Investigation of Yeast Selection and Pitching Rate for Fiero Coconut Rum Porter

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Abstract

This MQP worked with Purgatory Beer Co. to study the product quality and flavor inconsistencies in their Fiero Coconut Rum Porter. The project goal was to study how different yeast strains and pitching rates effected the flavor profile of the beer. Three different yeast strains and three different pitching rates were tested on a lab scale using the Fiero Coconut Rum Porter recipe. The alcohol by volume was measured and gas chromatography analysis was used to identify chemical compounds in the beer that contribute to the flavor profile, which was then used to compare the yeast strains and pitching rates. Results showed that inconsistencies in beer flavor are more likely to occur when using low pitching rates and the probability of inconsistency changes depending on the yeast strain used.

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Introduction

Beer is an alcoholic beverage consumed by various communities from around the world. In 2017, the U.S. sold approximately $111.4 billion worth of beer either from importation, commercialized domestic production, or private craft brewing production (Brewers Association, 2019). In the past few decades, America's fascination with beer has swept the nation. This has led to an increase of breweries, microbreweries, craft breweries, and home brewing amongst U.S. constituents everywhere. This boom of consumption inspired brewers to make beer better, tastier, and easier to make. New recipes are tested every day at local breweries as they compete against each other and national brands to brew the best beer, as they adapt to the changing culture. Although brewing is a form of art that can take years to perfect, this paper examines the scientific methods in which brewing can be studied and improved.

At the base of its core, the creation of beer is a scientific process. It involves the culmination of ingredients that spark various chemical processes to create alcohol, aromas, and flavors. Like any other chemical process, beer has reagents and products that can be examined and altered. Beer is like any other resource that can be purified, analyzed, and reinvented to fit a higher standard. This can vary from examining grain size, hops, and the carbonation of beer and beyond. This paper will focus on the fermentation of sugars by yeast in the brewing process and how it effects the flavor profile of a coconut rum porter beer. The analysis includes a look at different types of yeast strains as well as altering the yeast dosing, also referred to as the pitching rate. The concept behind this project is that altering the yeast pitching rate and strain will affect the chemical composition of beer after fermentation. Different chemical compositions indicate changes in the flavor profile of the porter in accordance with changes to yeast strain and pitching rate and can be related to the ability to brew consistently.

By conducting this analysis, it is possible to ascertain the necessary chemical compounds to create the desired flavor profile. It also indicates how these variables can affect the fermentation of beer and the consistency between batches. The goal of this analysis is to assist our sponsor, Purgatory Beer Co., in refining their Fiero Coconut Rum Porter.

Background

Brief History of Beer & Brewing

The art of brewing has been around for a very long time. Historians date brewing back to over 5,000 years ago during Mesopotamian times (Craft Beer & Brewing, n.d.). Beer has been an integral part of civilization since the very early years of man, providing nutrients and calories, while also creating mood-altering effects. Since its invention, it has flourished into the cultural phenomenon it is now in America. The U.S. claims to be the "brewing capital of the world" since it has more breweries than any other country (Great Fermentations, 2016). In 2017, approximately 74.90 liters of beer were consumed by the average American, which comes to about 211 standard drinks in a year (Armstrong, 2018). Beer is also economically vital with an industry employing nearly 2.23 million Americans either directly or indirectly (Beer Institute, 2017). Additionally, in 2016, the combined economic impact of the beer industry totaled around $350 billion (Beer Institute, 2017). With these statistics in mind, it seems impossible to ignore beer and everything it has to offer to the world. Given the history and popularity of beer, the art of brewing will continue to be practiced for years to come.

Breweries are constantly coming up with new and improved ways to make beer. This can range from experimenting with different flavors, altering the yeast, or improving the process. For most people, brewing follows the same steps anywhere you go. It starts with crushing malt, also known as grain, to release sugars and turn it into something called grist (von Siebenthal, 2014). The grist is then transferred to a vat of hot water and churned so that the malt's starches can be further broken down into sugar. Next, breweries will transfer the mixture into a lauter turn where the liquid is separated from the grain, this liquid is called wort. This sugary liquid is moved to a kettle where it is heated to a boil and hops are added. Hops are used to add bitterness to the beer to enhance and build the flavor profile. Once cooled, the wort is moved to a whirlpool where the malt and hops particles are separated. The wort is then ready for fermentation. Yeast is soon added to the wort to convert the sugars in the wort into alcohol, CO_2, and other compounds that provide the beer with its delicious flavor. The length of this process can vary depending on the type of yeast and desired smoothness of the beer (von Siebenthal, 2014). While the process may seem simple enough, breweries will spend years perfecting their recipes and altering them to come up with new beers.

Fermentation Process

Yeast Variations

Yeast is a single-celled microorganism that is classified as a fungus. It wasn't until the 17th century that yeast was identified by Antoni Van Leeuwenhoek for the first time. Throughout the 18th and 19th centuries chemists studied yeast and the role it played in beer and bread. In 1876, French chemist Louis Pasteur experimentally proved that living yeast can convert sugar into alcohol when oxygen is not present (Alba-Lois, L., & Segal-Kischinevzky, C., 2010). This changed the way people thought of brewing beer and inspired further investigation of yeast in the brewing process. In modern brewing, most brewers will purchase purified strains of yeast from mass manufacturers (All About Beer, n.d.). These mass manufacturers have identified the typical flavors produced by each strain and which strains lend themselves to typical styles such as IPAs, English ales, or porters and stouts. Brewing has transformed into an art and learning how the fermentation process works

and how different strains of yeast add flavor to each batch helps the brewer decide what sort of flavor profile they want to achieve.

There are two forms of beer that can be brewed: ales and lagers. These are the only two types of fermented beer, any other styles are subsets of each. Ale yeast is referred to as "top-fermented" yeast as it settles on top of the wort and is said to provide more flavor than a lager yeast. Lager yeast is referred to as "bottom-fermented" because it settles at the bottom of the beer (VineStaff, n.d.). Lager yeast fermentation occurs at a much lower temperature causing the fermentation reaction time to elongate. This type of beer has fewer complex flavors than ale yeast and provides a crisper and cleaner flavor profile (VineStaff, n.d.). Technically, wild yeast can also be used to brew craft beers to create new flavor profiles (VineStaff, n.d.).

