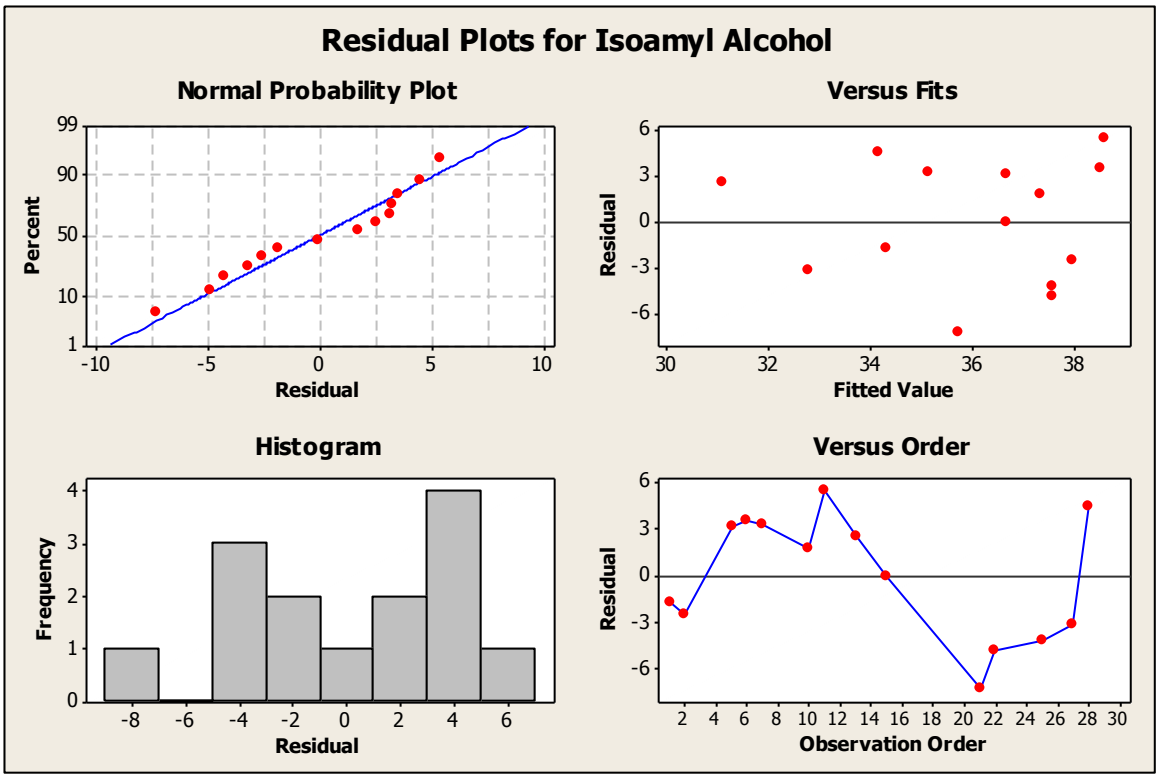
Analysis of Variance

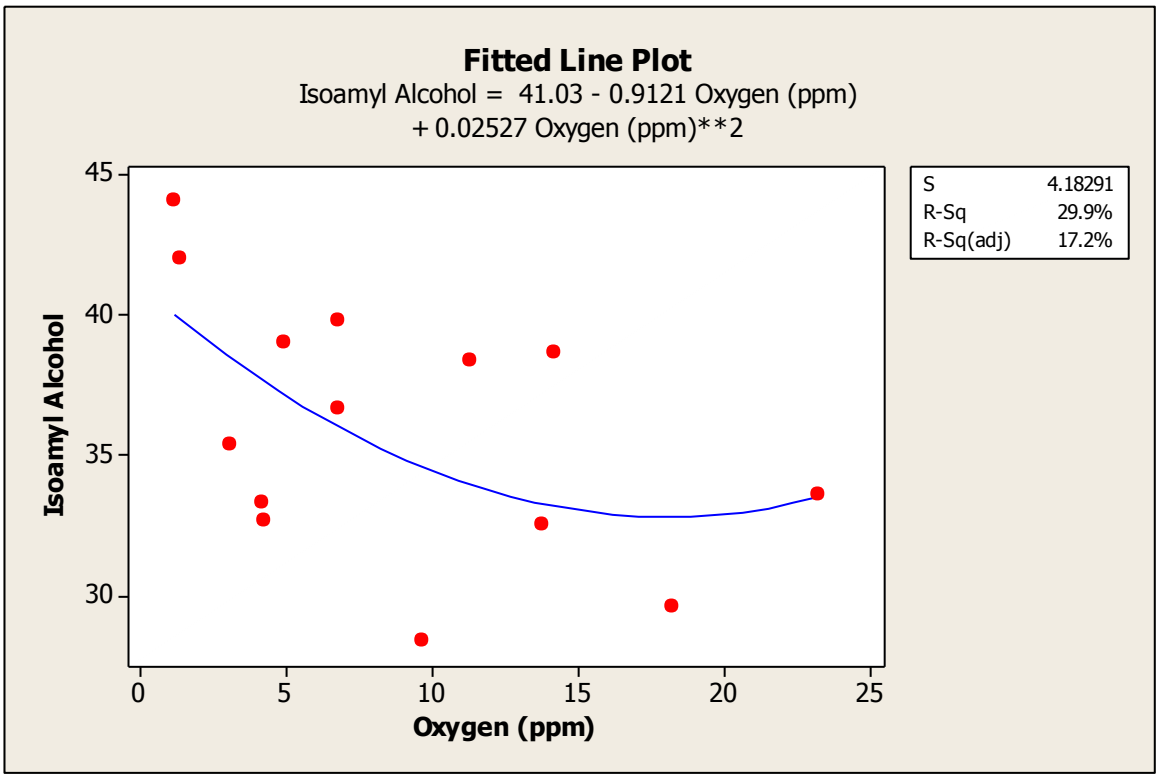
Source	DF	SS	MS	F	P
Regression	1	66.71	66.71	3.85	0.073
Residual Error	12	208.02	17.33		
Total	13	274.73			

Unusual Observations

Obs	Oxygen (ppm)	Isoamyl Alcohol	Fit	SE Fit	Residual	St Resid
13	23.3	33.58	31.06	2.75	2.52	0.81 X

X denotes an observation whose X value gives it large leverage.


