THE EFFECT OF TEMPERATURE ON SHORT TERM ETHYL ACETATE DEGRADATION IN BEER

Michelle Pather

Project Supervisor: Alisyn Nedoma

The University of Auckland, department of Chemical and Materials Engineering

Abstract

Macs Hop Rocker was subjected to short term ageing and the subsequent changes in flavour chemical concentrations were investigated. Three different ageing conditions - cold, room temperature, and heated were used. In particular, ethyl acetate, an ester, was investigated. Ethyl acetate provides a pleasant fruity flavour in beer, a decrease in this allows stale flavours to be more pronounced.

Gas chromatography combined with flame ionisation detection (GC-FID) was used to measure the changes in the level of ethyl acetate. The first step involved crafting a method for temperature changes in the GC-FID. Due to time constraints, once this method was developed, only three measurements were made over a one week period.

Previous research showed that for other beer types, ethyl acetate concentration decreased over time. From the results obtained in this research no trend was found. However, it is suggested that further research is done, involving more measurements over a longer time period, before any conclusions are made.

Table of Contents

Table of Tables

Table of Figures

1.0 Introduction

The beer industry

The beer manufacturing industry is continually growing, therefore consumers have a large variety of beer to choose from. This leads to expectations of high quality, putting pressure on brewers to produce premium product. Production costs for breweries are high, hence, a lot of value is put on the final product produced.

Beer stability

Beer is made up of hundreds of chemical compounds that offer different flavour profiles. Previous research has shown that the chemical compounds in beer will either increase or decrease in concentration over time due to chemical reactions still occurring (Vanderhaegen et al., 2003). Different types and brands of beer age at varying rates and therefore undergo different flavour changes.

Ethyl acetate was analysed in this project as it provides a common and distinguishable flavour change in beer. This degradation happens in a shorter time period compared to other flavour compounds, and so could be observed in the time period for this project.

Beer flavour stability over time is important to maintain customer satisfaction. However, long transportation times means that beer can undergo variable storage conditions before it reaches the consumer.

The variation of storage conditions, such as humidity, light, and temperature, have been shown to affect the chemical reactions in bottled beer. Temperature will be investigated in this report as its variability is more realistic for consumers. Temperature affects the reaction kinetics of chemicals in beer. Therefore, changes in temperature will affect whether these reactions occur or not, how fast they occur, and hence how fast a beer degrades.

1.1 Project objectives

Previous papers have investigated ethyl acetate degradation in beer. Over time its levels have been seen to decrease. However, most of these studies have been done on American and European beer, and none on Mac's brand beer. Different types of beer are produced by different hops, strains of yeast, and fermentation temperatures which leads to different chemical compounds being produced in varying amounts. Therefore, different results could be obtained.

Mac's Hop Rocker beer will be used for analysis in this study. It will be aged under three different temperature conditions – cold, room temperature, and heated. Initially in the investigation, a method of quantitative analysis of ethyl acetate in beer must be developed. The change in concentration of ethyl acetate will subsequently be investigated.

1.2 Acknowledgements

The author would like to recognise Alisyn Nedoma, Peter Martin, Matthew Sidford and Mitchel Woodhouse for their knowledge and guidance in this project. The author would also like to acknowledge support from the University of Auckland Chemical and Materials Engineering department.

2.0 Literature Review

2.1 The beer brewing process

Malted barley is heated by soaking in hot water to start the germination process. This means that malt sugars are released by the barley. This process is stopped part way through to ensure the barley does not fully seed. The malt sugar solution, called wort, is then boiled with hops, the flower from a hop plant. The hops adds bitter flavouring to beer which balances out the sweetness of the malt sugar (Coe, 2003).

Once this solution has cooled, yeast is added which ferments the malt sugars, breaking them down into alcohol and carbon dioxide. The top of the fermentation vessel is fitted with an airlock that is half filled with water. This releases carbon dioxide from the vessel while simultaneously preventing oxygen from entering (Palmer, 2006).

The fermentation stage should theoretically be completed after about 10 days. To test this, a hydrometer reading is taken from a sample of beer. This measures the specific gravity (SG) of the sample. The sugars and the alcohol in the sample have a higher and lower density than water respectively. When the SG reading is about 1.010 it means the correct ratio of sugar and alcohol has been reached and the beer is ready to be bottled.

The beer is then transferred to either kegs or bottles. To provide carbonation, carbonation drops or priming sugars are added. These sugars are again fermented by the yeast, however, this time the carbon dioxide is trapped in the vessel providing beer it's fizziness.

2.2 Flavour components of beer

There are hundreds of different favourable and unfavourable flavour combinations that combine to create the different types and brands of beer, each with their own distinct taste (Meilgaard, 1982).These are shown in the flavour wheel below.

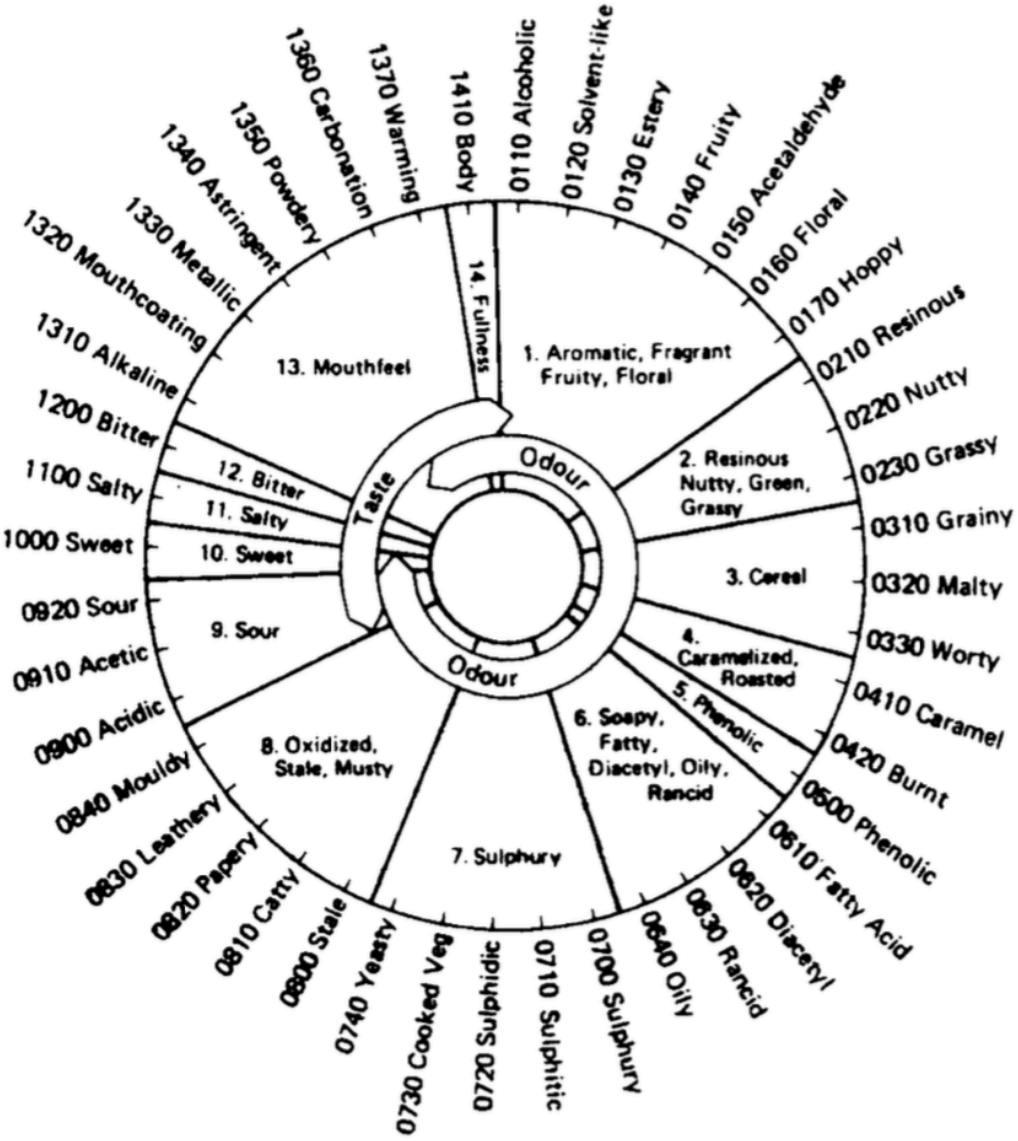
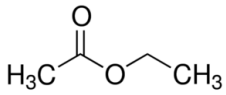
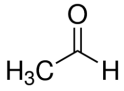
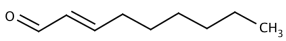


Figure 1: Beer flavour wheel (Hughes & Baxter, 2001).