Flavoring Effects of Yeast

The fermentation of beer is initiated and driven by pitching yeast. Pitching is the process of adding yeast to wort to start fermentation and pitching rate is the number of yeast cells added per volume of wort (The Oxford Companion to Beer, n.d.). Fermentation is the process during which yeast converts the glucose (sugar) in the wort into ethanol and carbon dioxide gas. This process gives the beer both its alcohol content and carbonation (Nice, 2019). Through the metabolic process of yeast, many different compounds are produced in beer that contribute to its complex flavor profile. Each individual strain produces different compounds characteristic of that strain because of their genetic makeup and metabolic pathways. Compounds that contribute to flavor, like higher alcohols are formed either by anabolism or catabolism of amino acids, while other chemicals like esters are formed "by enzymatic condensation of organic acids and alcohols" (Pires, 2014).

Many of these chemicals produced during yeast fermentations have significant effects on the flavor profile of the beer. For instance, esters tend to impart certain fruity flavors, such as apple and cherry. The esters that cause this flavor are sometimes the same as those found in the fruits themselves. In addition, higher alcohols also contribute significantly to flavor and often correspond to the flavor profiles of similar esters. For example, isoamyl alcohol and isoamyl acetate both are related to a fruity banana flavor and are very common among beers (Yeast, n.d.).

Another example of flavor inducing chemicals are phenols, which can give beer flavors reminiscent of smoky wood/burnt charcoal, and aromas such as peppercorn and clove. These are only a few examples of the types of compounds that give beer its flavor (Yeast, n.d.). There are many other factors that affect the flavor of beer, including the temperature of the fermentation vats, fermentation time, variety of yeast and the amount of yeast being used. This is explicitly seen when comparing between ale and lager yeast. Fermentation with ale yeast is generally performed at temperatures between 60 and 75 degrees and gives the beer a richer fruitier taste. Lager yeast is generally fermented between 45 and 60 degrees, and yields a crisp, clean and less complex flavor profile. The range of temperatures seen for the two yeasts is a great example of the variability of creating a unique flavor profile (Desalle., Tattersall, & Wynne, 2019).

Storage

Properly storing yeast is important, as poor yeast cultures will jeopardize the quality of the beer being produced. Yeast are living cells, and typically are stored in a chilled environment to minimize deterioration and slow down metabolism. The most important factor affecting the health and quality of the yeast is the yeasts glycogen reserves. The cells glycogen content indirectly affects its ability to absorb nutrients and perform a quality fermentation. Glycogen is what the yeast relies on for an energy source during periods of starvation. If metabolism is not slowed dramatically during storage, glycogen reserves quickly become depleted. This renders the yeast unsuitable for fermentation. For this reason, yeast should be kept below 34 degrees Fahrenheit as to slow the metabolism of the yeast and preserve the glycol within the cells. Other conditions to avoid during storage are exposure to oxygen and agitation, both of which stimulate yeast metabolism. Some other keys to yeast storage are to keep the yeast sealed to avoid oxygen exposure and keep in a place with little traffic to avoid agitating the yeast. (Yeast Storage, n.d.).

What Makes a Porter

A porter is a dark, malty style of beer which originated in England during the 1700s. It is classified as an ale. There are two main types of porters: an English Porter and an American Porter. English porters are either a brown style, with a more malty than hoppy flavor and an ABV of 4-5.4%, or a robust style with roasted or black malt, a heavy hop flavor, and ABV of 4.8-6.5% (VineStaff, n.d.). American porters are typically characterized by their dark brown, almost black color with an alcohol by volume (ABV) between 5% and 7.5% and are often characterized by warmer flavors such as dark chocolate, coffee, caramel, or licorice.

Purgatory Beer Company

The Purgatory Beer Company is a small craft brewery located in Whitinsville, MA and was founded in 2017 with one goal: make amazing beer. They are a small operation but are growing in their current popularity. One of the most popular beers they produce is the Fiero Coconut Rum Porter, which is a coconut, rum, and chocolate flavored beer. This beer has presented a challenge for Purgatory Beer Company, every time it brews it has been inconsistent in quality and taste. Despite its inconsistency, the Fiero Coconut Rum Porter is a staple in the tap room and one of their most popular beers.

To make the Fiero Coconut Rum Porter, a typical porter wort is fermented with Wyeast Irish Ale yeast for 2-3 weeks. The beer is flavored by adding a silk steeping bag filled with rum soaked roasted coconut and cacao nibs after 1-2 weeks of fermentation. The beer, like most typical porters, is fermented between 65 and 70°C. Fermenting at a temperature any higher than this could stress the yeast and cause further inconsistencies.

The Problem

The current problem with the Fiero Coconut Rum Porter that Purgatory Beer Co. is making is that the flavor is inconsistent with each batch which implies differences in the overall chemical composition. When at its best, the porter is full bodied and contains warm flavors of coconut, dark chocolate, and a hint of rum. Other times the porter can be overly sweet and has been compared to coca cola. We will be studying how different yeast pitching rates and strains impact the chemical

profile of the Fiero Coconut Rum Porter. This will allow us to determine which chemicals impact the flavor of the Fiero Coconut Rum Porter and whether our variables could be causing the inconsistencies between batches.

Methods

This project aimed to test varying parameters of the brewing process to discern a cause for the inconsistency in taste for the Fiero Coconut Rum Porter at Purgatory Beer Co.

Design of Experiments

The parameters chosen to analyze were yeast strain and pitching rate. Yeast is one of the main four components of beer and was selected as a parameter because the strain of yeast used for fermentation has a significant effect on the flavors present. For example, Wyeast Irish Ale 1084 produces a dry, crisp profile with subtle fruitiness while Wyeast London Ale III 1318 produces a fruity, very light and softly balanced palate that finishes slightly sweet. While both strains are commonly used for porters and stouts, they can make significant differences in the subtleties of the beer. The pitching rate was also selected as a parameter to investigate how precise the yeast measurements must be to maintain consistency in taste and alcohol content.

After selecting the parameters, the testing procedures were defined. Three yeasts traditionally used for porters and stouts were selected: Wyeast Irish Ale 1084, Wyeast London Ale 1028, and Wyeast London Ale III 1318. Each strain was used to brew at three doses as shown in Table 1.