Residual Plots for Isoamyl Alcohol
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Fitted Line Plot
Isoamyl Alcohol = 41.03 - 0.9121 Oxygen (ppm)
+ 0.02527 Oxygen (ppm)**2
S 4.18291
R-Sq 29.9%
R-Sq(adj) 17.2%
Isoamyl Alcohol
Oxygen (ppm)

Regression Analysis: 2-Methyl 1 Butanol versus Oxygen (ppm)

The regression equation is
2-Methyl 1 Butanol = 10.7 - 0.118 Oxygen (ppm)

14 cases used, 16 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	10.6759	0.4312	24.76	0.000
Oxygen (ppm)	-0.11835	0.03970	-2.98	0.011

S = 0.950370 R-Sq = 42.6% R-Sq(adj) = 37.8%

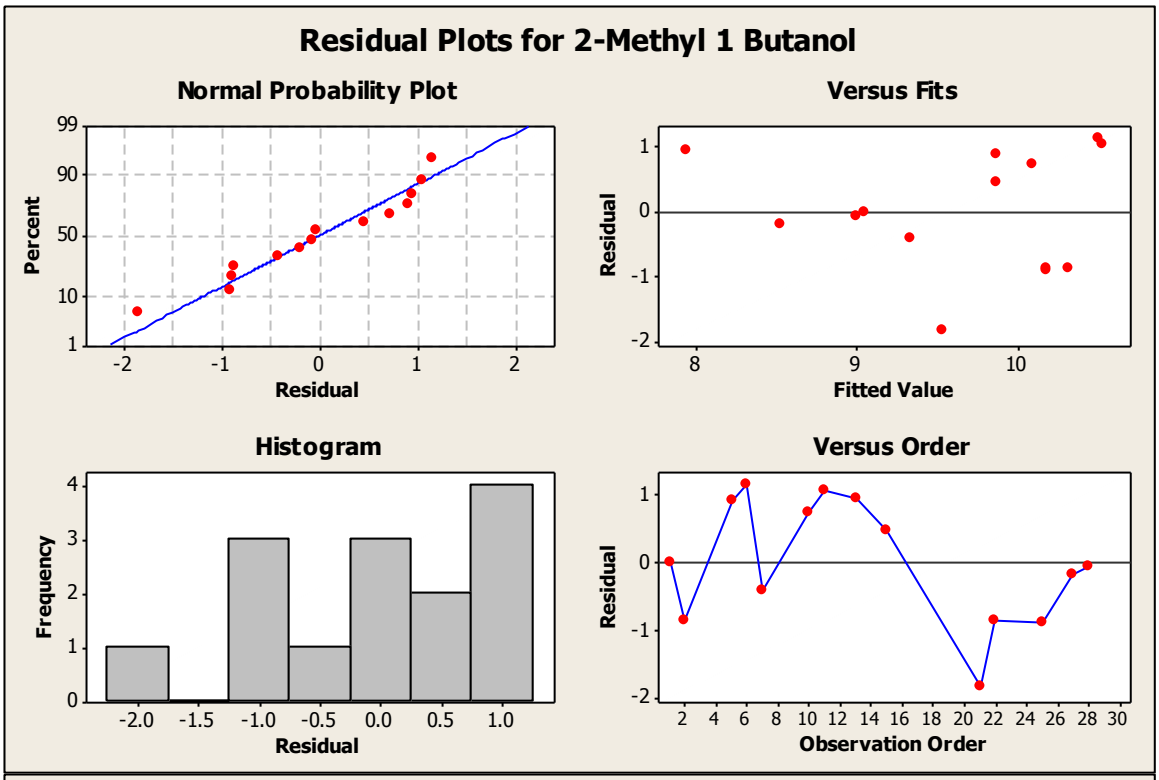
Analysis of Variance

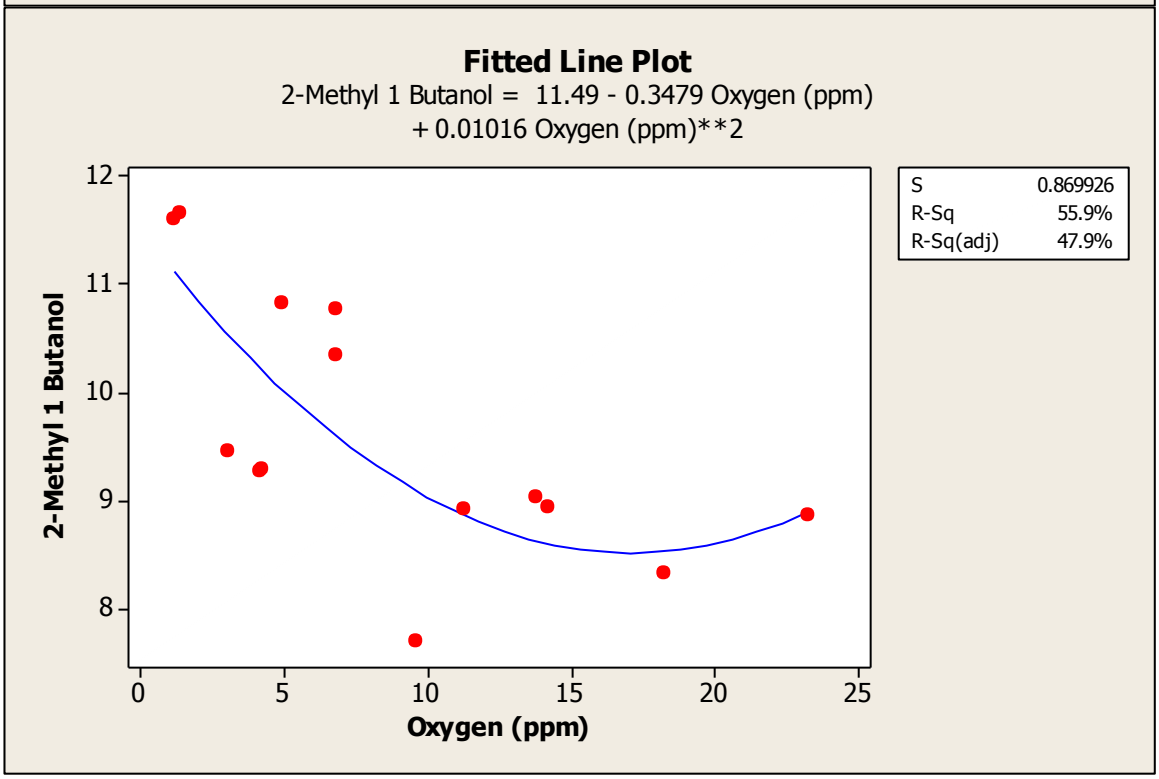
Source	DF	SS	MS	F	P
Regression	1	8.0278	8.0278	8.89	0.011
Residual Error	12	10.8384	0.9032		
Total	13	18.8662			

Unusual Observations

Obs	Oxygen (ppm)	2-Methyl 1 Butanol	Fit	SE Fit	Residual	St Resid
13	23.3	8.866	7.922	0.629	0.944	1.32 X
21	9.6	7.693	9.536	0.256	-1.843	-2.01R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.