2.3 Major flavour compounds in beer

The yeast used to ferment beer produces many other chemical compounds alongside ethanol and carbon dioxide. The flavour components shown in the flavour wheel shown in figure 1 are produced by different combinations of chemical compounds A brief overview of two significant types of compounds in beer, esters and aldehydes, has been provided in table 1 (Buiatti, 2009; Hough, Briggs, Stevens, & Young, 1982).

Table 1: Significant chemical compounds in beer

Type	Name	Chemical Structure	Description
Ester	Ethyl acetate		The main ester present in beer is ethyl acetate. At low levels ethyl acetate provides a fruity flavour, however, at higher levels it produces an unpleasant solvent like flavour.
Aldehyde	Acetaldehyde		This is the most common aldehyde found in beer – it is excreted by yeast into green beer during the first three days of fermentation. It gives beer a 'young', 'green' flavour. Young beer usually contains about 20-40mg/L, whereas final beer product contains about 5-15mg/L (Hough et al., 1982). Acetaldehyde can be reduced to ethanol, but can also be oxidised to acetic acid.
Aldehyde	Nonenal		Nonenal is regarded as one of the main sources of flavour degradation in beer. It is an alkyl aldehyde that gives beer a papery/wet cardboard taste. When a beer ages, changes in the level of nonenal causes beer to have a stale taste.(Lentini et al., 2015).

The production and degradation of ethyl acetate has been investigated further as it is the chemical compounds of interest in this research.

Production of ethyl acetate

Ethyl acetate is produced during yeast fermentation through esterification of ethanol and an acyl CoA compound (a long-chain fatty acid with a coenzyme attached to the end). A schematic of the chemical reaction pathways has been provided in figure 4 (García, García, & Díaz, 1994). This also shows the complexity of chemical reactions undergone during beer brewing.

An earlier paper shows that as ethanol concentration increased in fermentation, so did the concentration of ethyl acetate (Nordstrom, 1961). This can be seen in figure 2. As ethanol is readily available from the yeast fermentation, the concentration of esters is dependent on the fermentable sugars available.

The same paper by Garcia (1994) showed how an increase in temperature during fermentation increased production of ethyl acetate, as indicated in figure 3. (García et al., 1994)

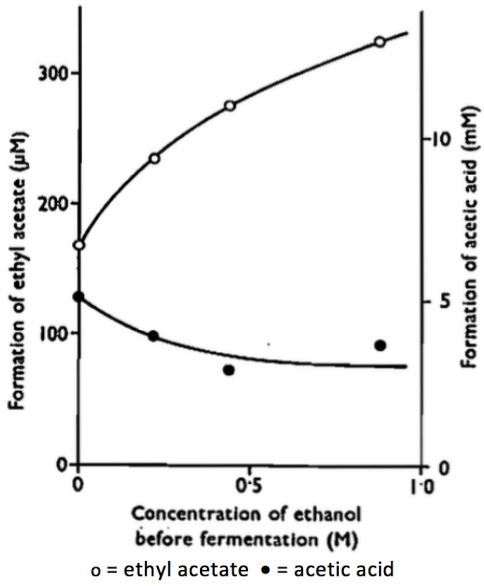


Figure 2: Effect of ethanol concentration on ethyl acetate formed

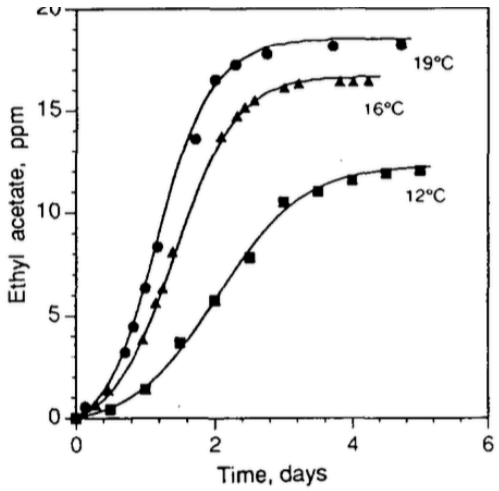


Figure 3: Effect of temperature on ethyl acetate formation

Degradation of ethyl acetate

Previous research has shown that as beer ages, the concentration of esters, including ethyl acetate, decreases (Vanderhaegen et al., 2003). Extracellular esterases from the yeast can break down esters, or they can be hydrolysed (broken down with water). This causes the fruity flavour of ethyl acetate to decrease and allows unfavourable flavours to become more pronounced, therefore altering the taste of the beer.

The same report investigated whether storage temperature and headspace gas affects the change in concentration of ethyl acetate during ageing. The measurements after six months of storage are shown in table 2. For all samples the concentration of ethyl acetate dropped. The largest decrease in concentration was seen in the high storage temperature samples.

Table 2: Ageing temperature effect on ethyl acetate levels

	Fresh beer	0ºC	20ºC		40ºC	
		CO_2	CO_2	Air	CO_2	Air
Concentration (μg/L)	28099	27578	27747	26711	22340	22650

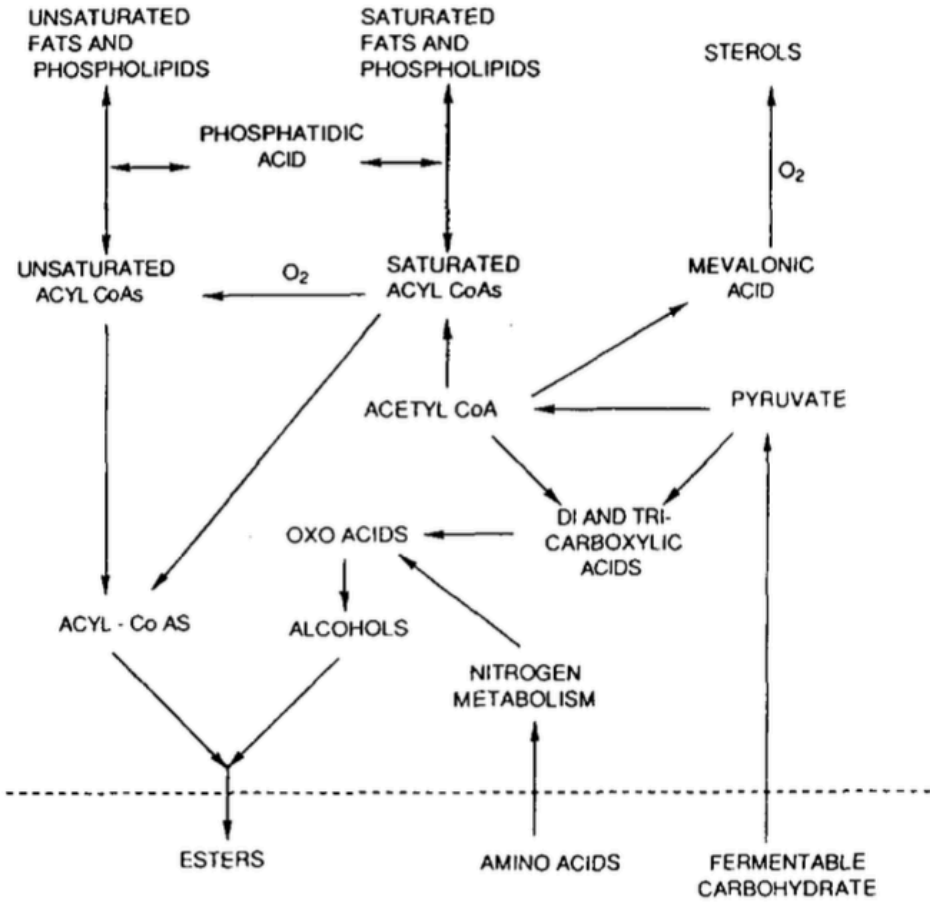


Figure 4: Reaction schematic for production of ethyl acetate

Vanderhaegen (2003) has done extensive research on how chemical compounds in beer change over time. One paper that focussed on an ale measured the levels of both ethyl acetate and nonenal over six months. Compared to other similar studies this time period is significant enough to see a difference in compound levels, but short enough to be a realistic time frame for a consumer to have stored beer. The beers studied in this paper were aged at varying temperatures which allows a comparison to be made between storing conditions.

