Analysis of beer aroma using purge-and-trap sampling and gas chromatography

by

Nardus Potgieter

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Magister Scientiae

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SUMMARY

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Fingerprint profiles generated through GC analysis, are powerful tools for quality control in the food and beverage industry. Beer aroma profiles can be used for the fingerprinting of specific beer brands or for trouble shooting purposes such as the identification of off-flavours.

The multichannel silicone rubber trap (MCT) lends itself perfectly to this task. The simplicity and robustness of the MCT makes it ideal for concentrating the volatile compounds that constitute the aroma of an alcoholic beverage such as beer. The retention of these substances by the silicone is based on dissolution into the polymer. This gives more intrinsic stability to the concentrated aroma compounds than traditionally used, adsorption based methods, so transport of the trap and contents should not be a problem.

Thermal desorption is used to introduce the trapped aroma compounds to the chromatographic instrument. This eliminates the need for high purity solvents and greatly reduces the sample preparation time. The easily identifiable and stable background peaks from the silicone matrix makes the MCT ideal for repeated use with a thermal desorber.

During this study the performance of the MCT as concentration device was investigated for its ability to concentrate aroma volatiles representing a wide range of volatilities. The sampling and desorption procedures were optimised in order to attain the required detection levels and repeatability of the analytical method based on this purge-and-trap sampling scheme

SAMEVATTING

Die analise van bier-aroma deur middlel van spoel-en-vang monsterneming en gaschromatografie

Deur

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Voorgelê ter vervulling van ‘n deel van die vereistes vir die graad Magister Scientiae
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Die sogenaamde ‘vingerafdruk-profiele’ wat deur gaschromatografiese analise gegenereer kan word, is baie handig vir gebruik in kwaliteitsbeheer in die voedsel- en drankbedryf. Bier-aromaprofiele kan gebruik word vir die uitkenning van spesifieke biersoorte en vir foutopsporingsdoeleindes soos die identifikasie van ongewenste smake in voedsel of drank.

Die *multi*-kanaal-silikoonrubber-konsentreerder (MSK) is ideal vir hierdie taak. Die eenvoud en robuustheid van die MSK maak dit uiters geskik om vlugtige verbindings, wat die aroma van ‘n produk soos bier uitmaak, te konsentreer. Hierdie aromaverbindings word met ‘n oplosproses in die silikoonrubber vasgevang. In teenstelling met meer tradisionele adsorpsie-metodes verleen hierdie meganisme groter stabiliteit aan die gekonsentreerde aromakomponente.

Termiese desorpsie word gebruik om die vasgevangde aromakomponente na die gaschromatograaf oor te dra. Dit elimineer die gebruik van duur, hoë-suiwerheid oplosmiddels en verkort die monster-voorbereidingstyd. Die herhaalbare en stabiele chromatografiese agtergrondpieke van die MSK vergemaklik die herhaalde gebruik daarvan met termiese desorpsie.

Gedurende hierdie studie is die toepaslikheid van die MSK as konsentreerder vir vlugtige aromakomponente ondersoek. Die monsternemings- en desorpsie-prosedure Is geoptimiseer en ge-evalueer in terme van chromatografiese herhaalbaarheid en waarneembaarheidsvlakke.

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“Barmhartig en genadig is die Here, lankmoedig en vol liefde.”
Psalm 103:8

The many facets of beer

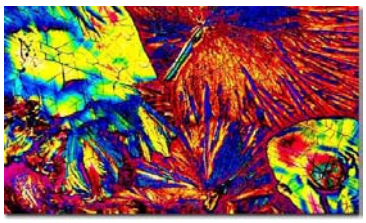
These images of different well-known beer brands are included at the start of every chapter, to show yet another of the many ways by which beer can be studied and enjoyed. The images are produced by the National High Magnetic Field Laboratory of the Florida State University. These beer images are as seen through a microscope and are captured through a photographic technique called photomicrography.

As discussed in this thesis, beer is a complex and heterogeneous mixture that contains a wide variety of both low and high molecular weight organic compounds. Differences in the amounts of polymers derived from the brewing process, such as polyphenols and yeast by-products, represent variations that serve as a "carbohydrate fingerprint" of individual beers. This fingerprint can be used to produce a wide spectrum of crystallization motifs, as seen in these images.

The images are reproduced with permission from Michael W. Davidson of the National High Magnetic Field Laboratory. For more information visit:

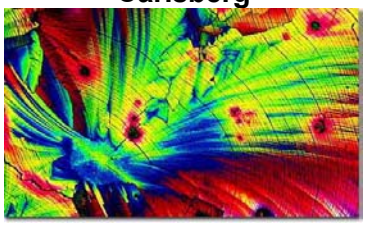
http://micro.magnet.fsu.edu/

and follow the "beershots" link.



Heineken

Carlsberg



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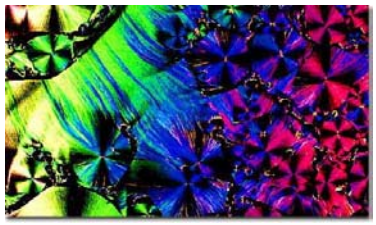

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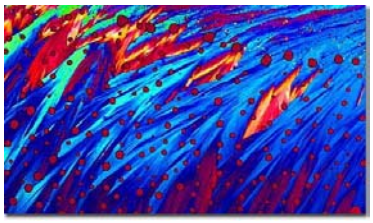

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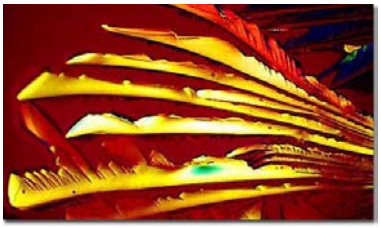
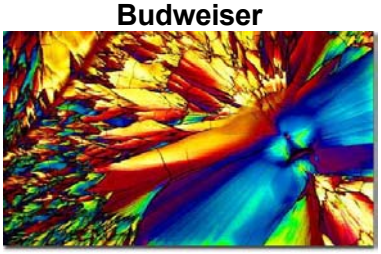


Miller Genuine Draft

Budweiser



Pilsner Urquel

CONTENTS

Chapter 3 Sample preparation

Chapter 4 Optimising sampling and analysis of beer aroma

Chapter 5 Repeatability

Chapter 6 Characterisation of beer aroma

Chapter 7 Application of the aroma sampling technique to GCxGC and GC-TOFMS

Chapter 8 Conclusion

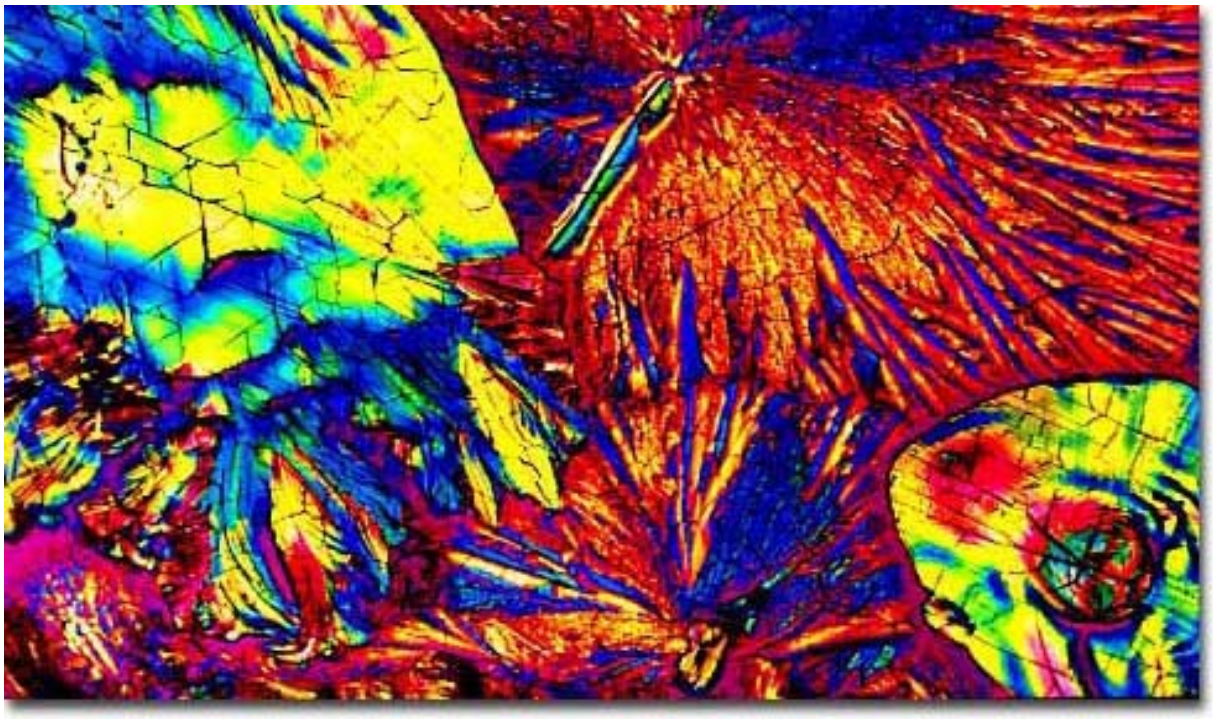
TABLE OF FIGURES

LIST OF TABLES

ABBREVIATIONS

CIS	-	Cooled Injection System
FU	-	Flavour Unit
GC	-	Gas Chromatography
FID	-	Flame Ionisation detector
MS	-	Mass Spactrometer
GC-O	-	Gas Chromatography-Olfactrometry
TOFMS	-	Time-of-Flight Mass Spectrometry
GCxGC	-	Multidimensional gas chromatography
MBT	-	3-methyl-2-butene-1-thiol
MCT	-	Multichannel Trap
OTT	-	Open Tubular Trapping
PCB	-	Polychlorinatedbyphenyls
PDMS	-	Polydimethylsiloxane
PTFE	-	Polytertafluoroethylene
PTV	-	Programmable Temperature Vaporizer
RSD	-	Relative Standard Deviation
RTL	-	Retention Time Locking
SABMiller	-	South African Breweries
SPE	-	Solid Phase Extraction
SBSE	-	Stir Bar Sorptive Extraction
SFE	-	Supercritical Fluid Extraction
SPME	-	Solid Phase Microextraction
TDS	-	Thermo Desorption System
TIC	-	Total Ion Chromatogram
Tg	-	Glass Transition Temperature
UV	-	Ultra Violet

Chapter 1



Heineken

Text from the bottle label: Heineken Lager Beer. Won the Grand Prix, Paris 1889. Brewed with natural ingredients. Brewed and bottled by Heineken Brouwerijen B.V., Amsterdam, Holland.

Chapter 1

INTRODUCTION

1. Background.

The modern consumer society demands a growing number of products and services at ever-increasing rates. For a manufacturer of consumer goods to be competitive in the marketplace it is essential to keep existing customers satisfied and interest new ones in its range of products. This can only be achieved if a consistent, good quality product is supplied to the customer at a reasonable price. Nowhere is this more important than in the food and beverage industry. The likelihood of a customer staying loyal to a specific brand of food or beverage is very low if there are batch-to-batch variations in quality or character of the product.

Quality control of products and raw materials is therefore an integral part of all food and beverage manufacturing processes. The quality of products is influenced by a number of factors such as; human error during manufacture, chemical contamination of raw materials and equipment, microbiological action, age, and exposure to light or oxygen.

When a variation in product quality is encountered the manufacturer is faced with the challenge of identifying the cause of the quality problem. In order to do this it is important to first identify the specific nature of the problem. In the food and beverage industry this is often done by sensory testing panel consisting of highly skilled people that are able to observe slight differences in the flavour of a product. Once it has been established that a product is not up to standard, the investigation into the causes can begin.

Against this background analytical chemistry is assuming an increasingly important function in the field of quality control. In the food and beverage industry the products consist mainly of organic compounds. Sub-standard tastes or aromas, so called ‘off-flavours’, are the result of changes in the composition or relative concentrations of these organic compounds. Chromatographic methods have played an important role in the understanding of how these compounds interact to produce a particular flavour.

Food and beverage samples are very complex in nature and the flavour producing compounds are often present only at trace level concentrations [1]. Various strategies can be employed for the trace level analysis of such samples, however, the steps in the analytical procedure should be carefully tailored to suit a specific sample. The choice of analytical strategy depends primarily on the nature of the analyte, the matrix in which it is found and the detection limit required.

Studies have shown that the organic compounds primarily responsible for flavour are volatile or semi-volatile in nature [2,3,4]. These compounds are therefore amenable to separation by means of gas chromatography (GC). GC has developed into a trusted and routinely used separation technique in most quality control laboratories. The development of reliable gas phase detectors, able to meet the levels of sensitivity and selectivity required in modern analytical science, allow for reliable trace level analysis of complex samples.

Food and beverage samples are typically not compatible with direct introduction into a gas chromatographic system. Of the three basic steps in any analytical procedure, namely; sample preparation, separation and detection, the sample preparation step is the most important consideration for ensuring a proper GC analysis of such samples. The basic premise of any sample preparation is the transfer of analytes of interest from their original surroundings (sample matrix) into a form more suitable for introduction into an analytical instrument [1]. This can be achieved by various techniques, each of which has its own advantages for specific analytes and analyte-matrix combinations. The preparation methods used for complex food and beverage samples are typically based on extraction of the analytes with large amounts of high-purity solvent. This is a time consuming and expensive process, which increases the risk of sub-standard products being overlooked.

Such problems have necessitated the development of solventless sample preparation techniques. Solventless techniques are becoming more popular and are finding their way into routine methods used for the analysis of food and beverage flavour.

2. Project outline

The project had two distinct aims. The first was to develop a solventless sampling technique that can be used for the collection and analysis of volatile aroma compounds. The second was to investigate the potential of this sampling technique for producing highly characteristic aroma chromatograms that can be used for quality control purposes.

The sampling technique was to be developed for sampling the aroma of alcoholic beverages, such as beer. It was to consist of purging the volatile aroma compounds from a beer sample and trapping it in silicone rubber tubes. The analytes are then thermally desorbed from the silicone rubber into a GC for further analysis. These results were to be used for determining the suitability of silicone rubber based traps for collecting and preserving an aroma sample under various conditions of handling and storage. Literature reported silicone rubber as being a successful extraction absorbent of both gas and aqueous phase organic compounds [5].

In the development of this technique a number of aspects were to be considered. The first would be that the technique should be as simple and robust as possible in order to produce repeatable results. With the technique one should also be able to collect the entire range of volatile compounds present in the vapour phase of the sample. From low molecular mass, very volatile compounds to high molecular mass compounds of lower volatility. Finally, the collected compounds should be stable in the selected sorbent material, so that no changes in concentration or composition of the compounds occur during storage or handling of the sample.

The optimised sampling technique would then be evaluated for the characterisation of different beer brands. The characterisation would primarily be done by one-dimensional GC-FID but also be demonstrated with the more powerful GC-MS and comprehensively coupled multi-dimensional gas chromatography (GCxGC). The multi-dimensional technique produces a two dimensional chromatogram wherein the constituents of a sample are separated orthogonally by means of two distinct methods of chromatographic separation. The result is a pattern where the compounds are sorted into homologous groups each appearing on a distinct area of the two-dimensional chromatographic separation plane. Any changes in the

composition of a sample can therefore easily be observed by means of visual inspection. The concepts mentioned above are discussed further in Chapters 2 and Chapter 7.

The objective was to apply the developed sampling technique as part of a method that can produce highly characteristic chromatographic aroma profiles by which different brands of a specific beverage can be distinguished. This would allow for the identification and chemical investigation of any uncharacteristic features in the aroma profile, which may be responsible for variations in product flavour and quality.

3. Approach and presentation

The work presented here will demonstrate the use of the multichannel silicone rubber trap, as a sample collection device, for the analysis of alcoholic beverage aroma volatiles. In this thesis, Chapter 2 will introduce the concepts of flavour and aroma. A description is given of how flavour originates and how it applies to food and beverage quality. Methods used for the analysis of flavour and aroma are discussed and the chapter is concluded with an introduction to beer aroma. Chapter 3 will review sample preparation techniques used in the food and beverage industry. The techniques are evaluated with special emphasis on their application to aqueous samples such as beer and compared to the multichannel silicone rubber trap for this purpose. Important parameters relating to the optimisation of gas phase sampling techniques are highlighted in terms of how they apply to the use of multichannel silicone rubber traps. The multichannel silicone rubber trap as extraction device for the quantitative analysis of aqueous samples has been a subject of research for a number of years. Various published papers and theses resulting from this work have been extensively used as background to this chapter.

The development and optimisation of the sampling method is described in Chapter 4. The methods of chemical analysis, in particular the GCxGC system, are briefly explained. Of particular interest is the use of the Gerstel CIS 4 thermal desorption system with the multichannel silicone rubber traps. The system was used to desorb the collected compounds from the multichannel silicone rubber traps and to cryogenically focus the sample before introduction into the GC. The special features of this system and optimisation of the desorption procedure is discussed.

Repeatability was one of the biggest challenges during the development of the technique. Results demonstrating the optimisation of sample preparation and analysis methods for repeatability are presented and discussed in Chapter 5.

Chapter 6 shows the use of the technique to produce characteristic aroma profiles of different beer brands. The stability of the collected aroma sample over time and under different conditions of storage was demonstrated. The aroma profiles of three different beer brands were compared and distinguished one from the other with a rudimentary method of pattern recognition, in order to demonstrate the practical application of the technique.

Application of the sampling technique to other methods of analysis and detection such as multidimensional gas chromatography (GCxGC) and mass spectrometry (MS) is demonstrated in Chapter 7. This aspect falls outside the original scope of this investigation, however, it was important to show that the technique was translatable to methods other than the one on which it was developed. Final remarks, including areas of future research on beer aroma with the aid of the silicone rubber trap are provided in the concluding Chapter 8.

Chapter 2

Carlsberg

Text from the bottle label: Carlsberg Beer - Brewed and bottled by the Carlsberg Breweries, Copenhagen, Denmark.

Chapter 2

FLAVOUR

1. Introduction

The perception of fragrance and aroma has strong influences on human behaviour. The senses of smell and taste are closely interrelated in the mechanism by which living organisms perceive their surroundings from the air they breathe or food they consume.

The term ‘odour’ is described by Etzweiler *et al* [6] as the combination of biological, physical and psychological responses, caused by the interaction between chemical stimuli (aromas and fragrances) and the receptors of the olfactory system of living creatures.

The study of odour perception and the chemicals that cause fragrance and aromas have found relevance in several fields of industry and science.

Perhaps the oldest and most well known is the perfume industry. For centuries pleasant smelling plants have been extracted in various ways and blended to produce perfumes and related products. The natural essential oils in the plants contain the fragrance compounds. The rarity of some plants, or possible toxic effects of these natural compounds, creates demand for synthetic alternatives. Determining which chemicals are responsible for the sought after fragrance is therefore essential for the production of synthetic fragrance ingredients [7].

For the biological sciences, the understanding of the emission dynamics and composition of natural-occurring volatile compounds is fundamental in the study of how animals and insects communicate through odour. Fragrance is also a factor in plant reproductive processes and certain organisms’ defence against predators [7].

Odour can be an important parameter in the detection of environmental pollution [7]. It serves as an early warning system when natural media like air and water are contaminated. Environmental scientists can often trace the origin of pollution by recognising the odour of certain chemicals.

For food science flavour is the most prominent parameter by which food is judged for quality and acceptability [7]. According to Sides and Rodards [8], the term “flavour” is used to collectively describe the multitude of sensory responses related to the desirable tastes and aromas from food. Understanding the way in which flavour is perceived and which chemicals play an active role in a specific flavour allows the production of better food and beverage products and aids the development of synthetic flavours.

For all of these applications the accurate identification and quantitative analysis of compounds responsible for the flavours is essential. In this chapter attention will be given to the study of flavours in foods and beverages. The origin and causes of food flavours will be discussed as well as the aspects that influence the analysis of flavour compounds. The chapter will conclude with a general description of the beer brewing process and brief description of the most prominent classes of compounds responsible for the characteristic flavour of beer.

2. Food flavours

The flavour perceived from a food or beverage is a series of sensory responses to a combination of both taste and aroma stimuli [2]. These stimuli result from a complex mixture of chemical components that are both volatile and non-volatile in nature. The particular flavour of foods and beverages originate from different combinations of these chemical components. There is a definite interaction between taste and aroma for the perception of flavour. Most people have had the experience of eating or drinking something while having a blocked nose. A familiar food then tastes totally different to that usually perceived.

Independently, the senses cannot accurately characterise a flavour. Laboratory studies have shown that individuals are prevented from distinguishing between different flavoured drinks by blocking the nasal opening [2]. So, both taste and smell is required for the perception of flavour. The question therefore arises which response, odour or taste should be considered dominant for the perception of flavour. In general, the more volatile compounds from a food or beverage constitute the component that would be perceived as odour. In turn the compounds responsible for stimulating the taste receptors are predominantly non-volatile in nature [3].

This particular question has been of interest to both flavour chemists and food scientists for a number of years. The interaction of taste and odour to give a particular flavour experience has been the subject of numerous research papers. In a review of *Taste vs. Aroma* literature by Ann Noble [2] she makes the statement that "the perception of flavour involves the integration of the separate sensations of smell, taste and touch and is influenced by their interactions".

Experiments discussed in Noble's review show these interactions are most pronounced when the associations are congruent [2]. In other words, if the taste and odour compliment each other, the overall flavour of a food or beverage is then enhanced. So, for instance, the odour of strawberries was found to enhance a sweet taste while the odour of peanuts did not. Conversely, an increase in the sweetness or acidity of a fruit juice enhanced its odour or aroma, and it was perceived as having a more fruity flavour.

If one now considers the volatile and non-volatile chemicals that cause the odour and taste responses, which group would be the most useful to characterise a particular flavour? As stated earlier, taste in general is a response to the non-volatile chemicals present in food [3]. These non-volatile chemicals are a combination of sugars, acids and salts all of which are relatively simple molecules and produce similar taste responses [9]. The experiments have shown that the taste can be enhanced by a complimentary odour. Noble further states that adding salt or acid (typical taste chemicals) to a beverage, increases the concentration of non-polar volatile compounds (typical odour chemicals) in the atmosphere above the liquid. This would further enhance the flavour perceived from a beverage.

A conclusion that can be drawn from this study is that a characteristic flavour would be the result of a particular combination of volatile odour chemicals which can be enhanced or masked by the presence of non-volatile taste chemicals. The complex flavour one experiences when tasting a beverage like wine or beer therefore has to be due to the volatile component of the flavour, of which a much larger variety has been identified from foods and beverages than the non-volatiles [10]. The compounds comprising the volatile component of the flavour can therefore be considered predominantly responsible for the characteristic flavour of a food or

beverage [11]. In numerous studies the analysis of these volatiles could be linked to the particular flavour of a sample as perceived by a taste-testing panel [2,3,4,12,13].

Another fact brought to the fore by the debate on *Taste vs. Aroma* is that the human senses of smell and taste alone, though powerful, are not reliable enough to be used for the study of flavour. This is because human senses are subjective and they can be 'fooled' into perceiving something different to the true nature of a sample. Chemical analysis is therefore required to identify and quantify the volatile compounds responsible for flavour.

Chemical analysis on the other hand cannot replace sensory testing for unravelling the character of a particular flavour [14]. Despite the fact that instrumental analysis can be used to identify and quantify the components of a sample, there is no way of knowing which of these components are responsible for the particular flavour, without assessment by human subjects.

Sensory testing methods and chemical analysis are therefore used in combination to separate and identify flavour compounds [10]. In order to develop meaningful information from these techniques it is necessary to identify compounds present at concentrations perceivable by a taste-testing panel [15]. The question therefore is how much of a compound needs to be present for it to be perceived. This is the concept of *threshold* and it is defined as the concentration of a substance that can just be perceived [15]. Related to this is the *difference threshold* defined by Meilgaard [15,16] as the change necessary in the concentration of a substance to produce a noticeable difference in the perception thereof.

These concepts, along with well-designed sensory testing methods, have been employed extensively in order to identify flavour active compounds [10,13,16]. Over 6000 flavour active compounds have been identified since 1990 [17]. Along with the knowledge of their particular contribution towards a flavour, this has aided flavour chemists and food scientists in the development of techniques for the characterisation of flavours. Characterising a flavour is, however, not such a simple task, since even a simple natural flavour can consist of hundreds of compounds.

The concept of threshold is used to solve this problem. Of the dozens of compounds that constitute a flavour only a few are present above the threshold level.

Furthermore, these so-called flavour active compounds can be monitored through chemical analysis and used to assess the quality or consistency of a particular flavour. The analysis of flavour can therefore be used as a means of quality control for both products and raw materials used for the manufacture of these products.

3. Analysis of flavour

There is no question that it is the flavour of foods or beverages that is the most important parameter that will determine whether it is considered agreeable or not. As discussed above, determining the chemical composition of flavour is the first step in understanding how compounds interact to produce a particular flavour. This is, however, not the only or even the most important reason for the analysis of flavour.

The need for food and beverage analysis arises primarily from concerns regarding the nutritional value and the benefits to health that can be derived from it [18]. Other reasons include process control, quality assurance, checking for food adulteration, identification of origin or looking for natural products that can be utilised in other purposes. Lehotay and Hajšlová [18], in their article on the analysis of food, identify three questions from which they believe all needs for analysis of food originate.

1. What is the natural composition of the food or beverage?
2. What chemicals appear in a food as an additive or by-product from intentional treatment, unintended exposure, or spoilage?
3. What changes occur in the food from natural or human-induced processes?

Finding answers to these questions is clearly not an easy task. Sensory testing of food and beverages, which historically can be seen as the first and oldest technique, cannot be regarded as objective or descriptive enough to give anything more than subjective information regarding the flavour, appearance and texture qualities of products.

In order to find information on the chemical composition of food, as well as the additives and transformation products present, one has to resort to scientific analytical techniques, in particular instrumental analysis. Instrumental analysis techniques, many of which found application in food and beverage analysis, are the

basis of modern analytical science. These techniques and their applications will be discussed further in a later section.

Full compositional analysis techniques involve drying steps by which water, which make up a large component of foods and beverages, is removed. During further operations like extraction and analysis the sample is basically destroyed. These exhaustive and time consuming sample preparation techniques, which will also have a more detailed introduction in Chapter 3, are currently the only means by which compositional information can be obtained.

For applications such as quality assurance and process control of food and beverages, time and labour intensive analytical techniques such as these, are not required. These applications are aimed at answering the second and third “questions” proposed by Lehotay and Hajšlová [18], and therefore require analysis which is much more routine in nature than compositional analysis.

Currently a number of the techniques employed for routine analyses are based on tedious sample preparation steps. Quality control, therefore, requires a lot of labour and financial input from a manufacturer. The time involved in these lengthy sample preparation procedures prevents them from being implemented in-line in production. This means that only the final product can be conveniently tested and any irregularities found may lead to the loss of a whole production run. However, as discussed in recent analytical and flavour literature, a possible solution to this problem can be found in the analysis of aroma.

The study of flavour, and in particular the volatile or aroma component, has received more attention in recent years because of its relation to the quality of foods and beverages. Aroma is particularly sensitive to any compositional alterations that may occur in these products [19]. The detection of these so-called off-flavours produced through the degradation of food has become an important tool for quality control and the identification of food that has gone bad [17]. The presence of other compounds such as; additives, preservatives and contaminants should in theory also impact the flavour and therefore be detectable in this way.

The analysis of volatile compounds (i.e. aroma) provides much faster sample preparation and analysis, as discussed later. This enables in-line analysis, thereby streamlining quality control throughout the manufacturing process [20].

However, before discussing the analytical applications further, one should first look at the problems associated with food and beverage samples. As identified by Augusto *et al* [7] the following inherent properties of aroma samples, pose an extreme challenge to their chemical analysis. These points also help to emphasize the importance of proper sample preparation techniques.

1. The concentration of flavour active volatile compounds in a sample can be very low. In Table 2.1, the threshold concentrations of some substances important in food and beverage aromas are shown. Some of these threshold level concentrations (concentration at which a human subject has a 50% chance of perceiving the compound), are well below 1 µg/L (1 ppb).

Table 2.1: Threshold levels of some aroma compounds

Substance	Concentration
ethyl-4-methyl-4-pentanoate	0.06 nL/L
2-iodophenol	1 µg/L
Butyric acid	240 nL/L
2-ethyl-3,5-dimethylpyrazine.	0.007 nL/L

Reproduced from [7]

   Analytical procedures used for aroma analysis therefore have to provide extremely high sensitivity in order to detect and quantify substances at such low levels.

2. Besides the low concentrations at which these compounds are typically present, most aromas are also very complex blends of different substances. In beer, for example, over 800 compounds have been identified [16]. Classes of flavour active compounds found in beer include esters, aldehydes, ketones, higher alcohols, terpenes, organic acids, sulphur compounds, all dissolved in an ethanol-water solution [21].

One can appreciate that the separation and identification of such a wide variety of compounds in a mixture would present an extreme challenge to any modern analytical technique.

3. The aroma compounds generated by biological sources, such as plants, present further complications because of the dynamic nature of these systems. The emissions from plants can be affected by factors such as light, temperature, stress and environmental pollutants. The results obtained from the analysis of aroma before storage and handling of the sample can therefore differ significantly from those obtained after such operations.

4. By far the biggest problem is that some flavour active compounds have limited chemical stability. These compounds dissociate or change as a result of photolysis, oxidation or high temperatures. For example the atmospheric chemical lifetime of mono-terpenes (some of which make up the hop flavour in beer) was estimated to range from less than 5 minutes to 3 hours only [7].

As a result the chemical characterization of an aroma demands state of the art techniques for analyte separation, detection, quantitation and, very importantly, sampling and sample preparation [21].

4. Methods used for flavour analysis

From the foregoing discussion it is clear that the chemical composition of food is highly complex and comprises both volatile and non-volatile substances. The volatile component (aroma) is usually responsible for the characteristic flavour of foods and beverages, and therefore has received the most attention in research [22, 23]. In early research the emphasis was on the development of methods to establish the chemical identity of aroma constituents. As shown above the analytical task is rather complicated considering the number and dilute nature of these volatile compounds.