Residual Plots for 2-Methyl 1 Butanol
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Fitted Line Plot
2-Methyl 1 Butanol = 11.49 - 0.3479 Oxygen (ppm)
+ 0.01016 Oxygen (ppm)**2
S 0.869926
R-Sq 55.9%
R-Sq(adj) 47.9%
2-Methyl 1 Butanol
Oxygen (ppm)

Regression Analysis: Isoamyl Acetate versus Oxygen (ppm)

The regression equation is
Isoamyl Acetate = 0.0218 - 0.000273 Oxygen (ppm)

14 cases used, 16 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	0.021766	0.002906	7.49	0.000
Oxygen (ppm)	-0.0002734	0.0002675	-1.02	0.327

S = 0.00640503 R-Sq = 8.0% R-Sq(adj) = 0.3%

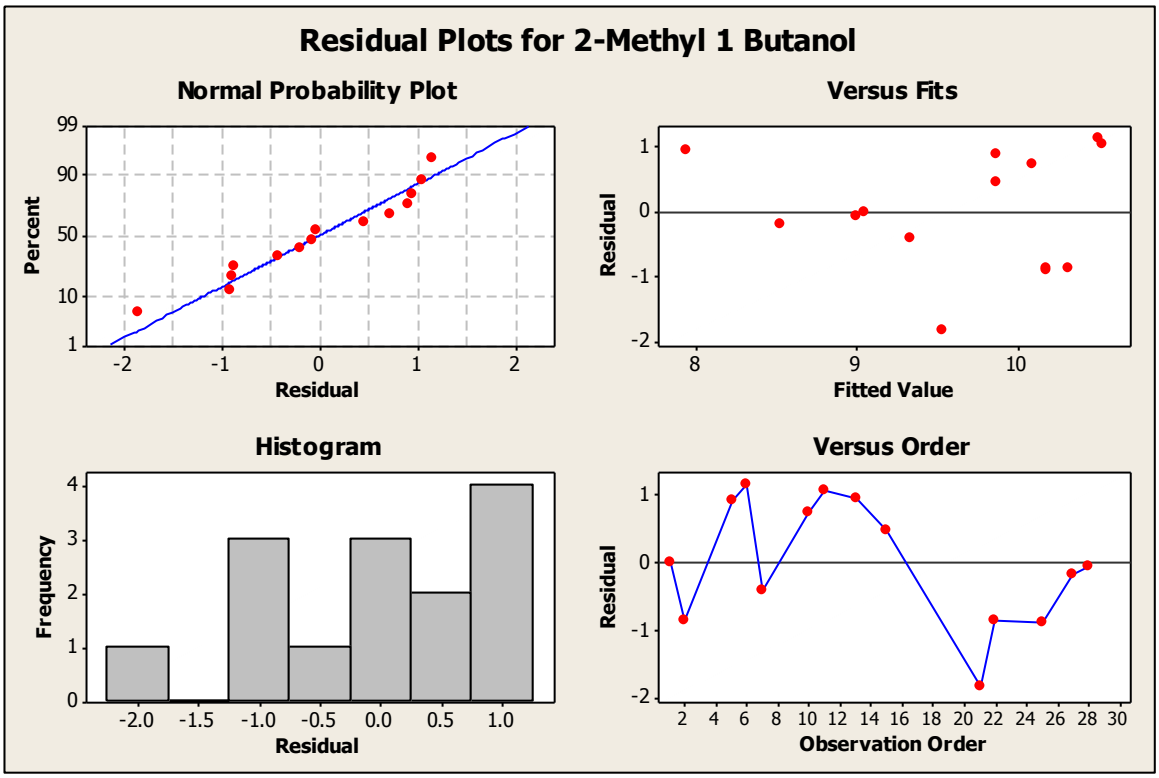
Analysis of Variance

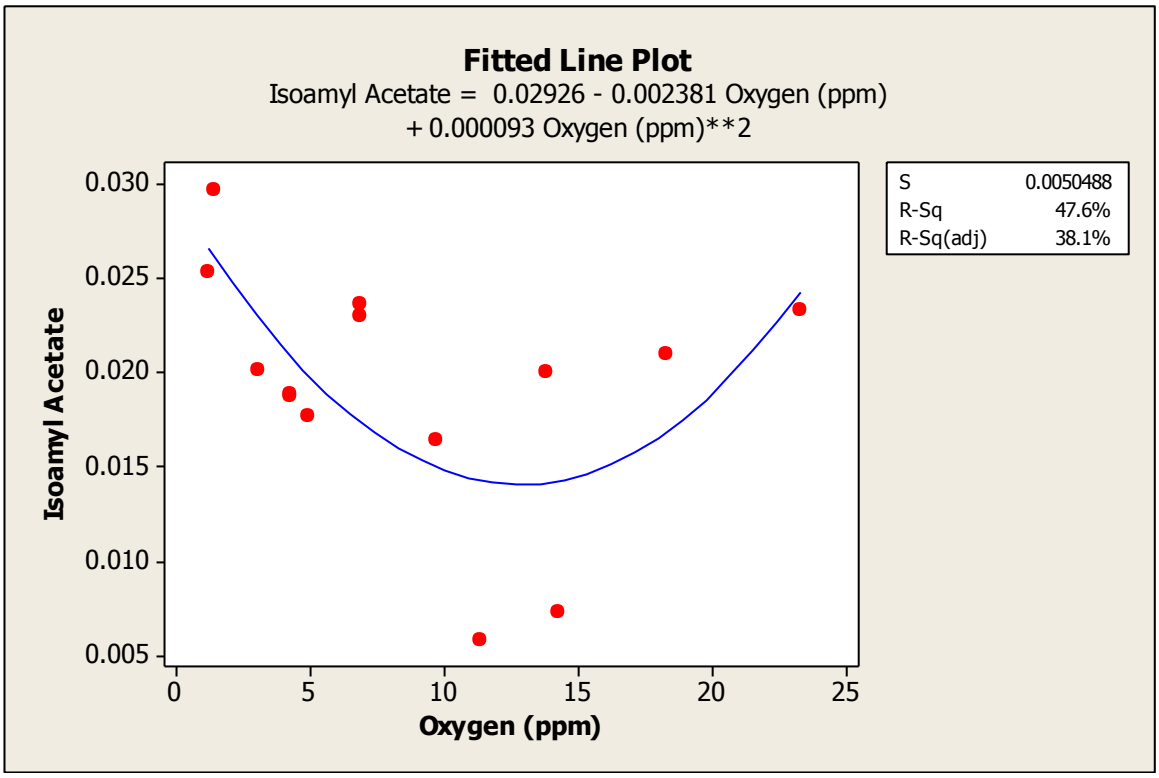
Source	DF	SS	MS	F	P
Regression	1	0.00004283	0.00004283	1.04	0.327
Residual Error	12	0.00049229	0.00004102		
Total	13	0.00053512			