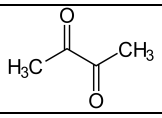
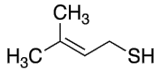
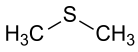
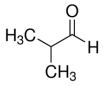
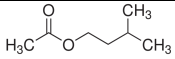
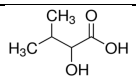
The ethyl acetate decreased in concentration in samples from all three conditions, with the greatest decrease in the elevated temperature sample. The nonenal concentration slightly decreased in the low temperature sample and slightly increased in both the other samples. This very slight decrease differs to traditional opinion that nonenal provides the unpleasant cardboard flavour of staled beer.

A later paper also headed by Vanderhaegen investigated the ageing effects on flavour compounds in eight different beers over one year. This meant that comparisons could be made between different beer types. As with his earlier paper, Vanderhaegen discusses how ester hydrolysis results in the decrease in concentration of most esters in beer. For all beer types, the concentration of ethyl acetate was shown to remain constant or decrease over the one year period (Vanderhaegen, Delvaux, Daenen, Verachtert, & Delvaux, 2007).

2.4 Unfavourable flavour compounds in beer

A summary of some common off flavours in beer are presented in table 3. To include all flavour compounds is beyond the scope of this research. Most of these off flavours arise due to brewing conditions, but degradation of these chemicals after bottling will also lead to flavour changes.

Table 3: Common off flavours in beer (Carr, 2016; Meilgaard, 1982)

Compound	**Chemical Structure**	**Boiling point**	**Flavour**	**Cause**
Diacetyl (2,3-butanedione)		88ºC	Buttery	Produced during fermentation by yeast
3-methyl-2-butene-1-thiol		128-135ºC	Skunky	Hop acids and riboflavin in the beer react with sunlight
Dimethyl Sulfide		37ºC	Sweetcorn/ Vegetables	Formed during malting from germination of grain where S-methyl methionine is broken down
Aldehydes, mainly isobutyraldehyde		63ºC	Fresh wheat/ grains	Compound released by malt when crushed, malted or soaked
Isoamyl Acetate		142ºC	Fruity/ bananas	Yeast produces esters during fermentation, an over production of these will produce this off flavour
Hydrogen Sulfide		-60ºC	Rotten eggs	Again, yeast produces hydrogen sulfide during fermentation, an over production of these will produce this off flavour
Isovaleric acid (3-Methylbutanoic acid)		175ºC	Cheesy/ dirty socks	When hop alpha acids are oxidised isovaleric acid is produced.

2.5 Sensory analysis

In industry, sensory analysis is used by tasting panels to identify flavours in beer. This is done to check the quality of the beer, and to ensure that different batches are identical. Flavour sensation is a combination of both gustatory (taste) and olfactory (odour) perceptions (Briggs, Boulton, Brookes, & Stevens, 2004).

Gustatory receptors are found in the mouth and are comprised of tastebuds and free nerve endings. The four main tastes are observed all over the tongue but most strongly in the specific areas shown in figure 5 (Briggs et al., 2004).

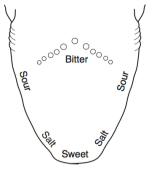


Figure 5: Taste areas of tongue

Odour is more complex than taste. There are olfactory receptors in the upper passages of the nostrils that pass signals to the olfactory lobe in the brain. The strength of an odour depends on the volatility of the compounds present (Dewey, 2011).

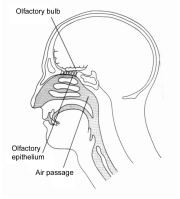


Figure 6: Olfactory system

Sensory tasting is a structured methodology for analysing food and beverage samples. A brief overview of its guidelines and operation procedure has been included in this research report as this is how flavour changes are quantified in industry. There are different thresholds used when measuring the flavour compositions of food and drink. The following definitions are used,

Table 4: Flavour thresholds for beer tasting (Briggs et al., 2004)

Detection threshold	The minimum value of a sensory stimulus needed to give a sensation
Difference threshold	The smallest perceptible change in the physical intensity of a stimulus
Recognition threshold	The minimum value of a sensory stimulus allowing identification of the sensation received
Terminal threshold	The maximum value of a sensory stimulus allowing identification of the sensation perceived.

Different tasters can be prone to bias, and will have different sensitivities to flavours. To minimise these factors sensory guidelines should be followed;

1. A tasting panel should comprise of 20-50 subjects.
2. Repeated tests should be done.
3. Individual test booths for tasters should be used.
4. Random number labels should be used instead of brand labels.

(Lawless & Heymann, 2010; Meilgaard, Carr, & Civille, 1999)

New technology has arisen to try and minimise taster bias. This includes instruments such as a quartz microbalance electric nose that mimics the human olfactory system to provide detection of an array of volatile compounds present in the samples (Berna et al., 2005). It is essentially headspace analysis with sensors that react differently depending on the volatile components it is exposed to. This has the advantage of doing continuous routine assessments, and has decreased influence from the environment and bias.

2.6 Methods of identifying chemical compounds in beer

There are a variety of methods to identify different chemical compounds in solutions. Each type has specialty uses.

- Gas chromatography, GC
- Mass spectrometry, MS
- Flame ionization detector, FID
- Electron capture detector, ECD
- High performance liquid chromatography, HPLC
- Sulfur chemiluminescence detector, SCD
- Ion chromatography, IC

(Coe, 2003)

For the scope of this report HPLC, SCD and IC have not been discussed as they are the least relevant methods for sampling beer for flavour compounds.

2.6.1 Gas chromatography

GC uses a capillary column to separate a sample into its individual chemical components. Not all samples are suitable for GC use, they must be sufficiently volatile and have some thermal stability to withstand the conditions in the GC.

GC analysis is done by injecting 1-5 μL of sample into the sampling port where it is instantly vaporised by the high temperature. The vapour (mobile phase) then moves up the thin capillary column that is about 30 m in length. The mobile phase interacts with the stationary phase that is internally coated on the column. The different compounds in the mobile phase stay on the stationary phase for different periods of time due to differences in their volatility, mass and level of interaction with the stationary phase. This means they take different periods of time to reach the end of the column where they are recorded, therefore separating the sample into its different components. (Coe, 2003; Jennings, 1987)

The 'method' for a GC is a description on the speed at which the instruments temperature is ramped up and how long these temperatures are held for. Trial and error is used to develop a GC method. It is recommended to start with a temperature just below the boiling point of the chemical of interest and ramp up the temperature slowly to above the boiling point and then hold the temperature. This method can then be tweaked from the resulting chromatogram produced by the GC software.

A chromatogram is an observable spectrum that displays different peaks that correspond to different chemical compounds eluted. An acceptable spectrum consists of narrow, separated, clear peaks to predict chemical compounds present. Changes to the GC method will result in different spectra due to altered chemical interactions and therefore a change in elution time.

GC column parameters

Listed below are some factors to consider when choosing an appropriate GC column for different types of samples,

- The stationary phase should be chosen in relation to the sample. The general rule is that the mobile phase will react with a stationary phase that is similar in chemical character.
- The film thickness of the stationary phase also needs to be considered. A thin film will release components quicker than a thick film will. High boiling point components will only require a thin film of about 0.2-0.5 mm. Thicker films of about 1-1.5 mm will be required for lower boiling point materials. Thicker films are also used for gases and solvents so that the interaction between them and the stationary phase is increased.
- Split or splitless injectors can be used. Capillary columns for GC can easily be overloaded with too much sample. One way of avoiding this is to use a split injector. If split injection is used then a

ratio is set to state the amount of sample entering the column versus the amount exiting through the vent. Split injection should be used when there is a highly-concentrated solution so that the column does not become overloaded. If splitless injection is used, the split line vent will be closed and all the injected sample will go into the column. Splitless injection should be used when there is a low concentration of solute in the sample and so a larger volume is needed. (Agilent Technolgy, n.d.; Chasteen, 2000)

Compounds eluted from a GC can be further processed by another device such as MS, FID or ECD, as discussed further below.

2.6.2 Mass Spectrometry

MS works by blasting the chemical components with electrons, breaking them up into charged fragments. These ions then go through an electromagnetic field that filters them by measuring their mass to charge ratios. The number of each ion is then counted and a mass spectrum created. The individual peaks can then be predicted from an inbuilt library on the GC-MS computer software (Vivian, Aoyagui, de Oliveira, & Catharino, 2016).