Initially, measurement of the total volatile composition of foods and beverages involved extraction and distillation methods in combination with gas chromatography (GC). Through these studies it was determined that the concentration of a volatile compound in a sample not necessarily reflects its concentration in the gas phase.

The concentration of these compounds in the gas phase is, however, the determining factor for the sensory perception of the aroma [22].

The so called headspace concentration was found to relate better to sensory properties, as the concentration of the aroma compounds in the gas phase depends on the interaction of the volatiles with the food matrix [4]. The focus of analysis therefore shifted from the determination of compounds inside foods and beverages to analysis of volatile compounds in the air around these samples (headspace). Various static and dynamic headspace techniques are extensively used today [1,7].

As the field of research progressed these and other techniques were developed for the investigation of many different aspects of flavour and aroma. Because of the nature of the volatile aroma compounds, gas chromatography is mostly used for analysis.

The combination of gas chromatography with mass spectrometry in the 1950's was a breakthrough in analytical aroma research and the beginning of the identification of a multitude of volatile aroma chemicals [10]. These and other similar detection schemes, such as infrared absorption spectrometry, are the techniques typically employed for aroma analysis

Only a few of the volatile compounds occurring in foods and beverages are present at concentrations exceeding the threshold level. In order to determine the contribution of these to the perceived aroma, the human nose, which is often more sensitive than most physico-chemical detectors, is utilised through the technique of gas chromatography-olfactrometry (GC-O) [24]. In this technique an aroma sample is separated by injection into a gas chromatographic column. A part of the effluent is split to the instrument detector while the rest is allowed to elute from the column into a device referred to as a sniff port.

Here a human subject then assesses the activity of each eluting compound by sniffing the effluent. The odour of each constituent in the sample is then related back to the corresponding chromatographic peak produced by the instrument detector [22]. This technique has aided the development of methods used for unravelling the complex and subtle flavours and aromas of beer, hops and malt. Aroma activities of

the many volatile compounds present in these products were established through groundbreaking work by Meilgaard [16] utilising GC-O methods.

In recent years research groups have developed methods for measuring the change in aroma profiles of products during consumption. These techniques represent a further development of the classic headspace methods [22,23] and involve collecting air at the nostrils of a subject consuming the product under investigation.

A new development in the field of separation science is the coupling of two gas chromatographic systems for the analysis of very complex samples. This technique, commonly referred to as GCxGC, is used to separate samples that are typically difficult to separate with a traditional one-dimensional system because compounds with similar physical or chemical properties (such as boiling point or polarity) would normally co-elute.

In comprehensive two-dimensional gas chromatography (GCxGC), the entire chromatogram eluting from one chromatographic column is submitted to a secondary column for a second independent separation. The resulting two-dimensional chromatogram has peaks scattered about a plane rather than along a line. The peak capacity (i.e. number of peaks it can separate) can be very large, thereby allowing more complete separation of complex mixtures such as petroleum products and aroma samples.

An added benefit of this technique is that the peaks in the two dimensional chromatogram are grouped together in homologous series of the compounds they represent. Consequently, a sample can have a characteristic pattern across the two-dimensional plane that can be utilised for identification or quality control purposes.

Besides the chromatographic techniques the use of electrochemical sensor arrays that are designed to emulate responses form the human sensing system have been growing in recent years. These “electronic nose” and “tongue” devices involve new materials such as piezoelectric crystals and synthetic conductive polymers as well as sophisticated data processing and chemometric techniques. These features allow the possibility of fast, direct qualitative and quantitative evaluation of specific compounds in a sample, with minimal or no sample preparation required.

These devices still have limited sensitivity and selectivity and do not provide true chemical analysis. They are, however, quite useful in rapidly detecting deviations from a chromatographic aroma profile to which they have been calibrated by statistical pattern recognition techniques.

5. Taste and flavour compounds in beer

As a conclusion to the chapter a brief introduction is given to the production of beer and the origin of beer aroma. The purpose of this section is to illustrate the complexity of a beer aroma sample and the diversity of the chemicals that contribute to the flavour. The information is largely sourced from the Wiley Encyclopedia of Food Science and Technology [25]

5.1 Beer brewing

Beer brewing involves three distinct but interrelated stages. The first stage is the preparation of an extract, referred to as sweet wort, obtained from the malted grains specially selected for the brewing process. The grain traditionally used to manufacture the malt used in beer brewing is barley. Barley is the grain of choice for brewing because among the cereals it is the only one that retains its husk during the germination process. The barley is selected from special strains that have been developed and grown especially for this purpose. The malted barley is the principal component in beer. It provides the starches and sugars, which will, partly or completely, become alcohol during fermentation; and the proteins and amino acids, which will supply the yeast with nutrition, as well as the colour, foam and flavour of the finished beer.

Barley malt is produced by soaking barley seeds in aerated water until they germinate. The germination produces a series of enzymes that modify the starch in the barley and prepare it for use in brewing. At a time when the modification is considered complete, the germinated barley is transferred to a kiln and dried. The temperature of this drying process determines the colour and partly, the so-called “malty flavour” of the beer. The dried product is called malt, and is stable and can be stored for extended periods of time until needed. Before it can be used the malt needs to be crushed. This is done usually just before use in order to keep it as fresh as possible.

The milled malt is then extracted with water in a process called mashing. This is a multistage process in which the mash (mixture of water and milled malt) is taken through a number of vessels at different temperatures, in order to produce the extract referred to as sweet wort. During mashing the temperature of the mixture is changed in a range of about 50 to 75°C. This is to enable the amolytic enzymes, which operate at different temperatures, to hydrolyse the starch from the grain into fermentable sugars.

After mashing, the malt residue (husks and fibres) is filtered out and the extract referred to as clear wort, is collected in a large vessel, the kettle, and boiled. Boiling the wort serves several functions:

- It stops the enzymatic reactions
- Sterilises the wort
- Concentrates the wort
- Removes the grainy odours present due to the malt and other grains used
- Darkens the wort and induces reactions between the simple sugars and the amino acids, which contribute to the flavour.

It is during the heating of the wort in the kettle that hops are added to the solution. The use of hops in beer only goes back some 500 years. Many plants and plant extracts have been used to flavour beer, until it was discovered that hops not only gives a pleasant flavour to beer but also controls the growth of spoilage bacteria. For this reason hops is now used in beer all around the world.

Hops contain a group of compounds referred to as humulones, which are very insoluble in water [25]. Boiling the hops with the wort serves to extract the oils and flavour compounds from the hops. The heat also induces certain reactions in the hop compounds that contribute significantly to the flavour of the finished beer [25].

Yonezawa and Fushiki [15] describe two distinct types of humulone compounds in hops oils, namely α– and β–acids, which structurally differ only in the fact that the β-

acid has an extra branched alkene chain instead of a hydroxyl group. There is, however, a big difference in the way the two compounds behave during the brewing process and also to the contribution of each to the bitter flavour.

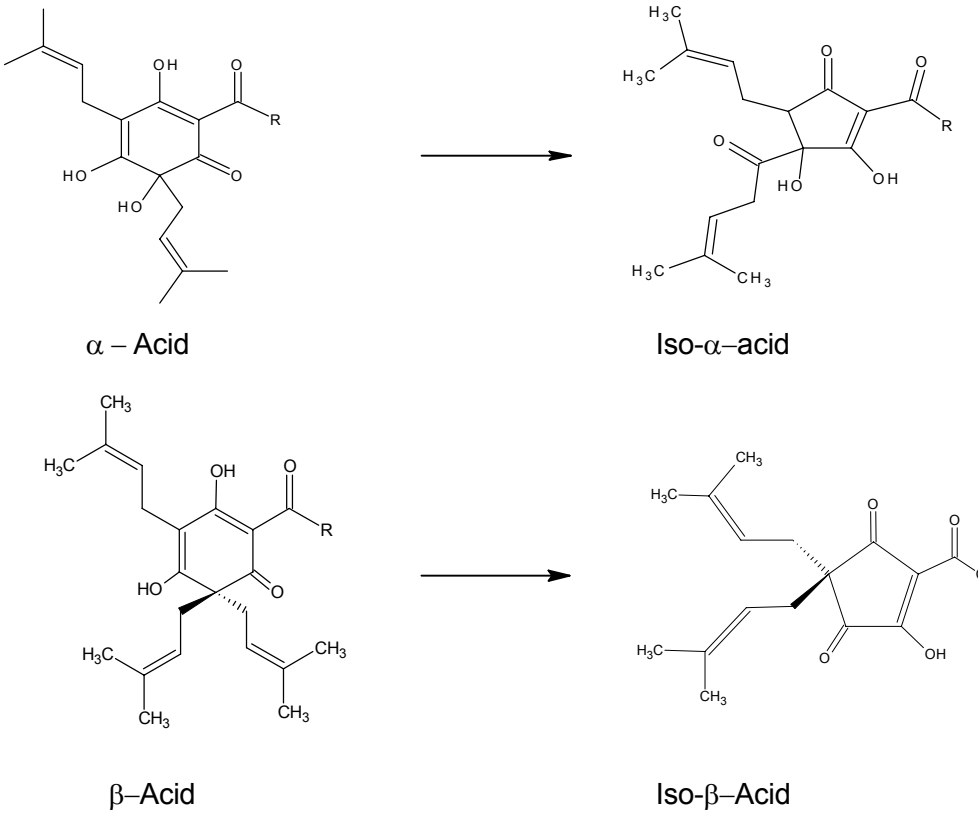


Figure 2.1: Chemical rearrangement of α- and β- acids during brewing [15]

During the brewing process these compounds undergo chemical rearrangement reactions (see Figure 2.1) to form an isomeric group of compounds called iso-humulones. The iso-humulones are soluble in water and impart to the beer the characteristic bitter taste. The α- and β-acids are not only made more soluble, but the thermal conversion is required for the activation of the hop bitterness. Table 2.2 lists the name of the compound and the conversion product, according to which alkane it has attached to the carbonyl group [26].

Hops also contain a volatile oil that consists of many odiferous compounds, some of which survive the brewing process and can be found in the final beer. The essential oil of hops is of terpenic origin, as is the case in all plants.

Table 2.2 List of humulone compounds and corresponding conversion product [26]

Compound group	Alkane group	Acid name	Conversion product
α-Acids	R = iso-butyl	Humulone	Isohumulone
	R = iso-propyl	Cohumulone	Isocohumulone
	R = sec-butyl	Adhumulone	Isoadhumulone
β-Acids	R = iso-butyl	Lupulone	Isohulupone
	R = iso-propyl	Colupulone	Isocohulupone
	R = sec-butyl	Adlupulone	isoadhulupone

The major terpenes present in the oil are the monoterpene myrcene, and the sesquiterpenes humulene and caryophyllene. The oxidation products of these terpenes namely, humaladienone and catyophyllene are more important to the flavour of beer than the base Terpenes [25].

Also present in the hops oil are alcohols like linalool and geraniol; ketones such as undecanone-2; and esters, namely geranyl butyrate. These also survive the brewing process and form an important part of the hoppy aroma of beer.

The second stage of beer production is the fermentation stage. At the end of the boil the wort is strained and settled to remove the hop leaves and any residue from the boil. The clear liquid is cooled and yeast is added. The particular strain of yeast added determines the type of beer that is produced.

The early beers were fermented by the yeast strain *Saccharomyces cereviciae* which is airborne yeast. It is a fairly hardy strain that survives in the atmosphere. This yeast is not added to the brew but enters the wort by means of exposure to the atmosphere. The resulting beer is known as ale, which can only be produced through this yeast strain. Besides ale this yeast is also used to make bread, wine and whiskey.

German brewers however discovered and made use of another strain of yeast found only in breweries and requiring cooler temperatures for survival. This yeast strain, *Saccharomyces carlsbergensis*, is used to brew the beer known as lager, which is produced all over the world today. This yeast is temperature sensitive and cannot survive in the atmosphere.

Through the metabolism of the sugars and amino acids in the wort, yeast cells reproduce and their numbers can multiply several fold during the fermentation

process. The yeast is responsible for converting the starches and sugars to alcohol and also for the effervescence of beer by splitting pyruvic acid and producing CO_2 as a by-product. The excess yeast produced in the process is removed from the bottom of the fermentation tank, which is where it settles after all the fermentable sugars have been depleted. Because the yeast enters a commercially sterile liquid, the wort, it can be used many times in successive brews without danger of contamination.

The last step in the brewing process is known as finishing. The fermented beer is transferred to another container while being chilled during the transfer. This stage called “ruh” (rest) gives the yeast particles still suspended in the beer time to settle out. During the period of rest the beer loses some of its harsher sulphury flavour and some of the other undesirable flavour compounds such as diacetyl, produced during the earlier fermentation. The finishing normally takes 7-14 days after which the final product is cold filtered through a medium such as diatomaceous earth and packed for distribution to consumers [25].

5.2 Beer flavour

The flavour of beer can be described as both subtle and complicated. Up to now it has not been possible to produce an artificial beer flavour. Producing a synthetic flavour is fairly simple if the key components of the characteristic flavour are known. For beer the problem lies in the fact that except for the bitter taste produced by the substances extracted from the hops no other peculiar taste components are found in beer [15].

The flavour of beer is the result of the combination of flavours produced through each of the operations involved in brewing. These flavours blend together in harmony with physical characteristics like foam, effervescence and colour to produce a particular beer flavour experience.

It is clear that characterising the flavour of beer is by no means a simple exercise. Pioneers in the field of beer flavour study, notably Morten C. Meilgaard [16], conducted numerous studies in order to unravel the complexities associated with beer flavour. His experiments were designed to not only identify the specific compounds that feature prominently in an analysis but rather linking all the compounds involved in the flavour and their interactions in terms of concentration

and threshold. The concept of threshold has been introduced earlier in the chapter. It defines the perceivable concentration of a substance.

The first step in Meilgaard's characterisation of beer flavour was to determine and quantify all the compounds present in beer. By means of sensory testing the threshold of each of these compounds were determined. The problem is now to relate the threshold of a compound to its concentration in the beer in order to earmark it as playing an active role in the perceived overall flavour. A relation known as the Flavour Unit (FU) developed by Meilgaard was used in order to classify the complex multitude of compounds into different classes of flavour activity [26].

The Flavour Unit (FU) of a compound is defined as the concentration of that compound divided by the difference threshold of the compound [15, 16]. This gives a value that links the significance of the compound to both parameters and not concentration alone. In other words, even if the compound is only present in a small quantity, if it has a small difference threshold then it can have a large FU value and be considered highly 'flavour active'.

Meilgaard suggested as a rule of thumb that compounds present in quantities greater than 2 FU can be considered highly flavour active. Those between 1 and 2 FU are flavour active while compounds present in lower concentrations of between 1 and 0.5 FU, would only be perceived through their interaction with other similarly flavoured compounds or by individuals that are sensitive to the compounds. Compounds present below 0.5 FU are unlikely to be flavour active and are usually disregarded.

The following sections discuss the most important compounds identified and researched in beer. It is by no means a complete reference of all the compounds identified but merely aims to show the diversity of compounds found in beer and how each contributes to the complexity and subtlety of beer aroma. By applying the information presented below one can predict the flavour character of a beer as alcoholic/aromatic, estery/fruity, or fatty/rancid, just like analysing humulones would be an estimation of the bitterness. Important flavours such as the malty, hoppy and oxidised flavours have, however, not yet been fully explained [15].

5.2.1 Alcohols

Besides water, ethanol and other higher or fusel alcohols make up the largest constituent of beer. In general alcohols have very high flavour thresholds and therefore have very weak flavour. Some higher alcohols may contribute nutty or flowery flavours but on the whole the flavour contribution is plainly alcoholic [15,16].

5.2.2 Flavanoids

Flavanoids are large polyphenolic molecules that originate from the raw materials used in the brewing process. According to Delcour [27] these molecules have received considerable interest in brewing research and in his article a number of these molecules identified from barley malt, hops and beer is shown. Besides the fact that flavanoids contribute to the formation of beer haze, recent studies, sited by Delcour, have shown that they have no impact on flavour or flavour stability.

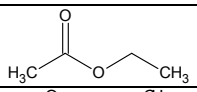
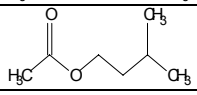
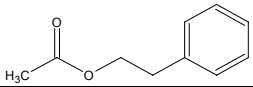
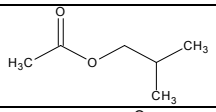
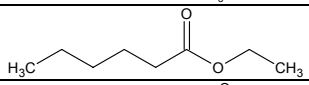
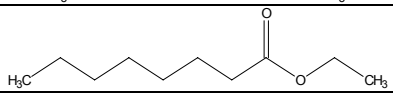
5.2.3 Acids

Most of the 110 acids that have been reported in beer are not flavour active because they take part in human metabolism and therefore occur in large amounts in our mucus membranes [16]. The less polar long chain aliphatic acids are more flavour active and have a unique rancid smell described as goat-like or body odour. The dissolved carbon dioxide, present in beer as by-product of the fermentation process, contributes to the acidity and gives the pleasant sparkling carbonation effect [15].

5.2.4 Esters

Volatile esters are only trace compounds in fermented beverages such as beer, but are extremely important for the flavour profile of these drinks. The most important flavour active esters in beer are the acetate esters, shown in Table 2.3 [28]. As will be shown in later chapters, esters comprise most of the volatile component of beer aroma. These compounds show thresholds 10 times below the corresponding acids and are very flavour active. They contribute fruity and flowery aromas to beer [16].

Table 2.3: Ester compounds commonly found in beer [28]

Component	Structure	Flavour description
Ethyl acetate		Solvent like
Isoamyl acetate		Fruity, banana
Phenethyl acetate		Flowery, roses, honey
Isobutyl acetate		Butterscotch
Ethyl caproate		Sour apple
Ethyl caprylate		Sour apple

The amount of volatile esters formed in modern high gravity fermentation methods can have detrimental effects on the flavour character of beer. The ester balance resulting from this technique performed in tall cylindroconical vessels, are often not optimal. Effort is therefore being made to understand these processes and the influence it has on beer quality.

5.2.5 Aldehydes and ketones

Most aldehydes show very low flavour thresholds and have very unpleasant flavours that become worse as the chain length increases. Ketones have thresholds comparable to that of alcohols and are of no importance to beer flavour because of the low concentrations at which they are present [16]. Diacetyl gives the unpleasant butterscotch flavour and trans-2-nonenal has a cardboard-like flavour, both typical of oxidised beer [15]. Aldehydic and ketonic flavour compounds can therefore be used as indicators of beer quality. Ketones are peculiar in the sense that unlike other compounds the flavour gets weaker but more pleasant as molecular size increases for the vicinal diketones.

5.2.6 Sulphur compounds

Sulphur compounds, particularly the volatile compounds of low molecular weight, are extremely flavour active even at very low concentration [15,29]. The flavour of mercaptans varies from rotten egg or onion to rotten fish, and can present severe quality problems to breweries because of the low concentrations at which these

compounds are active [16]. Volatile sulphur compounds usually account for less than 1% of the total amount of sulphur compounds in beer [29]. Although the link between sulphur compounds and off-flavours have been established in the late 1800's, analysis was difficult [30].

It is only with the advent of concentration techniques and highly sensitive and selective methods of detection that problems with sulphury flavours in breweries could start to be unravelled. Most sulphur compounds in beer are in fact involatile such as inorganic sulphates, amino acids, and proteins. However, these substances are directly involved as precursors for the volatile sulphury flavours [29].

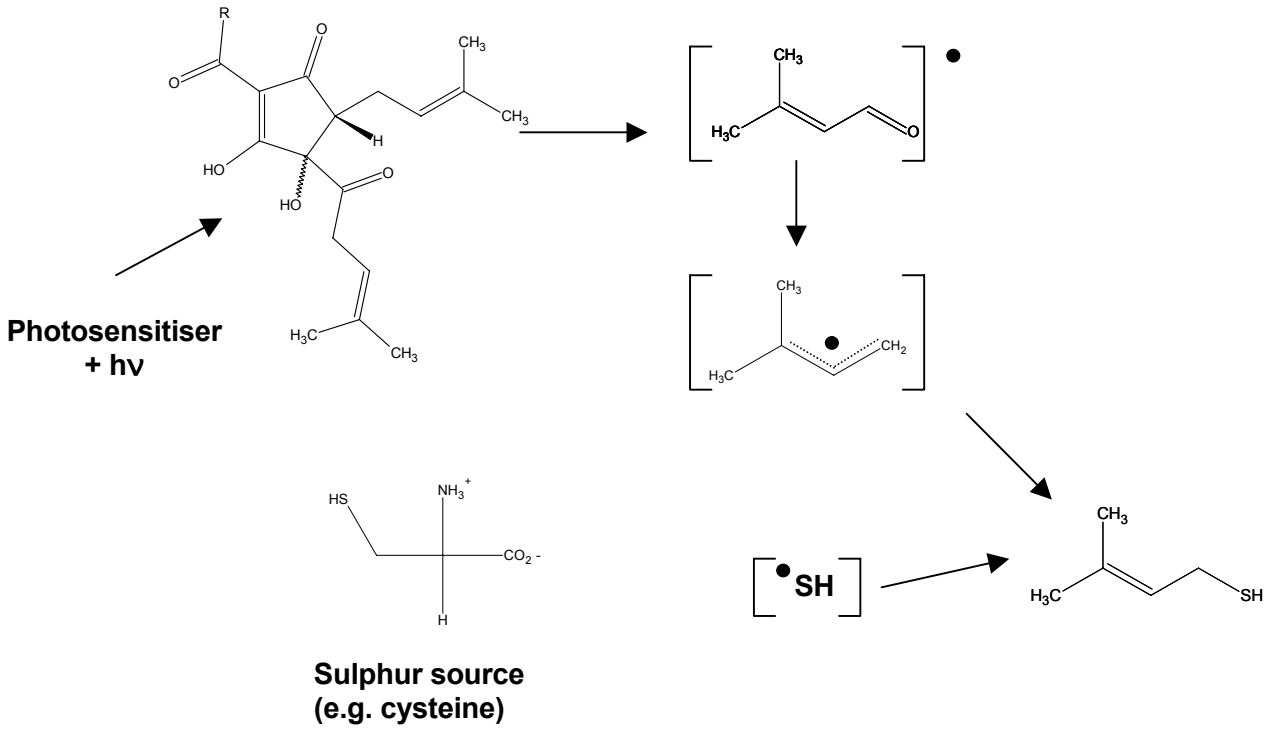


Figure 2.2: Formation of "lightstruck" flavour [31]

The photolytic transformation of a hop derived iso- α -acid compound to 3-methyl-2-butene-1-thiol, which is responsible for the objectionable flavour of beer exposed to light.

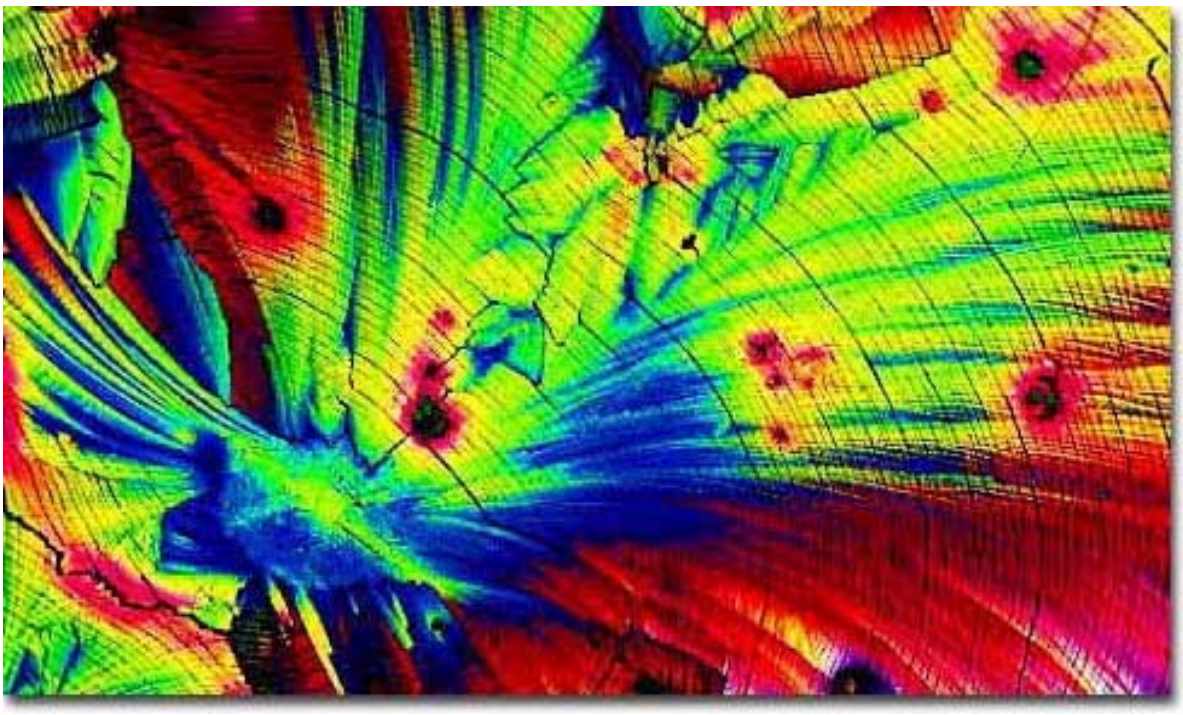
The most prominent is an objectionable "skunky" or so-called lightstruck or sunstruck aroma. This aroma is attributed to the compound *3-methyl-2-butene-1-thiol* (MBT) formed when beer is exposed to light in the 350 to 500 nm region [30,31]. An iso-α-acid compound from the hops is degraded to form MBT. The α-acids cannot, however, absorb the low wavelength light so a photosensitiser, which in beer was found to be riboflavin, is required to transfer the energy for the degradation to the

acid molecule. Iso-α-acid also does not contain sulphur atoms, so a sulphur source in the form of cysteine (demonstrated by Verzele & De Kukeleire [32]) needs to be present. The resulting mechanism for the formation of MBT is illustrated in Figure 2.2 [31].

6. Conclusion

The literature reviewed in this chapter provided a background of proof that volatile compounds are largely responsible for the aroma perceived from foods and beverages and can therefore be used for the characterisation of flavours and aromas. For beer, the classes of characteristic aroma compounds indicated in Section 5, can be chemically analysed with techniques based on chromatography. The chemical analysis of these aroma compounds is complex due to certain aspects related to the very volatile nature and low concentration of these compounds in food and beverage samples. For analysis proper concentration of these chemicals are therefore required. Chapter 3 describes the techniques that can be used for the preparation of aroma samples for chromatographic analysis, and discusses the aspects relevant to aqueous samples in particular.

Chapter 3



Guinness stout

Text from the bottle label: Guinness Stout - Guinness Extra Stout, St. James's Gate, Dublin. Brewed in Ireland by Arth Guinness Son and Co.

Chapter 3

SAMPLE PREPARATION

1. Introduction

In Chapter 2 the contribution of highly sensitive detection and separation techniques, like mass spectrometry (MS), detector arrays (electronic nose/tongue) and gas chromatography (GC), to the understanding and analysis of flavour was introduced. Modern separation and analysis techniques such as these are capable of separating extremely complex mixtures and also quantifying and identifying constituents at femtogram levels or below [33]. Understandably, technological advances and expansions in the application of these techniques dominate the progress in this field of analytical chemistry [33]. Unfortunately most of these instruments cannot contend with the complex matrices in which flavour compounds naturally occur i.e. aqueous mixtures or moist solids. It is therefore necessary to transfer the analytes (e.g. aroma compounds) either into a more acceptable phase, like an organic solvent or gas, and, for very dilute samples, to concentrate the analytes in order to make detection possible [7, 34].

Proper sample preparation therefore forms an integral part of aroma analysis. Unfortunately, the importance of sample preparation to the success of an analysis is, in general, often overlooked. Recent articles on the subject describe how this area of analytical chemistry was neglected because it was seen as less “glamorous” than other steps [8,33]. Fortunately the importance of sample preparation is now being reflected in literature due to advances like solid-phase extraction, pre-concentration, sample enrichment and sample handling techniques [7,17,35,36,].

The development of sampling as it applies to flavour and aroma analysis will be briefly discussed in this chapter. The focus will be on the sorptive techniques as used for the pre-concentration of volatile compounds in the headspace of a sample. The central theme of this project was the development of a sample preparation technique and its application to the analysis of beer aroma. Through this work it is demonstrated how the complexity of aroma samples and dilute nature of the

components make sample preparation and concentration essential to the successful analysis of flavour. [7,8].

2. Sample preparation methods

The basic concept of all sample preparation methods is the transfer of analytes of interest from their original surroundings (sample matrix) into a form more suitable for introduction into the analytical instrument. This can be achieved by various techniques, each of which has its own specific strengths for specific analytes and analyte-matrix combinations [1,37]. Problems common to all sampling techniques used in the analysis of aroma are the potential destruction of analytes and the possible production of artefacts.

Sample extraction and concentration techniques used for aroma analysis can be divided into four broad categories namely distillation methods, chemical methods, liquid extraction methods based on solubility, and solid phase extraction or sorption based methods [14]. The following are brief discussions of each as it is applied to aqueous samples such as beer, with particular focus on the sorption-based methods.

2.1 Distillation methods

Distillation is a physical separation technique based on the differences in volatility of compounds in complex samples like foods and beverages. It is one of the oldest techniques of separating compounds and indeed in sample preparation. It is mostly used for the concentration of volatile and semi-volatile compounds from solid samples like foods or plant material. Several techniques that operate on this principle have been developed for different applications, a few are discussed in the following sections.