Unusual Observations

Obs	Oxygen (ppm)	Isoamyl Acetate	Fit	SE Fit	Residual	St Resid
7	11.3	0.00584	0.01868	0.00184	-0.01283	-2.09R
13	23.3	0.02336	0.01541	0.00424	0.00796	1.66 X

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage


Residual Plots for 2-Methyl 1 Butanol
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Fitted Line Plot
Isoamyl Acetate = 0.02926 - 0.002381 Oxygen (ppm)
+ 0.000093 Oxygen (ppm)**2
S 0.0050488
R-Sq 47.6%
R-Sq(adj) 38.1%
Isoamyl Acetate
Oxygen (ppm)

Regression Analysis: Ethyl Hexanoate versus Oxygen (ppm)

The regression equation is
Ethyl Hexanoate = 0.00337 - 0.000086 Oxygen (ppm)

14 cases used, 16 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	0.0033657	0.0003833	8.78	0.000
Oxygen (ppm)	-0.00008590	0.00003530	-2.43	0.032

S = 0.000844982 R-Sq = 33.0% R-Sq(adj) = 27.5%

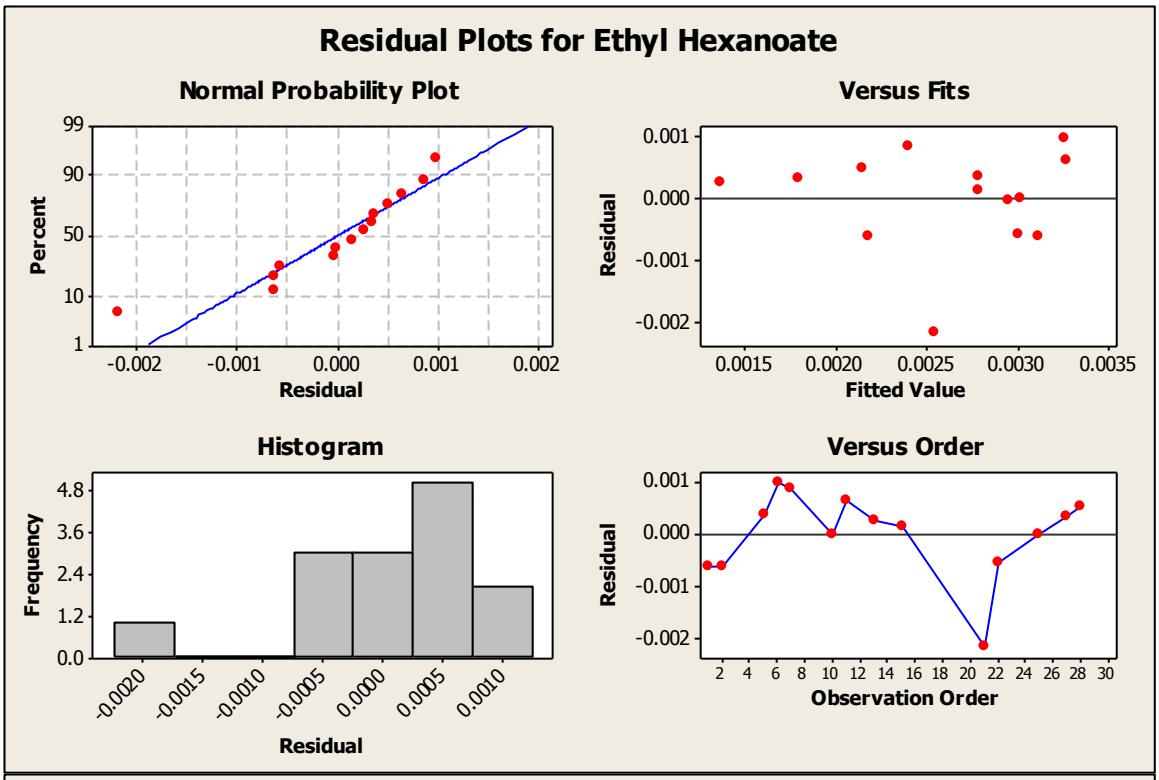
Analysis of Variance

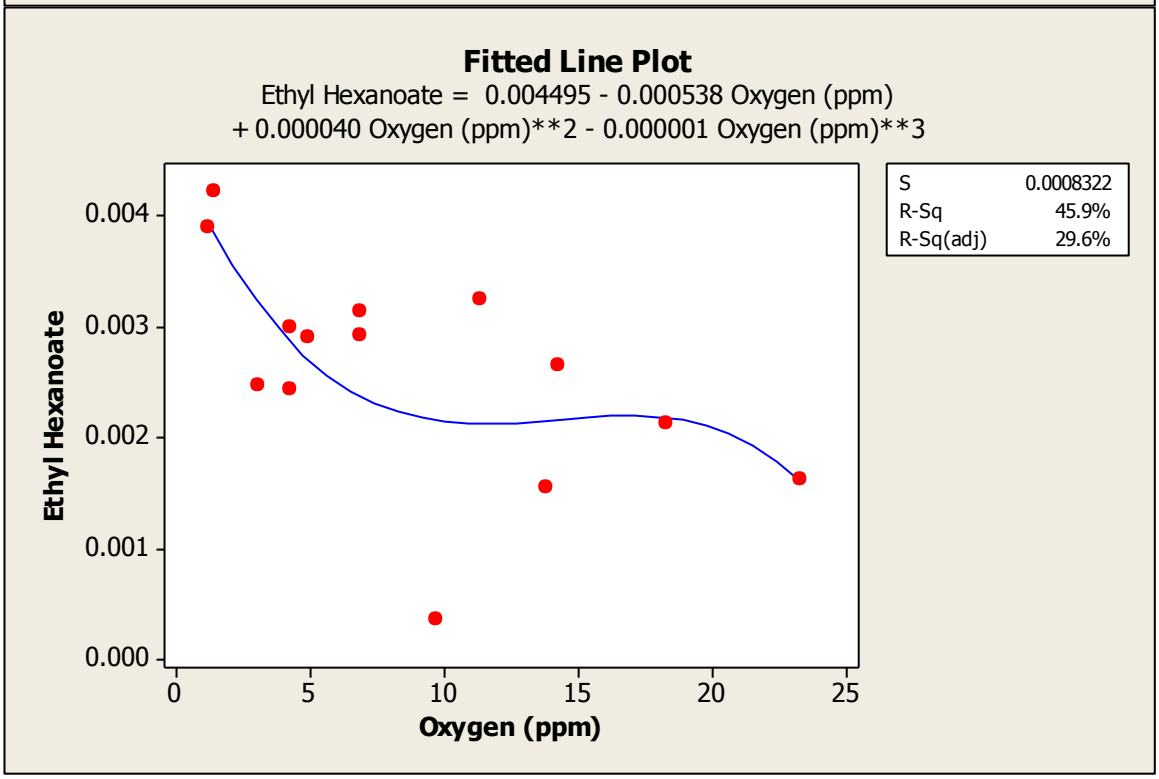
Source	DF	SS	MS	F	P
Regression	1	4.22864E-06	4.22864E-06	5.92	0.032
Residual Error	12	8.56794E-06	7.13995E-07		
Total	13	1.27966E-05			

Unusual Observations

Obs	Oxygen (ppm)	Ethyl Hexanoate	Fit	SE Fit	Residual	St Resid