2.6.3 Flame ionization detector

FID works by passing the sample through a hydrogen/air flame which ionises the molecules. They are then detected using a metal collector that has a high voltage deliberately applied across it. The current can therefore be measured, which is indicative of the number of ions and therefore the concentration of the chemical compound. FID is a very sensitive GC detector for organic compounds. For this research, this is not a limitation as ethyl acetate is an organic compound and is present in very small amounts in beer.

2.6.4 Electron capture detector

ECD bombards the eluted stream from the GC with beta particles. When these beta particles impact with molecules in the makeup gas, electrons are released. The electrons are drawn to a positively charged anode and are measured as a current. Some organic molecules will capture these electrons. This results in a reduction in the current being measured. These absorptions can be translated into peaks that can be read as the type of compound present. In comparison to the other methods ECD is very sensitive, even in comparison to FID. This makes it ideal for measuring samples that contain very low levels of contaminants.

Quantitative measurements with GC

An external standard needs to be used when making GC quantitative measurements. At least three solutions of varying concentration of the pure chemical substance of interest should be used to create a calibration curve. The R^2 value generated by the GC computer software gives an indication of how well the plotted points fit the linear curve. An R^2 value of >0.99 is generally acceptable. Once the calibration curve has been generated the samples of unknown concentration are run and are compared to the calibration curve. From this a concentration value of the chemical compound of interest can be evaluated.

2.7 Problem statement

This literature review has shown that the chemical compositions in beer change over time, resulting in unfavourable flavour changes. These chemical compositions will be different depending on the specific type and brand of beer. In particular, ethyl acetate has been shown to decrease in concentration over time in a range of different beers. This ageing effect will be investigated in Mac's Hop Rocker beer. The effects of different ageing temperatures will also be investigated. It is hypothesised that the concentration of ethyl acetate will decrease over time. This decrease will be more pronounced in the elevated storage temperature sample, and less pronounced in the cooled sample due to the temperature effects on degradation reaction rate.

3.0 Semester one project work

Lion brewery in East Tamaki agreed to offer support for this research project. A meeting with engineers there resulted in a multitude of research ideas and industry insight. This visit and a tour of their testing laboratories, sensory and tasting lab, pilot plant, and new product development areas provided an insight into the issues that a brewery faces every day, and the solutions they came up with to solve these. It also showed the significance of this research project and its real-life applications. By understanding flavour degradation in beer, storage conditions could be tailored to each beer type to maximise their quality for a longer period of time.

Most of semester one was spent defining a project to undertake with the help of Lion brewery. The following list of project ideas were researched as potential projects.

- The ageing effect of yeast in bottled beer.
- Home brewing of four identical ales, another different ale, one IPA, one stout, and one lager. The effect of different storage conditions such as temperature, light and oxygen on beer flavour would then be investigated.
- The effect of dissolved oxygen during production of beer, with a cost benefit analysis to see if changing production techniques would be economically beneficial.
- The effect of UV light on skunky flavour in beer.
- The effect of enzymes in the mash profile, and how temperature variation would affect this.
- The effect of yeast on different flavour compounds produced. This would be investigated using different yeast strains in four identical batches of wort.
- Measurement of hop content in beer to create a standard that future beer batches can be compared against so that a consistent product is made.

3.1 Experimental Procedure

The experimental procedure has been split into two sections. The earlier project work focussed on trials of whiskey, store bought beer and home brew beer batches in the GC-MS, and developing a GC method for beer analysis. The second section however, used the GC method developed in the first section and focussed on the Mac's Hop Rocker measurements from GC-FID. The second section is most relevant to this research and is discussed further in the results.

3.1.1 Major equipment Used

- The GC-MS used for all the experiments in this report was a Shimadzu GC-QP2010 Ultra, with an Agilent Technologies column HP-5MS, 30 m x 0.320 mm.
- The GC-FID used for the Mac's Hop Rocker beer was a Shimadzu GC-2010, with an Agilent Technologies column, HP-INNOWAX, 30 m x 0.320 mm.

3.1.2 Earlier Experimental work

GC-MS Whiskey run

In order to practice using the GC-MS and its software, store bought whiskey was run with the two methods outlined in table 5.

Table 5: Whiskey GC-MS methods

Method Number	Method
1	50 ºC, hold for 10 min 50-180 ºC at 1 ºC/min, hold for 3 min
2	A split ratio of 25:1 35 ºC, hold for 5 min 35-100 ºC at 10 ºC/min, hold for 1.5 min 100-220 ºC at 15 ºC/min, hold for 3 min 220-250 ºC at 25 ºC/min, hold for 2.8 min

The methods in table 5 and 6 were used for the following reasons.

- The boiling point of ethyl acetate is 77 ºC. The GC temperature starts below this boiling point so that the temperature can ramp up through it.
- The initial holding temperature and time was reduced from method one to method two in table 5 to improve the resolution of the earlier eluting peaks as the less volatile compounds can interact with the stationary phase longer.
- The temperature ramp rate affects the resolution of the peaks. For the whiskey runs in table 5, the resolution was sufficient, so the ramp rate was increased so that analysis time decreased. If the resolution was poor, the ramp rate should be decreased. This would allow more time for interactions between the mobile phase and the stationary phase, increasing resolution. However, a balance is required, as increasing the ramp rate also means that more volatile compounds elute very quickly, sometimes resulting in peaks joining together (Agilent Technologies, 2007).
- A mid ramp hold, as shown for method two in table 5, is used to try and improve the resolution of the peaks in the middle of this ramp
- The holding time at the end of the run tries remove the remaining compounds on the column. This holding time can be reduced if all solutes come off the column in a short amount of time.

GC-MS Store bought beer run

To further practice using the GC-MS and to adapt a GC method for beer analysis, store bought beer was trialled. Beck's (a German pilsner), Mac's 3 Wolves (a pale ale), Mac's Springtide (a lower carb lager), and Mac's Sassy Red (an amber ale) were run through the GC-MS.

The method used for these beers was;
65 ºC, hold for 5 min
65-150 ºC at 4 ºC/min, hold for 5 min.

The initial spectra produced from the whiskey and beer runs were unclear and unusable as they showed many wide peaks layered over each other. Changes to the GC-MS method were required, as discussed later in this section.

GC-MS Home brew run

Initially, home brewed beer was going to be used for analysis in this project instead of store bought beer. Therefore, two batches of Mangrove Jack's pale ale were brewed in 23 L batches from malt extracts, and stored in amber bottles in lightproof boxes. Unfortunately, variation in the concentration of yeast by-products, such as esters and aldehydes, can arise in homebrew. Therefore, commercially produced beer was used for this research.

Two bottles from the home brew batch were selected at random to be trialled with the GC-MS. Three samples of 2 mL were drawn from one bottle and filtered through 0.45 μm filters into clean vials. Two samples were taken from the second bottle via the same process. Repeated samples of the same beer were used so that the spectra results could be compared.

The method used for these beers was;
50 ºC, hold for 5 min
50-250 ºC at 3ºC/min, hold for 5 min.

This method was used because it was believed that a long, slow ramping through a large range of temperatures could show more chemical compounds. However, as shown previously, the results from the GC-MS software indicated unclear and indistinguishable peaks. This indicated that more work was required to refine the GC-MS method.

Preparation of standard solutions

To perform quantitative analysis, standard solutions were required. Multiple volumetric flasks were prepared by thoroughly rinsing with ethanol, to get rid of organic molecules. They were then rinsed with milli-Q (ultrapure, Type 1 water) water followed by acetone. The glassware was then dried with nitrogen gas to ensure all residue was removed.

The accuracy of the pipette used in solution preparation was tested by weighing the water drawn up the pipette with an electronic scale to see if they were the same value. Only pipettes that gave accurate readings were used for solution preparation.

The solubility of ethyl acetate in water is 8.3 g/100 mL. Therefore, a standard solution of 83,000ppm (parts per million) was produced in a 100mL volumetric flask. This solution was then diluted ten times to get an 830ppm solution, and so on, down to 8.3ppm. This 8.3ppm solution was used so as not to overload the GC-MS. The same pure ethyl acetate solution was used to make all the standard solutions. This reduced variation due to preparation as there was no difference in initial solution concentration or purity.

GC-MS methods with Mac's Hop Rocker

The 8.3ppm solution of ethyl acetate and a room temperature sample of Mac's Hop Rocker were run through the GC-MS using the methods in table 6.