Table 1: Yeast Pitching Rates

Dose	Pitch Rate (million cells /mL of wort)
1	0.1127
2	0.2208
3	0.4417

Brewing Method

All brewing was executed using 16 oz mason jars outfitted with a water trap sealed to the lid of the jar to allow for CO_2 to leave the system without allowing outside bacteria or other contaminants in. Each jar was filled with 12 oz of refrigerated wort and the designated dosing of yeast. The water traps were filled with sink water to just below the “max” lines and the lids were sealed. The jars were left to ferment for 14-20 days at 60-70 °F. After 7-10 days of fermentation the flavoring components were added. To prepare the flavoring, roasted unsweetened coconut and roasted cacao nibs were soaked in white rum for 3 days. The coconut and cacao nibs were then drained and partitioned into steeping bags to be tied off and placed in the brewing jars. Once the steeping bags were added to the jars, they continued fermenting for 3-4 more days. After the beer finished fermenting, it was tested to determine the alcohol content and chemical composition.

Testing Methods

Two tests were conducted on the beer to measure the alcohol by volume (ABV) test and gas chromatography (GC) test. The specific gravity of the beer was taken before and after fermentation to determine the ABV and GC tests were taken after fermentation to determine the chemical composition of the beer, See Appendix I: Procedures for ABV and GC testing procedures.

Results & Discussion

Common Compounds and Flavors

Analysis of the GC results obtained throughout this project have showed that there were 14 chemical compounds that were common to each of the trials. Data were inconsistent on two of these compounds. Data for the other 12 compounds were selected because they were found in all the trials, which makes them a good measure of consistency. These 12 compounds were used to compare trials to each other as well as to the control samples of good and bad beer given to us by Purgatory Beer Co.

The first component that was studied was the flavor profile of the beer created by these compounds. Chemical compounds have different flavors and aromas based on what kind of organic compound they are, and the amount of compound present. Of the 12 compounds identified, five were identified to relate to a common flavor or aroma. The first four of these five compounds were all higher alcohols, also known as fusel alcohols. The first chemical compound analyzed was isoamyl alcohol. Isoamyl alcohol contributes to both the flavor and aroma of the beer. It is known for giving off either a fusel whiskey or molasses aroma and giving the beer a cognac or fermented flavor. Cognac is a flavor that can be described as tasting like earthy black truffle or vanilla. The second compound analyzed was phenylethyl alcohol which can give beer a floral or bready aroma and taste. Another compound identified was isobutanol, which can give the beer an ethereal winey or fusel whiskey taste. Active amyl alcohol was another common compound and can contribute a balsamic or sweet aroma to the beer. It gives the beer a fermented and bready taste (Flavor, Fragrance, Food and Cosmetics Ingredients Information). The final compound analyzed was ethyl acetate. This compound is an ester and contributes a fruity “solvent like” flavor. The “solvent like” flavor has three sub flavors, those being “plastic like”, "can-liner" and "acetone like" flavors. Ethyl acetate doesn’t contribute much to the aroma of the beer.

Only five of the 12 common compounds identified were able to be related to flavors and aromas. The other seven compounds were not present in a high enough concentration to be able to determine what kinds of flavors they contributed to the beer, if any. This may also be due to the testing method used. Typically, the GC is very good at distinguishing higher alcohols, but maybe not other compounds that are also contributing to the flavor profile. As seen in the paragraph above, many compounds have more than one flavor associated with them. Some flavors have sub flavors as well. The means to determine which specific chemical resulted in which specific flavor or sub flavor was in the beer produced was not feasible for this project.

Yeast Strain

Comparing yeast strain is important because the metabolic pathways of each yeast strain are different and therefore produce different flavor inducing compounds. The yeast strains, Irish Ale, London Ale, and London Ale III were chosen because all are typically used to brew porters and stouts. All three are characterized by their mild fruitiness and esters produced during fermentation. According to Wyeast, the Irish Ale strain produces a crisp, dry flavor when fermented at temperatures below 64°F, but when fermented at temperatures above 64°F, stronger fruit and ester flavors are detected. The London Ale is described to produce “a rich mineral profile that is bold

and crisp with some fruitiness." Lastly, the London Ale III is used for a malt and hop profile with a light and soft fruitiness and slightly sweet finish.

Trial three was used to compare yeast strains because the compounds found in that trial were most closely related to the "new beer" that we used as the good control of the Fiero Coconut Rum Porter. Chemical compounds that showed up exclusively in the three pitching rates tested of each strain were identified and used to characterize the flavor profile of the beer in relation to the Fiero Coconut Rum Porter. See **Error! Reference source not found.** below for the compounds uniquely identified in each of the yeast strains.

Table 2: Organic Compounds Unique to each Yeast Strain and Their Associated Flavor in Trial 3

Yeast	Compound	Description
Irish Ale	Amylene hydrate (tert-amyl alcohol)	An amyl Alcohol which is a higher alcohol and increase the solvent and fruitiness taste.
	L-Proline, 5-oxo-, methyl ester	An ester derived from an amino acid; l-proline derivative and alpha-amino acid ester.
	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	An ester
London Ale	2-Isopropyl-5-methyl-1-heptanol	A primary alcohol in the isopropyl group and derives from a hydride of a heptane. It has role in human metabolite and is an anti-fungal compound.
	Hexane, 2,3,4-trimethyl-	A branched alkane found in fats and oils, isolated from the sunflower.
London Ale III	1-Heptanol, 2,4-diethyl-	A higher alcohol
	Octane, 3,3-dimethyl-	An alkane
	Propyne	An organic compound sometimes used as a rocket fuel. Has a sweet odor.

London Ale III did not show any new ester formations specific to that strain, but it increased in higher alcohol content, specifically in 1-Heptanol, 2,4-diethyl-. It also showed an increase in alkane and other organic compounds with Octane, 3,3-dimethyl- and Propyne. Research did not indicate that Octane, 3,3-dimethyl- had a flavor profile but while propyne has a sweet odor, it was identified to be an organic compound found in jet fuel (National Center for Biotechnology Information, n.d.).

The London Ale I showed an increase in higher alcohols and alkanes. 2-Isopropyl-5-methyl-1-heptanol is an alcohol that plays a role in human metabolite and is known to have medicinal, anti-fungal properties (Mannaa, Mohamed & Kim, Ki Deok, 2018). Hexane, 2,3,4-trimethyl- is a branched alkane found in fats and oils and can be isolated from the sunflower (Human Metabolome Database. 2019).