2.1.1 Steam distillation

Steam distillation is one of the earliest methods used for the extraction of volatiles. The sample is dispersed in water that is then heated to boiling point. The steam is collected and the resulting condensate is further extracted with solvents to concentrate the volatiles. One drawback of this method is that due to the heating of the mixture, some of the thermally labile compounds may decompose. These “fragments” of the original molecules, referred to as artefacts, complicate the

identification of compounds originally extracted from the sample and result in a lower concentration of the original compounds. Furthermore, the large volumes of solvent required to extract the condensate may also contain trace contaminants and contribute to the artefacts detected [14].

2.1.2 Vacuum distillation

An improvement on the method described above is to perform the distillation under reduced pressure. This lowers the boiling point of the system and thereby reduces the time and risk of artefact formation. Vacuum distillation is typically performed at pressures below 20 torr, and the use of pressures as low as 1 torr has been reported. Liquid distillation carried out under high vacuum may be followed by the collection of the resulting volatiles in traps cooled by liquid nitrogen. This method is called cold-finger distillation [14].

2.1.3 Simultaneous distillation-extraction (SDE)

A widespread distillation-based method used for sample preparation in flavour analysis is simultaneous distillation-extraction [7]. This method combines the distillation, extraction and concentration in one continuous step. This reduces the amount of solvent required because the same solvent is recycled in the system, which in turn keeps the solvent contribution to the artefacts to a minimum [14].

Other distillation methods include:

- A technique called flash distillation where steam is injected directly into a liquefied food sample. This method is effective for extremely volatile compounds but cannot be considered quantitative [14].

- Fractional distillation, using a distillation column and cold traps for recovering the volatiles [14].

2.2 Chemical procedures

Flavour samples are typically very complex; this may result in poor separation or the masking of minor but important compounds by other compounds present in high concentration [14]. In such cases the use of the entire collected sample is not practical so it may be desirable to reduce the number of components by splitting it

into different fractions [38]. Various wet chemistry methods utilising appropriate reagents, solvents and/or pH adjustment can be used to achieve very selective compound class separations. Examples include: 1) fractioning the volatile phase of a food sample into acidic, basic or neutral fractions by simple adjustments to the pH of a solution, 2) transforming a class like carbonyl compounds into stable derivatives for introduction into a GC analytical column [14] or 3) separation of a sample into different polarities by using a short bed of chromatographic packing [38].

Another interesting technique is the use of on-line chemical abstractors. These abstractors are organic or inorganic compounds that react selectively with a specific type of functional group thereby transforming it into a non-volatile derivative. This compound is placed on a solid support material before or after a GC column to remove interfering compound classes [14].

Limitation to the method is that a relatively large amount of sample is required, the quantitativeness of the method is questionable, and the methods are very time consuming.

2.3 Liquid extraction based methods

Direct liquid extraction is the most widespread sample preparation method utilised for separating analytes of interest from a sample matrix using a solvent [33]. This technique relies on the difference in solubility of compounds in the sample matrix and in a particular solvent. If the difference favours the solvent, then the compounds can be separated from the matrix. A solvent is selected with an optimum yield and selectivity, so that as few potential interfering species (artifacts) as possible are carried through to the analytical separation stage [33]. These extractions can be performed either in a batch or continuous manner and have been used for many years in analytical laboratories [34].

Liquid extraction methods include; solvent extraction from solids and liquid–liquid extraction from solutions. The solvents used for these may be organic liquids, supercritical fluids and superheated liquids or the extraction liquid may be bonded to a support material [8, 33].

Liquid extraction techniques have been used for volatile compounds, but are most often used for semi-volatile compounds. This together with the very low levels at which the flavour and aroma compounds are present in foods compared to the level of co-extracted matrix components, has generally restricted the use of direct extraction as a sample preparation technique [8]. Despite these limitations it is still a widely used technique in the food and beverage industry.

Liquid extraction techniques are discussed briefly in the following sections.

2.3.1 Liquid extraction of solid samples

For the preparation of any sample it is necessary to extract close to 100% of the analyte of interest out of a residual matrix without destroying the compounds or introducing artifacts. The easiest way to extract compounds of interest from a solid sample is to dissolve the solid material completely and then extract the solution with an appropriate solvent [33]. This is, however, not always practically possible with all solid food samples.

Alternatively, multiple extraction methods are used where exhaustive extraction is obtained by continuously recycling the solvent through the sample for some hours (e.g. a Soxhlet system). However, the analyte must be stable in the refluxing, boiling solvent, which once again may limit its use. Less efficient methods include immersing and stirring the sample in hot or cold solvents for prolonged periods [8].

All these processes can be quite slow and require the use of significant amounts of sample and large volumes of pure organic solvents to ensure complete extraction. The subsequent work-up employs solvent evaporation and concentration of the sample, which can be slow and manually laborious [8]. The added disadvantage is that any impurities in the extraction solvent are also concentrated through these procedures [33].

A solid/liquid extraction technique that can be used for the analysis of labile food flavour compounds is microwave-assisted extraction. In this technique microwaves are used for the extraction of plant or animal tissue into a microwave transparent solvent. Internal heating at the microscopic scale breaks the cells but the contents immediately spill into the cool surrounding solvent so that thermal degradation of

labile compounds is negligible [17]. This technique makes use of smaller amounts of solvent than other liquid extraction based methods.

2.3.2 Liquid-liquid extractions

The simplest method of extracting compounds from aqueous samples is to do a liquid-liquid extraction by shaking the sample in question in an immiscible water/organic solvent system. Compounds that are more soluble in the less polar solvent can then be efficiently separated and concentrated. Commonly available solvents can be used and the operation can be performed using a separating funnel in a batch or continuous fashion or at elevated temperatures by making use of a Soxhlet apparatus [14].

The operational parameters considered during the optimisation of a batch liquid-liquid extraction process are mainly choice of solvent combination and the ratio in which these are used. For continuous methods the stability of the sample matrix and flavour compounds in the boiling solvent is the major concern.

The liquid-liquid extraction technique has been used for volatile compounds, but is most often used for semi volatile compounds. After the compounds of interest have been extracted, a solvent concentration step is required (i.e. rotary evaporation, Kuderna-Danish evaporation etc.) to selectively remove the solvent. Heating and pressure reduction is usually employed during this procedure [14], which may result in the loss of volatile compounds. An alternative to this is using the liquid-liquid extraction as a technique for separating compounds from the matrix (sample clean-up) and then using a selective adsorbent to recover compounds of interest from the extract.

In spite of the simplicity that direct liquid extractions offer, the current tendency is to replace these by alternative techniques because of the high purity solvents required for trace analysis and the need to reduce the environmental and health risks associated with their use [7]. Direct liquid based extractions also cannot be applied to live samples [7].

2.3.3 Supercritical fluid extraction (SFE)

This is a more recent development in which a gas, (usually CO_2) held above its critical temperature and pressure, is used as extractant [8,39]. The enhanced mass transport properties of this supercritical fluid allow higher extraction fluxes and shorter extraction times than is obtainable with conventional methods. These systems are extremely efficient, quantitative and result in little or no artefact formation since low temperatures are used [14].

SFE is most often used for the recovery of volatile or semi-volatile compounds. The recovery of polar analytes can be a problem due to the non-polar nature of the liquefied gases most commonly used. The addition of organic modifiers or use of a more polar solvent such as supercritical water promises to extend the array of applications of SFE in flavour analysis, something that is a constant challenge to the field [8]. The major limitation to the widespread use of SFE is the fact that sophisticated equipment capable of withstanding high pressure is required for this technique [14].

2.4 Sorption based methods

Because of demands for trace analysis, the dilute nature of typical flavour compounds and the ever-increasing sensitivity of analytical instruments, more selective sampling techniques that separate and concentrate flavour compounds are required.

Currently the most favoured sampling techniques used in flavour and aroma analysis relies on trapping analytes of interest with an adsorbant material from either gas, liquid or solid samples. An adsorbant with a strong affinity towards organic compounds will retain and concentrate these compounds from very dilute samples [40]. The adsorbed compounds are then released by either thermal or chemical desorption methods followed by analysis [1].

The adsorbants used for trapping analytes are porous materials with high internal surface area such as activated carbon [1]. Recent advances in the manufacture of porous polymers have extended these techniques to a wider range of sample analyte combinations. The advantages of these polymeric adsorbents are their ability to

selectively retain organic compounds, while having very little affinity for water [14]. Numerous types of adsorbant materials with various surface characteristics and polarities are available for different applications.

A current trend is the use of materials such as silicone rubber that rely on the dissolution of the analytes into a liquid polymeric material rather than adsorption onto a surface. It is a much more inert means of solute retention which overcomes some of the limitations encountered with adsorbants. This technique is the basis of methods such as solid phase microextraction, stir bar sorptive extraction and the multichannel silicone rubber trap [1,5,40].

In a review article, Baltussen *et al*. [1] describes the differences between materials used for adsorptive and absorptive sample preparation. The primary difference is based on the mechanism by which the compounds are retained by the different materials and the consequence thereof for subsequent desorption. The following discussion is based on this review on sorptive sample preparation.

2.4.1 Adsorptive sample preparation

Adsorbent materials can be grouped into three categories. The first category includes inorganic carbon based materials such as carbon blacks, carbon molecular sieves, and activated carbon. These materials have very high affinity for organic compounds and can be heated to very high temperatures (400-450°C) without degradation [1].

Second is alumina or silica based materials that can be used to trap analytes from gaseous or liquid samples. These materials are used for larger molecules than are the carbon based materials and can be heated to 600°C. The surface of these materials can be covered with organic groups for selective enrichment of compounds from liquid samples, however, these organic coats are only thermally stable up to about 100°C [1].

The third category is the largest and most diverse and includes the commonly used polymeric adsorbents such as Tenax, Chromasorb, etc. These materials are copolymers of synthetic building blocks like styrene. These materials have excellent adsorptive properties and combinations of different building blocks or materials can be tailored for the concentration of specific analytes [1].

The primary disadvantage of these synthetic materials is that during heating (i.e. thermal desorption) depolymerisation occurs, releasing monomeric compounds and reaction products thereof. These, unfortunately, include many target compounds such as benzene, aldehydes and ketones such as benzaldehyde, acetophenone, 2,6-diphenyl-p-benzoquinone and 2,6-diphenyl-p-hydroquinone etc., particularly from Tenax the most commonly used material [41]. The contribution of these breakdown products to the chromatographic background can lead to false positives and is enhanced when traces of water or oxygen is present during heating of the materials.

The surface of an adsorbent material contains active groups that interact with analytes and bond them to the surface. This interaction can range from ionic bonding to weak van der Waals interactions, depending on the nature of the adsorbent material and analyte. The process required for desorption of the analytes from the adsorbent surface is determined by the strength of this interaction. Thermal desorption, for instance, can only overcome the weaker van der Waals type bonds while desorption with a solvent is required to overcome stronger interactions [1]. Due to this, thermal desorption of analytes after adsorptive sampling may result in poor recoveries even at very high desorption temperatures.

High temperatures result in degradation products of the adsorbent materials and may promote catalytic breakdown of the trapped analytes. Liquid desorption is most often combined with adsorptive sampling. Thermal desorption of these materials is thus often limited to very non-polar analytes such as alkanes, alkenes and hydrocarbons.

2.4.2 Absorptive sample preparation

Absorptive materials are polymers with their glass transition temperature (T_g) below that temperature at which they will be utilised for sampling-storage-desorption. At a temperature above the T_g the polymeric material behaves like a gum or even liquid-like material and not a solid. In this state the material has properties like diffusion and distribution coefficients similar to those of organic solvents [1].

These absorbents are in essence homogeneous, non-porous materials into which analytes can actually dissolve. The analytes do not undergo real temporary bonding, as is the case with adsorptive media, but are rather retained by dissolution. The dissolution of analytes is usually based on the so-called like-like principle. A

substance will always have a higher affinity for a phase that has similar properties to the substance itself. The analytes diffuse into the polymeric material and are concentrated and retained there because of a higher affinity for this material than the surrounding sample matrix [1].

The material most commonly used as absorbent is the non-polar polydimethylsiloxane (PDMS) also commonly used as stationary phase in capillary columns. This material is so popular because of the fact that it is very inert and so minimises the risk of irreversible bonding or catalytic reactions of the analyte. Retention data for many compounds on PDMS is available in literature, so the behaviour of analytes can be predicted. Synthesis of PDMS is fairly simple and as a result leads to very consistent properties and reproducible products between manufacturers.

Alternative materials used alone or in combination with PDMS for the concentration of more polar compounds include: poly(butyl)acrylates, waxes (i.e. Carbowax), cyanopropyl derivatives, Carboxen, divinylbenzene, etc. These compounds improve the range of application of sorption-based methods but have limitations in certain situations as described in the section on SPME later on. To ensure that the sorptive phase retains it shape during heating the polymers are crosslinked for mechanical stability [1].

When sampling is performed with adsorbent based methods the analytes are retained on adsorptive sites on the surface of a material. The surface contains a fixed number of these sites so equilibrium is reached between the analytes present in the sample and the analytes adsorbed. With a high sample concentration all the active sites will be occupied and an increase in sample concentration will not lead to an increase in the amount of compounds adsorbed. A further implication of this is that matrix compounds present in high concentrations can occupy active sites thereby preventing the adsorption of target analytes. For absorption based methods, analytes are retained by dissolution into these phases where they can freely diffuse throughout the sorbent [1].

Absorptive sample preparation methods provide a means of overcoming the limitations of adsorptive sampling, however, both these methods find application in a

number of modern sampling techniques. These techniques are the focus of the rest of this chapter, in particular those techniques used with samples in the gas phase. A few techniques commonly used for the analysis of aroma are discussed in the section following.

2.5 Modern sampling techniques

2.5.1 Solid phase extraction (SPE)

In this technique solid adsorbent materials are used to extract and concentrate compounds from aqueous samples. The sorbent material is packed in a small column and held in place between porous metal or plastic frits. This SPE “cartridge” is available in different sizes with volumes as large as 30 ml and can hold 20 mg to10 g of sorbent material [34].

The liquid sample is pumped through the cartridge and by selecting an appropriate solid phase, the aroma compounds from an extract can be retained on the sorbent material while the interfering materials are eluted. The reverse is also possible, where the interfering materials are retained and the target analytes remain in the liquid, however, the benefit of concentrating the extract is thereby lost [8]. With the former, the cartridge is dried and the retained aroma compounds are eluted with a suitable solvent [34]. The sorbant materials used for SPE cannot be reconditioned so the cartridge can only be used once and is discarded after use.

When this technique was first introduced it had many problems such as poor reproducibility due to large batch-to-batch variation in the cartridges and low recoveries resulting from sorption of analytes on the cartridge walls. Many of these limitations have since been overcome allowing SPE to be widely used in the field of aroma analysis. It is best suited to the recovery of semi volatile compounds and is mostly used for sample cleanup [8].

2.5.2 Adsorbent packed traps

This technique utilises solid granular adsorbents such as Tenax, activated carbon, etc. or combinations of these materials packed into tubes. A gaseous or liquid sample is blown or poured through the tube and target analytes are retained by the chosen adsorbent while the rest of the sample runs out of the tube [34].

Excess moisture or undesirable sample components can be blown off or selectively eluted from the trap before the analytes are released through thermal or liquid desorption methods. For thermal desorption, tubes of different sizes, ready-packed with various adsorbents or adsorbent combinations, are commercially available. These are usually manufactured for use with a specific model of thermal desorber and are made to fit this device only.

The materials used as adsorbent are manufactured to be thermally stable and retain their shape and can therefore be reconditioned by thermal means so that the traps can be used repeatedly without risk of carry-over.

The limitations of this technique are mainly related to the adsorbent material used, and to the sample capacity of the traps which is defined by the breakthrough volume, a concept explained in Section 3.6.6 on open tubular trapping.

2.5.3 Solid Phase Microextraction (SPME)

Solid Phase Microextraction, an absorption based method, was developed to address the need for rapid sample preparation. Pawliszyn *et al*. [40] introduced it in the early 1990's and SPME has since become a very popular technique for sample preparation, mainly because of its simplicity and the fact that no additional instrumentation is required for its use. It consists of a fused silica fiber with an outer diameter of, typically, 150 µm that is coated with an absorbent layer of 5 to 100 µm thick. This fiber is inserted directly into a liquid or gaseous sample and the compounds of interest then dissolve in the absorbent coating. The extracted compounds are desorbed from the fibre by inserting it into the heated inlet of a gas chromatograph [1,40,42].

The small size and round shape of the SPME fiber allow it to fit inside the needle of a syringe-like device. The fused silica fiber has very low mechanical strength. To prevent breakage it is attached to the plunger of the syringe-like device and this allows it to be retracted into the needle for storage or protection during piercing of septa (i.e. insertion into a GC inlet). By pushing the plunger in, the fiber is exposed for sampling or desorption [40].

Fibres are available with different types and thickness of coatings. These include copolymers of polydimethhylsiloxane (PDMS) with more polar compounds such as divinylbenzene and Carbowax and a mixture of PDMS with Carboxen. These combinations extend the range of compounds SPME can be used with, however, it still does not solve the lack of sensitivity, which is the most important disadvantage of SPME [1].

The thin absorbent PDMS coating has a very low analyte capacity. By adding other materials the capacity is improved, however, these materials are not pure polymeric materials and the absorption mechanism is thereby lost [1]. This means that more polar compounds like salts, acids, proteins, etc, present in complex samples, now compete for adsorbent sites with target analytes. This leads to irreproducible results and prohibits quantitation [1].

SPME has nevertheless found wide application and numerous studies of flavour compounds have been conducted with the aid of this device. Examples include, analysis of aroma compounds in vinegar (Marin *et al*. [43]), determination of sulphur compounds in beer (Hill & Smith [44]), and characterisation of whiskey (Fitzgerald *et al*. [45]).

2.5.4 Stir bar sorptive extraction (SBSE)

A recently introduced, novel approach to absorbent-based sample enrichment is Stir Bar Sorptive Extraction. It was noticed that non-polar compounds ($K_{OW} > 10^5$) were strongly sorbed onto the surface of glass vials used for SPME and the surface of the PTFE covered magnetic stir bars used to agitate solutions [1]. From this observation a technique was developed whereby a magnetic stir bar incorporated in a glass jacket, commonly used for agitating solutions in the laboratory, is covered with a thin film of silicone rubber (PDMS) typically 0.5 – 2 mm thick [1,46]. The coated stir bar is either inserted directly into the sample solution and stirred for a period of time, or suspended in the gas phase above the sample. The stir bar is subsequently removed and desorbed either by means of thermal desorption, which provides high sensitivity, or by a solvent for improved selectivity.

This technique provides an answer to some of the limitations of the other techniques discussed above. The thick film of PDMS coating results in a higher phase ratio and

subsequent increase in sensitivity compared to SPME. Loss of volatile compounds, common to techniques where drying of the extracting phase is required, is also avoided because the stir bar can be desorbed without drying [1].

SBSE has found wide application, examples include determination of preservatives in beverages (Ochiai *et al*.[46]), bitter compounds in beer (Sandra *et al*.[47]), and PCB's in water (David *et al*.[48]). Volatile aroma compounds present at trace amounts are, however, not as well concentrated with this technique, especially the more polar compounds (i.e. low K_{OW}) like esters, alcohols and acids that make up the aroma of beer. It is therefore not as easily applied to the characterisation of fragrances and aromas.

2.5.5 Membrane extraction

This technique was developed for use with mass spectrometry as a method of cleanup, but has also been applied to general sample preparation for low molecular weight compounds from aqueous samples, such as sulphur compounds in beer. It consists of a membrane that is in physical contact with the sample. The membrane is made from a non-polar hydrophobic material through which the low molecular weight compounds diffuse (i.e. PDMS). The compounds transported through the membrane are stripped by a carrier gas on the other side and transported to an intermediate sorbant trap or directly into a cooled GC inlet [40].

In the past supported membrane sheets were used, these were later replaced by hollow membrane tubes [49], which are self-supporting and provide larger surface to volume ratio for more efficient stripping. The tubes can be looped and put into a liquid sample with the open ends connected to the carrier gas inlet and receiving trap or instrument respectively. The compounds diffuse into the tube through which the carrier gas is flowing. The hydrophobic character of the membrane material prevents moisture from entering the carrier gas [40].

2.5.6 Open tubular trapping (OTT)

Open tubular traps consisting of capillary columns coated with sorbent materials, similar to GC capillary columns, have been used for the trapping of analytes from aqueous samples [34]. The water sample is forced through the trap and analytes are

then trapped in the capillary wall coating. Although the sorbent material used in OTT is similar to the active part used in SBSE and SPME, the extraction is fundamentally different in that it is not a single equilibrium mechanism. As the analyte molecules move through the trap they are subject to multiple extractions like in a chromatographic process. The compounds are released from the trap by heating the traps i.e. thermal desorption.

The efficiency of trapping is greatly dependent on the flow rate of the sample through the trap. Generally, the lower the flow rate the greater the residence time and therefore the better the chance of extracting trace compounds into the sorbent material [34]. If one relates this to what happens in a chromatographic column where, according to the van Deemter curve, an increase in flow rate past the optimum will decrease the number of plates, one can understand that a slower flow will result in a greater number of interactions over the length of the trap and therefore result in an increased residence time i.e. trapping of the analyte.

The breakthrough volume is defined as the volume above which the components retained in the trap start to elute from the trap. It is given by the following equation:

$$V_b = V_o(1+k)\left(1-\frac{3}{\sqrt{N}}\right) \qquad (3.1)$$

Where V_o is the void volume of the trapping column, k is the capacity factor of the sorbent in the trap and N is the number of plates in the trapping column (with N > 9) [50]. From Equation 3.1 it is clear that increasing the capacity factor increases the breakthrough volume, so a greater sample volume can be passed through the trap. The capacity factor is defined as $k = K/\beta$, where K is the concentration distribution constant and β the phase ratio. The phase ratio is defined as $\beta = V_m/V_s$, from which it follows that an increase in the volume of the stationary phase (V_s) will enhance the breakthrough characteristics of the trap. So, apart from increasing the length of the trap (i.e. larger V_o and more plates), increasing the film thickness will also increase the retention power of the traps [1,34]. In order to increase the capacity of the traps a number of processes for producing films of up to 145 µm thick were developed [1].

Open tubular traps have been used for the analysis of gaseous samples [1,34]. The diffuse nature of most gaseous samples, however, requires the use of traps with high

breakthrough volume. This implies either a very long thin film trap or a shorter very thick film trap. This may present problems because of the way sampling is performed. For sampling with an open tubular trap, a sample is either sucked or blown through the trap by means of a pump. The restriction of flow, as a result of the trap, should not be to high because this will make sampling times very long due to very low flow rates.

A new open tubular trap devised by Ortner *et al*. [5,34] overcame these difficulties by replacing the long thick film traps with a shorter multichannel configuration. This trap consists of a number of silicone rubber tubes positioned in parallel inside a glass tube (see Figure 3.1).

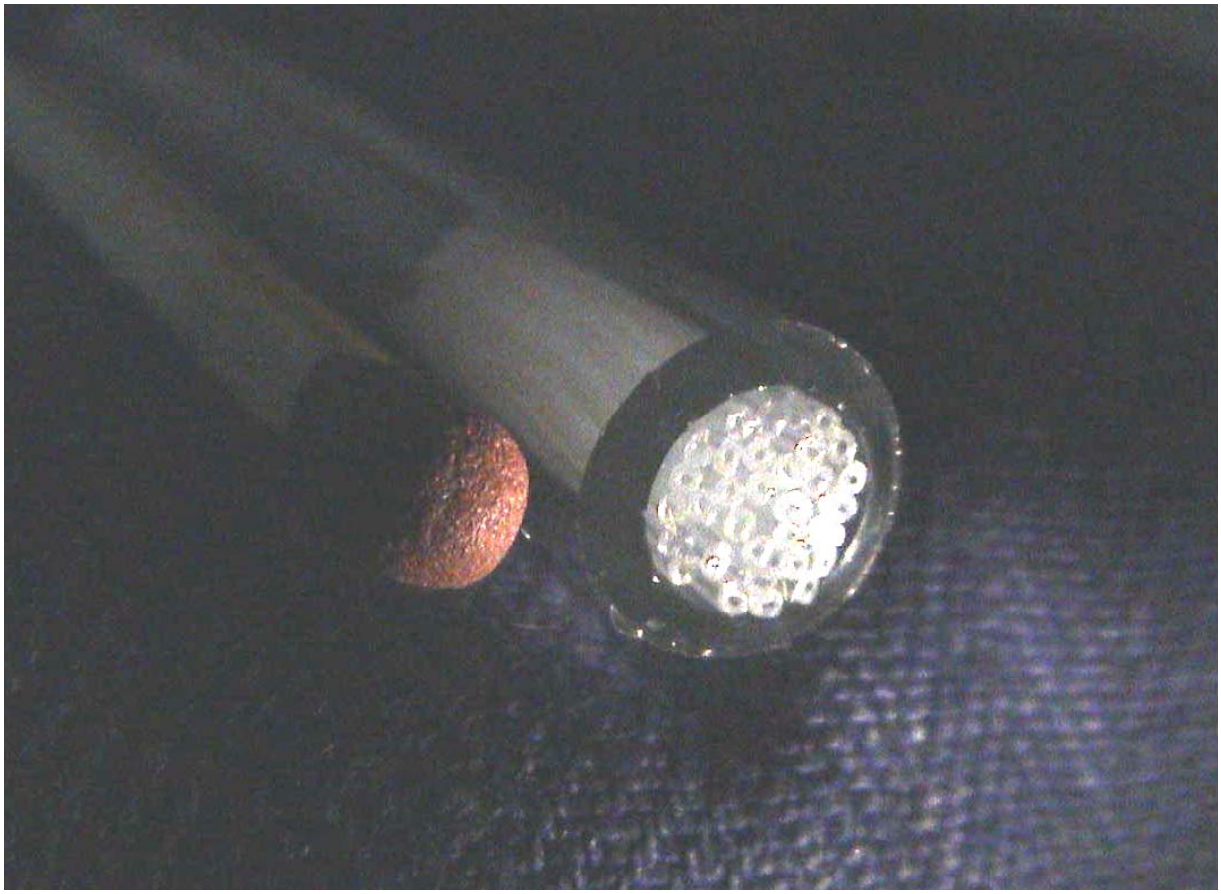


Figure 3.1: Silicone rubber tubes packed in a "multichannel" configuration

The photo shows a cross section of the multichannel silicone rubber trap. This particular trap contains 32 silicone rubber tubes and next to it is a matchstick for size comparison.

By comparing the total length of silicone in the multichannel configuration with a single channel tube of the same silicone length, roughly the same phase ratio is obtained. The other advantage of this configuration is that at the same volume flow

rate, the linear velocity through the multichannel trap is much lower than through a single channel trap. As explained earlier, a lower velocity increases the number of plates so a similar number of plates is attained as that of a long single channel trap.

This more compact configuration enables the multichannel trap to be manufactured to fit into commercially available GC desorption devices. This greatly simplifies the use and application of open tubular traps in the routine analysis of beer aroma samples.

3. Gas phase sampling theory

As described in Chapter 2, volatile compounds are primarily responsible for the characteristic aroma of food and beverages. Analysis of aroma therefore involves introduction of gas phase samples into a gas chromatograph.

This gas phase is generally referred to as the headspace [51] and is defined as the volatile components from a solid or liquid sample, placed into a closed container (like a capped glass vial), that will partition into the free gas phase over the sample. If the sample is kept at a constant temperature these volatile components will reach equilibrium between the gas and solid or liquid phase.

Methods for introducing the headspace into a gas chromatograph for analysis employ mainly two approaches. The first and simplest way is to directly analyse a portion of the headspace [7].

For direct methods the main consideration is that gas samples can only be introduced into a gas chromatograph if the pressure of the sample exceeds the head pressure of the column [34]. This is achieved through the use of a gas tight syringe or large volume injector whereby a sample of the headspace is injected under pressure into the column for analysis. Specialised headspace samplers pressurise the headspace vial with an inert gas prior to expansion of the sample into the sampling loop of a gas-sampling valve [34]. Many such direct methods have been developed for measuring the equilibrium concentrations of compounds above the sample.

With these methods a dilute sample of the headspace, usually containing water vapour, is transferred to the GC. Considering the problems associated with moisture on GC stationary phases and the trace levels of components in flavour samples, it is

usually not feasible to apply direct methods to this type of analysis because of the low sensitivity achievable [7].

Indirect methods on the other hand involve the intermediate concentration of analytes onto an ad- or absorbent material prior to desorption and analysis. The advantage of techniques utilising this approach is that sorbed volatiles are concentrated, thereby increasing the sensitivity. The samples can also be easily transported and stored in a stable form and then later desorbed by either thermal or liquid desorption.

Another advantage of the preconcentration step is that, after trapping of the analytes, the trap can be dried or purged with an inert gas stream. Because most sorbent materials have little affinity for water, this would remove any moisture that was present in the headspace from the trap, thereby preventing it from going into the chromatographic column. Techniques such as solid phase micro extraction (SPME), adsorbent packed traps, and open tubular trapping can be used in this approach. These techniques can be used as part of either a static or dynamic sampling method. The following discussion of these concepts was adopted from the review on sorptive sample preparation by Baltussen *et al*. [1].

3.1 Static sampling

In static sampling techniques all the extractant is in contact with the sample, so neither is renewed during the extraction. These techniques rely on the diffusion of the analyte into the extractant and ultimately the goal is that equilibrium be reached between the two phases. Shaking, stirring and sonification is often used to promote diffusion of the analyte into the gas phase, however, it should be noted that these operations only affect the time that the extraction takes and not the equilibrium of the extraction.