Table 6: Store bought beer GC-MS methods

Method Number	Method
1	60 ºC for 4 min 60-200 ºC at 6 ºC/min 200 ºC for 2 min
2	35 ºC, hold for 5 min 35-100 ºC at 10 ºC/min, hold for 1.3 min 100-200 ºC at 15 ºC/min, hold for 3 min 220-250 ºC at 25 ºC/min, hold for 3 min
3	60 ºC, hold for 5 min 60-200 ºC at 6 ºC/min, hold for 2 min
4	35 ºC, hold for 5 min 35-150 ºC at 2 ºC/min, hold for 5 min
5	25 ºC, hold for 5 min 25-100 ºC at 2 ºC/min, hold for 5 min

As explained for table 5, the methods in table 6 were adapted after viewing the spectra from the previous run. The initial hold time was reduced from method one through five in table 6. This was to try to increase the resolution of the initial peaks, as ethyl acetate is one of the first compounds to elute. The temperature ramp rate was also reduced to try and improve the peak resolution.

The first method was suggested by Agilent Technologies (Technologies, n.d.). This method was suggested for alcohol beverage standards, including detection of ethyl acetate. However, the column used for this analysis was HP-FFAP which differed from the column in the laboratories. This meant that different chemical compounds in the sample would interact differently with the column and therefore will not produce the same spectra of results.

As previously, these methods resulted in spectra that had wide and indistinguishable peaks, meaning chemical compounds could not be predicted. Therefore, GC-FID was used for the rest of this research project to try and detect ethyl acetate and obtain readable results. The column on the GC-MS differs from that on the GC-FID. This means ethyl acetate will have a different affinity with the chemicals of the GC-FID's stationary phase resulting in a different elution time.

3.1.3 Later Experimental work

Storage conditions of store bought beer

After the initial unsuccessful GC-MS testing was done, the Mac's Hop Rocker was placed in different storage conditions and left to age for one week before the first GC-FID measurement. Two bottles of the beer were placed in the laboratory fridge that was set at 4 ºC. Two more bottles were placed in the chemical analysis room that was kept at room temperature of 19 ºC. The last two bottles were placed in an elevated temperature environment on top of an electronic water boiler. The elevated temperature of this could not be measured, but heated the beer over 30 ºC. This variable elevated temperature could have been a source of error in measurements as the beer was kept at an unknown elevated temperature. If future experiments were to be done, the water incubator in the laboratory should be used as this would give a more reliable elevated storage condition.

GC-FID standard solution preparation

Volumetric flasks (three 5 mL, two 25 mL, one 50 mL, and one 100 mL) were cleaned as described above. 0.111 mL of ethyl acetate was pipetted into the 100 mL volumetric flask and topped with milli-Q water to make a 1000ppm solution. This 1000ppm solution was then used to produce 500, 200, 100, 40, 20 and 10ppm solutions in the other volumetric flasks which can be seen in figure 7

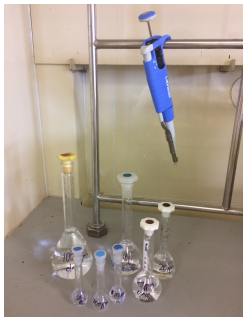


Figure 7: Ethyl acetate standard solutions and pipette

1 mL of each of the standard solutions was syringed through a 0.45 µm PTFE filter into sampling bottles. A 1 mL sample of cold, room temperature and elevated temperature Mac's Hop Rocker beer were also filtered through the 0.45 µm PTFE filter into separate sampling bottles. These were placed in the GC-FID as shown in figure 8.

Figure 8: Ethyl acetate and beer samples in GC-FID

Only 5 mL of beer from the bottles was used for this sampling, the rest was poured into plastic vessels and kept under the same storage conditions until the next sample was taken. This could have affected results as after a beer bottle is opened the beer is exposed to oxygen in the air which can begin to break down some chemical compounds. In real life applications, in contrast to this experiment, beer would be aged while in an unopened bottle.

The samples were run through the GC-FID in the following order; all standard solutions, water sample, beer samples. This order is to try and reduce any effect of build-up of chemicals in the column, called 'carry over'. These could be eluted in the beer sample and therefore give inaccurate measurements. To further try and reduce carry over, each beer sample was run through twice. The first sample should clear out any residual chemicals left on the column so that these did not contaminate the next run. An average of these two runs was used in the results.

Figure 9: GC-FID machine used for measurements

The following methods, shown in table 7, were then input into the GC-FID.

Table 7: Mac's Hop Rocker beer GC-FID methods used

Method Number	Method
1	25 ºC, hold for 20 min 25-100 ºC at 2 ºC/min, hold for 5 min
2	27 ºC, hold for 20 min 27-100 ºC at 2 ºC/min, hold for 5 min

An injection temperature of 250 ºC, and injection volume of 3 µL was used for both methods.
The method was changed to 27 ºC from 25 ºC because the GC-FID struggled to reach 25 ºC. This had no effect on the results obtained.

The standard solutions, water and beer samples were run through the GC-MS three times within one week. The same standard solutions were used for the first and second run, with fresh ones produced for the third run.

Analysis of GC-FID results

After the samples were run through GC-FID, spectra were produced and displayed on the post run analysis screen. Manual integration was used to analyse the peaks by selecting the base each side of the peak. The function on the GC-FID software then calculated the area of the ethyl acetate peak in each of the standard solutions. These areas were then plotted against their concentrations to form a calibration curve.

The time at which the ethyl acetate in the solutions elutes is also noted by the GC-FID. When the beer samples are run, a peak at the same time interval is expected to be observed, this is likely to be ethyl acetate. The area of this peak is calculated and compared against the calibration curve to give the concentration of ethyl acetate in the sample.

The concentration in the sample is found in ppm. To convert this to a percentage of the bottle of beer 10,000ppm = 1%. Therefore, 25ppm = 0.0025%.

4.0 Results and Discussion

The post run analysis software for the GC-FID produces a spectrum for each sample run. The spectrum in figure 10 is from the 200ppm standard solution. A narrow ethyl acetate peak can be seen at an elution time of 6.2 minutes. The noise from 35-40 minutes can be ignored.

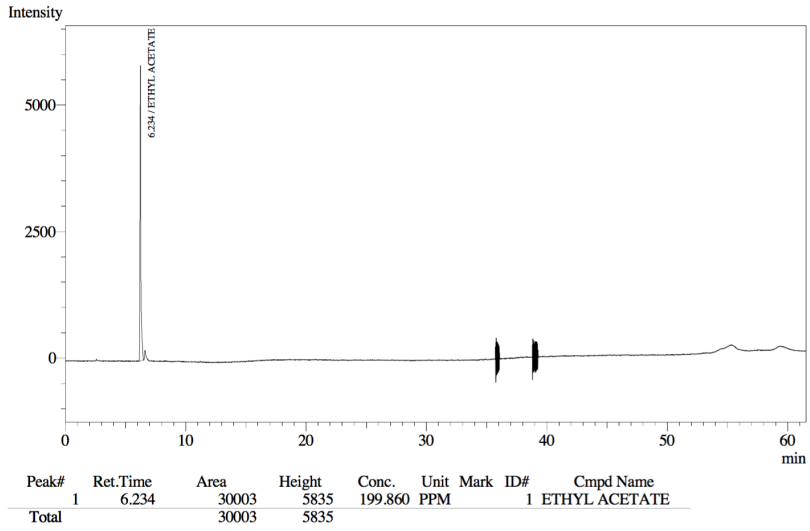
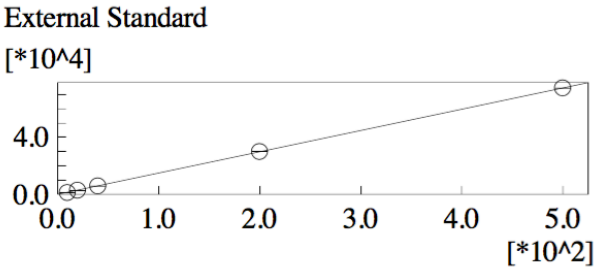


Figure 10: Spectrum from GC-FID for 200ppm ethyl acetate standard solution

These standard solution areas are plotted against the appropriate concentrations to produce a calibration curve. The calibration curve used for the first and second trial is shown in figure 11. The R^2 value for this curve is 0.99998, meaning that the concentrations of the ethyl acetate solutions were sufficiently accurate, and can be used for quantitative analysis. A second calibration curve was produced for the third trial, it had an R^2 value of 0.99992. This can be found in appendix A.