The Irish Ale produced two additional esters as indicated by the GC, L-Proline, 5-oxo-, methyl ester and pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl ester. In addition, amylene hydrate was found to be significant to the Irish Ale. Amylene hydrate is an amyl alcohol, a higher alcohol, that

increases in solvent and fruity flavor profiles. The L-Proline, 5-oxo-, methyl ester is most likely produced from the metabolic pathway of the Irish Ale, contributing to the unique flavor profile of the strain. The significant amount of increased ester compounds in the Irish Ale compared to the other yeast strains, with the addition to the amyl alcohol, adds complexity to the flavor of the beer. This additional complexity could indicate why there were large variations in Fiero Coconut Rum Porters brewed at Purgatory Beer Co. between batches.

Pitching rate

One of the variables studied was the yeast pitching rate. Each strain of yeast was pitched at three rates of 0.1127, 0.2208, and 0.4417 million cells per mL of wort. Note that Purgatory Beer Co. uses a pitching rate of 0.3997 million cells per mL of wort.

According to Wyeast, the recommended pitching rate is 5.25-6 million cells per mL of wort for an Original Gravity below 1.060 (WyeastLabs.com, n.d.). Other sources recommend even higher doses, especially when aiming for a higher ABV (Brewersfriend.com, 2012; Dawson, n.d.). At the current size of operation, 132.2 gallons of wort per batch, this would require 30 packages of 125mL Wyeast Activator smack-packs. All our trials were completed at or near the pitching rate used by Purgatory Beer Co.

Literature indicated that a lower pitching rate would allow the yeast to have a longer growth phase and a higher rate of reproduction which would produce more esters and therefore a fruity, "funky", and "yeastier" flavor profile (Dawson, n.d.) but also more inconsistencies between batches (Brewersfrend.com, 2012). Higher pitch rates were expected to have a cleaner flavor profile and more consistent products due to lower growth rates and less reproduction, leading to older cells which can produce off flavors (Brewersfriend.com, 2012; Dawson, n.d.; Kucharczyk & Tuszynski, 2015).

Across the three rates tested, there were no discernable trends in the GC data. The inconsistency between batches was expected based on the literature and Purgatory Beer Co.'s experiences. This was consistent across all yeast strains, except for 1-butanol-3-methyl produced by London Ale I yeast. In this instance, the area under the curve increased as the pitching rate increased across all three trials.

As previously mentioned, many of the identified compounds which were connected to flavors were higher alcohols. The GC data of these compounds did not indicate a correlation between the concentration and yeast pitching rate. This correlates to similar studies by Verbelen et al, 2009 and Kucharczyk & Tuszynski, 2015 because the synthesis of higher alcohols is related to the growth of yeast and amino acid metabolism through the Ehrlich pathway (Hazelwood, Daran, van Maris, Pronk, & Dickinson, 2008). There may be trends present in compounds which were not registered by the GC test. Other compounds could be analyzed by using a different solvent for GC, or solid-phase microextraction (Horák, 2010).

Steeping During and After Fermentation

One hypothesis for why the Fiero Coconut Rum Porter wasn't brewing consistently was that undesired reactions occurred between the fermenting yeast and the coconut, rum, and cacao nibs while it steeped. The flavors were steeped while yeast fermented for all yeasts and pitching rates.

For trial 2 and 3 an extra batch was created at dose 2 where the rum soaked coconut and cacao nibs were steeped after removing the yeast. This extra batch fermented for the same length of time as all other batches within the trial. Figure 1-Figure 3 show the concentrations of each compound when brewed with yeast dose 2. Trial 1 had consistently higher concentrations of each compound across all three yeast strains. This could be because trial 1 fermented for the longest length of time, 18 days. This trial also had the highest ABV, so we can assume that the other trials could have reached higher concentrations had they fermented longer.

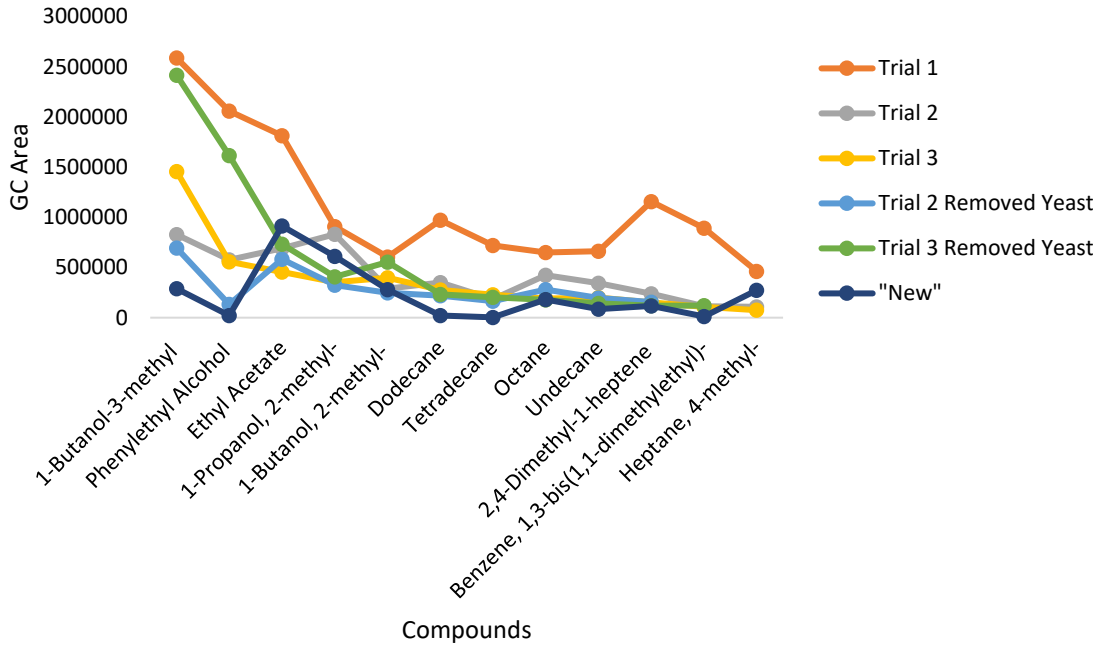

Figure 1: Comparison of area under the GC curve for all D2 trials with London Ale I Yeast

For the London Ale I yeast, trial 2 with removed yeast had consistently lower GC data than other trials, but the contour of that trial matches most closely with the “new” batch brewed by Purgatory Beer Co.