13	23.3	0.001622	0.001367	0.000559	0.000255	0.40 X
21	9.6	0.000355	0.002538	0.000228	-0.002183	-2.68R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.


Residual Plots for Ethyl Hexanoate
Normal Probability Plot
Percent
99
90
50
10
1
-0.002
-0.001
0.000
0.001
0.002
Residual
Versus Fits
Residual
0.001
0.000
-0.001
-0.002
0.0015
0.0020
0.0025
0.0030
0.0035
Fitted Value
Histogram
Frequency
4.8
3.6
2.4
1.2
0.0
-0.0020
-0.0015
-0.0010
-0.0005
0.0000
0.0005
0.0010
Residual
Versus Order
Residual
0.001
0.000
-0.001
-0.002
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Observation Order


Fitted Line Plot
Ethyl Hexanoate = 0.004495 - 0.000538 Oxygen (ppm)
+ 0.000040 Oxygen (ppm)**2 - 0.000001 Oxygen (ppm)**3
S 0.0008322
R-Sq 45.9%
R-Sq(adj) 29.6%
Ethyl Hexanoate
0.004
0.003
0.002
0.001
0.000
0
5
10
15
20
25
Oxygen (ppm)

Regression Analysis: Phenyl Ethanol versus Oxygen (ppm)

The regression equation is
Phenyl Ethanol = 6.31 - 0.0319 Oxygen (ppm)

14 cases used, 16 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	6.3078	0.6169	10.22	0.000
Oxygen (ppm)	-0.03190	0.05680	-0.56	0.585