No.	Conc.	Area
1	10.000	1124
2	20.000	2851
3	40.000	5930
5	200.000	30003
6	500.000	74491

Figure 11: Calibration curve produced from ethyl acetate standard solutions

For the first successful use of the GC-FID only one room temperature beer sample was tested. 25.4ppm of ethyl acetate was predicted to be present in this sample from comparison against the calibration curve. This is about 0.00254%. This likens to Vanderhaegen's paper (2003) where 28 ppm of ethyl acetate was measured in fresh beer.

The first run with a cold sample, a room temperature sample and heated sample was done on the 20th September. The same standards were run through the GC-FID and a similar calibration curve was produced. There may have been some degradation of the ethyl acetate when it was stored for the two days. The cold, room temperature, and heated beer all had slightly different spectra. The spectrum for the first sample of the room temperature beer from the first trial is shown in figure 12. The ethyl acetate peak can be identified as it elutes from the GC at the same time as the ethyl acetate in the standard solutions.

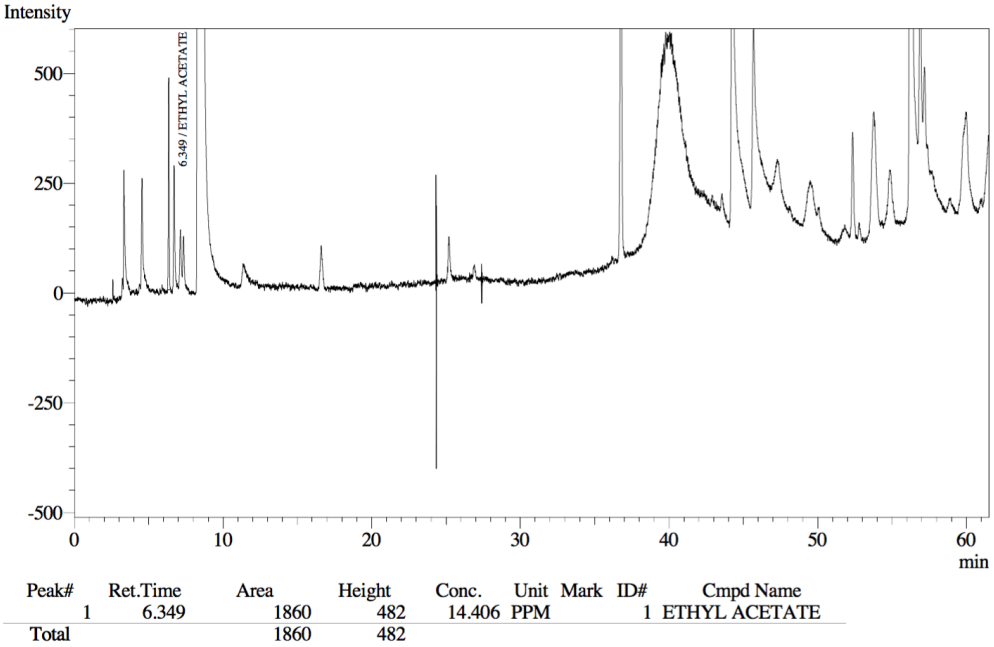


Figure 12: Room temperature beer sample GC-FID spectrum

For the second and third trial on the 25th and 26th of September duplicate samples of the beer at each storage condition were put through the GC-FID. The GC ran samples from the same vial twice. However, the first and duplicate samples did not have identical results, so an average of the two was taken. These can all be seen in table 8.

Table 8: Measured ethyl acetate concentrations from GC-FID

		First sample (ppm) (mg/L)	Duplicate sample (ppm) (mg/L)	Average (ppm) (mg/L)
1st trial 20th September	Cold	14.4	14.7	14.5
	Room temperature	14.4	14.5	14.5
	Heated	12.0	13.5	12.7
2nd trial 25th September	Cold	32.8	31.2	32.0
	Room temperature	30.2	31.6	30.9
	Heated	31.1	32.0	31.6
3rd trial 26th September	Cold	21.1	19.2	20.2
	Room temperature	17.7	18.7	18.2
	Heated	19.0	17.2	18.2

The difference in ethyl acetate concentration between the first sample and the duplicate sample could be due to residual chemicals left on the GC column. This could be from the ethyl acetate standard solutions, or other chemicals left by researchers using the same GC-FID. Some of these compounds could have stayed on the column wall and eluted with one of the samples. It was originally thought that these chemicals would come off in the first sample, and not affect the duplicate sample. However, for six out of the nine runs the duplicate sample had a higher level of ethyl acetate compared to the first sample.

Furthermore, the water sample spectrum from the third trial is shown in figure 13. The water sample was run after the all standard solutions but before any beer samples. It can be seen that there is still an ethyl acetate peak and peaks from other compounds present. These chemicals would not have

been present in the Milli-Q water used. This indicates that there was ethyl acetate and other chemical compounds left on the GC column from previous runs. If this water run did not flush out all of the residual chemicals then they could have eluted in the subsequent beer sample runs.

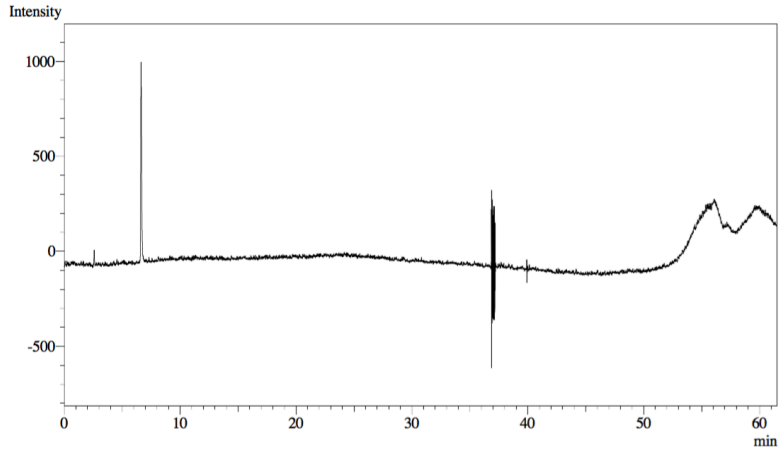


Figure 13: Third trial water sample spectrum from GC-FID

This therefore indicates that there could be errors in the GC-FID readings as other chemicals that are not present in the samples were detected and recorded. To reduce residual chemicals on the column, multiple water runs could be done until the chemical responses recorded reduces to zero. Another possible cleaning method is to hold the column at a high temperature so that the chemicals get burnt off.

The level of ethyl acetate at the three different storage temperatures for each trial has been plotted on the graph shown in figure 14.