The physical process that governs the partitioning of the analytes from the sample into the extractant is analogous to partitioning process responsible for chromatographic separation. The factors that define the processes can therefore also be defined in the same way. For static extraction the most important controlling factor is the distribution coefficient K (referred to as the partition coefficient in chromatography). K is defined as the equilibrium ratio of the analyte concentrations in equal amounts of the sample and extractant respectively [1].

$$K = \frac{C_E}{C_S} = \frac{m_E}{m_S} \times \frac{V_S}{V_E} = \frac{m_E}{m_S} \times \beta \qquad (3.2)$$

With: C_E and C_S the equilibrium concentration in $g.L^{-1}$ of the analyte in the extractant and sample respectively. Which can also be written as the product of the analyte mass (in g) in each phase and the volume (in L) of the phases. The ratio of the volumes of the two phases is defined as the phase ratio (β) of a static extraction system.

$$\beta = \frac{V_S}{V_E} \qquad (3.3)$$

The phase ratio is used in chromatography to characterise a column. For a static extraction system this term can also be used for characterisation and, along with K, to predict the recovery of an analyte by an extraction system. This is defined by the extraction efficiency η.

$$\eta = \frac{1}{\frac{\beta}{K} + 1} \qquad (3.4)$$

Analytes with $K_i = 0$ will not be retained by the extractant, so it is important to match the choice of extractant to the analytes in question in order to achieve extraction. From Equation 3.4 it is clear that a large distribution coefficient will produce a high recovery. However, a large phase ratio (small volume of extractant relative to the sample volume) will lead to low recovery. In practice K is more or less a fixed property of the system and is chosen for a specific recovery with the minimum extractant. Care should be taken to make sure that the distribution constant of the static system is always kept constant in a set of extractions. This is difficult because temperature strongly effects equilibrium and distribution constants. Temperature control of 1-2°C is required to ensure repeatable results [1].

3.2 Dynamic sampling

In dynamic sampling all of the extractant is not brought into contact with the sample immediately. Gas phase or liquid samples are pumped through the extractant, which can be the packed bed of an adsorbent trap or sorbant film of an open tubular trap. The concentration of an analyte will decrease in the sample as it moves through the

medium, (i.e the analyte partitions from the sample phase into the extractant phase). At the exit of the trap the concentration of the analyte will be zero initially but will increase after a period of time. This is because the capacity of the trap for this analyte has been reached. The volume of sample that can be passed through the trap before this happens is the breakthrough volume as defined in Section 2.5.6 [1].

The breakthrough volume depends on what is considered an acceptable loss of analyte and is a function of the trap retention factor (k) the void volume in the trap and a factor taking into account the acceptable analyte loss. If the sampling is stopped before the first analyte of interest starts to elute from the trap, it is called breakthrough sampling.

If sampling is continued beyond the breakthrough point until all the analytes are in equilibrium with the sorption phase the technique is referred to as equilibrium sampling [1]. This is a very simple way of ensuring the collection of constituents in the sample for which the breakthrough volume is very high. The sample must, however, be large enough to prevent constituents with low breakthrough volumes from being lost due to depletion of these from the sample.

As with direct or indirect methods the dilute nature of food and beverage headspace samples and the very low concentration of the target analytes must be considered when choosing static or dynamic methods for sample preparation. Examples of dynamic sampling techniques include purge-and-trap and closed-loop stripping.

4. Purge-and-trap sampling technique

Purge-and-trap is a dynamic gas phase sampling technique commonly used for the preparation of flavour samples from aqueous matrices. The technique involves an inert gas that is bubbled through a liquid sample in order to strip volatile and semi-volatile compounds from the liquid phase. As the purge gas bubbles leave the liquid sample it carries with it the stripped compounds into the gas phase (headspace). This technique is usually performed in a closed container, which allows the headspace to be pushed through a sorbant based concentration device by the purge gas flow. An example of a purge-and-trap sampling arrangement is shown in Figure 4.2 (Chapter 4).

Once the sample is collected on the concentration device it is usually released from the sorptive media for analysis by means of thermal desorption. Thermal desorption involves the concentration device being heated while a stream of inert gas is blown over the sorptive media in the opposite direction to the purge gas flow used for collection of the sample. The desorbed analytes partition into the desorption gas stream and are carried into the gas chromatographic (GC) column for analysis. Before it enters the GC column, however, most desorption techniques use a means of collecting the analytes into a narrow “plug” for introduction in order to prevent peak broadening. The technique most often used is cold trapping. In this technique the sample laden desorption gas is passed through a cryogenically cooled tube in which the analytes are either precipitated onto the walls of the tubes or onto the granules of a packing material placed inside this tube. After desorption this cold trap is heated very rapidly to release the analytes in a very short time period into the GC column.

The purge-and-trap technique of sample preparation was selected for the analysis of beer samples. The following is a description of the parameters and concepts important to the optimisation of a purge-and-trap sampling technique.

4.1 Sample volume

The volume of the sample used for dynamic sample preparation methods, such as purge-and-trap sampling, only affects the amount of these components that can be collected if the sample is too small to provide enough of a specific component. Such a component is then depleted as it is purged from the sample and as it elutes from the trap it is not replenished and the amount of it retained on the trap (per unit time) therefore gets less.

4.2 Sample temperature

The temperature of a sample determines the amount of volatiles present in the vapour phase. It also has an effect on compound stability, so care should be taken not to increase the temperature to a value that will cause breakdown products, which lead to sample contamination. Sample temperature is also an important parameter when specific compounds in the sample are preferred to others. In beer, for instance, ethanol is a major component of the vapour phase, however, it is not an important compound for the investigation of the aroma. In order to prevent trapping high

amounts of ethanol, the temperature of the beer can be kept very low at close to 0°C. This prevents highly volatile components, such as ethanol, from saturating the vapour phase.

4.3 Ionic strength of sample solution

By increasing the ionic strength of a aqueous solution containing volatile organic chemicals, the concentration of these chemicals in the vapour phase can be increased. A simple way of doing this is to add a water-soluble salt, like NaCl, to the solution. This method is particularly useful where heating can cause sample degradation or excessive amounts of water vapour.

4.4 Temperature of concentration device relative to sample

Water is a definite problem if one works with cryogenically focused desorption. Water freezes in the cold trap and prevents a high flow through the sampling tube being desorbed. One method of minimizing the water captured during sampling is to keep the sampling tube a few degrees warmer than the sample. This prevents water vapour, which enters the tube as part of the headspace being sampled, from condensing in the tube. A drawback of this method is that very volatile components may also be lost if the tube is too warm.

4.5 Sampling volume

Sampling volume refers to the total volume of gas passed through the sampling tube during sampling. This will determine the sensitivity of the method for those compounds that have not yet exceeded their breakthrough volumes. Sampling volume consequently is a far more fundamental parameter than flow rate. At first approximation the sampling flow rate will not have any effect on the amount of a compound adsorbed on a sampling tube. (it will only affect N in the Equation 3.1 according to the van Deemter curve for the trap)

4.6 Sampling time

The time over which a sample is collected can influence other sampling parameters like sample temperature, sample integrity and sampling flow rate. Most of these parameters are, however, not of concern at relatively short sampling times and, like sample temperature, can easily be controlled. In conjunction with sampling flow rate,

sampling time determines the volume of sample collected and is important for dynamic sampling methods like purge and trap which have to consider trap breakthrough.

4.7 Desorption volume

Just like the sampling flow rate the desorption flow rate will not have an effect on the amount of a compound desorbed. However, the volume of gas required for the desorption of a specific compound is always the same at a specific temperature because it is a function of that compound's volatility and solubility in the stationary phase.

In general, a higher volume of gas is required for the desorption of a compound than is used to collect it. This is due to intermolecular forces between the analyte molecules and the adsorbent material. Using higher temperatures during desorption helps to overcome these forces resulting in a faster desorption

Desorption volume is the product of desorption time and flow rate, however, a specific volume is usually selected that is known to totally desorb all of the components off the trap.

4.8 Desorption time

The desorption time should be selected according to the flow rate so that the optimum desorption volume is achieved. It should, however, be long enough to compensate for the time lost through heating of the trap to the final desorption temperature required to volatilise all the captured components. So, a very high flow with a short desorption time is not necessarily the best method.

4.9 Desorption flow rate

In addition to the considerations affecting desorption volume, the flow rate of desorption will have an effect on whether a component of specific volatility will be captured in the trap used for refocusing the components before injecting into the GC column. If the flow rate is too high the very volatile components are 'blown through' the focusing trap. A flow for which no components are lost in this way should be optimised.

Chapter 4

Beck's

Text from the bottle label: Beck's is brewed in strict accordance with the "Reinheitsgebot", the German purity law of 1516, which stipulates that only the natural ingredients of barley-malt, hops, yeast and brewing water be used in the brewing process. Brewed and bottled by Beck and Company Brauerei, Bremen, Germany.

Chapter 4

OPTIMISING SAMPLING AND ANALYSIS OF BEER AROMA

1. Introduction

As described in Chapter 1, the aim of this project was to develop a method of sampling beer aroma suitable for quality control purposes. The requirements for the method is that it must collect the entire range of compounds present in a beer aroma in a repeatable manner so that aroma profiles can be compared directly for the identification of possible variations. The resulting aroma profiles should ideally represent the consumer's perception of a particular brand of beer. Furthermore, the collected compounds must be stable enough for transportation to a central laboratory for analysis, from anywhere in the world, without any changes to the aroma profile.

From the discussions in the foregoing chapters it is clear that headspace sampling is the approach best suited to beer aroma analysis. Any method employing liquid sample extraction procedures could alter the aroma profile to such an extent that subtle differences in the concentration of components in the headspace may be overlooked.

Because of the aqueous nature of a beer sample, the headspace contains water vapour and a sample preparation method that incorporates water elimination is required.

The chemical stability requirement means that the method of concentration should preferably be absorption based because the retention of solutes by dissolution provides much more stability to the concentrated compounds than adsorption based methods.

And, finally, a dynamic sampling method that efficiently extracts aroma components out of the aqueous sample matrix into the headspace is best suited to a beer aroma sample. Such a method helps to concentrate the trace components present in the dilute beer aroma headspace and improves the sensitivity of the method [16].

Considering all these requirements and limitations, the multichannel silicone rubber trap (MCT) seemed ideal for this application and was selected as concentration

device. With silicone rubber as sorptive medium, the dissolution mechanism utilised for retention should provide the stability required for the sample.

The non-polar nature of the silicone rubber and low affinity for water should prevent the concentration of the moisture present in the headspace. (Water collecting in the trap, as demonstrated later on, was still found to be a problem inherent to the sampling method, regardless of the positive attributes silicone rubber has in this regard.)

Furthermore, the open tubular design of the MCT is ideal for use with a dynamic means of sample collection that further aids the concentration of diffuse analytes in the headspace.

In this chapter the use of the multichannel silicone rubber trap for beer aroma analysis is demonstrated and the optimum conditions of the sampling and desorption procedures are investigated.

2. Experimental

2.1 Collection of a beer aroma sample

The gaseous headspace is collected on multichannel silicone rubber traps consisting of 32 polysiloxane rubber tubes (0.65 mm o.d. x 0.30 mm i.d., Silastic, medical grade tubing, Dow Corning, Midlands, MI, USA), of approximately 65 mm long packed parallel to each other as a bundle inside a 6 mm i.d. x 180 mm long glass tube.

The beer aroma sample is concentrated onto the MCT by making use of a purge-and-trap sampling scheme. Figure 4.1 shows the general arrangement of this scheme. The beer sample is placed inside a round flask and closed with an open ended hollow stopper that is equipped with a glass purge tube penetrating into the beer sample.

By means of this purge tube nitrogen gas is bubbled through the beer sample at a volume flow rate of 25 ml/min. The selection of this flow rate is based on work done by the South African Breweries (SABMiller) with similar equipment where the adsorbent (Tenax) was used in a packed trap. To directly compare the performance of the MCT with that of a solid sorbent filled tube it was decided to initially use the

same conditions of sample collection and analysis. The purge flow is set using an empty sampling arrangement and a bubble flow meter.

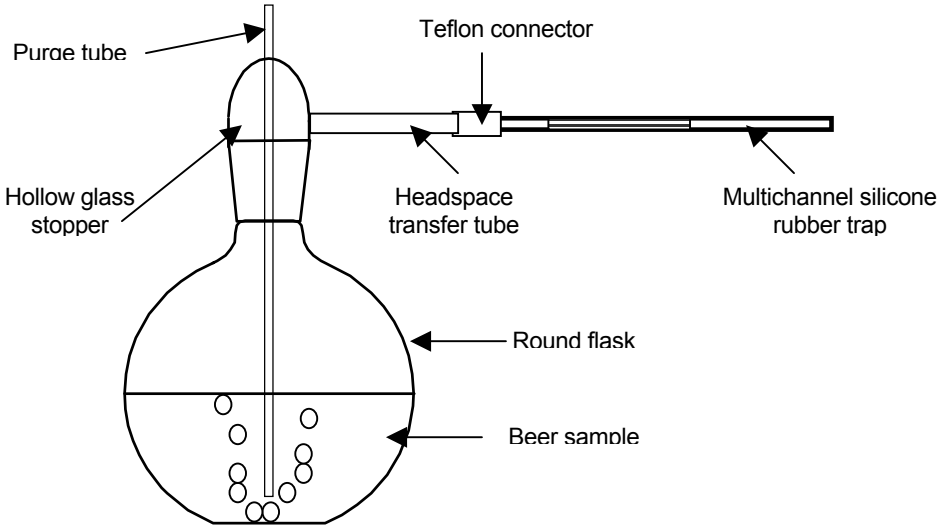


Figure 4.1: The purge-and-trap sampling arrangement

The diagram shows the purge-and-trap sampling arrangement used for the concentration of beer aroma volatiles onto the MCT.

The volatile aroma compounds are purged from the beer sample by the nitrogen gas stream. These compounds go into the vapour phase and are carried up by the nitrogen flow through a second glass tube, protruding from the hollow glass stopper, to a connected multichannel silicone rubber trap.

After sampling the MCT is disconnected from the purge-and-trap arrangement and capped. It is capped both to prevent the loss of collected compounds from the trap and also to avoid compounds present in the ambient laboratory air from contaminating the sample. The cap consist of Teflon™ tubing and a short glass rod of which one end is flame polished to fit snugly onto the open end of the trap.

2.2 Analytical instrumentation

All analyses are performed on a (Agilent Technologies 6890 model) gas chromatograph equipped with flame ionization detection. The instrument is equipped with a PTV inlet (CIS4, Gerstel) and a Thermal Desorption unit (TDS2, Gerstel). The thermal desorption unit, shown in Figure 4.2, is used for desorption of the MCT.

For desorption the caps are removed from the sample laden trap (MCT) and it is inserted into the horizontal desorption tube (TDS). A stream of carrier gas (H_2) is blown at a flow rate of 50 ml/min through the MCT from the rear (in the reverse direction to the sampling flow). The desorption-gas flow is towards the heated transfer line of which the top end protrudes into the front end of the MCT. The TDS is heated and this causes the compounds to desorb from the silicone rubber and partition into the carrier gas stream. The desorbed compounds are transferred through the heated transfer tube to the cooled injection system (CIS4), also referred to as the cold trap, where it is cryogenically refocused before injection into the analytical capillary column by rapid heating.

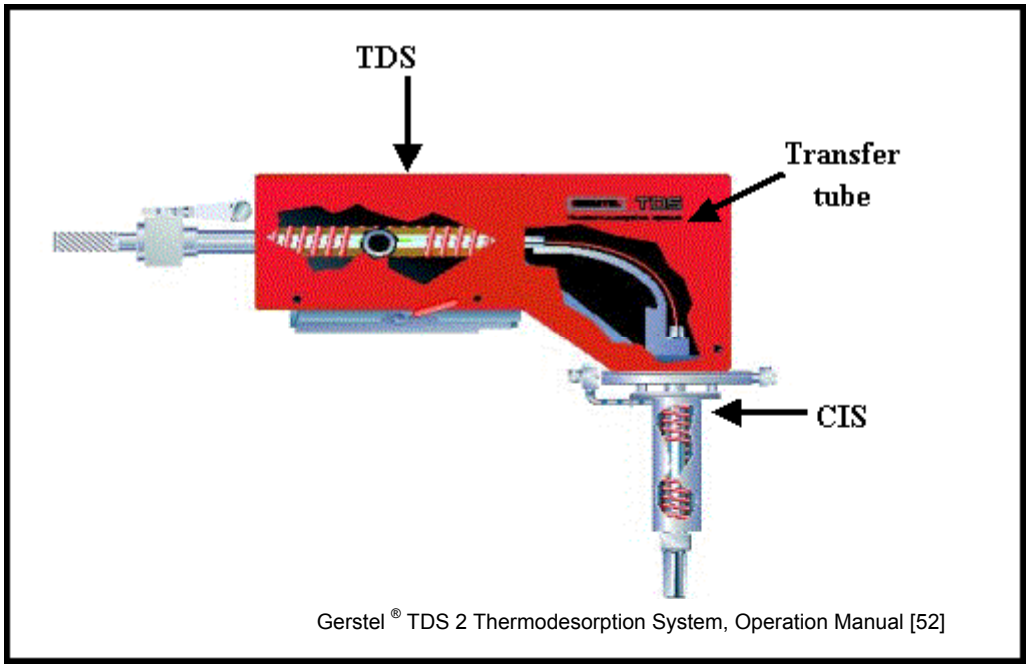


Figure 4.2: **Diagram of the Gerstel TDS 2 Thermodesorption system**

The cooled injection system or cold trap (CIS) is designed to form part of the GC inlet system so the focussed compounds are injected directly into the column without having to move through a second transfer tube.

2.3 Analysis Conditions

Prior to desorption, the TDS is held at 30°C and the sample purged for 3 minutes in solvent vent mode. The solvent vent mode is turned off and analytes are desorbed at 210°C for 10 minutes with a 50 ml/min gas flow and cold trapped in the CIS 4 inlet using a baffled, unpacked liner at –100°C. At the end of the desorption cycle analytes are transferred to the GC column with a 3 minute CIS heating cycle, in the split mode (split injection) (1:5). The column used is a 30 m x 0.25 mm x 0.25 µm HP-1 (Agilent).

Hydrogen is used as carrier gas at constant pressure (47.0 kPa). The GC oven temperature program is; 10°C (3 min), 5°C/min to 50°C, 10°C/min to 220°C (5 min).

3. Results and discussion

3.1 Optimisation of desorption conditions.

The initial conditions used for desorption of the beer aroma sample were very different to the conditions described above. As stated earlier the idea was to keep the experimental conditions as close as possible to those used for the work done by SABMiller. This was to see whether both volatile and less volatile components of the beer aroma could be sampled onto the multichannel silicone rubber trap and observed on one chromatogram, as was the case with the packed trap used by SABMiller.

These initial conditions include transfer of the analytes to the cold trap from where it is injected with a 40 ml/min (1:26) split flow. The following chromatogram (Figure 4.3) shows the first test run of beer volatiles collected onto the MCT.

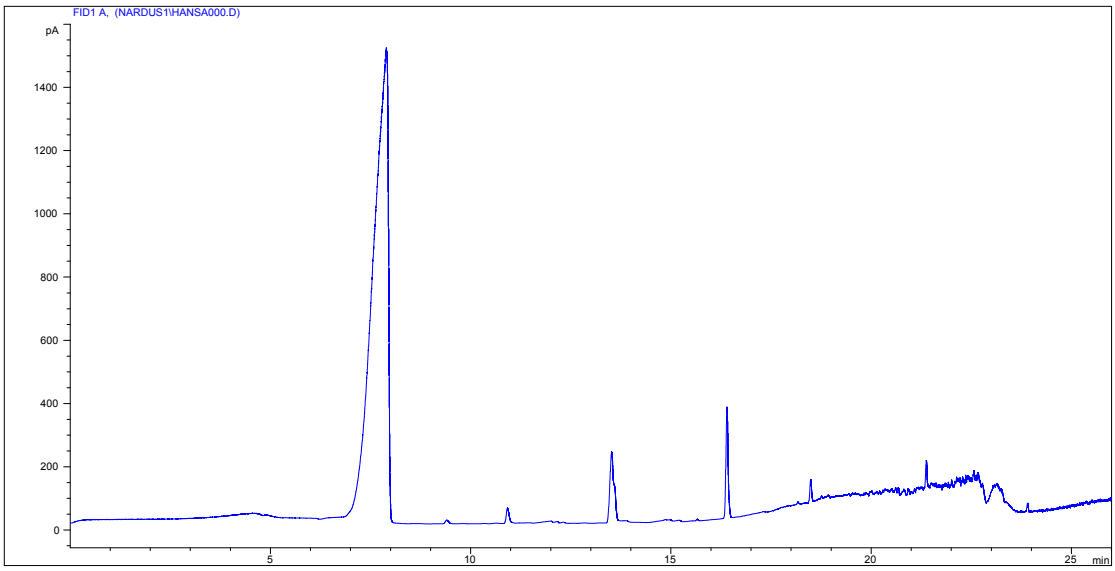


Figure 4.3: Chromatogram of the first test run of beer aroma sampled onto silicone.

During injection of this first sample a 40 ml/min split flow was used. Notice that not many peaks are observed, compared to the SABMiller chromatogram below.

Not many peaks are present in this result and the pattern expected for a beer aroma sample, from the results provided by SABMiller (Figure 4.4), is not visible.

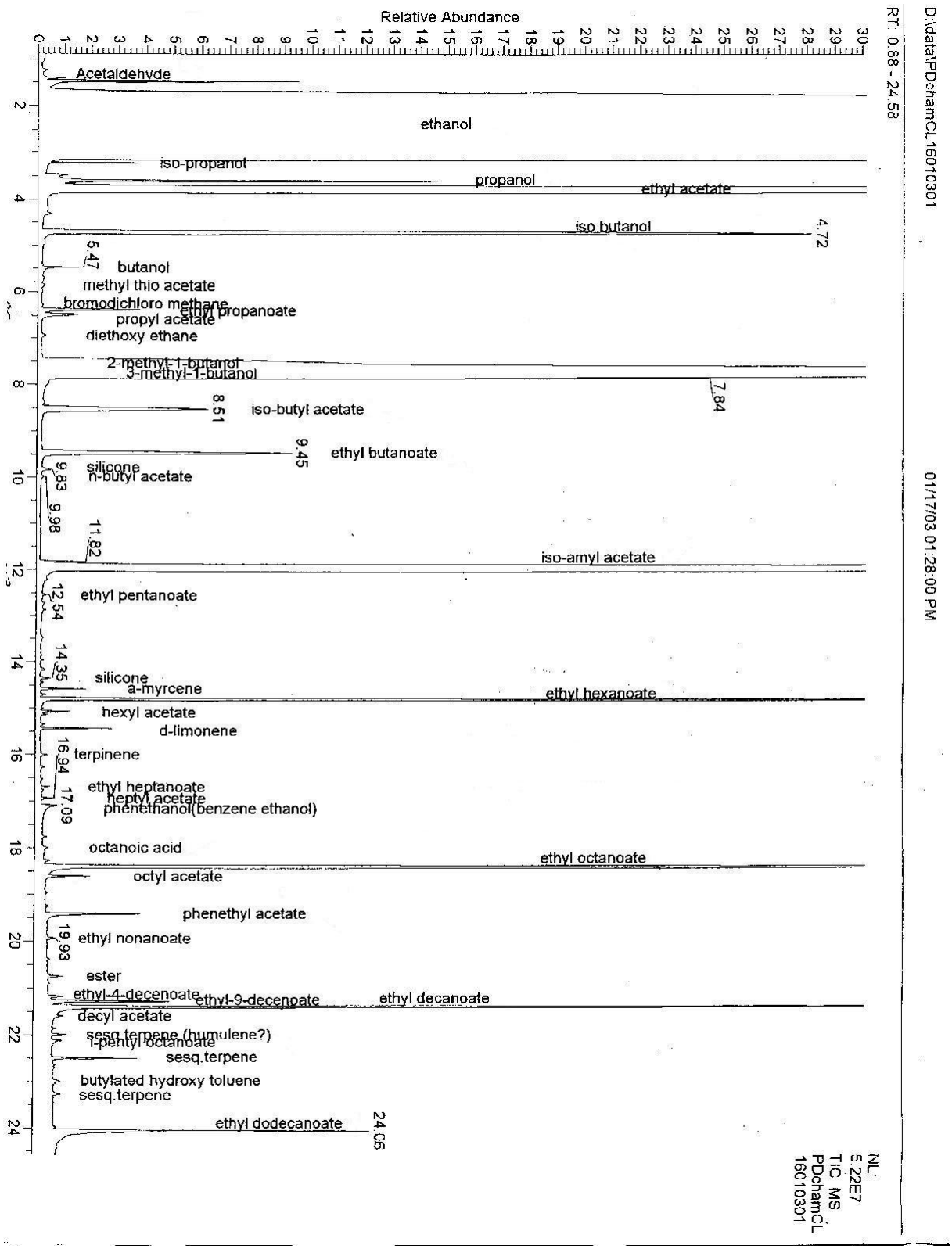


Figure 4.4: Beer aroma chromatogram obtained from the SABMiller

This chromatogram was produced by the South African Breweries research laboratory from a sample of beer aroma volatiles collected onto a Tenax trap.

By reducing the split flow to 10 ml/min (1:6), an immediate improvement in the amount and area of the later peaks was observed (Fig 4.5). The result shows the major peaks observed in the SABMiller result. This is an indication that the trap and the thermal desorption system is compatible and that it works in a similar manner to the desorption system used by SABMiller. It was decided at this point that a split flow of no higher than 10 ml/min will be used, to minimise the loss of minor components from the sample.

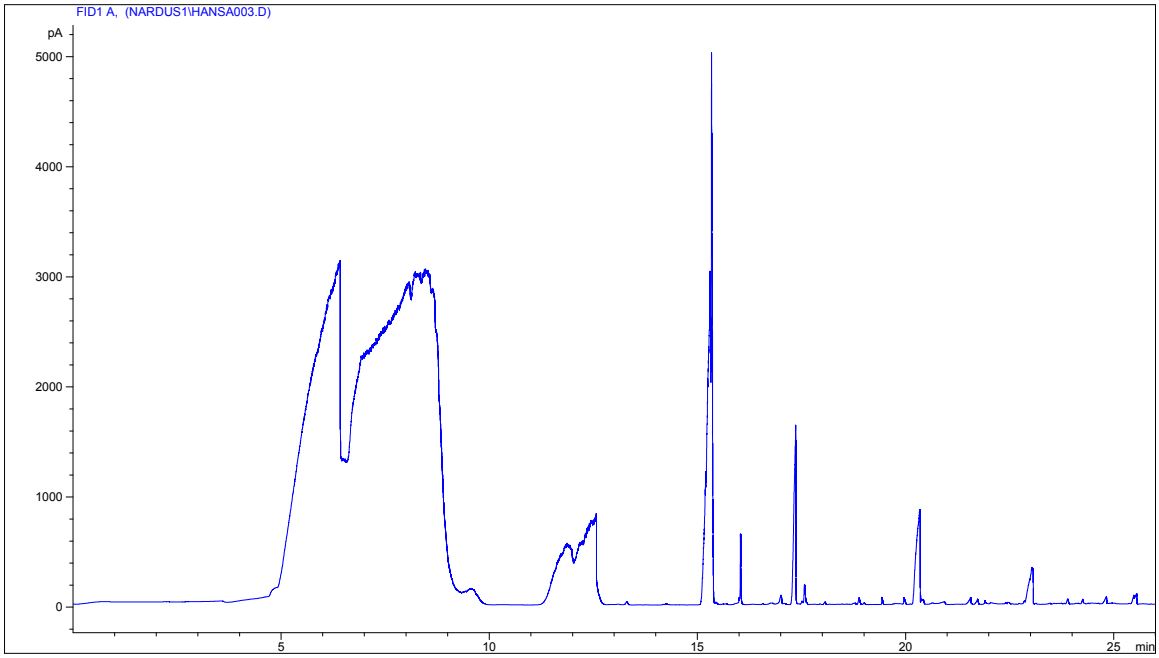


Figure 4.5: The beer aroma profile obtained with modified desorption conditions

The sample of beer aroma sampled onto a MCT was injected with a split flow reduced to 10 ml/min. The result shows the major peaks observed in the SABMiller chromatogram (Fig 4.4).

A major feature of the earlier results was the broad unresolved peaks at the beginning of the chromatogram. During the desorption phase of these runs the inlet pressure sporadically increased, on occasion to such an extent that the GC instrument underwent a pressure safety shutdown.

A literature search revealed that this broad peak and observed pressure increase is in fact due to moisture accumulating in the cold trap (CIS) during sampling. In his review on headspace sampling, Bruno Kolb [53] states that the water problem associated with the analysis of headspace samples by GC is immediately obvious when a capillary is blocked by ice formation, which explains the dramatic increase in head pressure. A less apparent detrimental effect of trapped water, according to

Kolb, is peak distortion, particularly if it occurs in the early part of the chromatogram where highly volatile compounds are eluted with water.

Although water is present in the headspace sample of both static and dynamic techniques, it is in dynamic headspace techniques (such as the purge-and-trap procedure) where the water problem is much more prominent [53]. This is because during the long time an aqueous sample is purged, the stripped gas remains permanently saturated with water vapour while the concentration of analytes decrease exponentially. A high amount of water is therefore accumulated in the final gas extract [53]. With the accumulated water highly volatile polar compounds such as ethanol, which is miscible in the water, is also concentrated in large amounts.

Nevertheless, it was surprising to find that water was being accumulated in spite of the non-polar nature of silicone rubber and the efforts made to prevent moisture from condensating in the MCT. The most likely reason for this observation is that the silica powder, used as filler during the manufacture of silicone rubber, selectively adsorbs water, leading to increased amounts of moisture being collected.

The removal of accumulated water can generally be achieved by blowing an inert gas such as nitrogen through the trap for a period of time, before the trap is placed in the thermal desorption unit. The disadvantage of drying the tube in this way is that volatile analyte can be lost in the process.

For removal of the collected water the thermal desorption system itself was used to vent the moisture from the trap at room temperature before desorption is started. With this method one has more control over the flow and temperature, thereby ensuring a more repeatable result. With the Gerstel TDS system, a small part of the vented gas still goes to the cold trap (CIS) even if a 1:50 or even a 1:100 split ratio is used, so not all volatile analytes are lost. It was found that purging for 3 minutes at 30°C prior to thermal desorption of the silicone trap prevented freezing in the cold trap (CIS) and other associated moisture problems such as the flame of the FID being extinguished. During this solvent venting period the cold trap was held at –100°C.