S = 1.35983 R-Sq = 2.6% R-Sq(adj) = 0.0%

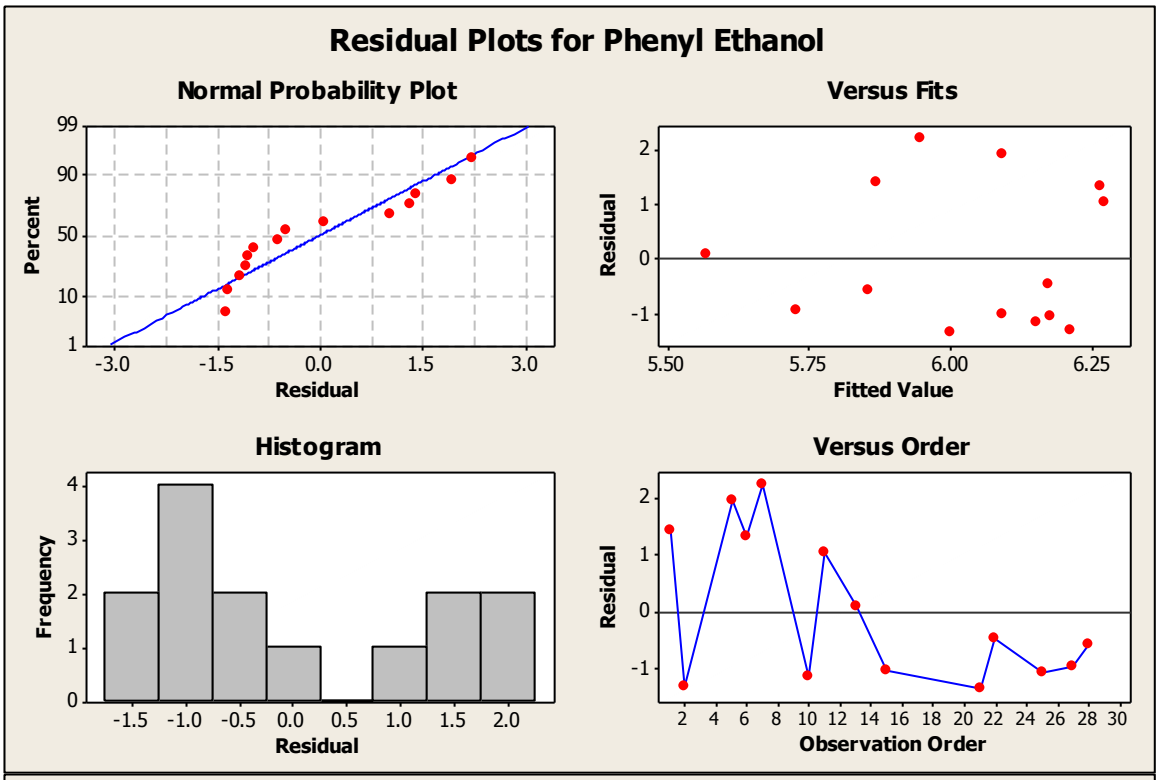
Analysis of Variance

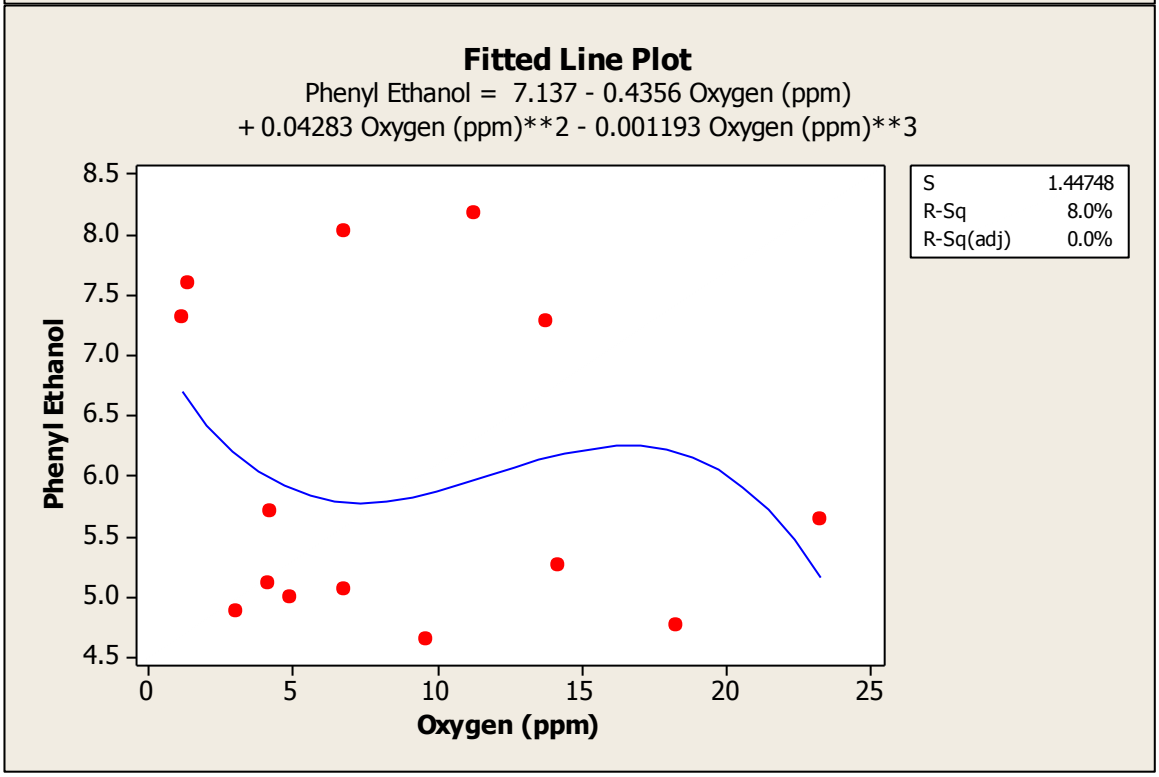
Source	DF	SS	MS	F	P
Regression	1	0.583	0.583	0.32	0.585
Residual Error	12	22.190	1.849		
Total	13	22.773			

Unusual Observations

Obs	Oxygen (ppm)	Phenyl Ethanol	Fit	SE Fit	Residual	St Resid
13	23.3	5.640	5.566	0.900	0.074	0.07 X

X denotes an observation whose X value gives it large leverage.


Residual Plots for Phenyl Ethanol
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Fitted Line Plot
Phenyl Ethanol = 7.137 - 0.4356 Oxygen (ppm)
+ 0.04283 Oxygen (ppm)**2 - 0.001193 Oxygen (ppm)**3
S 1.44748
R-Sq 8.0%
R-Sq(adj) 0.0%
Phenyl Ethanol
Oxygen (ppm)

Regression Analysis: Ethyl Octanoate versus Oxygen (ppm)

The regression equation is
Ethyl Octanoate = 0.00535 - 0.000029 Oxygen (ppm)

14 cases used, 16 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	0.005355	0.001161	4.61	0.001
Oxygen (ppm)	-0.0000287	0.0001069	-0.27	0.793