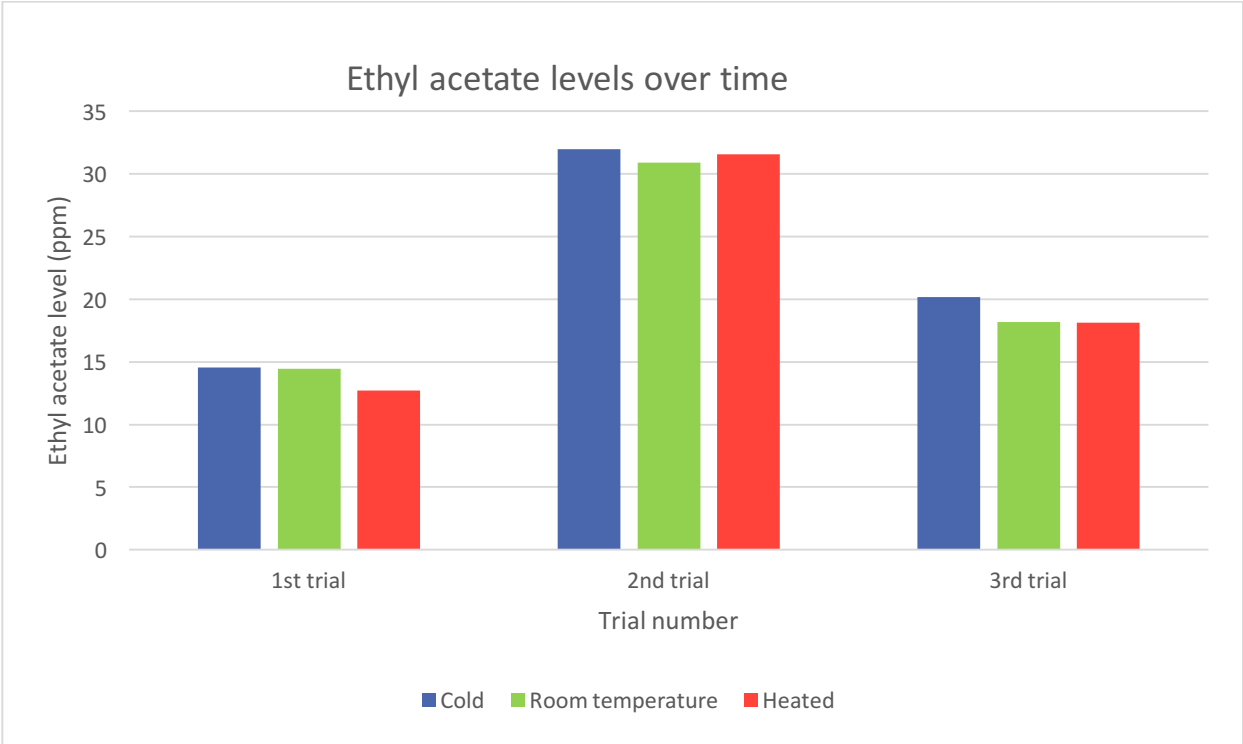


Figure 14: Ethyl acetate levels over time with different storage temperatures

Figure 14 shows no trend for change in ethyl acetate levels in Mac's Hop Rocker with respect to time. However, this research was conducted over a very short time period of less than a week, and only three data points were measured. Therefore, this data is not statistically significant as the sample size is far too small. To improve this experimental method and get statistically significant results where a trend could potentially be seen, it is recommended to measure ethyl acetate levels over a six month time period. This is a realistic time period for a fourth year project and also a realistic time frame for ageing beer.

The beer used for this sampling was bought four months before the first ethyl acetate measurement was made. It was thought that ethyl acetate could have degraded exponentially over time. Therefore the measurements made should not show much variation as they are late in the degradation stages. However, Vanderhaegen (2003) measured ethyl acetate concentration over two hundred days and found a linear decrease in ethyl acetate.

For all three trials, the cold beer sample showed higher levels of ethyl acetate than both the room temperature and heated samples. This is possibly due to the lower temperature reducing the rate at which the esterases break down the ethyl acetate, and reducing the rate of the hydrolysis reaction of ethyl acetate. However, due to the small number of measurements this conclusion cannot be made confidently.

Figure 14 shows large variation between trials, but little variation within trials. It is unlikely that the concentration of ethyl acetate increased significantly (12.7ppm to 31.6 ppm) in 5 days and then decreased significantly again one day later (13.6 ppm to 18.2 ppm). This could indicate error due to the GC-FID equipment used. Each trial was run over a full day. Therefore, it is possible that on different days the GC gave inaccurate results for all three samples of a single trial.

The first two trials used the exact same standard solutions, whereas the third trial used fresh standard solutions. This could have affected the results as a different calibration curve was produced for the third trial. However, using the same standards for the second trial five days after they were produced could also have affected the results. This is because the standards could have degraded and changed in composition over time, therefore resulting in an inaccurate calibration curve.

5.0 Future developments

If any future research is done in relation to this project, the following is recommended,

- There are many flavour chemical compounds in beer that change over time. Any further research on Mac's Hop Rocker beer could also include analysis of other compounds, such as nonenal.
- No conclusions can be made from the results of this research as too few measurements were made over too short a time period. Any future research on ethyl acetate degradation should be done over a longer time period, for example, six months.
- The stationary solution on the GC column is important in column selection as it depends on the application the GC column is used for. Stationary phases have varying interactions with different types of chemicals. A different stationary phase may separate two compounds that co-elute in another column. The GC-FID column available in the labs, HP-INNOWAX, was used during this project but was not ideally suited for analysis of flavours and fragrances in the food and alcoholic beverage industry. For future researchers, it is recommended to use an SPB or CP-WAX-52-CB column (Sigma Aldrich, 2010).
- The elevated temperature beer samples were not kept in a constant heated environment. To improve the accuracy of the results, the beer samples should be placed in the water incubator in the laboratories where they will be kept at a constant temperature.

6.0 Conclusions

The concentration of ethyl acetate in Mac's Hop Rocker beer was not constant with time. However, due to the limited number of measurements made, no trend could be found with the results. Further research is required to find the effect of ageing temperature on ethyl acetate concentration.

The main conclusions that were made from this research project are summarised below,

- The results obtained in this research are not statistically significant as the sample size was too small. No trend could be seen from the results as the time period this research was conducted in was too short.
- Theoretically, compared to similar results from different beer types, the level of ethyl acetate should decrease in concentration as beer ages as it is broken down.
- At elevated temperatures, this decrease in concentration should be accelerated due to the increased rate of breakdown reaction. At lower temperatures, the opposite is true.
- Future testing should be done over a longer time period of about six months so that an ageing trend can be seen.

7.0 References

Agilent Technolgy. (n.d.). Choosing the right column. Retrieved from http://www.chem.agilent.com/cag/cabu/gccolchoose.htm

Agilent Technologies. (2007). *GC Method Development*. *GC Method Development*. Retrieved from www.chem.agilent.com

Berna, A. Z., Buysens, S., Di Natale, C., Grün, I. U., Lammertyn, J., & Nicolaï, B. M. (2005). Relating sensory analysis with electronic nose and headspace fingerprint MS for tomato aroma profiling. *Postharvest Biology and Technology*, *36*(2), 143–155. https://doi.org/10.1016/j.postharvbio.2004.12.006

Briggs, D. E., Boulton, C. A., Brookes, P. A., & Stevens, R. (2004). Beer flavour and sensory assessment. In *Brewing Science and Practice* (pp. 716–758).

Buiatti, S. (2009). Beer Composition: An Overview. In *Beer in Health and Disease Prevention* (pp. 213–225). Elsevier Inc. https://doi.org/10.1016/B978-0-12-373891-2.00020-1

Carr, N. (2016). 18 Common "Off" Flavors In Beer. Retrieved from https://learn.kegerator.com/off-flavors-in-beer/

Chasteen, T. G. (2000). Split / Splitless Gas Chromatography Injection. *Department of Chemistry, Sam Houston State University*.

Coe, M. (2003). IGB Exam.

Dewey, R. (2011). Olfaction. Retrieved from http://www.intropsych.com/ch04_senses/04nasal.jpg

García, A. I., García, L. A., & Díaz, M. (1994). Prediction of ester production in industrial beer fermentation. *Enzyme and Microbial Technology*, *16*(1), 66–71. https://doi.org/10.1016/0141-0229(94)90111-2

Hough, J. S., Briggs, D. E., Stevens, R., & Young, T. W. (1982). Chemical and Physical Properties of Beer. In *Malting and Brewing Science* (pp. 776–838). https://doi.org/10.1007/978-1-4615-1799-3_11

Hughes, P. S., & Baxter, E. D. (2001). *Beer : Quality, Safety and Nutritional Aspects*. The Royal Society of Chemistry. https://doi.org/10.1039/9781847550224

Jennings, W. (1987). *Analytical Gas Chromatography*. Academic Press Inc.

Lawless, H. T., & Heymann, H. (2010). Introduction. In *Sensory evaluation of food principles and practices*. https://doi.org/10.1007/978-1-4419-6488-5

Lentini, A., Nischwitz, R., Rigoni, P., Goldsmith, M., Duan, D., Fogarty, L., & Rogers, P. (2015). Further Studies on the Impact of Commercial Malting and Brewing Practices on Beer Flavour Stability, (November), 1–16.

Meilgaard, M. C. (1982). Prediction of flavor differences between beers from their chemical composition. *Journal of Agricultural and Food Chemistry*, *30*, 1009–1017. https://doi.org/10.1021/jf00114a002

Meilgaard, M. C., Carr, B. T., & Civille, G. V. (1999). Introduction to Sensory Techniques. In *Sensory Evaluation Techniques* (pp. 1–6). https://doi.org/doi:10.1201/9781439832271.ch1

Nordstrom, K. (1961). Formation of Ethyl Acetate in Fermentation Kinetics of Formation From Ethanol and, 188–196.

Palmer, J. J. (2006). *How to Brew: Everything You Need to Know to Brew Beer Right the First Time*. Retrieved from http://howtobrew.com/book/introduction

Sigma Aldrich. (2010). GC Column Selection Guide, 1–24.

Technologies, A. (n.d.). *GC and GC/MS*.