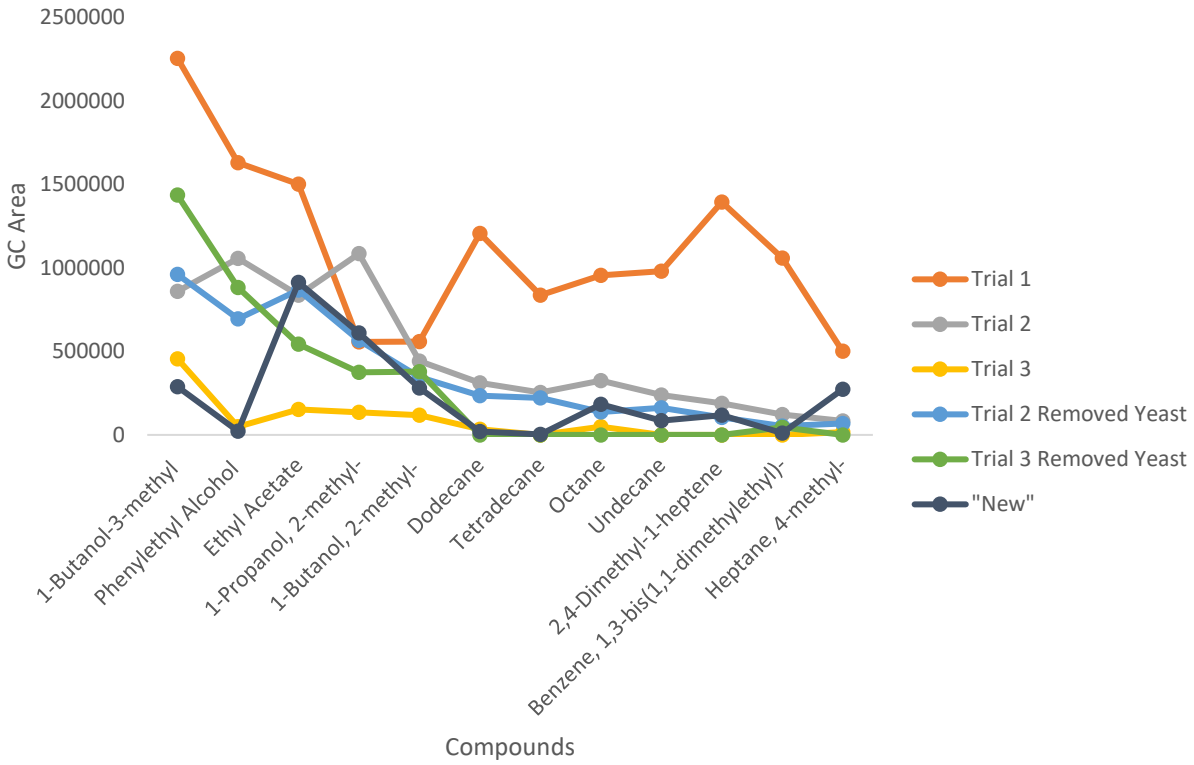

Figure 2: Comparison of area under the GC curve for all D2 trials with London Ale III Yeast

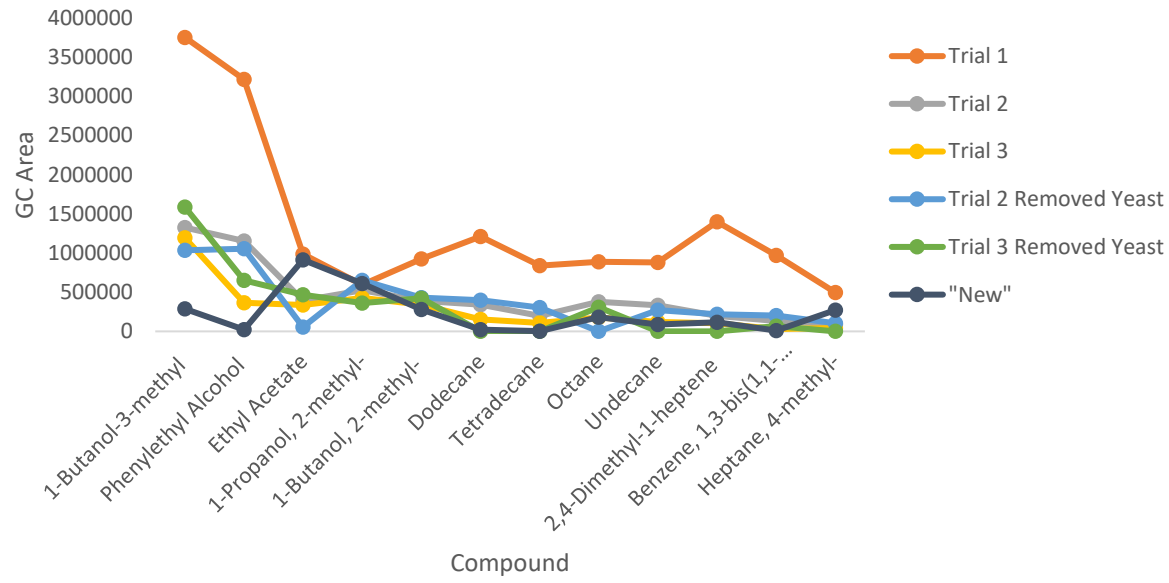

Figure 3: Comparison of area under the GC curve for all D2 trials with Irish Ale Yeast

There was a lack of consistency among all trials and no clear trends emerged through removing the yeast prior to steeping flavoring. This could be because there are fewer compounds produced

exclusively while the yeast consumed the sugars from the flavoring. Although these trials did not indicate a trend, further research could be done to evaluate how removing the yeast prior to flavoring could affect the reusability of yeast. Previously, Purgatory Beer Co. could not reuse the yeast from the Fiero Coconut Rum Porter because the yeast showed decrease in growth and metabolism after ingesting the non-traditional sugars introduced by the flavoring. If removing yeast has minimal effect on the flavor of the beer, it could result in lower material costs through reuse of yeast for future brews.