S = 0.00255895 R-Sq = 0.6% R-Sq(adj) = 0.0%

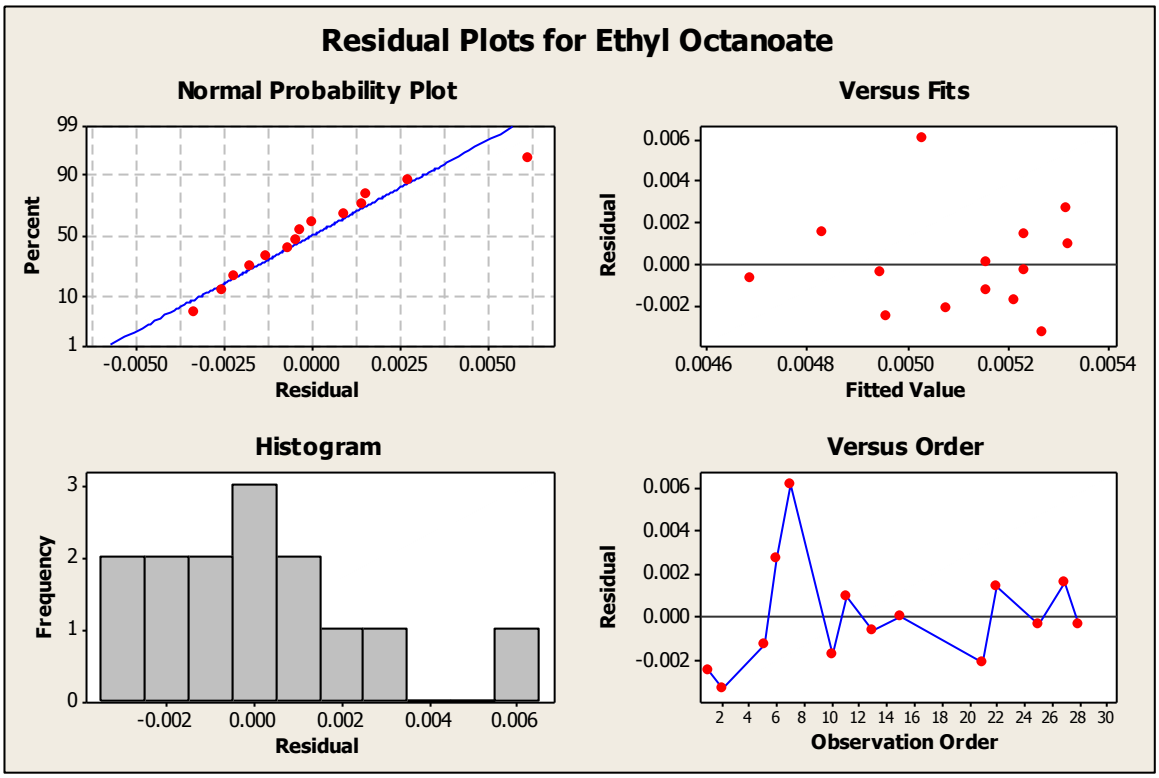
Analysis of Variance

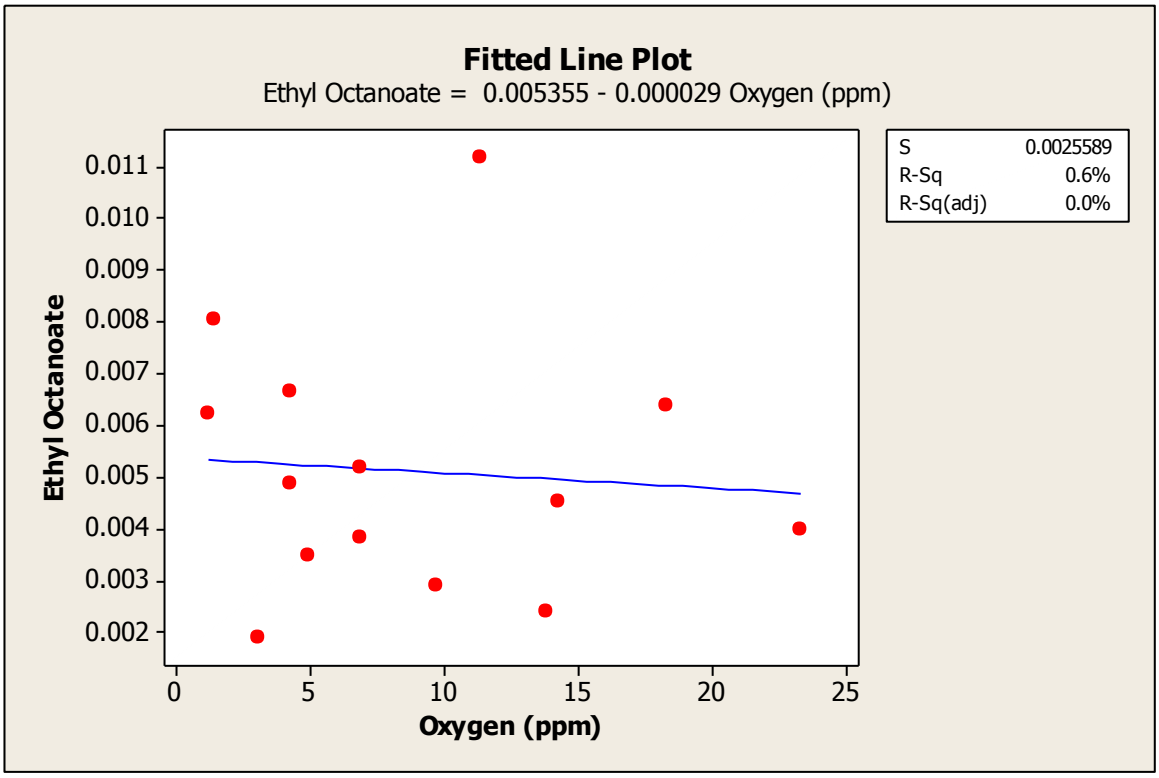
Source	DF	SS	MS	F	P
Regression	1	0.000000474	0.000000474	0.07	0.793
Residual Error	12	0.000078579	0.000006548		
Total	13	0.000079052			

Unusual Observations

Obs	Oxygen (ppm)	Ethyl Octanoate	Fit	SE Fit	Residual	St Resid
7	11.3	0.011154	0.005030	0.000735	0.006124	2.50R
13	23.3	0.003993	0.004686	0.001693	-0.000693	-0.36 X

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.


Residual Plots for Ethyl Octanoate
Normal Probability Plot
Percent
99
90
50
10
1
-0.0050
-0.0025
0.0000
0.0025
0.0050
Residual
Versus Fits
Residual
0.006
0.004
0.002
0.000
-0.002
0.0046
0.0048
0.0050
0.0052
0.0054
Fitted Value
Histogram
Frequency
3
2
1
0
-0.002
0.000
0.002
0.004
0.006
Residual
Versus Order
Residual
0.006
0.004
0.002
0.000
-0.002
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Observation Order


Fitted Line Plot
Ethyl Octanoate = 0.005355 - 0.000029 Oxygen (ppm)
S 0.0025589
R-Sq 0.6%
R-Sq(adj) 0.0%
Ethyl Octanoate
0.011
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0
5
10
15
20
25
Oxygen (ppm)

Regression Analysis: Ethyl Decanoate versus Oxygen (ppm)

The regression equation is
Ethyl Decanoate = 0.00185 - 0.000047 Oxygen (ppm)

14 cases used, 16 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	0.0018515	0.0002336	7.93	0.000
Oxygen (ppm)	-0.00004654	0.00002151	-2.16	0.051