A concern was, however, that solvent venting in addition to the 10 ml/min injection split (initially used to reduce the amount of moisture and ethanol entering the column) would reduce the concentration of the other analytes, resulting in very small peaks. Therefore, the experiments utilising a solvent venting step were initially performed without the additional injection split flow.

A series of experiments were carried out to ascertain the effect of increasing the solvent vent flows and times of the TDS during the first few minutes of purging before thermal desorption. Figure 4.6 (a to d) shows how the shape of the broad volatile peaks improve with increasing TDS vent flow and time. Unfortunately, the lower-volatility compound peaks also showed a decrease in peak height.

Because of this reduction in the peak height of later eluting compounds it was decided that the optimum of 3 minutes at 50 ml/min, which did not significantly change the later peak heights, was to be used to prevent the excess moisture from interfering with the instrument operation.

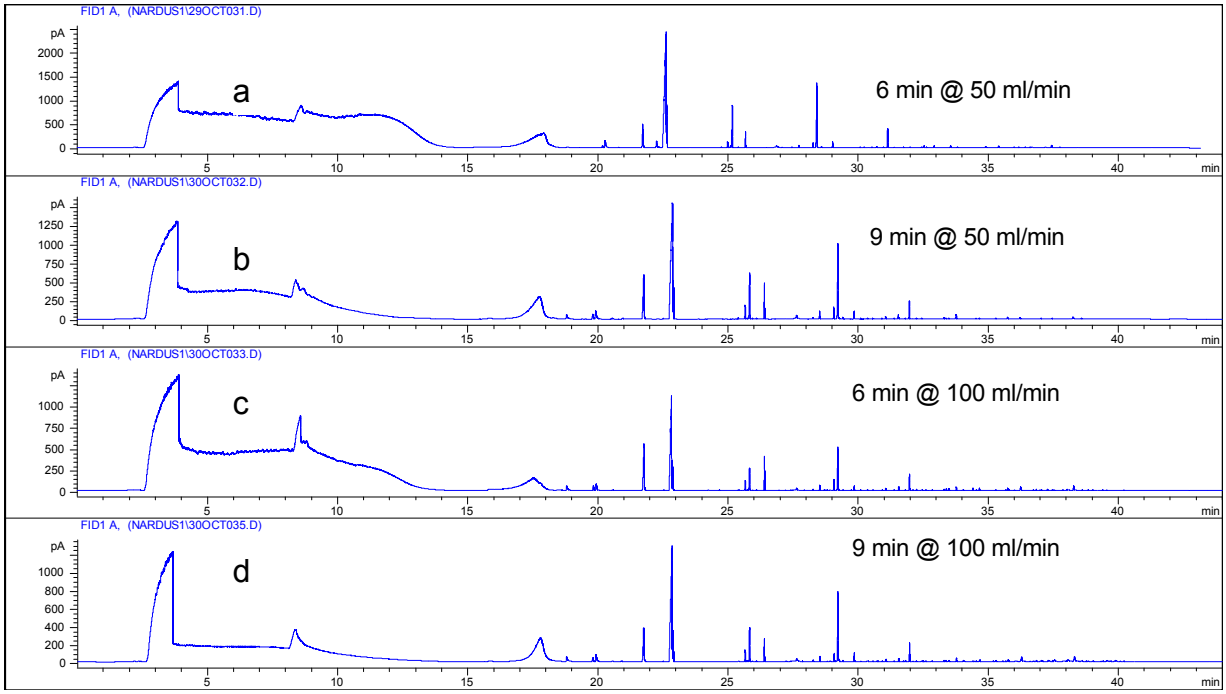


Figure 4.6: Effect of solvent vent time on observed peaks.

Notice how the shapes of the first set of peaks improve with an increase in the volume of purge gas blown through the trap, before thermal desorption starts. Four volumes; 300 ml, 450 ml, 600 ml, and 900 ml were evaluated.

The problem of a broad unresolved peak early in the chromatogram, however, still persists. Two further courses of action were considered for solving this problem. The first was to further reduce the amount of moisture (and associated ethanol) focused onto the cold trap (CIS) in such a way that the components of lower volatility (later peaks) are not affected.

Alternatively, it could be considered to increase the amounts of the lower volatility components (relative to the amount of ethanol) collected on the MCT trap so that a split injection would still provide significant peak area for these components. A combination of these two options was eventually employed which required an optimisation of the sampling procedure, as discussed in the following section.

3.2 Optimisation of purge-and-trap sampling technique

The aim now was to optimise the sampling technique in order to get maximum collection of less volatile compounds with the minimum amount of water and ethanol being retained.

Because the ethanol/volatiles (and water) combination peak at the front of the chromatogram is so much higher than those of the later compounds, the possibility was considered that too little of these lower volatility components was being collected in the short collection time used for sampling.

The effect of an increase in the sampling time on peak areas of all components was therefore investigated to evaluate how the ratio of the ethanol peak area to that of the other components could be adjusted to favour the less volatile compounds. Figure 4.7 shows three chromatograms that illustrate the effect of increased sampling time.

These chromatograms show an increase in the peak area as the sampling time is increased from 10 to 30 minutes in increments of 10 minutes. This observation prompted further study into the breakthrough characteristics of the beer compounds when sampled for extended periods.

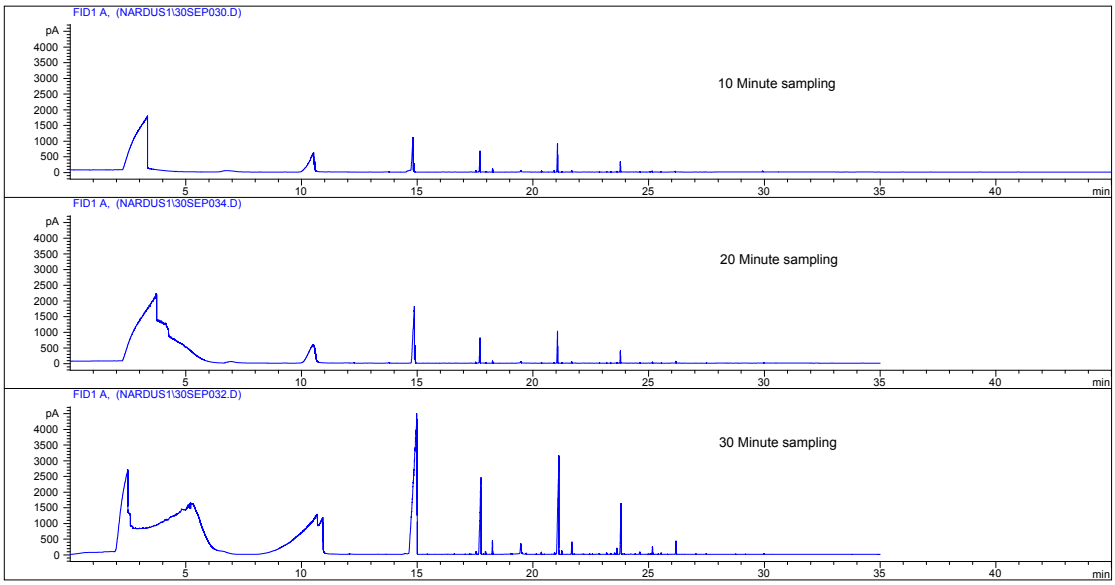


Figure 4.7: Effect on peak area of increased sampling time

Chromatograms are plotted on the same y-axis scale for direct quantitative comparison. Notice the drastic increase in peak area for the less volatile compounds after 30 minutes of sampling.

A number of parameters are important in the optimisation of a purge-and-trap based sampling technique, as were discussed in Chapter 3. The three parameters considered with these experiments are; sample volume, purge volume and sample temperature. Initially only the effect of sample and purge volume were to be investigated; however, during these experiments the importance of sample temperature was realised.

3.2.1 Optimisation of beer sample volume and purge volume.

For the experiments two sample volumes namely 10 ml and 250 ml were selected. This choice is based on the size of the available sampling equipment, and aims to compare a small sample volume to a large one. Sampling times for both volumes were extended progressively at a constant purge gas flow rate, thereby increasing the purge volume sampled.

Tables 4.1 and 4.2 show the results of these experiments. For each analysis, peak areas of a number of selected compounds were monitored. The peaks monitored were the five most prominent ester peaks (marked in Figure 4.8) that cover most of the volatility scale of the compounds analysed in beer aroma. Monitoring these five esters gave a good indication of the purge volume needed to accumulate all compounds of interest in the required amounts.

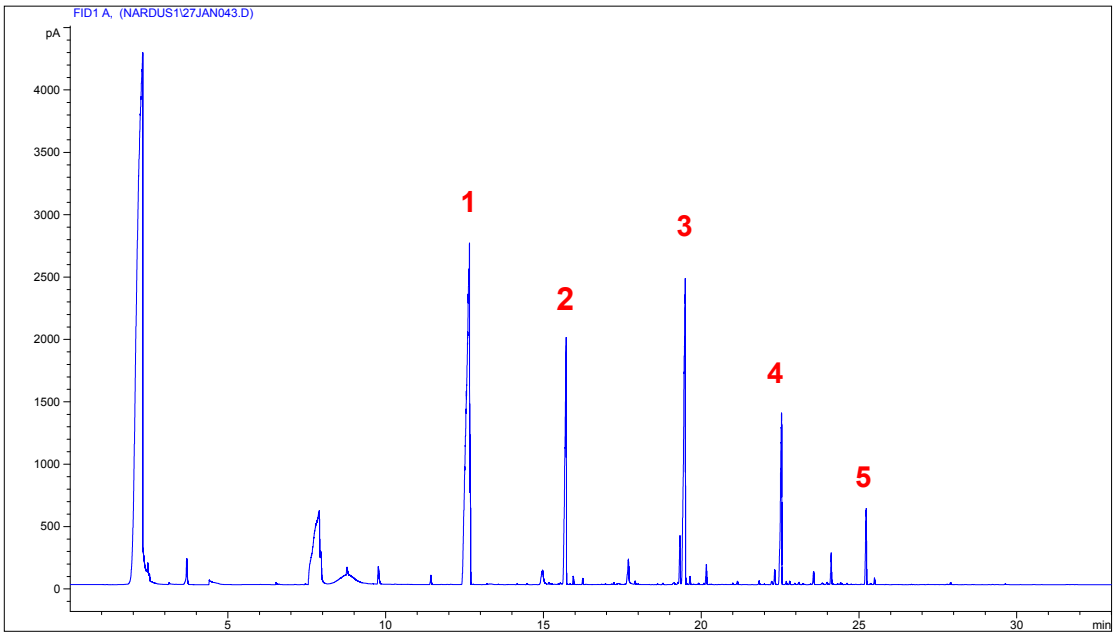


Figure 4.8: Five ester peaks used to monitor effect of sample volume

The areas of these five largest and most stable ester peaks were used to follow the effects observed from changes in sampling parameters. These ester compounds include; isoamyl acetate (peak # 1) and ethyl esters with respectively 6-, 8-, 10- and 12-carbon alkyl groups (peaks # 2 - # 5).

By monitoring the change in the chromatographic peak area of a compound as the purge volume is increased, either of two situations, as illustrated in Figures 4.9 & 4.10, can be observed.

Theoretically a larger purge volume should concentrate more of a certain compound into the MCT, and therefore increase the area of the corresponding chromatographic peak. This increase in chromatographic peak area can only continue up to a certain point after which the compounds start eluting from the MCT. The purge volume at which this occurs is the breakthrough volume as defined in Chapter 3. This point is indicated in the sample collection profiles shown in Figure 4.9.

After breakthrough has occurred, the amount of compound collected in the MCT usually stays constant (Figure 4.9), representing an equilibrium-sampling situation. The breakthrough volume is compound specific and is determined by the solubility of the compound in the silicone rubber. It is therefore related to the retention volume of the compound on a polydimethylsiloxane (DB1, SE30) stationary phase.

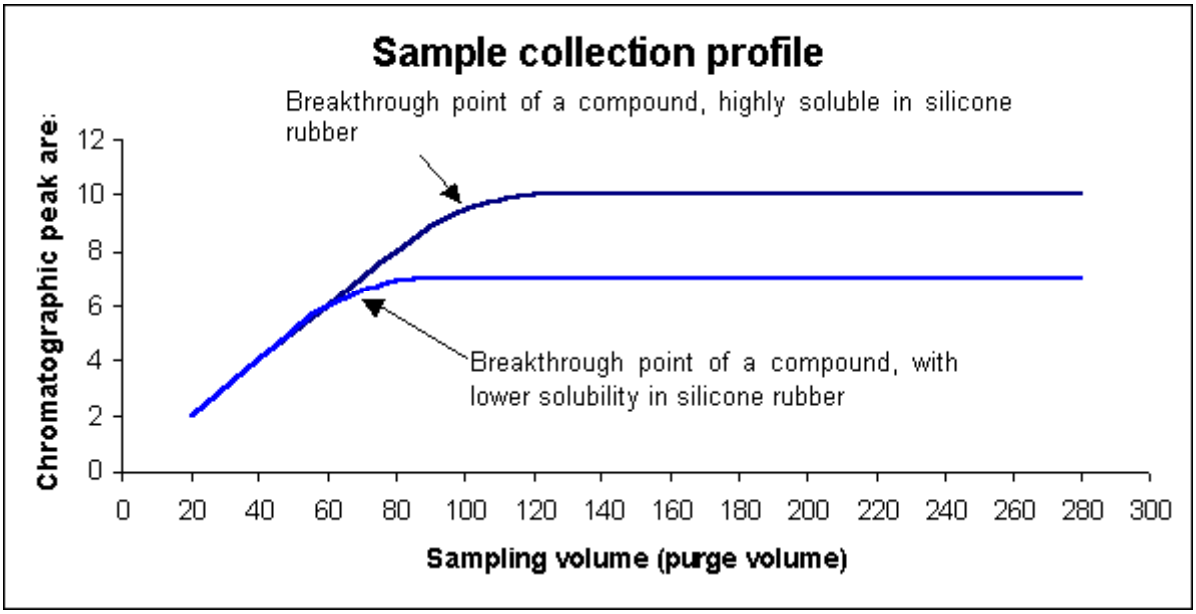


Figure 4.9: **Diagrammatic collection profile representing accumulative and equilibrium sampling from an infinite liquid sample**

Two breakthrough curves showing the effect of increased sampling volume on the chromatographic peak size of two organic compounds with different solubility in silicone rubber.

In many complex samples, however, the compounds of interest have widely different combinations of solubility and volatility. If a small volume of a complex sample, such as beer, is purged for an extended period of time, some of the more volatile compounds with low solubility in the beer sample, may be exhausted.

If the purge volume used was greater than the breakthrough volume for these components on the MCT, a drop in chromatographic peak area may be observed (Figure 4.10). The tempo at which the decrease in chromatographic peak area occurs after breakthrough is independent of the concentration of the compounds in the sample, but is a function of the volatility of the compounds (how much partitions into the gas phase from the aqueous medium).

Purging beyond this point of exhaustion, (Figure 4.10), would result in compounds being lost from the analysis for finite sample volumes. The smaller the sample volume and the higher the volatility of the compound, the more pronounced the effect.

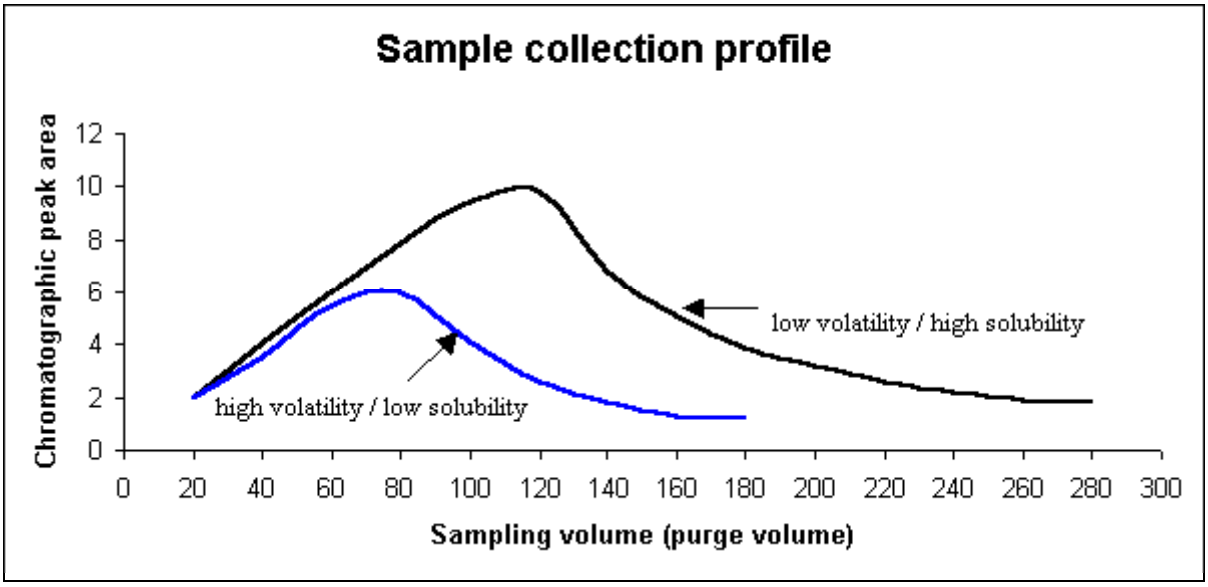


Figure 4.10: **Diagrammatic collection profile representing sampling to exhaustion for two organic compounds from a limited sample size**

The two curves show the effect of increased sampling volume on the chromatographic peak area of two organic compounds with different volatility and solubility in both silicone rubber and the aqueous sample.

Breakthrough characteristics of compounds on a specific sampling device should consequently be studied with samples large enough to represent an 'infinite' sample volume relative to the purge volumes utilised.

Table 4.1: **The effect of increased purge volume on the area of selected peaks of a 10ml beer sample. Purge flow rate 25 ml/min.**

Sample Volume (ml)	Purge Volume (ml)	Ester Peak Area				
		Isoamyl acetate	Ethyl Esters			
			C6	C8	C10	C12
10	250	1822	244	527	179	54
10	750	5481	778	2231	759	135
10	1250	10196	1793	2692	1115	242
10	1750	15827	2677	3292	1393	824
10	2250	15823	2992	5436	1893	474
10	3750	7738	2881	2604	842	686

Table 4.1 shows results of the experiments done on a 10 ml sample of beer. The experiment was terminated at a purge time of 150 minutes, which at a flow of 25 ml/min represents a total purge volume of 3750 ml. At this point a decrease in the ester compounds up to C10 was observed. The C12 ethyl ester still appears to be increasing. This is due to the low volatility of the large C12 ethyl ester compared to that of the smaller compounds.

Table 4.2 shows the results for a 250 ml sample of beer. Even though the scatter in some of the results is high (temperature control of samples was introduced only later in the study) the trend is clear. Even the volatile ester compounds are accumulated for a longer time before exhaustion occurs, showing that a larger sample is required to fully utilise the capacity of the MCT.

Table 4.2: The effect of increased purge volume on the area of selected peaks of a 250 ml beer sample. Purge flow rate 25 ml/min.

Sample Volume (ml)	Purge Volume (ml)	Ester Peak Area				
		Isoamyl acetate	Ethyl Esters			
			C6	C8	C10	C12
250	250	2010	329	531	204	25
250	750	7439	1007	3250	1206	349
250	1250	11870	1939	2977	1471	371
250	1750	11608	2202	3815	2097	939
250	2250	35125	6582	15518	7059	1057
250	3750	32524	8076	11034	5152	1795
250	4250	36427	9667	13609	5865	2148
250	4750	36185	14157	23597	10422	2830
250	5250	49264	15311	31969	13980	2402
250	5750	33656	16182	24444	9925	3470
250	6250	37527	18530	38898	16043	3354
250	9000	31293	22401	32283	10913	4928
250	10500	25205	20387	49860	15244	4807
250	12000	22701	16732	43468	13051	6552

The isoamyl acetate as well as the C6 ethyl esters shows exhaustion between 6250 and 9000 ml purge gas, while the C8 to C12 ethyl esters are still accumulating at the maximum purge volume. The C10 ethyl ester seems to show exhaustion, this, however, is a result of the scatter in the results, as it is unlikely that this ester would be exhausted while the more volatile C8 ester is still accumulating.

The curve representing the isoamyl acetate (Figure 4.11) shows a sharp increase at around the 9 litres purge volume, the peak area decreases rapidly, while the C8 area is still increasing. Consequently, there seems to be far less isoamyl acetate compound present in the sample than there is C8 ethyl ester. In fact there is probably 10 times as much of the isoamyl acetate in the headspace of the sample than there is of the C8 compound.

The reason for this observation is the higher volatility of the isoamyl acetate and corresponding low breakthrough volume on the MCT. Its higher volatility also accounts for its lower solubility in the beer, resulting in earlier depletion from the beer sample compared to the C8 ethyl ester. Because of the lower breakthrough volume, the isoamyl acetate is already being sampled under breakthrough conditions while the C8 ethyl ester is still accumulating in the trap. Most of the isoamyl acetate compound, therefore, breaks through the trap and is lost to the analysis at higher purge volumes.

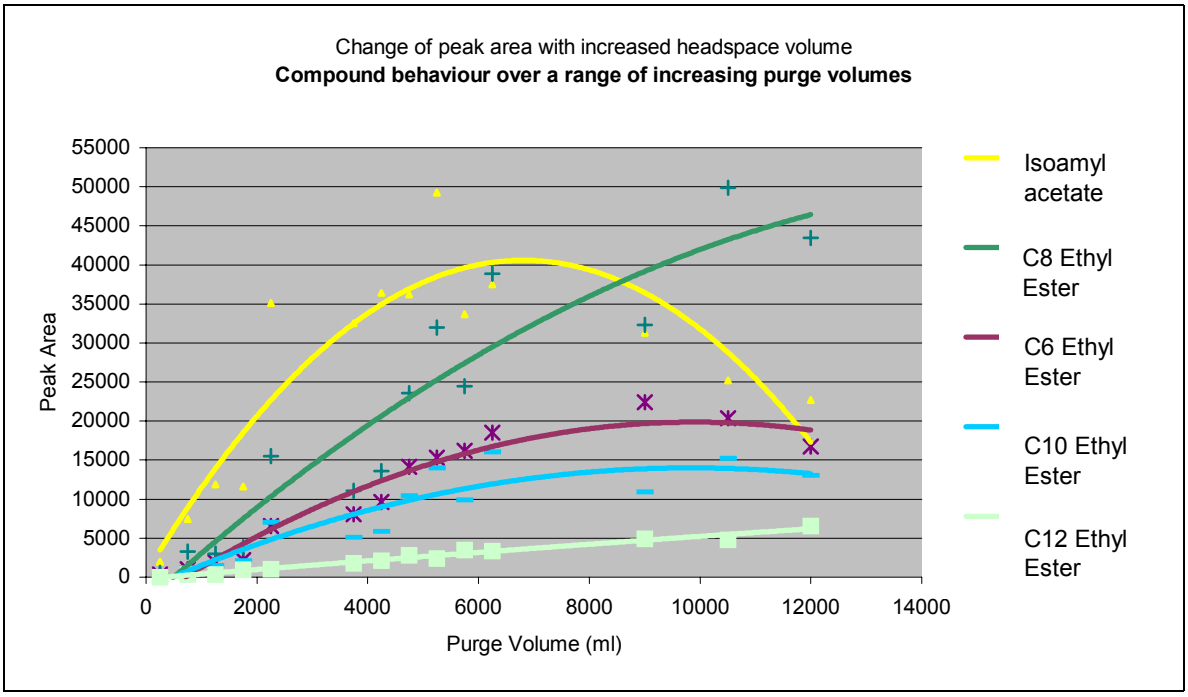


Figure 4.11: Effect of increased purge volume on ester peak areas observed

The results depicted here were obtained from 250 ml samples of beer.

By plotting these values one can follow the trend of the less volatile compounds slowly concentrating and the more volatile compounds being exhausted from the sample and blown off the trap (Figure 4.11). From the results it was decided that a purge volume no larger than 750 ml would be used for future experiments. At this volume all compounds less volatile than isoamyl acetate should be in the accumulation phase before breakthrough has occurred. This would give the best indication of relative concentrations in the headspace. At this purge volume even smaller beer samples should contain enough of the different compounds not to be exhausted prematurely.

3.2.2 Optimisation of temperature

The general trend of peak areas for increasing purge volumes is clear from Figure 4.11, however, individual curves revealed the importance of sample temperature (Figure 4.12), the third sample parameter referred to earlier. The erratic behaviour of the values was surprising at first until the temperatures of the sample, measured before and after sampling, was plotted alongside (thick pink and blue lines). As can be seen from Figure 4.12, the peak area measured for the more volatile esters has strong temperature dependence.

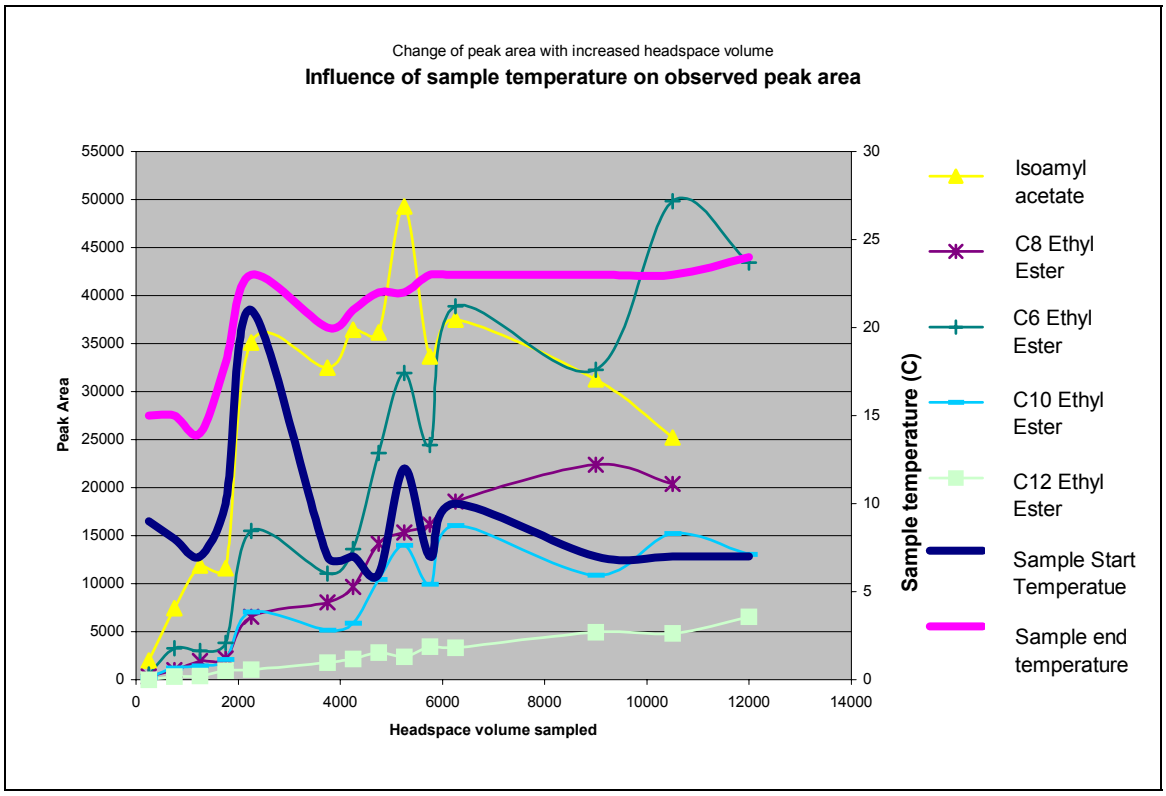


Figure 4.12: Effect of increased purge volume and sample temperature on ester peak areas observed

The thick, pink and dark blue curves are the temperatures of the samples measured at the beginning and end of sampling. Notice how closely the observed peak areas for the different ester compounds follow in particular the end temperature (dark blue) curve.

The measured values changed depending on whether the sample was collected just after it was removed from the refrigerator or whether it had time to equilibrate in the laboratory at room temperature before sampling.

The main problem was that, even if the beer was taken from a refrigerator in the lab, it still heated up during the extended periods of sampling. Depending on the weather

(i.e. temperature in the lab) large temperature differences between the beginning and end of sampling were observed. A possible solution to this was to keep the beer cold during sampling. This option was considered because it had the possibility of reducing the amount of moisture and ethanol in the vapour phase. Unfortunately, the low temperature (approximately 5°C) reduced the volatility of all compounds and consequently the recovery (amount sampled onto the MCT).

It was subsequently decided to sample beer at room temperature, as this would be an easier temperature to maintain fairly constant (in an air-conditioned laboratory) and would result in higher amounts of compounds available in the vapour phase. However, this would also contribute to the problem of water condensation in the trap due to more water and ethanol vapour present in the headspace.

The simplest way of reducing the amount of water vapour that condenses in the MCT is to have the trap at a slightly higher temperature than the sample. For this purpose a sampling tube heating device, which uses water kept at a constant temperature, was designed. The device consists of a large outer tube into which a tube with a smaller diameter is inserted. The ends of the larger tube are then sealed around the inner tube to form a water proof cell through which warm water is pumped. The inner tube was selected so that it fits snugly around the sampling tube in order to have effective heat transfer. Figure 4.13 shows a cross section of this device with a multichannel silicone rubber trap inserted.