Comparison of "New" and "Old" Beer Samples

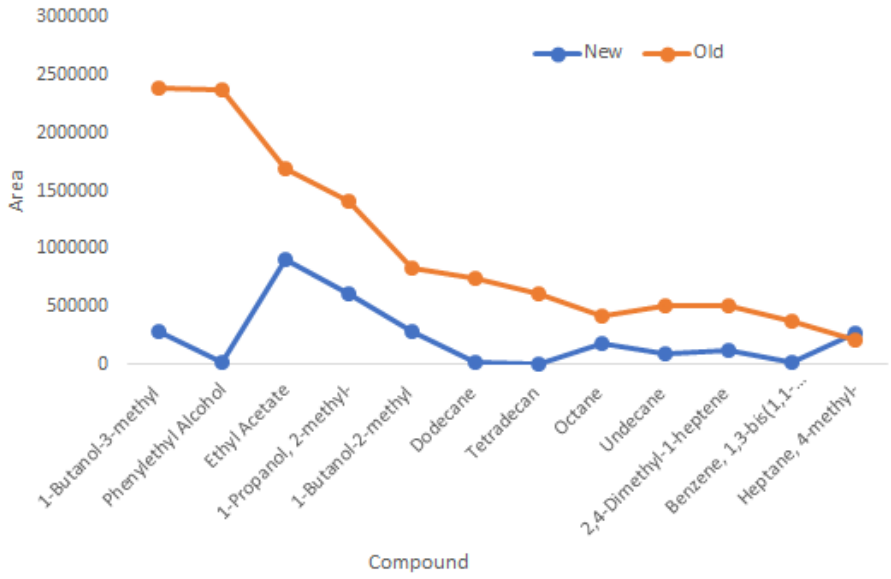

Figure 4: Trend of how the new and old beer samples compared to one another during GC analysis to identify various chemical compounds.

During experimentation, beer samples received from Purgatory Beer Co. were labeled as the "new" and the "old" beer. The "new" beer was qualitatively identified by Purgatory Beer Co. as a "good" batch of beer whose flavor profile appropriately matched the desired Fiero Coconut Rum Porter. The other sample received was the "old" beer sample which was identified as a "bad" batch of beer. During GC analysis, the old and new were analyzed along with the recreated beer batches which included parameter changes in pitching rate and yeast strain. Without individual concentration curves the actual concentration of each compound could not be discerned from the GC data. Instead, the difference between areas under each peak in the GC was used to determine the similarity between samples. In addition to comparing the homebrewed batches of the "new" and "old" beer samples, the new and old samples were compared to each other in Figure 4 above. It seemed that the biggest discrepancy between the samples was the concentration of each compounds in the respective samples. For instance, it appeared that the "old" had larger peaks of every chemical compound compared than the "new" batch. This could indicate that the reason the sample was deemed a "bad" batch was the higher concentrations of each compound could create a less desirable flavor profile. From this data, the recreated beer samples could be compared to the

"new" beer sample to find similar trends. Through this comparison, the homebrewed beer samples with lower concentrations of compounds similar to the amounts in the "new" beer sample could be deemed as "good" beer samples.

As mentioned previously, the GC data collected from the homebrewed beer were compared to the GC data collected from the "new" beer. This was done to determine which yeast strain and trial were most similar to the "good" batch brewed by Purgatory Beer Co. **Error! Reference source not found.** below shows the GC data for each yeast strain that are averaged for each chemical over the three homebrewed trials. The green highlighted cells represent the averages that appeared within ±15% of the "new" sample area of that chemical compound. It appeared that the Irish Ale (I) and London Ale I (L1) yeast strains were most closely related to the "new" sample areas that were analyzed.

Table 3: Comparison of GC Data between Each Yeast Strain Dose 3 and "New" Beer Sample

Compound	Homebrewed Yeast Strain (Area Under the Peak for Dose 3)			"New" (Area Under the Peak)
	I	L3	L1	
1-Butanol-3-methyl (Isoamyl Alcohol)	1529514	1358905.67	1863278	288930
Phenylethyl Alcohol	410813.3	847795.3	1588089.67	21997
Ethyl Acetate	572199.3	648482.3	791254.3	913180
1-Propanol, 2-methyl	532425.3	483674	550174.67	611215
1-Butanol, 2-methyl-	426981.3	397660.3	522788.67	280919
Dodecane6	226720	381477.67	699149.3	20568
Tetradecane	144643.67	293781	531467.67	2988
Octane	159113	478629.67	375490.67	182229
Undecane	159782.67	391653	494601	86040
2,4-Dimethyl-1-heptene	129884	349626	554809	117478
Benzene, 1,3-bis(1,1-dimethylethyl)-	71917.3	234006.3	410454.67	10594
Heptane, 4-methyl-	63436	135515.3	223155.3	272261

The Irish Ale appeared slightly more than the London Ale I, making it the most prevalent of all the yeast strains tested. This finding was aligned with our hypothesized yeast strain due to that the yeast used for the "new" beer was the Irish Ale. This is the yeast strain currently used by Purgatory Beer Co. to make the Fiero Coconut Rum Porter. What was unexpected was how similar the London Ale I chemical makeup was to the "new" beer sample. This finding indicates that the London Ale I could be a comparable strain to use instead of the Irish Ale. It is interesting to note that the London Ale III did not appear similar to the compound amounts that were present in the "new" beer sample. Testing on the yeast themselves could identify the reason for why this is, in addition to future analysis of how the London Ale III differs from the Irish Ale and London Ale I yeast strains. Recommendations for future testing are included in Appendix II: Recommendations for Further Projects to provide further details for this discrepancy.

Table 4: Comparison of GC Data by Trial to "New" Sample

Trial	Frequency of Appearance Closest to "new" sample
1	3
2	9
3	9
None	15

From the GC results, the areas of every batch of each strain and each chemical were examined and compared to the "new' sample areas. Since there were three different areas to compare to the "new" sample, the area of each batch that was *closest* to the "new" sample was picked and counted. This was done for every batch of every strain for each chemical compound. The full chart of these results can be seen in Appendix III. It appeared that Trial 2 and 3 had the most occurrences of areas being closest to the "new" sample areas. The value of this information is important to include in the recommendations section as the methodology for these batches will be the procedure that garnered the most similar results in a good batch of beer. A closer look at the area of chemical compounds compared between the "new" sample and Trial 2 and 3 can be seen below in Figure 1Figure 5.