S = 0.000514871 R-Sq = 28.1% R-Sq(adj) = 22.1%

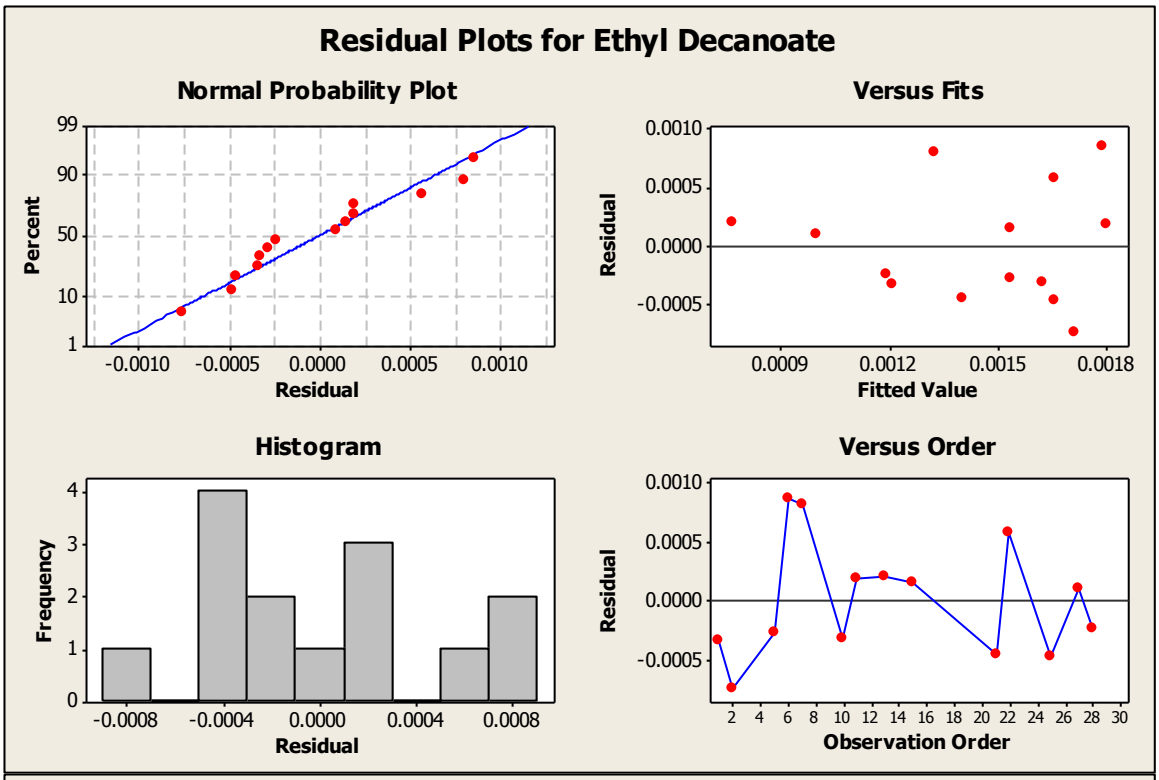
Analysis of Variance

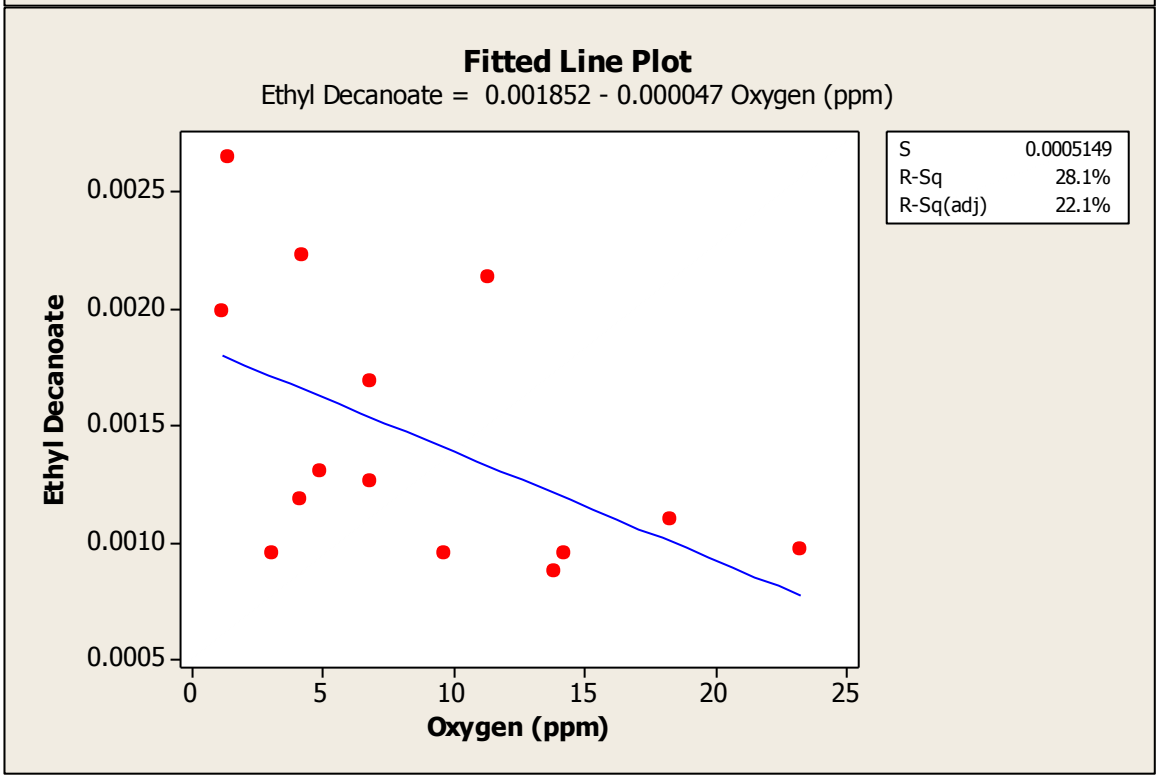
Source	DF	SS	MS	F	P
Regression	1	1.24118E-06	1.24118E-06	4.68	0.051
Residual Error	12	3.18111E-06	2.65093E-07		
Total	13	4.42229E-06			

Unusual Observations

Obs	Oxygen (ppm)	Ethyl Decanoate	Fit	SE Fit	Residual	St Resid	
13	23.3	0.000963	0.000769	0.000341	0.000195	0.50	X

X denotes an observation whose X value gives it large leverage.





General Notes:
On my third analysis it has become increasingly apparent that some of these volatiles do not belong in this category and that outliers of them may not have any effect on the actual

esters that I am viewing. I will run a best subsets on the entire group, as well as look at the general chemistry of the volatiles to determine their categorization.

Should probably remove 7(11.3)

Categories
Alcohols:

- 1-Propanol
- Isoamyl Alcohol
- 2-Methyl-1-butanol
- Phenyl Ethanol