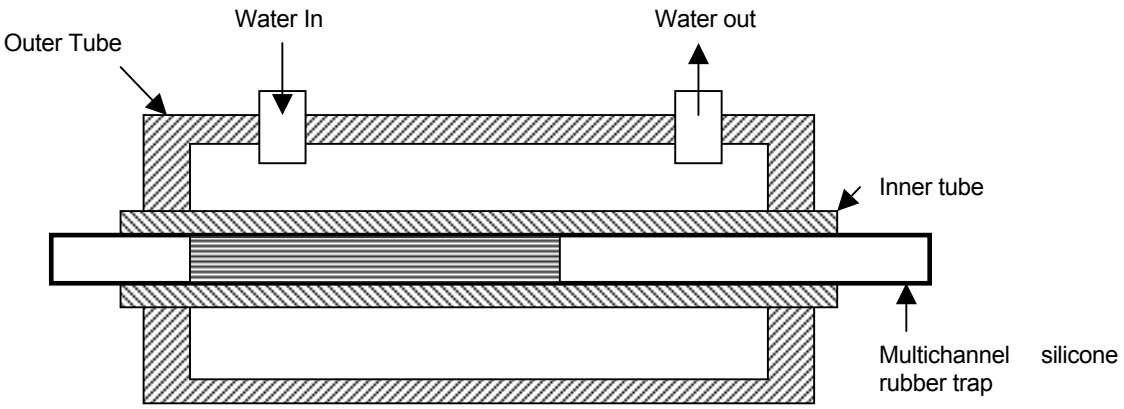


Figure 4.13: Sampling tube heating device

This simple water mantle was used to control the temperature of the MCT during sampling.

The sampling tube (MCT) is inserted into the heating device approximately 5 minutes before the sampling procedure begins, for equilibration of the temperature. The end caps of the trap are then removed and it is connected to the sampling arrangement. A diagram of this arrangement is shown in Figure 4.14.

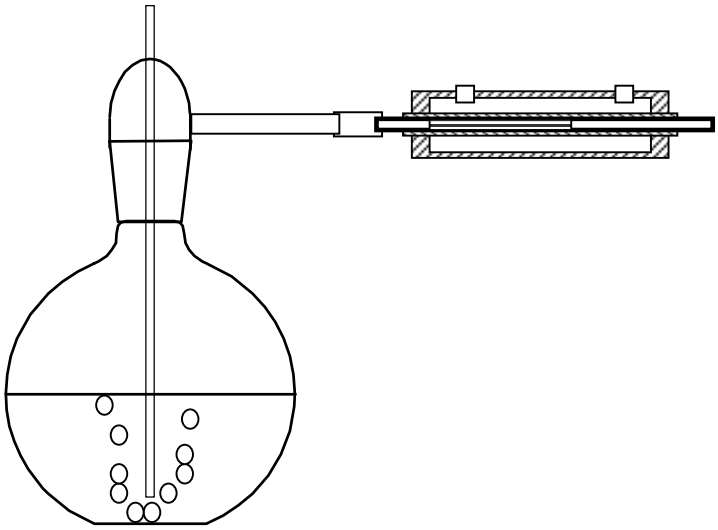


Figure 4.14: **The purge-&-trap sampling arrangement with sampling tube heating device**
For detail see Figures 4.1 and 4.13

An arbitrary temperature difference of approximately 25°C between the sample and sampling tube (MCT), was selected for the first experiment. The results show the reduced ethanol peak size due to less condensation in the MCT. The initial broad peaks are now separated into sharp peaks and the smaller peaks following the large ethanol peak are clearly visible. Figure 4.15 shows one of the results obtained with this method.

The sample was at approximately 21°C (room temperature) and was collected for 30 minutes at a purge gas flow rate of 25 ml/min. The sampling tube was at approximately 47°C. Except for the very volatile acetaldehyde, all of the peaks anticipated for this sample were present, so none of the expected components were lost due to heating of the trap.

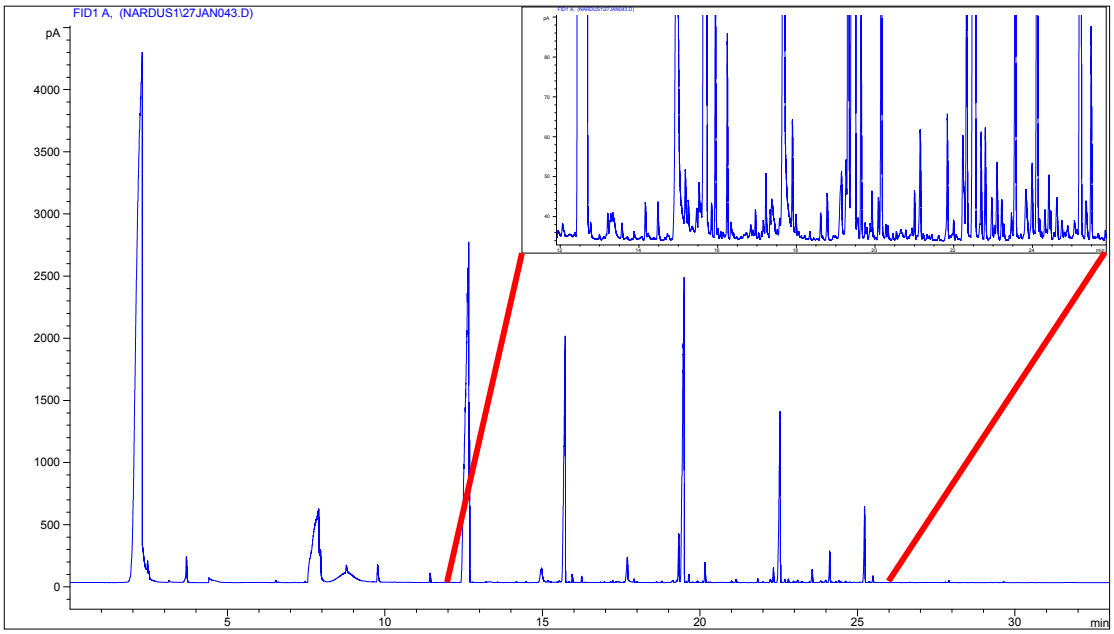


Figure 4.15: A result obtained with the heated trap sampling device

Note the much improved ethanol peak shape and the amount of detail that can be observed between the major compounds. The zoomed-in section shown is from 12 to 26 minutes.

The results obtained through these optimisations proved that the MCT and a purge-and-trap sampling method could be used to successfully sample beer aroma. Further optimisation of these parameters would only be necessary if analytes with volatilities beyond those of the range of test compounds are investigated. For beer samples such a situation is not expected. Further investigations into the relevant chromatographic parameters were undertaken.

3.3 Chromatographic background

As part of the optimisation of the sampling method it is necessary to determine which peaks observed are beer aroma components and which constitute the chromatographic background. A number of system, trap and method blank runs were recorded to identify this background.

A system blank involves doing a full desorption on an empty glass tube and a chromatographic run on the same temperature program used for the analysis of samples. The result is a profile of peaks due to impurities in carrier gas or instrumental noise. Figure 4.16 shows the result of such a system blank.

Definite peaks are visible, however, the highest of these are only 6 pA above the baseline and have a very small area.

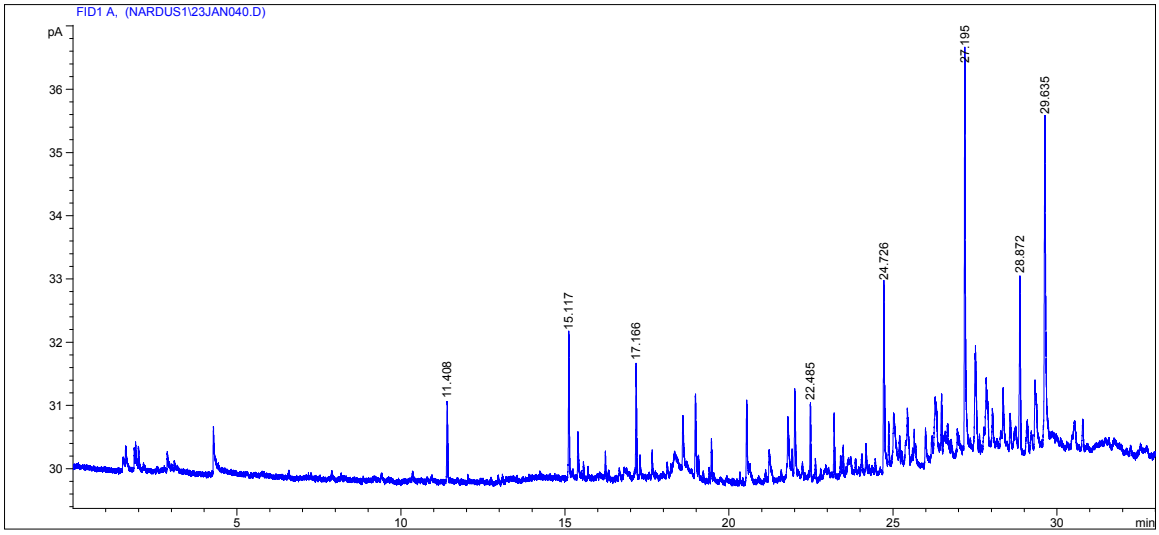


Figure 4.16: A system blank

The result of a desorption of an empty glass tube and a chromatographic run at the temperature program used for analysis of beer aroma, showing the trace contaminants in the desorption and chromatographic system.

Similar to the system blank, a trap blank is a full desorption of the multichannel silicone rubber trap with no compounds deliberately sampled onto it. The trap then releases only volatile silicone components from the silicone rubber tubes used as absorbent, thereby establishing the contribution of the trap to the chromatographic background.

Figure 4.17 shows a chromatogram of a trap blank. The three most prominent silicone peaks are clearly visible. These compounds make a far higher contribution to the overall chromatographic background compared to the system blank. The silicone profile is reproducible and the peaks are easily distinguished from the aroma compound peaks in the chromatogram of a desorbed sample.

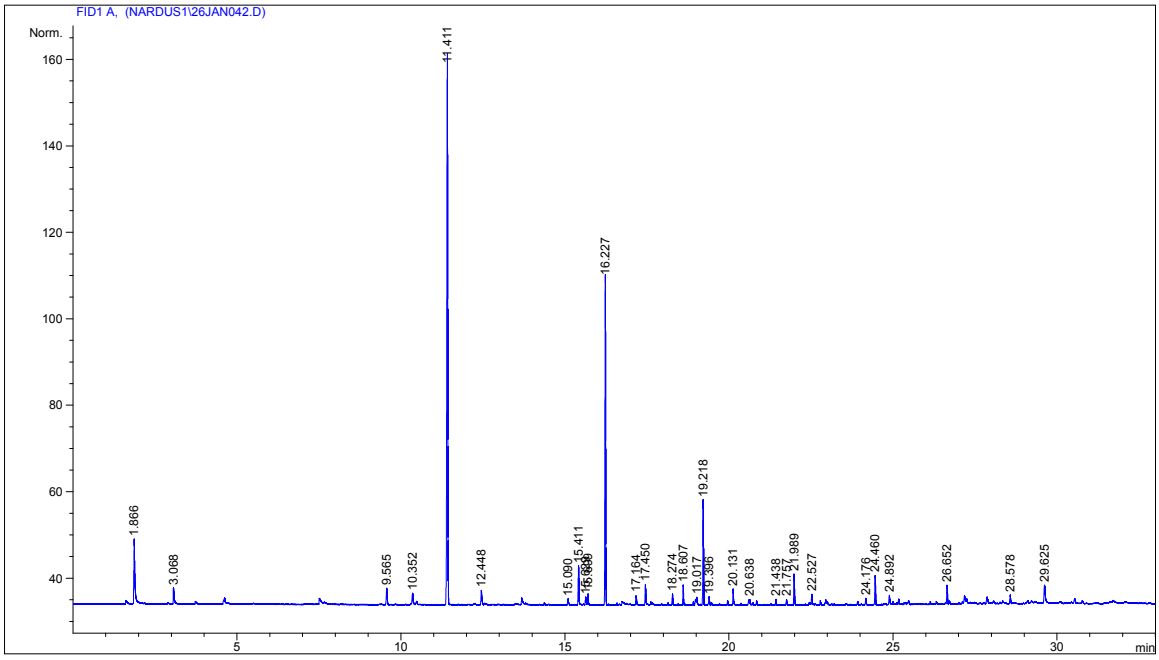


Figure 4.17: A trap blank

The result of a desorption of an un-sampled MCT. Note the regular intervals of the silicone compound peaks released by the silicone rubber in the trap.

In order to identify peaks due to contaminants in the purge gas or sampling equipment, a cleanly desorbed trap is connected to the empty sampling equipment that contains no sample.

The purge gas is turned on and a complete sampling of the equipment is performed. Peaks appearing in this method blank chromatogram, in addition to those already observed in the system and trap blanks, can then be attributed to the sampling equipment.

In the chromatograms shown in Figure 4.18 a trap blank (red line) is overlain with a method blank (blue line). The extra peaks are clearly visible as indicated. These peaks are also fairly low when compared to the aroma compound peaks or the silicone peaks. Unfortunately this profile is not as reproducible as the silicone profile and can be expected to change over time as shown in Figure 4.19.

The same comparison as in Figure 4.18 is made between the silicone (red line) and method (blue line) blanks. The peaks decreased significantly in number and height after 10 sampling sessions with the equipment.

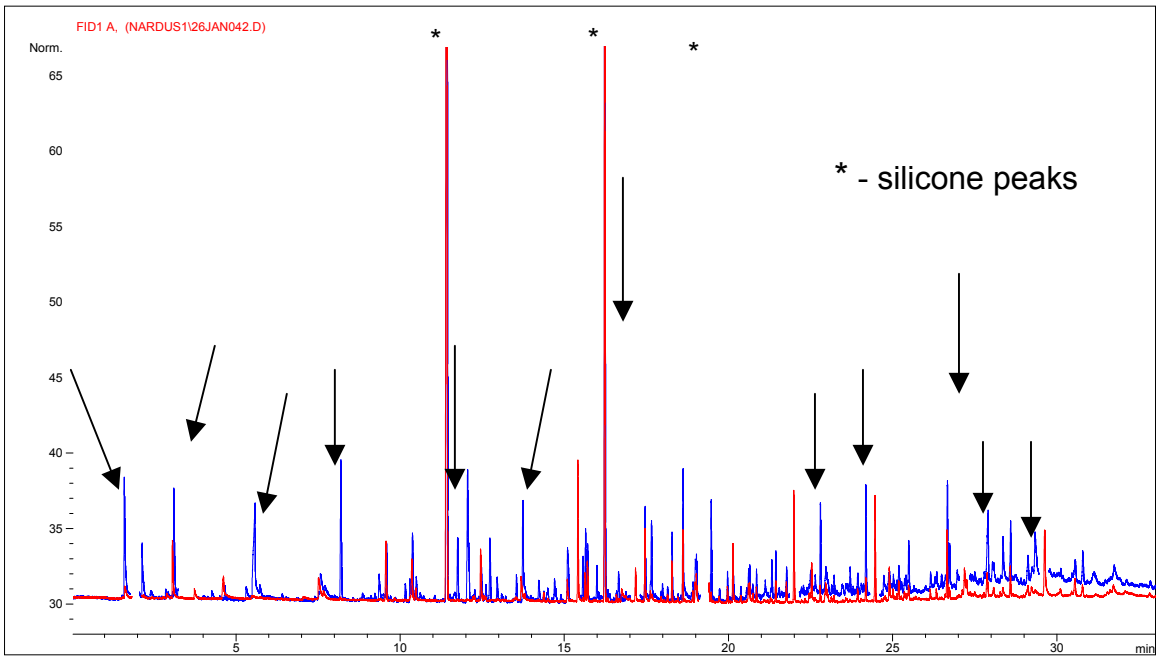


Figure 4.18: **Overlain trap blank (red line) and method blank (blue line)**

This shows the contribution to the chromatographic background from the sampling system (arrows).

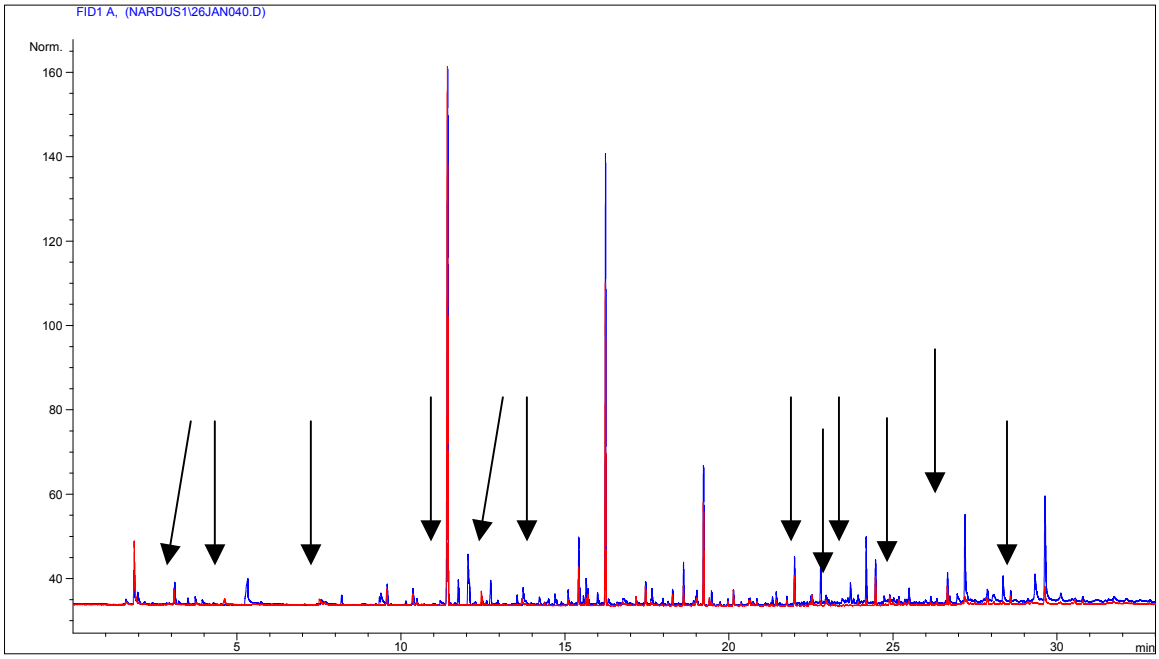


Figure 4.19: **Overlain trap blank (red line) and method blank (blue line)**

A second method blank shows how the background peaks from the desorption system (arrows) decrease over time and makes no significant contribution to the overall chromatogram

When comparing the aroma compound chromatogram of a beer sample to a system blank one can easily identify the background peaks (Figure 4.20). It is therefore essential that a blank run be done before a sample is collected and after a sample is

analysed in order to accurately identify changes in the sample profile. The blank also serves to condition the trap for collection of the next sample.

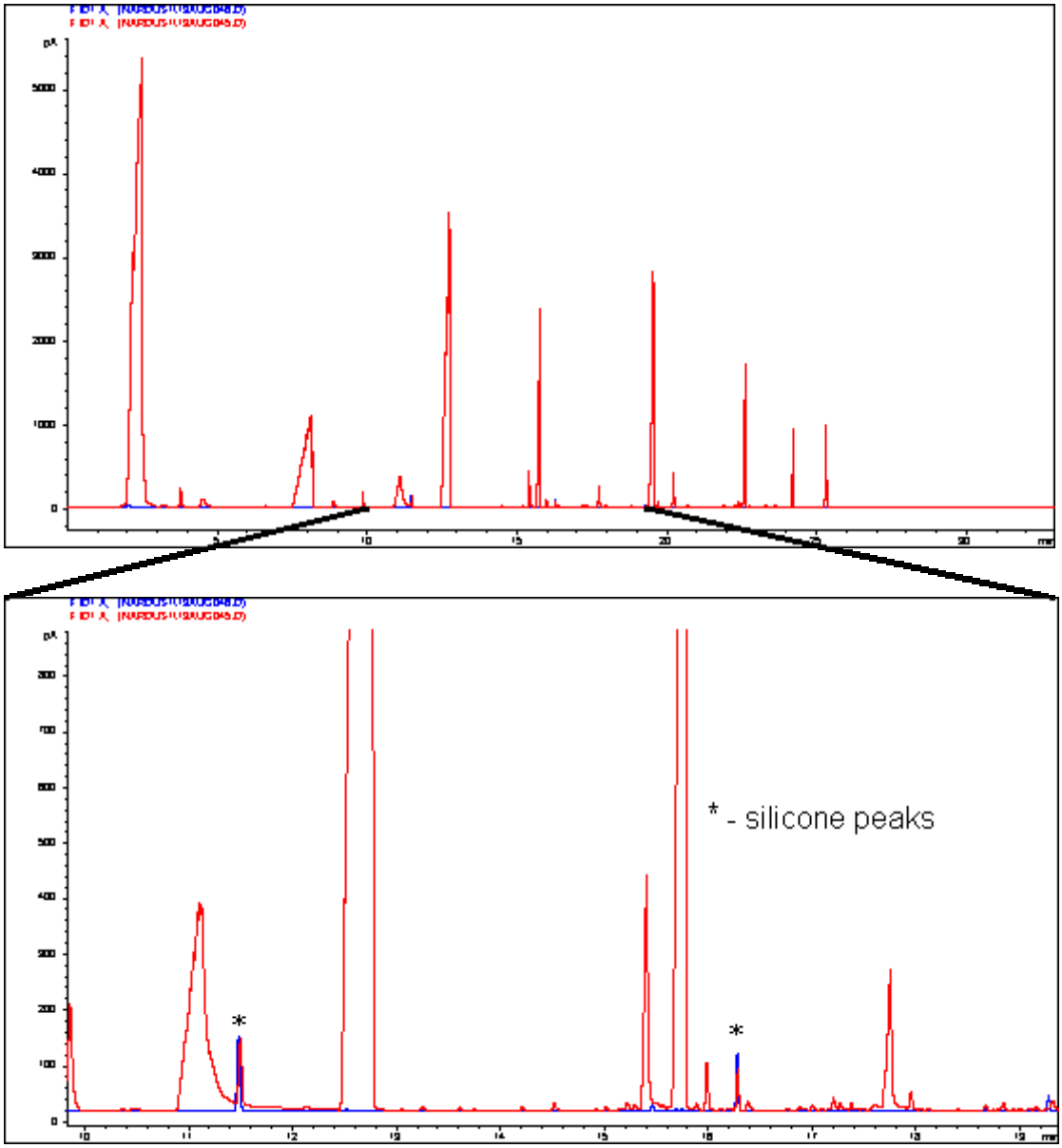
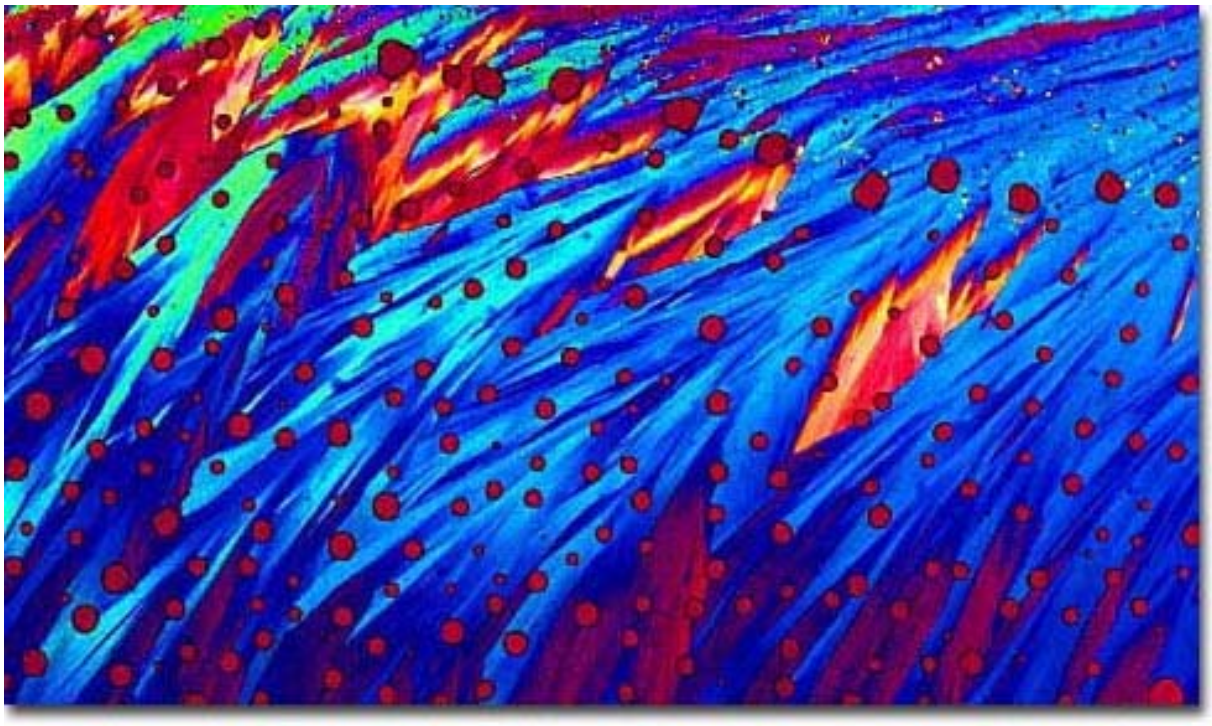


Figure 4.20: Overlain trap blank (blue line) and aroma profile (red line)

Notice that the silicone background peaks from the MCT are easily distinguishable from the beer aroma compounds.

Chapter 5



Corona extra

Text from the bottle label: Corona Extra - La Cerveza mas Fina. Brewed and bottled by Cerveceria Modelo, S. A. De C. V. Mexico, D. F.

Chapter 5

REPEATABILITY

1. Introduction

In the previous chapter the optimisation of sampling parameters produced results proving that the chosen combination of the multichannel silicone rubber trap and the purge-and-trap sampling method is capable of collecting a full spectrum of beer aroma compounds. The next part of the study now focuses on achieving repeatable results with this sampling method. It is necessary to establish a very repeatable pattern for the aroma profile of a specific beer brand before an attempt can be made to recognise different brands or off-flavours on the basis of differences observed from this profile.

The aim therefore is to produce results from one brand of beer for which the chromatographic profiles are as close to identical as possible. Only then can small differences in the aroma profile be considered significant. Without a high degree of repeatability, differences in the aroma profile cannot with any certainty be correlated with differences in perceived aromass of the beer sampled.

2. Experimental

Instrumental conditions as described in Chapter 4 were used for the experiments. As samples, two brands of local beer were purchased from retail outlets in the form of 340 ml commercially sold glass bottles. The bottles were all kept in the laboratory at room temperature until they were opened for use. The sample volume used for the analysis was 250 ml beer poured into a 500 ml round bottom flask and sampled with the arrangement shown in Figure 4.14 the beer sample was at room temperature (approximately 22°C) and the MCT was heated to 47°C with the trap heating-device. Before sampling started the nitrogen purge gas was set to a flow rate of 25 ml/minute. The beer sample was purged for a period of 30 minutes resulting in a total headspace volume of 750 ml being sampled through the trap.

3. Results and discussion

For the repeatability experiments beer flavour samples of one particular beer brand were collected, desorbed and analysed according to the optimised method described in Chapter 4.

Visually the chromatographic profiles seemed fairly close to one another. Figure 5.1 shows results from consecutive analysis of two samples of one brand of beer, analysed on the same day under identical conditions as described above.

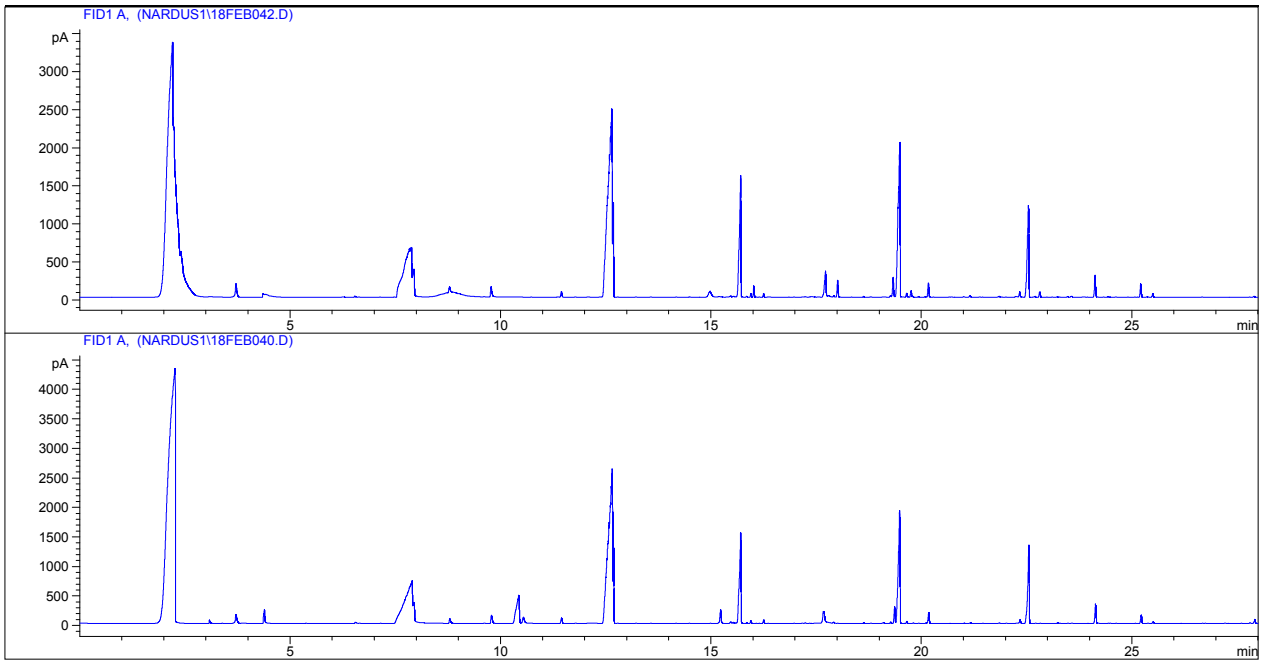


Figure 5.1: Variability in consecutive results from one beer brand

Note the additional peaks visible in the top chromatogram particularly in the section between 15 and 25 minutes.

In general the peak shape and relative peak heights seem consistent. However, at closer inspection it is apparent that not all peaks are repeated in both the results. The heights of matching peaks are clearly not the same and the scales of the chromatograms are also different, with one showing the maximum at 4000 pA and the other at 3000 pA.