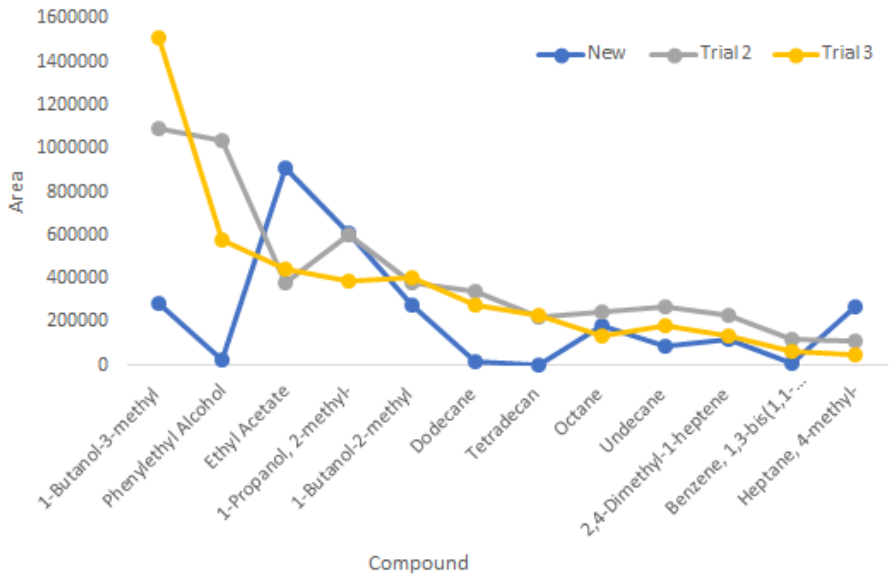

Figure 5: Comparison of GC Data between the Average Areas throughout each Strain in Batch 2 and 3 to GC Data of "New" Sample

After determining that Trial 2 and 3's GC data appeared most similar to the "new" sample, Figure 5 above was created to further compare the data. As seen above, Trial 2 and 3 overlap consistently with the "new" beer sample data. This is especially prevalent in the last four compounds where the areas are significantly closer. The initial trend, while not overlapping, is similar across the first

two compounds in Figure 5. The trend across the compounds phenylethyl alcohol, ethyl acetate, and 1-propanol, 2-methyl showed the greatest disparity between the two samples. However, the trend between both Trial 2 and 3 are extremely similar with 1-Propanol, 2-methyl- being the biggest disparity between the two. It would be recommended to further investigate these chemical compounds and how they can be improved upon in future testing to better match the trend line of the “new” beer sample.

Conclusion

The data collected throughout the project suggest that low yeast pitching rates and the consistency of the ingredients used are most likely to affect the consistency of the flavor profile. Using enough yeast ensures that most of the sugars in the wort are converted to alcohol and carbon dioxide. Keeping the ingredients consistent between batches is important, as using different ingredients could cause different chemical compounds to form and change the flavor profile of the beer. The yeast strain that was more likely to give inconstant results was the Irish Ale because it had greater variation in its flavor profile compounds. Lastly, taking the yeast out before steeping was inconclusive and no concrete findings can be proven in how it affects the brewing process.

Recommendations

1. From our research we recommend increasing the yeast pitching rate to a minimum of 6 million cells per mL of wort to minimize inconsistencies in the flavor profile between batches.
2. It was prevalent that throughout the process of recreating the Fiero Coconut Rum Porter that *consistency* in recipe was most important above all else. To test the beer appropriately and make concrete conclusions, the recipe must stay the same during the process. Changing coconut brands or types can affect the process and the number of chemical compounds that will be present. Additionally, changing the amount of the steeping product, pitching rates, yeast doses, or the temperature range will all affect the way in which the beer will turn out. Therefore, having a standardized recipe that does not change will make it easier to identify when things go poorly in the brewing process.

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Appendix

Appendix I: Procedures

Brewing

Set Up:

1. Drill a 1cm hole at the center of the lid to each mason jar.
2. Fix a plastic water trap to the hole using a water proof sealant such as gorilla glue or caulk.
3. Wash and sanitize jar, lid, screw cap, and water traps.

Procedure:

1. Pour 12oz of wort into each jar.
2. Using an automatic pipette, pipette the designated amount of yeast into the jar.

Dose	Pitch Rate (million cells /mL of wort)	mL of liquid yeast
1	0.1127	0.050
2	0.2208	0.098
3	0.4417	0.196

Repeat each dose once per yeast strain.

3. Fill and cap the water traps before sealing the jar.
4. Leave jars to ferment between 60 and 70°F for 14-20 days.
5. After 7-10 days, add steeping flavorings. To prepare to steep:

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5.1 OR pour beer into a fresh jar to separate from yeast before adding steeping ingredients.

a. Remove yeast and add flavoring on the same day that all other jars are done fermenting.

6. Reseal the jar and continue fermenting for 3-4 more days.
7. After fermentation is done, remove steeping bag and prepare beer for testing.

Alcohol By Volume

1. Using a graduated cylinder, place and read a hydrometer in 100mL of wort or beer.
2. The hydrometer reading in wort is the Original Gravity and the reading in the beer is the Final Gravity.
3. Use equation 1 to calculate the alcohol by volume (ABV).

$$ABV = (Original\ Gravity - Final\ Gravity) \times 131.25 \quad (1)$$

4. Test the final gravity of every jar. The same original gravity can be used for each jar with the same initial wort.

Gas Chromatography

1. Mix 7mL each of beer, dichloromethane, and deionized water with 3.15g of table salt in a 50mL centrifuge tube. Repeat for each jar.
2. Shake each tube for 10 minutes.
3. Centrifuge at 3500 RPM and 25°C for 10 minutes.
4. Extract the bottom layer using a syringe and filter through a polytetrafluoroethylene (PTFE) filter with 0.45μm pores.
5. Put filtered organic material into GC vials with porous caps and run GC. With the following settings:
    a. GC Settings:
        i. Injection port & injection heat port: INJ1
        ii. Injection Mode: Split
        iii. Column oven Temp: 30°C
        iv. Injection Temp: 290°C
        v. Carrier Gas: Helium
        vi. Prim Press: 500-900
        vii. Flow Control Mode: Pressure
        viii. Pressure: 14 kPa
        ix. Total Flow: 19 mL/min
        x. Column Flow: 0.62 mL/min
        xi. Linear Velocity: 28.1 cm/sec
        xii. Purge Flow: 3mL/min
        xiii. Split Ratio: 25
    b. Sampler Settings:
        i. # Rinses with Solvent Pre Run: 0
        ii. # Rinses with Solvent Post Run: 1
        iii. # Rinses with Sample: 2
        iv. Plunger Speed (Suction): High
        v. Viscosity Comp. Time: 0.2 sec
        vi. Plunger Speed (Injection): High

vii. Syringe Insertion Speed: High
viii. Injection Mode: 0:Normal

c. MS Settings:
    i. Ion Source Temp: 200°C
    ii. Interface Temp: 80°C
    iii. Solvent Cut Time: 0.5 min
    iv. Micro Scan Width: 0 u
    v. Detector Voltage: 0kV Relative to the Tuning Result
    vi. Threshold: 0
    vii. GC Program Time: 94 min