Vanderhaegen, B., Delvaux, F., Daenen, L., Verachtert, H., & Delvaux, F. R. (2007). Aging characteristics of different beer types. *Food Chemistry*, *103*(2), 404–412. https://doi.org/10.1016/j.foodchem.2006.07.062

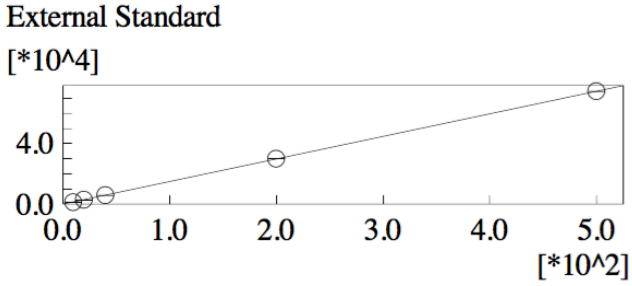
Vanderhaegen, B., Neven, H., Coghe, S., Verstrepen, K. J., Verachtert, H., & Derdelinckx, G. (2003). Evolution of Chemical and Sensory Properties during Aging of Top-Fermented Beer. *Journal of Agricultural and Food Chemistry*, *51*(23), 6782–6790. https://doi.org/10.1021/jf034631z

Vivian, A. F., Aoyagui, C. T., de Oliveira, D. N., & Catharino, R. R. (2016). Mass spectrometry for the characterization of brewing process. *Food Research International*, *89*, 281–288. https://doi.org/10.1016/j.foodres.2016.08.008

8.0 Appendices

8.1 Appendix A: Ethyl acetate concentration calibration curves

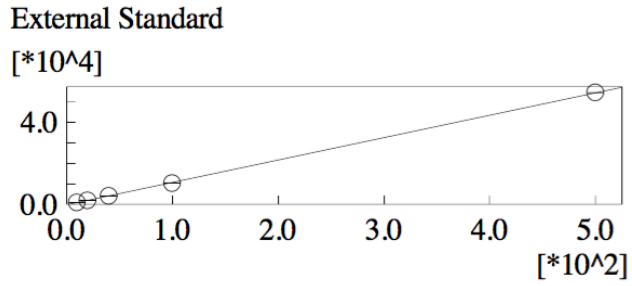
First sample (only room temperature beer) calibration curve. $R^2 = 0.99997$



No.	Conc.	Area
1	10.000	1124
2	20.000	2851
3	40.000	5930
5	200.000	30003
6	500.000	74491

Figure 15: First ethyl acetate sample calibration curve

Third trial calibration curve. $R^2 = 0.99992$

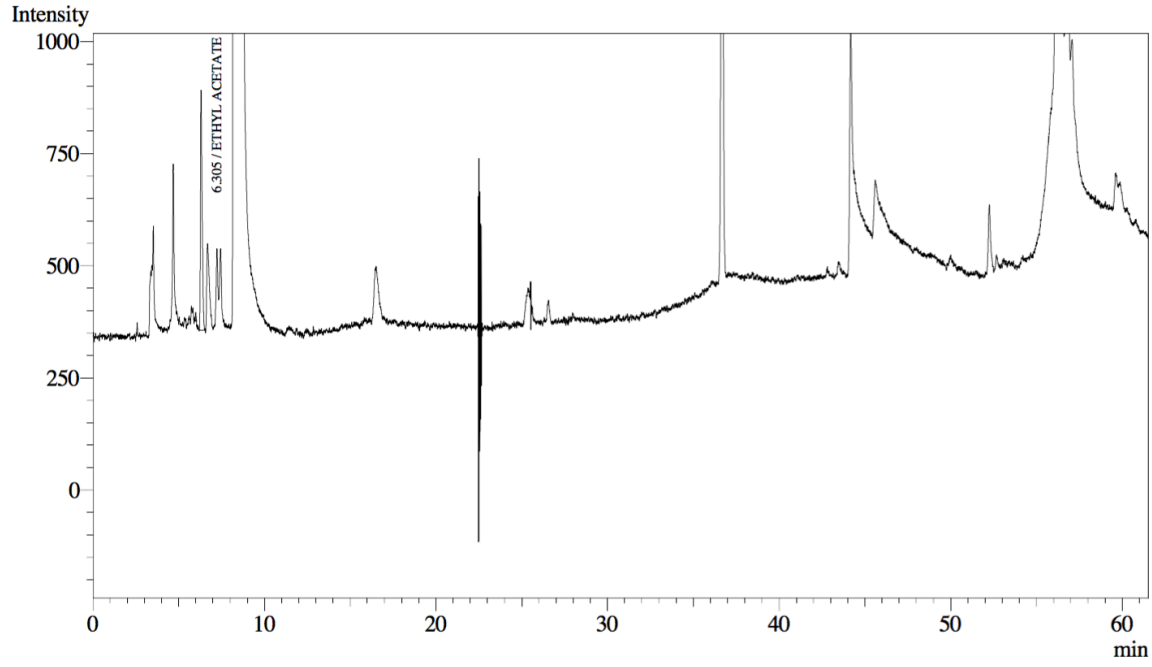


No.	Conc.	Area
1	10.000	964
2	20.000	1952
3	40.000	4189
4	100.000	10318
6	500.000	54605

Figure 16: Third trial calibration curve

8.2 Appendix B: GC-FID spectrum

GC-FID spectrum of first successful measurement with only room temperature beer measurement. This shows an ethyl acetate concentration of 25.4ppm.



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	6.305	3656	535	25.374	PPM		1	ETHYL ACETATE
Total		3656	535					

Figure 17: First room temperature GC-FID spectrum

8.3 Appendix C: Safety & Laboratory Use

HAZOP

Project: Short term ethyl acetate degradation in beer and effect of temperature

Project Objectives: To investigate the change in concentration of ethyl acetate in Mac's Hop Rocker beer over time after it has been aged in three different temperature conditions – cold, room temperature, and heated.

Equipment used:

1. Shimadzu GC-QP2010 Ultra GC-MS
2. Shimadzu GC-2010 GC-FID
3. Glass volumetric flasks, pipette
4. 5mL plastic syringes
5. 0.45µL filters
6. Small glass vials

Chemicals used:

1. Ethyl acetate
2. Milli-Q water
3. Acetone
4. Ethanol
5. Beer

Experimental procedure:

1. Produce ethyl acetate standard solutions by first rinsing volumetric flasks with ethanol, milli-Q water, and acetone.
2. Pipette correct proportions of ethyl acetate and milli-Q water into volumetric flasks.
3. Syringe standard solutions and beer samples through filter and into small glass vials, to then be put in the GC-MS or GC-FID.
4. Input method and run GC-MS or GC-FID throughout day, or overnight.

Personal protective equipment (PPE) required:

1. Lab coat
2. Safety glasses
3. Covered shoes

Emergency shut down procedure: Turn off machine with power button on bottom right. Switch power off at wall.

HAZOP analysis

Table 9: Beer degradation HAZOP analysis

Potential Hazard	Consequence	Suggestions
Electrocution from mixture of water and electrical equipment	Electrocution	Keep water away from GC equipment
Prolonged exposure to chemicals (Beer samples do not carry this hazard, only ethyl acetate)	Fatigue, headaches, or nausea	Chemical sampling to be performed in fume hood
Spray of chemicals from syringe onto face/skin/eyes due to pressure applied to push sample through filter (Beer samples do not carry this hazard, only ethyl acetate)	Irritation and possible damage to skin and eyes	Wear safety glasses and lab coat at all times
Broken glassware	Minor cuts to skin	Use first aid kit and dispose of broken glass carefully

CHEMMAT 751 A/B - PROJECT COMPLETION SIGN OFF – LABORATORY USE

(The completion of this section is required before your final mark is processed. Scan and attach the completed form to your Appendix section of your final report)

Name of Student: Michelle Pather

Research Project Title: Effect of Temperature on Short term ethyl acetate degradation in beer.

Supervisor's name: Alisyn J. Nedoma

List the labs used for this project: ~~239~~ 207B

(If you did not use any of the department labs, simply write 'NA' and obtain your supervisor's signature)

Lab Room(s)	Name of PIC	Area Left Tidy? (Y/N/NA)	Equipment dismantled/returned? (Y/N/NA)	Chemicals handover/disposed safely? (Y/N/NA)	PIC signature
207B	Matthew Sidford	Y	N/A	N/A	

Supervisor's signature: Alisyn J. Nedoma

(Note to Supervisors: Please ensure the student has correctly included ALL the lab facilities used)