A statistical evaluation was performed on the absolute peak areas of all compounds not identified as part of the chromatographic background. The percentage relative standard deviation (RSD), for the areas of matching peaks from consecutive analysis of five identical samples of one beer brand, was used for the evaluation. The values

for peaks with closely matching retention times in the first part of the chromatogram up to 16 minutes are shown in Table 5.1. The results show deviations of up to 80 per cent for some peaks.

Table 5.1: Statistical evaluation of matching peaks for five identical samples of beer

Retention time (min)	Areas of Matching Peaks (5 samples)					%RSD
1.771	91.4	98.9	124.7	247.9	73.1	55.0
2.234	39942.6	36578.2	34429	33962	33348.7	7.5
3.731	434.2	464.8	359.8	330.4	435.9	14.1
4.413	234.5	358.8	334.1	330.7	265.7	17.1
6.572	33.1	97.7	28.9	29.2	33.7	67.0
7.886	1088.5	1894.3	3156.2	9015.9	9202.4	80.9
7.938	1140.6	1289.9	1288.5	1083.1	1295	8.2
8.813	835.5	232.3	155.3	697.5	162.5	77.9
9.806	325.8	433.3	293.1	296.3	312.8	17.5
10.326	6.4	7.3	4.8	6.3	6.4	14.4
11.463	128.1	114.6	148.8	174.4	124.7	17.2
11.794	5	13.2	3.3	4.6	3.9	68.0
12.092	13.5	10.6	11.1	8.5	10.7	16.4
12.659	18871.5	21907.8	17265.6	17253.8	16943.5	11.3
12.689	1572	1699.6	1493.1	1489.9	1479.2	6.0
12.789	5.5	6.9	6.6	7.3	5.7	12.1
13.009	2.3	2.4	2.2	2.2	2.4	4.3
13.227	9.7	10.2	8.6	8.8	8.4	8.5
13.585	5.3	6.1	7.2	5.6	5.4	13.2
14.499	13.6	15.6	12.3	11.1	12.3	13.2
14.862	475.1	449.2	440.4	429.5	403.6	6.0
15.197	25.5	19.1	15.8	22.9	13.5	25.5
15.274	10.6	9.7	15.3	10.4	10.8	19.7
15.489	4.6	9.2	8.5	7.2	6.4	25.2
15.545	22.2	21.2	28.4	17.6	27.3	19.2
15.716	5138	4789.6	4652.4	4259.9	4039.7	9.5

However, at closer inspection it was noticed that a number of peaks showed good repeatability and statistical values under 10% RSD was achieved. These peaks were scattered all across the volatility scale (as represented in a beer chromatogram), which confirmed that a high degree of repeatability was possible and that the variability was not inherent to the method.

In spite of this encouraging result the fact that such a great deal of variability resulted from samples expected to give identical results, meant that the method still required further investigation.

In this chapter the systematic investigation of possible causes of this variability is presented. The aspects considered here are:

- errors in the handling of the sample;
- possible bottle-to-bottle variability in the samples themselves;
- differences in sample temperature during sampling; and
- errors during the desorption and analysis.

3.1 Sample handling

The first possible cause of variability investigated was handling of the sample before collection of the flavour volatiles onto the MCT. The rationale is that pouring beer out of its original container may cause differences due to air exposure and foaming. As CO_2 is released from the foam it carries with it volatile components, thereby causing variability in the concentration of some volatile components. The clear glass container the beer is poured into may also allow exposure to UV light from the fluorescent lighting used in the laboratory, resulting in chemical changes in some of the components of the sample.

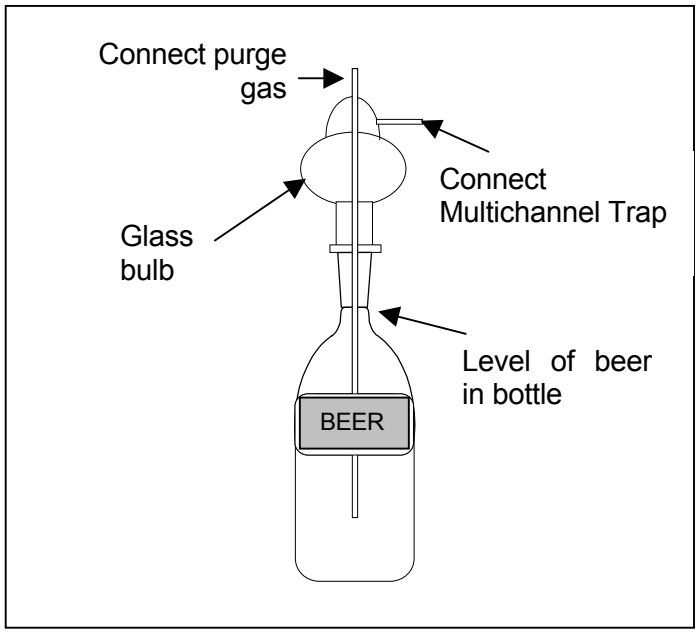


Figure 5.2: On-bottle sampling device

A diagram showing the sampling device developed for sampling directly from a beer bottle in order to investigate variability introduced by sample handling.

In order to decrease the variability introduced through handling of the sample, an on - bottle sampling technique was developed. This technique is aimed at eliminating the need for the beer to be transferred to another flask. For this a piece of glassware was designed which seals onto the neck of a beer bottle and to which the purge gas and MCT trap can be connected. The device, on top of a bottle, is shown in Figure 5.2. A glass flange at the base of the glass bulb is sealed onto the neck of the bottle by means of a tightly fitting Schott ™ clip.

The on-bottle sampling device makes sample preparation much faster and reduces the number of surfaces with which the beer comes into contact, thus reducing the risk of contamination. Figure 5.3 shows the on-bottle sampling arrangement with a MCT connected and heated with the trap heating- device.

Figure 5.3: The on-bottle sampler and trap heating device

The sampler was specifically designed with a bulb to contain any possible foam resulting from purging of the beer sample and prevent it from entering the MCT.

The on-bottle sampling technique produced good results, with a well focused ethanol peak and a number of smaller peaks following the ethanol clearly visible. Figure 5.4 shows results of two consecutive beer samples.

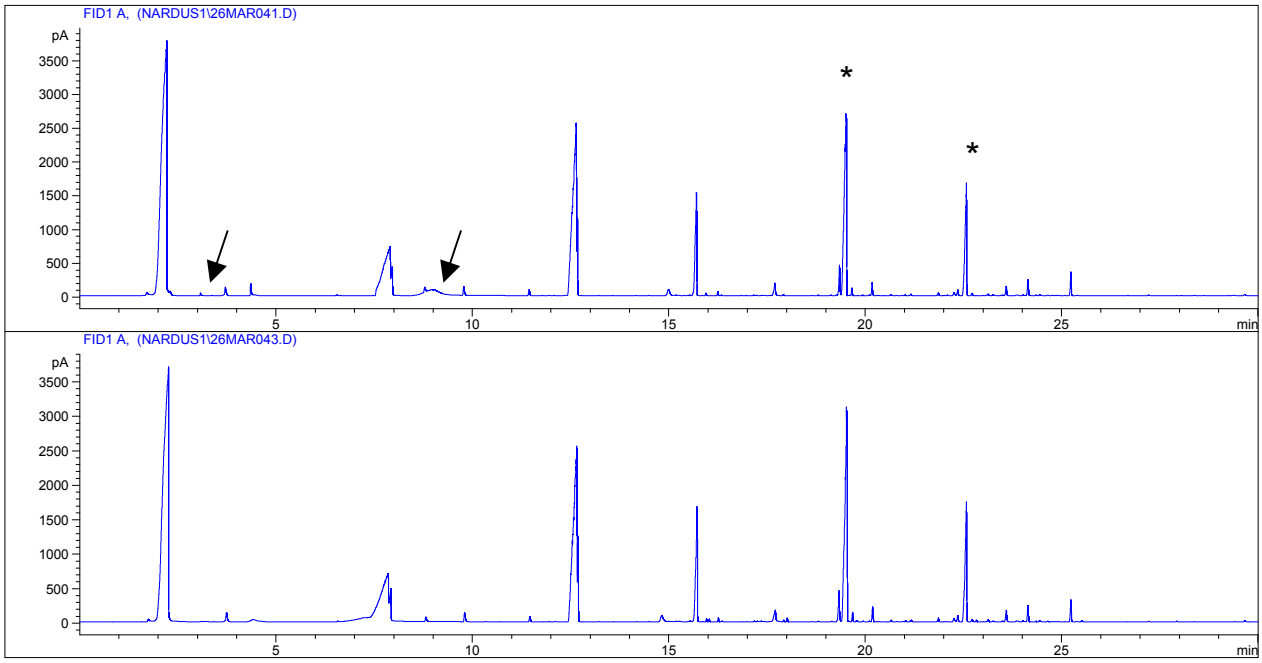


Figure 5.4: Results obtained with the on-bottle sampling device

The on-bottle device improved the similarity between the chromatograms of consecutive beer samples of the same brand. Some variations (as marked above) was still observed.

A definite improvement in repeatability was noticed in terms of peak shape and relative intensities (compare Figure 5.1). Notice that some peaks (marked the first half of the first chromatogram) are not present in the second chromatogram and the height of the two large peaks indicated at approximately 19.5 minutes and 22.5 minutes seems to increase in the second chromatogram. These small differences present in the chromatographic profiles meant that the results were not yet good enough for direct visual comparison. The results also still showed variability on statistical evaluation of absolute peak areas. Sampling was, however, simplified a great deal by this method and it was used for sample preparation of all future analysis.

3.2 Differences in the sample

Besides the success of the on-bottle sampling technique, the visible variations and inconsistent number of peaks still presented a problem. It was then considered that the variability might be inherent to the samples because these were bought from different retail stores and at different times, as required.

Beer samples were then collected from the brewery in order to ensure that all of the bottles sampled are from the same batch. The repeatability of the method was

evaluated again using the new samples and on-bottle sampling method. This, however, did not improve the repeatability.

A decision was made to try and collect more than one sample from one bottle of beer so that any possible "between-bottle" variation could be identified. A number of different ways was attempted and the one that gave the most reliable result was to divide one sample in two and then sample each portion separately.

The results unfortunately showed no change and were still not reproducible enough for direct comparison, so the investigation of the source of variation continued.

3.3 Sample temperature

Results of the investigation into the effect of increased sample volume on peak area discussed in Chapter 4 (Section 3.2) showed the temperature of the sample to have a strong influence on the observed peak area. It was subsequently decided to have the sample at room temperature before and during sampling, as this would be an easy temperature to maintain in an air-conditioned laboratory. For all repeatability experiment thus far, room temperature was still the temperature used for sampling, however, with the Chapter 4 results in mind it was realised that equilibration of the sample to "room temperature" before sampling may not be adequate temperature control. Depending on season and time of day this temperature may vary. As a result it was decided to use a thermostated water bath to keep the sample temperature constant during the sampling.

A closer inspection of the peak area data collected as part of the repeatability investigation revealed that the largest variation was observed for small peaks. This is because a difference of 1 unit for a total peak area of 3 would represent more than a 30 per cent variation. The simplest way to increase the area of the smaller peaks is to increase the amount of those compounds going into the headspace and on into the MCT. The technique chosen for this was to increase the sample temperature, which is the same method used during the optimisation of the sampling method.

An experiment was done to determine at which temperature the sample would provide an adequate recovery of the aroma compounds. For this the temperature of

the trap (MCT) was held constant while increasing the water bath temperature in increments of 10°C. Figure 5.5 shows the result of these experiments.

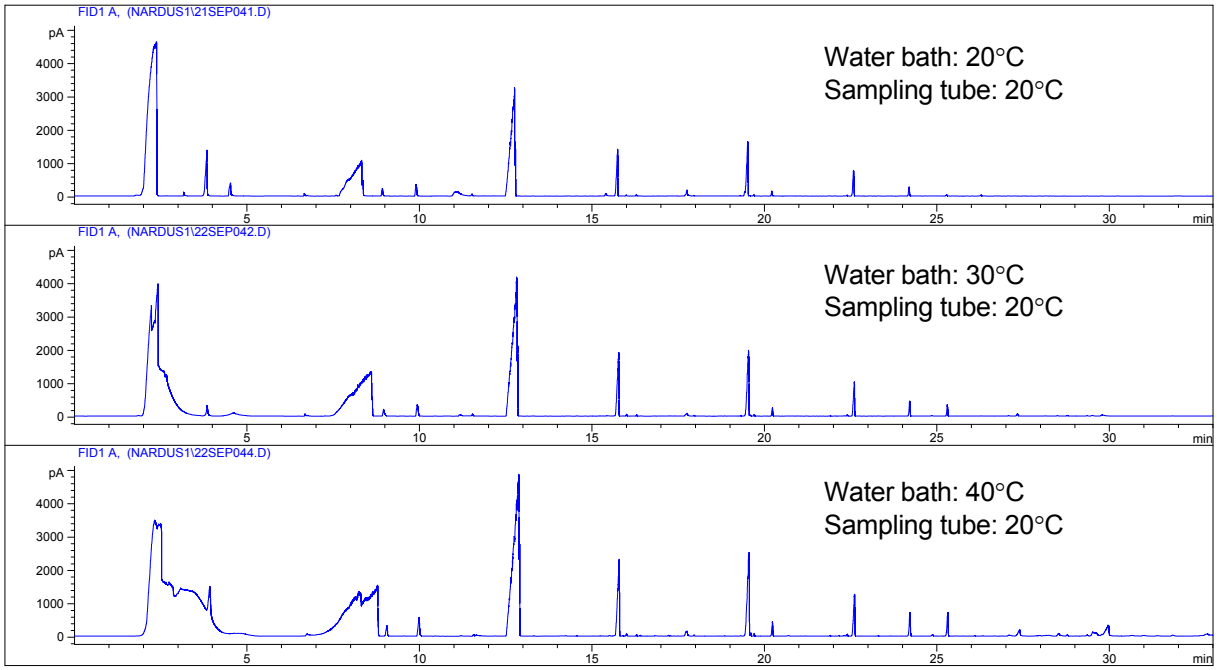


Figure 5.5: Optimisation of sample temperature

The three graphs show the effect of increasing the beer sample temperature on the observed peak area of the compounds. The sampling tube temperature was held constant during the experiments.

A definite improvement in the general peak height is observed, with again the broadening of the ethanol peak due to increased moisture in the headspace. Notice the asymmetric “fronting” peak shape of the isoamyl acetate peak at 12 minutes, due to concentration overloading and the resulting non-linear chromatography. At a temperature of 50°C the excess moisture caused freezing of the cold trap on the desorber system with the associated increase in inlet pressure.

The problem of excess moisture accumulating was resolved simply by increasing the temperature of the trap heating-device to 10°C above that of the beer sample. This result is shown in Figure 5.6.

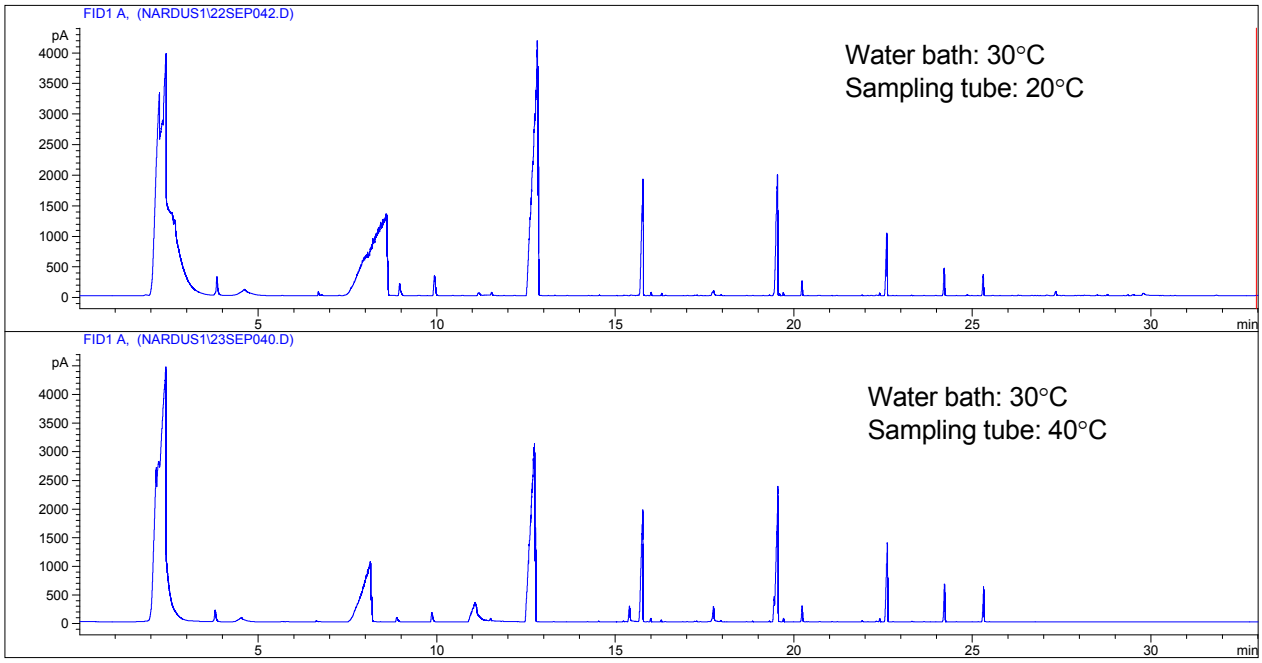


Figure 5.6: Reduction of accumulated moisture with trap heating device

Note that by increasing the temperature of the trap relative to that of the sample the moisture accumulation is reduced without a drastic reduction in the peak height of the later eluting compounds.

Notice that a reduction in the peak sizes is observed only for the first set of peaks up to the third large peak (eluting between 10 and 15 minutes). This is an indication that the higher temperature sampling conditions has slightly exceeded the retention volumes of these compounds. Later peak heights actually seem to be enhanced by the reduction in moisture and volatile compounds like ethanol. A water bath temperature of 40°C and a trap heating-device temperature of 50°C were selected for the remainder of the experiments.

By sampling the beer at a constant higher temperature the visual repeatability did show a marked improvement. Peak areas and peak shapes became more or less visually comparable (Figure 5.7), however, statistically the results still showed unacceptable variability (Table 5.2).

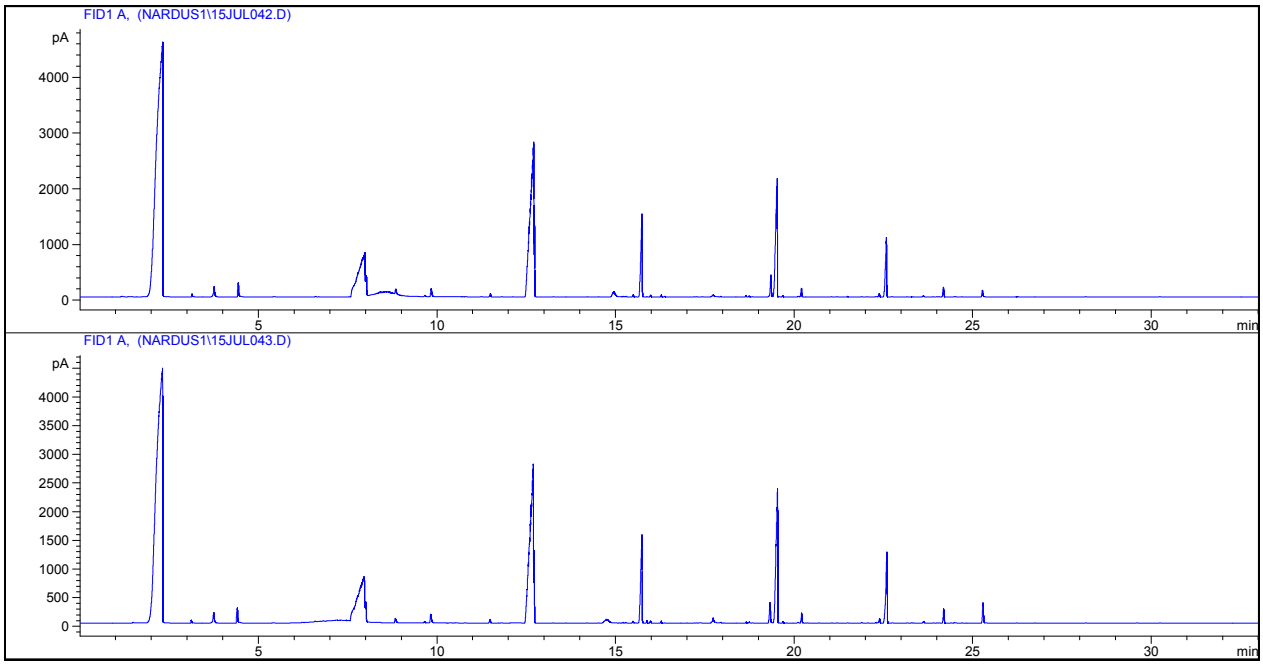


Figure 5.7: Improved repeatability due to sample heating

Table 5.2 shows peak areas for peaks up to 19.5 minutes, which were selected based on closely matching retention times, to illustrate the point.

Table 5.2 Statistical evaluation of matching peaks for ten identical samples of beer

Retention time (min)	Areas of Matching Peaks (10 samples)										%RSD
2.3	61452.2	54638.2	52314.0	63694.2	54899.5	69381.9	66739.8	57542.9	65060.3	71573.3	10.8
3.1	82.2	77.3	71.1	87.9	76.7	95.0	89.2	80.2	91.5	100.2	10.7
3.4	5.0	4.7	5.1	5.0	4.5	4.2	4.4	3.8	4.2	4.8	9.3
3.7	526.9	454.6	460.1	421.2	473.9	474.2	441.1	382.3	460.9	472.6	8.3
4.4	497.6	461.2	428.1	526.7	467.7	558.9	515.2	476.7	537.4	578.2	9.3
6.6	40.2	32.5	32.3	32.4	33.8	38.8	35.7	33.0	35.9	40.2	9.1
6.7	17.4	14.8	14.1	14.6	14.5	17.2	15.3	13.4	16.8	18.5	10.8
8.8	208.8	175.7	173.0	181.8	184.7	546.0	202.4	190.2	1090.6	212.7	92.9
11.5	135.2	124.9	151.6	139.7	125.8	118.4	112.0	106.3	104.8	152.8	13.7
11.8	8.5	9.0	7.6	9.0	7.6	7.9	8.5	8.2	9.7	7.7	8.4
12.7	25887.4	23651.5	20418.5	21671.6	22977.3	24459.3	23781.9	22139.1	23959.4	27443.2	8.6
13.3	14.0	12.8	11.4	12.3	14.1	14.9	12.8	14.8	14.5	17.5	12.3
14.2	9.9	9.7	9.3	8.4	9.5	8.7	8.3	7.6	9.2	10.4	9.3
14.5	16.5	15.3	12.9	13.8	13.7	13.0	14.7	13.5	16.3	18.2	11.9
15.5	27.9	25.5	22.6	19.9	20.3	17.4	20.3	17.0	15.6	47.0	39.1
15.6	12.3	10.6	10.8	10.0	8.0	7.3	17.2	8.6	8.1	22.7	42.0
15.8	5869.5	5783.8	4456.6	4913.3	5306.9	5050.9	5372.3	4876.3	4804.7	6398.8	11.1
15.9	14.5	15.2	14.2	20.2	9.2	9.8	11.6	9.2	14.6	26.5	37.3
16.0	92.7	92.5	70.0	75.0	83.7	77.3	83.2	72.1	76.7	97.9	11.6
16.3	64.7	60.7	69.2	66.9	63.4	59.6	57.4	54.7	54.6	59.3	8.1

Retention time (min)	Areas of Matching Peaks (10 samples)										%RSD
16.4	28.3	29.0	36.5	23.6	27.1	19.7	15.8	21.5	45.1	20.5	32.7
16.9	5.6	5.0	3.6	2.8	2.9	2.7	3.7	2.7	3.1	6.8	36.7
17.0	13.4	14.6	10.1	11.2	12.2	11.1	12.8	10.5	9.3	13.3	14.2
17.2	2.7	2.7	2.5	2.3	2.0	1.8	3.6	2.3	2.0	3.3	22.9
17.2	17.8	20.1	12.8	13.1	15.6	11.6	8.7	10.7	12.7	12.8	24.9
17.3	17.7	17.8	12.4	15.4	14.0	17.7	18.3	16.5	15.8	20.7	14.2
17.4	31.4	34.5	17.9	27.5	18.5	15.7	20.2	17.0	21.1	26.1	28.3
17.6	2.0	1.9	59.4	75.7	66.2	54.6	64.7	54.2	65.7	71.4	52.3
17.7	219.6	338.7	111.8	103.5	66.2	115.8	122.3	94.7	94.1	166.2	56.5
17.9	32.5	32.7	23.0	28.1	32.1	25.4	27.3	24.8	30.4	32.5	12.6
18.0	5.5	8.1	4.2	6.7	9.6	27.1	21.7	7.2	7.8	15.0	67.3
19.5	9330.4	9380.5	6352.4	7635.8	8191.7	7111.7	7779.4	7005.1	6934.2	8800.3	13.4

3.4 Accurate retention times

In most of the beer aroma chromatograms produced during this project a strange unfocused peak was observed.

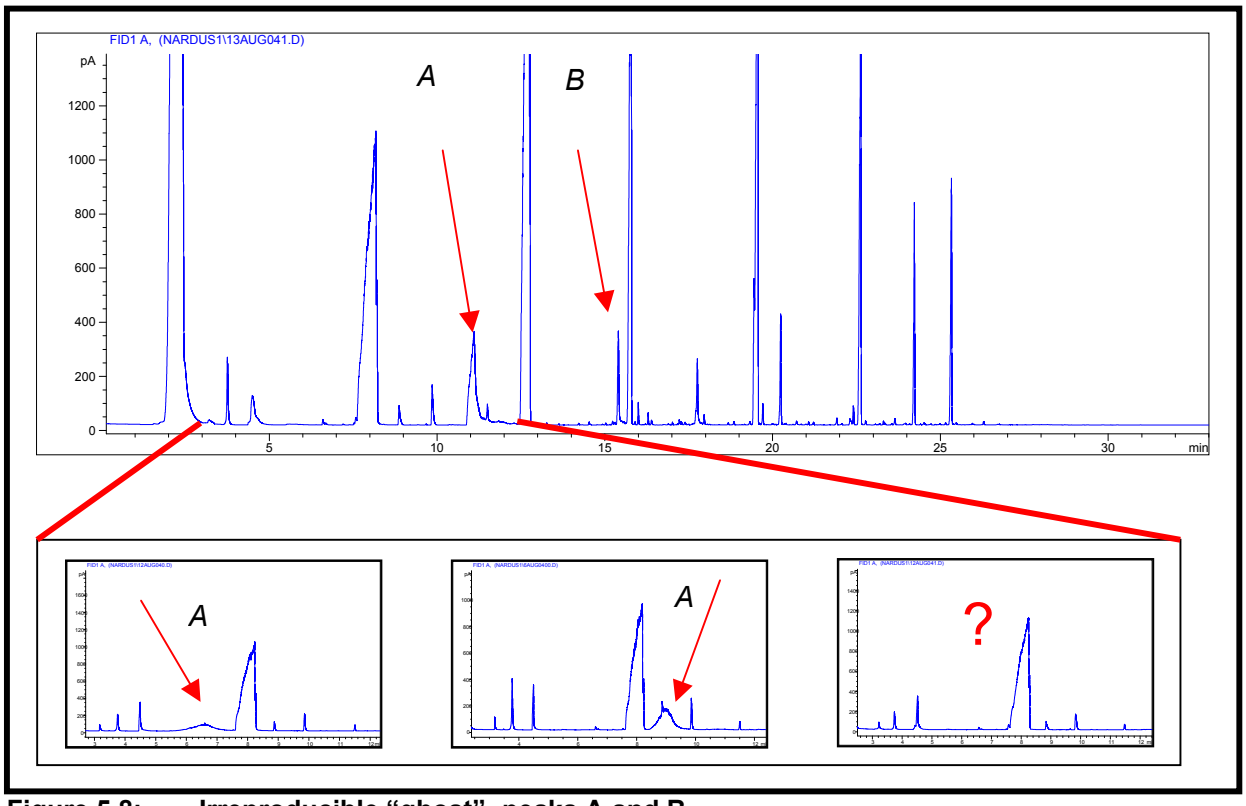


Figure 5.8: Irreproducible "ghost"- peaks A and B

The peak indicated as A seemed to move relative to other peaks in the chromatogram and on some occasions disappear from the chromatogram. A second peak B, showing similar retention time variations, was later discovered, however, the movement of this peak was not as pronounced as for peak A

As shown in Figure 5.8 this peak also eluted at different retention times while the retention times of the peaks around it stayed constant. At later inspection a second peak (indicated as B, Figure 5.8) was discovered, that showed the same behaviour. The retention time shift of this peak was, however, less pronounced, so it was not so apparent at first.

Initially these peaks were attributed to carryover due to incomplete desorption of the MCT, however, blanks of the silicone rubber trap immediately following the desorption of a sample showed no residual aroma compounds. In order to establish the cause of these peaks a mass spectrum of a beer aroma was obtained. It showed the peaks to be ester compounds of the same type as the large peak immediately following it.

Because these “ghost” peaks elute prior to the large ester peak it cannot be attributed to dead volume in the system, as peaks caused by dead volume elute after the corresponding “parent” peak. This suggested that these extra, unfocussed peaks could be the result of cold-trap-break through of the two volatile ester compounds during desorption, before injection into the chromatographic column. A number of tests were done to confirm this suspicion. First the effect of the GC oven temperatures during desorption was investigated. It was realised that the GC oven temperature was never regulated during desorption of the MCT. The GC oven was only equilibrated to the starting temperature of the analysis (10°C) just before the sample was introduced into the column (i.e. heating of the CIS cold trap). To test the effect of this the GC oven temperature was kept constant at different settings during the first 11 minutes of desorption after which it was cooled to the regular 10°C.