Appendix II: Recommendations for Further Projects

1. To maintain consistency during testing, the use of fresh materials and freshly cleaned equipment is critical. Yeast will stay active when kept refrigerated for a few weeks to months, but it may not be as effective or efficient as when it was first opened.
2. Due to the wide variation seen in the data collected compared to the "new" beer sample, it's recommended that more samples be collected for qualitatively "good" beer. There should be multiple beers brewed that are considered adequate that can be analyzed and compared to one another before the process is recreated by testers. This would reduce the assumptions made in the data collected and provide a larger range of possibility for which the data can be analyzed.
3. For future testing, start as early as possible, as more data will statistically improve the results collected and allow for more precise analysis while comparing batches and yeast strain to one another.
4. Additionally, testing for pH and analyzing the yeast culture may prove to be more informative as to why certain compounds form in the brewing process. The pH analysis would provide further insight into how the pitching rate or yeast strains affected the pH of the beer and how that could affect the flavor profile. The pH could also be used to relate or compare different trials and act as an indicator for consistency.
5. It is important that the fermentation process happens in a reasonably controlled environment. It is important for the yeast to be near optimal temperature for the duration of the fermentation, and changes in temperature or temperatures outside of the allotted range may affect the production of alcohol and carbon dioxide in the beer.

Appendix III: Raw Data

Table 5: Comparison of GC Data of Each Compound between "new", "old", and Dose 3 from Each Trial and Yeast Strain

Compound	Yeast Strain	Batch	"New"	"Bad"	D3 by Batch
1-Butanol-3-methyl (Isoamyl Alcohol)	L1	1	288930	2392373	1819507
		2			1077396
		3			2692931
	L3	1			1319747
		2			1032748
		3			1724222
	I	1			1391052
		2			1176638
		3			2020852
Phenylethyl Alcohol (2-phenylethanol)	L1	1	21997	2381827	939410
		2			1367461
		3			2457398
	L3	1			299401
		2			1013141
		3			1230844
	I	1			501441
		2			730999
		3			0
Ethyl Acetate	L1	1	913180	1695243	536726
		2			806471
		3			1030566
	L3	1			437347
		2			216864
		3			1291236
	I	1			351180
		2			121883
		3			1243535
1-Propanol, 2-methyl- (Isobutanol)	L1	1	611215	1407231	368990
		2			620342
		3			661192
	L3	1			317089
		2			640949
		3			492984
	I	1			477369
		2			542816
		3			577091
1-Butanol, 2-methyl- (active amyl alcohol)	L1	1	280919	831444	463083
		2			414401
		3			690882
	L3	1			354754
		2			374540
		3			463687
	I	1			396010
		2			358745
		3			526190

Dodecane	L1	1	20568	749382	259333
		2			462166
		3			1375949
	L3	1			220604
		2			233396
		3			690433
	I	1			354417
		2			325743
		3			0
Tetradecane	L1	1	2988	602572	225211
		2			352746
		3			1016446
	L3	1			193019
		2			166950
		3			521374
	I	1			277469
		2			156462
		3			0
Octane	L1	1	182229	415189	0
		2			282183
		3			844289
	L3	1			192732
		2			212667
		3			1030490
	I	1			224929
		2			252410
		3			0
Undecane	L1	1	86040	505288	162708
		2			348207
		3			972888
	L3	1			169594
		2			196448
		3			808917
	I	1			217298
		2			262050
		3			0
2,4-Dimethyl-1-heptene	L1	1	117478	508586	126025
		2			273776
		3			1264627
	L3	1			137359
		2			163872
		3			747647
	I	1			134176
		2			255476
		3			0

Benzene, 1,3-bis(1,1-dimethylethyl)-	L1	1	10594	378966	30704
		2			132758
		3			1067902
	L3	1			52435
		2			106050
		3			543534
	I	1			101320
		2			114432
		3			0
Heptane, 4-methyl	L1	1	272261	208799	63381
		2			116332
		3			489753
	L3	1			37166
		2			71565
		3			297816
	I	1			52869
		2			137439
		3			0

Table 6: Comparison of GC Data of Each Compound between "new", "old", Trial 2, and Trial 3 averages

Compound	Yeast Strain	"New"	"Bad"	Trial 2 Averages	Trial 3 Averages
1-Butanol-3-methyl (Isoamyl Alcohol)	L1	288930	2392373	1095594	1510102
	L3				
	I				
Phenylethyl Alcohol (2-phenylethanol)	L1	21997	2381827	1037200.3	580084
	L3				
	I				
Ethyl Acetate	L1	913180	1695243	381739.3	441751
	L3				
	I				
1-Propanol, 2-methyl- (Isobutanol)	L1	611215	1407231	601369	387816
	L3				
	I				
1-Butanol, 2-methyl- (active amyl alcohol)	L1	280919	831444	382562	404615.67
	L3				
	I				
Dodecane	L1	20568	749382	340435	278118
	L3				
	I				
Tetradecane	L1	2988	602572	225386	231899.67
	L3				
	I				
Octane	L1	182229	415189	249086.67	139220.3
	L3				
	I				
Undecane	L1	86040	505288	268901.67	183200
	L3				
	I				
2,4-Dimethyl-1-heptene	L1	117478	508586	231041.3	132520
	L3				
	I				
Benzene, 1,3-bis(1,1-dimethylethyl)-	L1	10594	378966	117746.67	61486.3
	L3				
	I				
Heptane, 4-methyl	L1	272261	208799	108445.3	51138.67
	L3				
	I				