As indicated in Figure 5.9 the GC oven was at 10°C, 30°C and 50°C respectively, during the MCT desorption. The observed shift in retention time with increasing oven temperature confirmed that the peaks were indeed breakthrough entering the column before the actual injection.

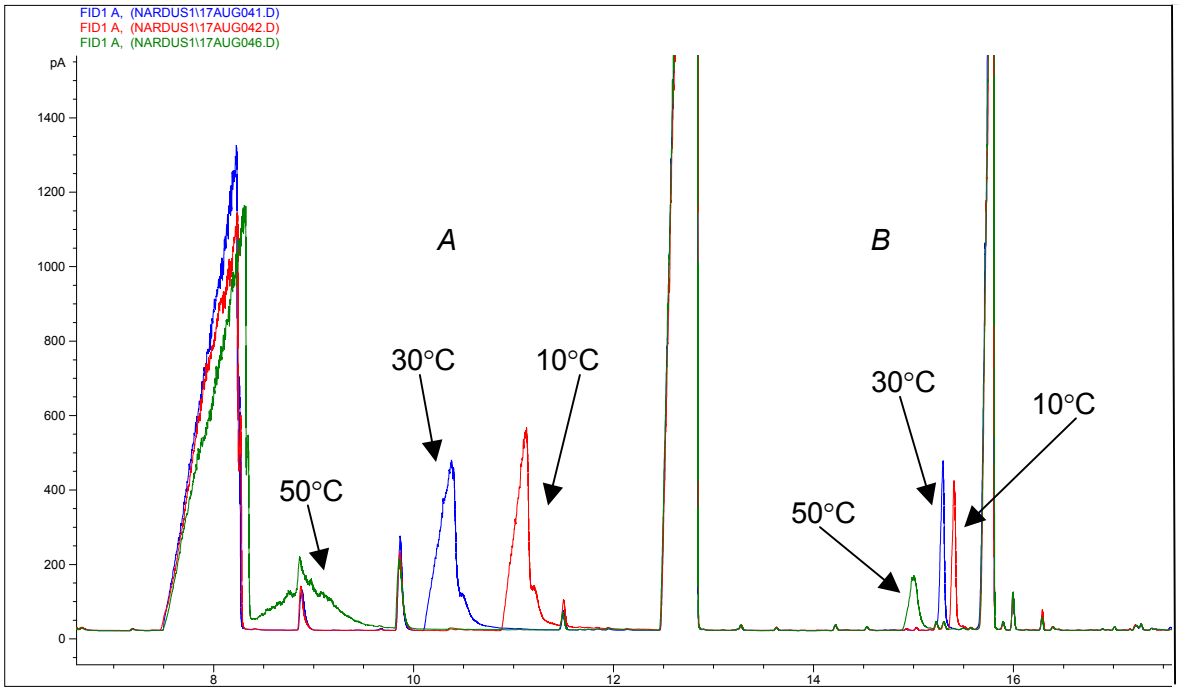


Figure 5.9: Effect of oven temperature on suspected breakthrough peaks

The retention time of the "ghost" peaks (A&B) shifted depending on the temperature at which the GC oven was held during the desorption phase, before the injection of the sample from the CIS.

The temperature of the column during the first part of the desorption phase, determines the speed at which these breakthrough compounds elute. Inconsistencies in the oven temperature during the desorption phase caused the retention time of the peak to shift, the oven temperature was, however, never high enough, or the period between desorption and injection long enough, to fully elute these compounds before the run started. The shift for the first ester compound is more pronounced because the compound is more volatile and therefore moves further during the 11-minute desorption time window than the second ester compound.

The next step was to determine the nature and a possible cause of the breakthrough in the cold trap (CIS). For this the CIS (cold injection system) was held at different temperatures during desorption of the MCT. The ratio of the breakthrough peak area to that of the parent peak was measured and plotted (Figure 5.10).

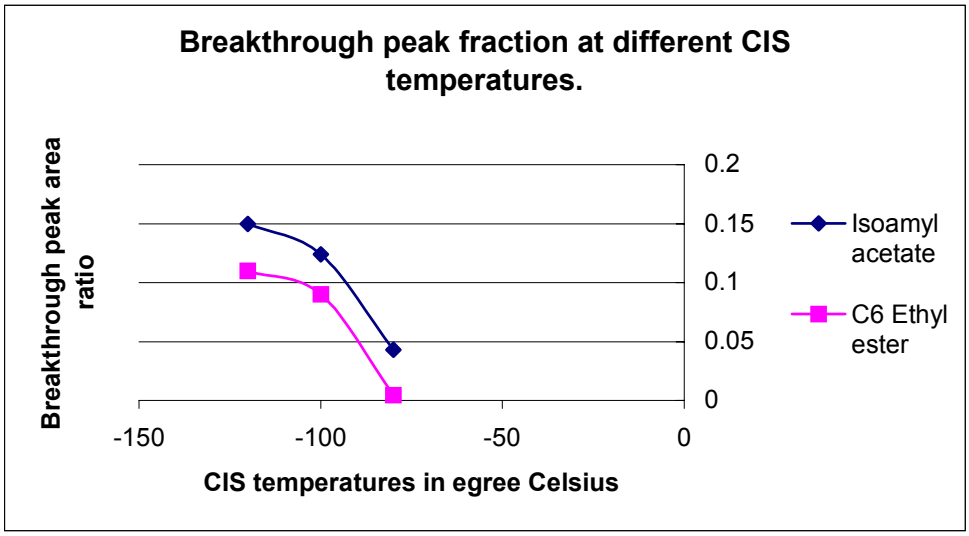


Figure 5.10; Graph of breakthrough peak area ratios

The graphs show that an increase in the peak area of the "ghost" peaks correlate with a lower cold trap (CIS) temperature. This indicates that the compounds break through the CIS as droplets rather than discrete molecules.

This downward trend observed at higher CIS temperatures (Figure 5.6), suggest that these compounds break through as tiny droplets or an aerosol rather than as discreet gas phase molecules. The latter type of breakthrough would show an inverse temperature relationship [54].

At lower CIS temperatures larger and/or more aerosol droplets (composed of mostly ethanol or ethanol/water) are formed. These larger droplets exhibit slow diffusion characteristics, preventing them from reaching the cold surface [54] during the short time available while moving through the CIS inlet. A portion of these never reaches the cold surface and is swept into the column with the gas stream leaving the CIS. Some of the more polar compounds like the esters are dissolved in these ethanol droplets and are also carried into the column. These compounds then start to partition inside the column as soon as the temperature is high enough to do so. The compounds therefore have an advantage over the other compounds in terms of time already spent within the column, and consequently elute slightly earlier. The distance between the breakthrough peak and the parent peak increases (a) as volatility of the compound increases, and (b) as the column temperature increases. This is simply due to the fact that more volatile compounds would move faster, and therefore further into the column during the isothermal period in the GC oven.

Based on these results it was decided that all future analysis would be performed with the GC oven kept at a constant temperature of 10°C during the desorption phase before injection

3.5 Attainment of repeatability

The benefit of the investigation into the source of the ghost peaks was that a dramatic increase in the retention time repeatability of all compounds was observed due to the oven being kept at a constant temperature during the desorption phase before injection. Evaluation of the peak areas revealed an increase in the absolute repeatability of the method making it possible for chromatograms of the same beer brand to fit on top of one another almost perfectly.

Figure 5.11 below shows the results of eight consecutive analyses of a particular beer brand, plotted on top of one another.

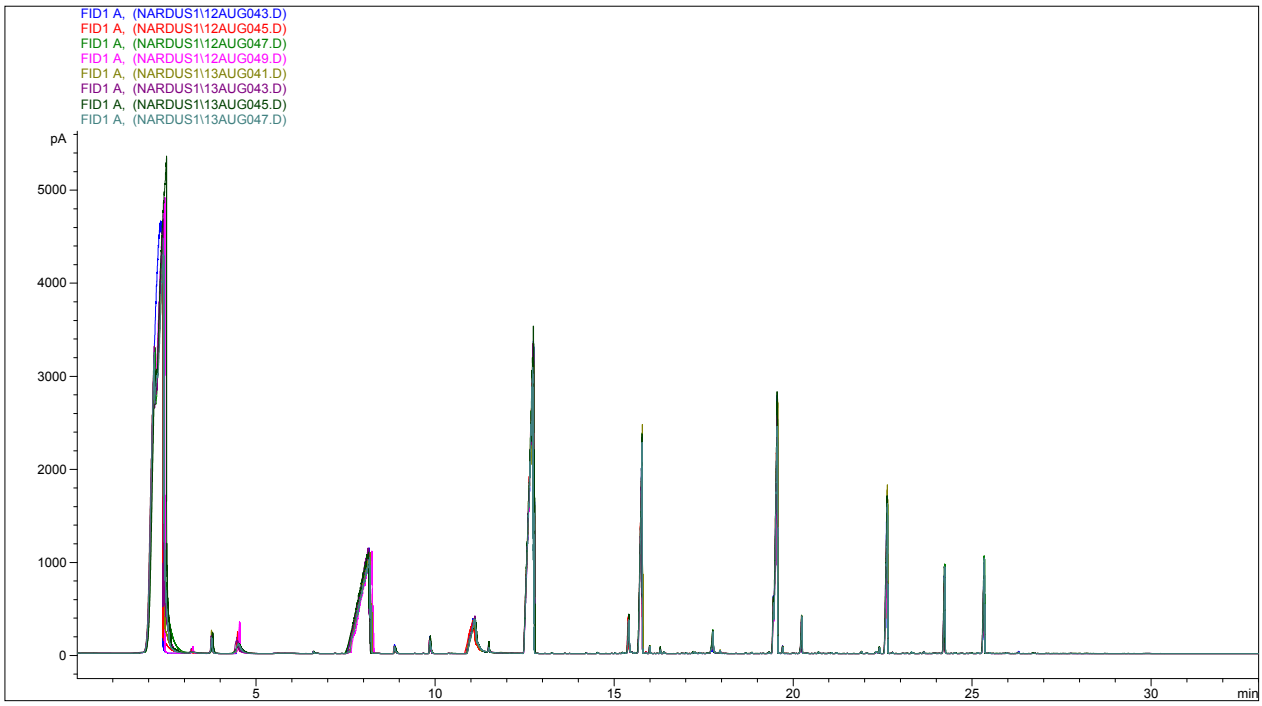


Figure 5.11: Eight overlaid chromatograms demonstrating repeatability of sampling method
For detail on smaller peaks see Figures 5.12 and 5.13.

Excellent repeatability was achieved, even for trace compounds (small peaks) as demonstrated by the extractions of the mono- and sesqui-terpene sections of the chromatogram shown in Figures 5.12 & 5.13. Notice the differences in the relative intensities of the peaks. In Figure 5.11 the peak heights range between 1000 and

4000 pA in height while the peaks shown in the two terpene sections, are on average 40 to 100 pA in height.

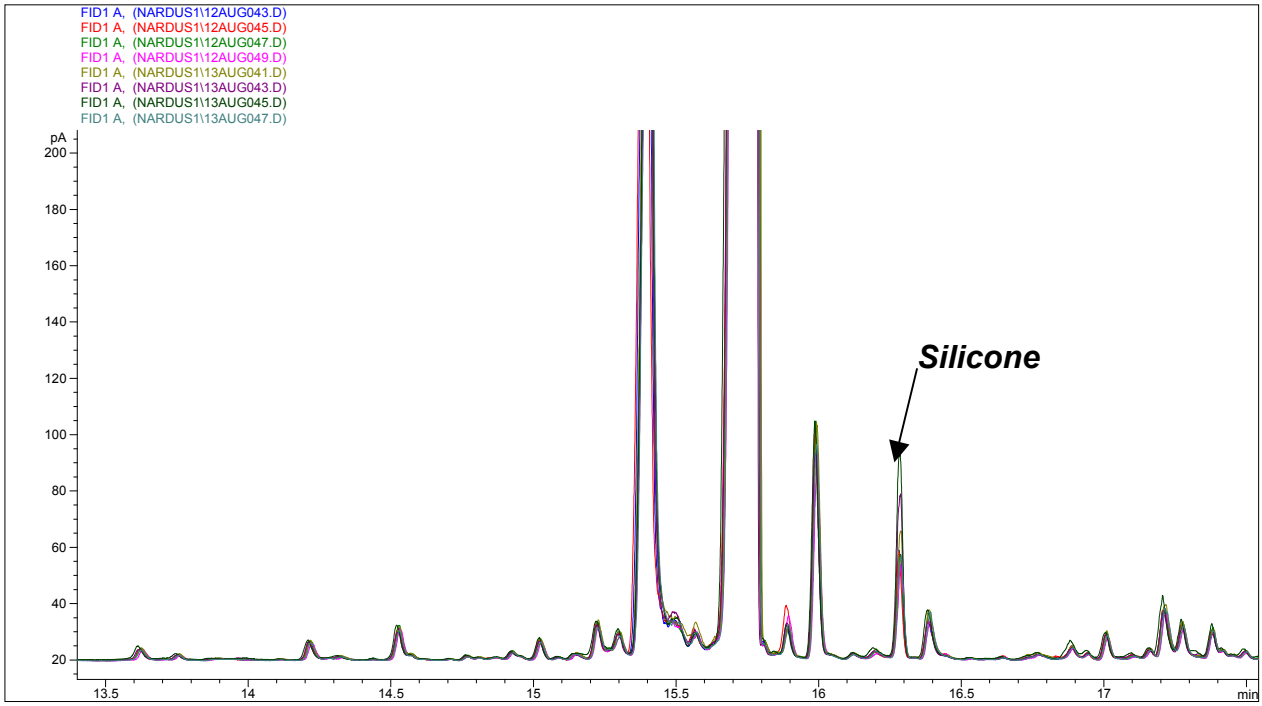


Figure 5.12: Overlay of eight chromatograms displaying reproducibility of smaller peaks including the mono-terpenes

The mono- and sesqui-terpenes are compounds derived from the hops added to the beer. The presence of these compounds in the sample was confirmed by means of mass spectrometry (see Chapter 7).

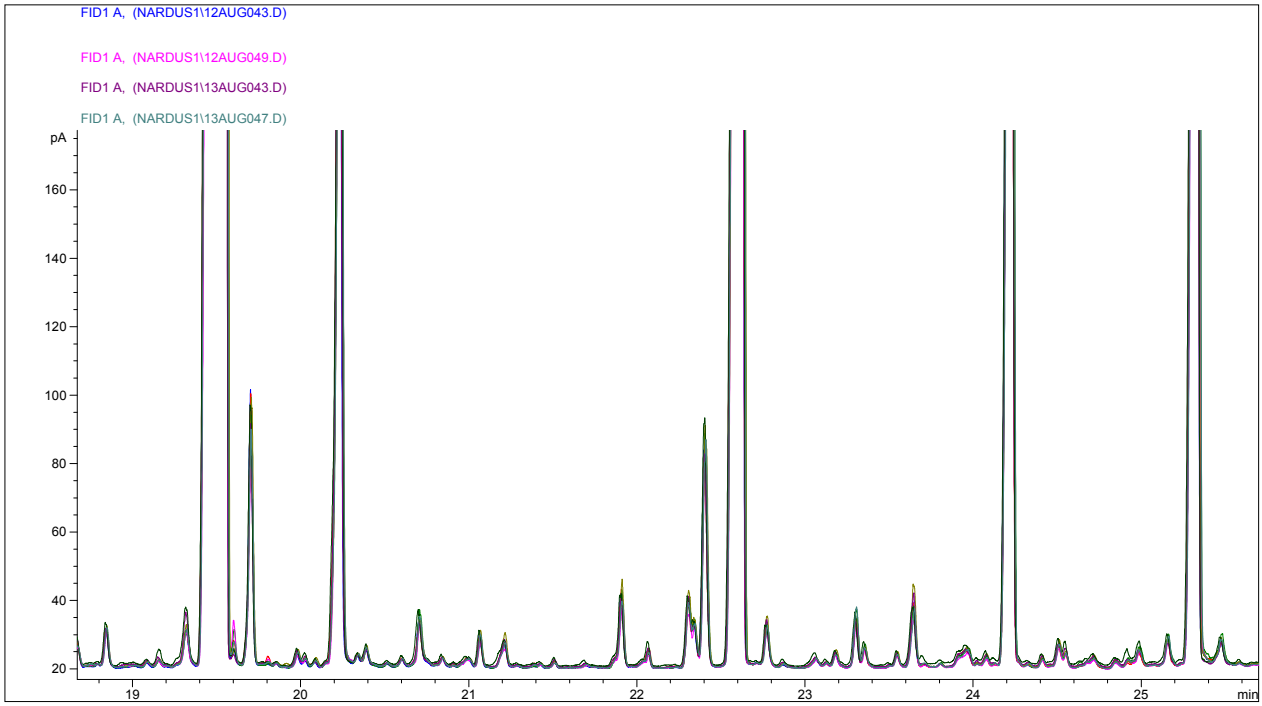


Figure 5.13: Overlay of eight chromatograms displaying reproducibility of smaller peaks including the sesqui-terpenes

At this point it was important to determine which of the peaks observed were beer aroma compounds and which were part of the chromatographic background such as the silicone peak indicated in Figure 5.12. Especially for the trace compounds it was important to confirm that the highly repeatable peaks in the chromatogram were beer aroma compounds. In Figures 5.14 and 5.15 a beer aroma sample (blue line) and a method blank (red line), as defined in Chapter 4 (Section 3.3), is compared. The figures show the same detailed sections that include the peaks indicated in Figures 5.12 and 5.13.

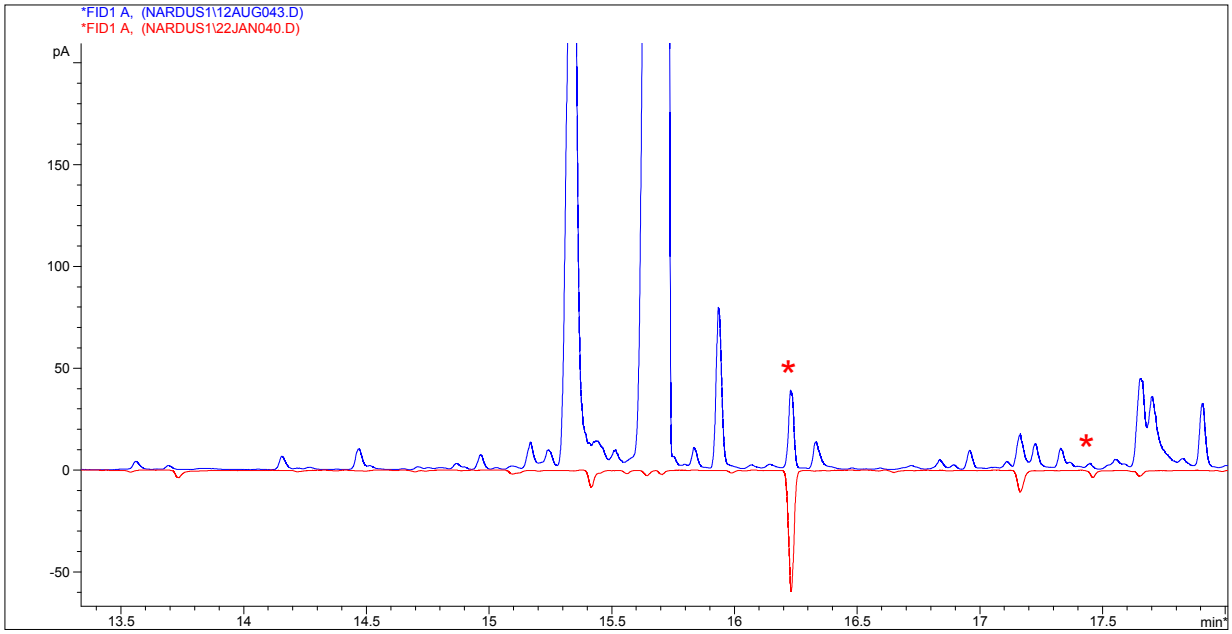


Figure 5.14: Comparison of method blank (red line) with section of chromatogram containing mono-terpene peaks (blue line)

Notice that there are very few peaks from the method blank that match up with peaks from these two sections of the beer chromatogram. Peaks indicated are silicone peaks.

Very few peaks in the method blank matched the peaks observed from the beer aroma sample. Only peaks that are the same size or larger in the method blank are considered as significant. The peaks marked in Figures 5.14 and 5.15 were identified as silicone peaks. Over 80 visually reproducible peaks could positively be distinguished from the chromatographic background (method blank). Statistical peak area reproducibility was confirmed on a subset of 63 of these peaks. The 63 peaks were selected from the automated peak area listing, based on clearly matching peaks in the consecutive chromatograms evaluated.

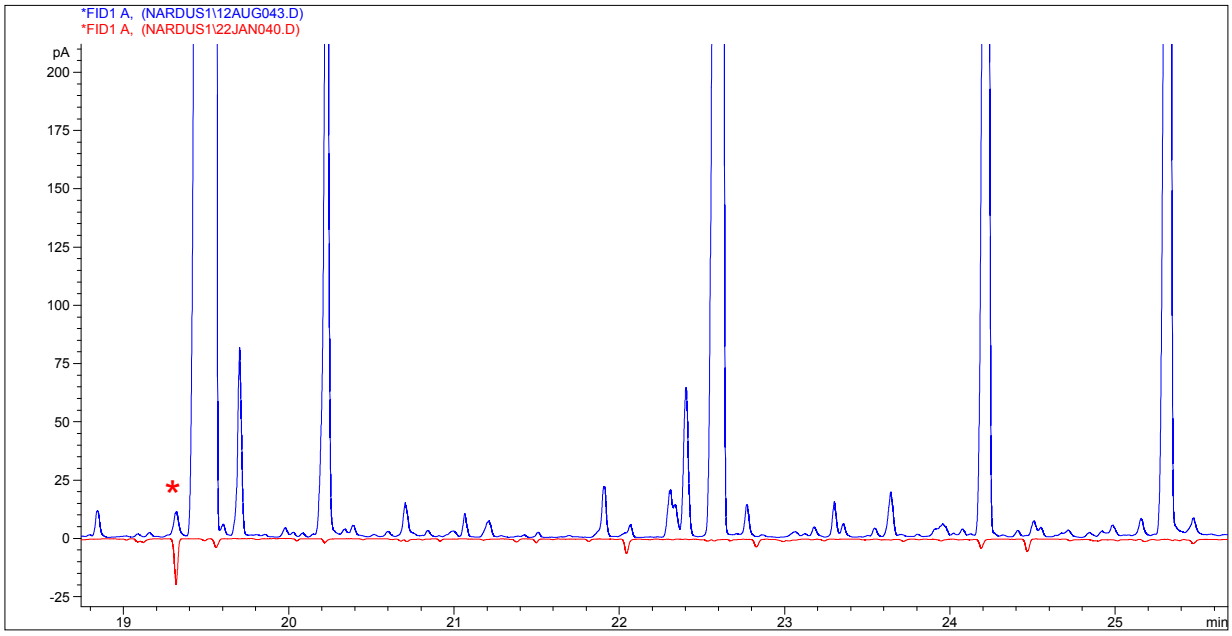


Figure 5.15: Comparison of method blank (red line) with section of chromatogram containing sesqui-terpene peaks (blue line)

Statistical evaluation of the absolute peak areas of the 63 peaks across the entire chromatogram revealed that a high degree of repeatability was being achieved. The results show that for over 80 per cent of the compounds evaluated show a percentage relative standard deviation (RSD) of below 15%, and over half of the peaks have an RSD of below 10%. The following table shows the results for the consecutive analysis of eight samples of one beer brand. The five major ester peaks are indicated.

Table 5.1: Statistical evaluation of matching peaks for eight identical samples of beer

	Retention time (min)	Areas of matching peaks (8 samples)								%RSD
	3.8	557.2	435.6	504.4	552.3	666.6	517.7	628.4	460.4	14.6
	4.5	824.4	859.9	989.6	1006.6	937.4	990.9	1143.9	933.2	10.2
	6.6	48.3	46.2	46.9	52.0	47.9	51.0	57.6	46.5	7.8
	6.7	18.7	16.7	17.5	18.6	18.0	18.6	21.4	16.9	8.1
	7.2	7.3	7.1	7.7	8.1	7.8	8.3	9.3	7.9	8.5
	8.9	233.3	221.5	231.5	245.0	224.2	232.8	260.1	214.8	6.1
	9.8	468.2	441.4	463.3	476.7	451.4	459.2	513.1	421.3	5.8
Ester	12.7	27960.5	26554.3	28020.8	26869.6	27598.5	26287.7	28962.5	24017.9	5.6
	12.8	1894.0	1833.9	1880.9	1900.6	1833.0	1887.3	1930.7	1744.6	3.1
	12.8	7.5	7.9	8.3	8.3	6.8	6.0	9.0	7.4	12.4
	13.1	3.1	3.3	3.7	3.5	3.5	3.4	3.8	3.0	8.1
	13.3	15.7	15.1	16.0	14.8	16.2	15.2	16.3	14.2	4.8
	13.6	7.5	7.4	7.9	6.9	7.9	7.4	9.3	6.9	10.0
	13.8	3.2	3.1	3.2	3.0	3.1	2.8	3.4	2.9	6.1
	14.2	11.1	10.8	11.8	10.1	11.8	10.6	12.3	9.9	7.8

	Retention time (min)	Areas of matching peaks (8 samples)								%RSD
	14.5	21.9	21.7	25.5	21.5	23.4	20.2	24.5	20.2	8.7
	15.0	11.1	11.4	11.6	10.5	11.3	10.0	11.9	10.2	6.3
	15.2	25.9	20.9	24.9	22.1	28.9	25.2	33.9	21.1	17.3
	15.3	22.3	16.7	24.8	22.5	21.7	20.1	22.5	22.0	10.9
Ester	15.4	998.6	949.6	932.6	955.5	983.2	924.6	1031.4	952.4	3.7
	15.8	8751.0	8770.7	8974.7	8026.9	9217.9	8040.8	9170.5	8182.0	5.7
	15.9	18.8	31.8	24.1	32.7	23.2	24.1	18.9	24.1	20.9
	16.0	129.0	122.9	131.6	117.8	134.2	115.4	134.6	120.1	6.1
	16.1	5.2	3.7	5.0	4.6	4.4	3.9	4.6	3.4	14.5
	16.4	29.3	31.2	34.0	32.2	37.0	29.3	38.2	34.3	10.0
	17.0	14.0	15.1	15.1	12.8	16.2	12.6	15.7	14.0	9.1
	17.2	33.3	37.3	39.8	32.0	41.0	31.9	39.2	36.6	9.9
	17.3	22.9	24.6	24.0	20.5	26.8	20.1	20.1	20.7	11.2
	17.4	22.1	25.9	23.5	20.3	25.9	19.3	20.6	18.1	13.3
	17.5	4.6	4.8	4.9	4.8	7.4	4.6	5.0	3.2	23.5
	17.7	108.7	590.9	770.2	581.8	682.6	530.2	735.3	530.8	36.3
	18.0	56.6	65.0	62.9	56.9	66.1	54.1	61.1	46.3	11.2
	18.4	2.6	2.8	2.4	2.7	3.0	3.4	3.7	2.8	14.7
	18.7	12.4	11.3	10.4	9.5	11.8	12.9	13.9	10.0	13.2
	18.8	20.3	19.8	19.9	17.3	21.4	18.3	21.9	19.2	7.7
	19.3	23.2	23.2	23.6	21.6	27.0	33.4	44.6	23.6	28.5
	19.4	1192.3	1158.0	1072.2	946.8	1128.1	1040.5	1251.4	1081.6	8.6
Ester	19.6	9638.5	9562.9	9508.5	8124.5	10256.8	8665.7	9967.0	9060.3	7.5
	19.7	132.1	134.5	126.3	110.8	137.9	117.9	132.5	119.3	7.5
	20.2	669.2	798.8	814.3	730.0	856.0	743.3	918.2	829.1	9.9
	20.3	7.5	6.9	7.5	6.7	7.0	7.1	8.3	7.7	7.1
	20.4	10.9	11.5	11.6	10.1	11.9	11.0	15.3	12.3	13.2
	20.7	29.4	28.1	39.1	25.9	33.1	26.2	43.5	31.9	19.5
	21.1	15.5	14.5	16.0	12.5	21.4	18.2	20.7	16.8	17.9
	21.9	42.8	40.5	43.1	34.5	46.0	40.4	44.9	39.4	8.7
	22.1	10.1	9.4	9.0	5.0	9.1	9.0	11.2	6.6	22.7
	22.4	126.8	118.5	137.9	107.1	137.9	117.6	139.2	128.2	9.2
Ester	22.6	4486.4	4246.4	5218.7	3871.0	5221.1	4415.6	5069.9	4703.9	10.5
	22.8	22.8	21.5	25.5	17.1	28.9	24.7	26.1	22.3	15.0
	23.1	9.7	7.4	8.5	6.0	9.4	6.9	10.8	8.4	18.8
	23.2	9.1	7.8	8.6	7.8	10.3	10.7	10.3	8.1	13.2
	23.3	25.1	22.1	31.2	23.5	25.8	22.3	27.5	32.1	14.6
	23.4	10.6	10.0	13.1	9.6	12.0	10.4	13.0	11.3	11.9
	23.5	6.8	6.3	7.7	5.6	7.3	6.0	6.4	6.5	10.3
	23.6	37.6	36.4	33.9	27.2	47.5	41.4	37.5	32.5	16.5
	24.0	21.2	20.9	25.6	18.8	25.0	21.2	22.2	21.6	10.1
	24.1	9.2	8.6	7.9	6.1	9.6	10.2	9.1	8.9	14.4
	24.2	1783.1	1649.0	2118.5	1698.7	1717.1	1505.2	1999.7	1919.1	11.2
	24.4	5.8	5.3	6.4	4.1	6.8	5.7	6.3	5.4	14.6
	24.5	14.5	13.8	16.5	11.6	16.4	14.2	14.6	13.8	10.8
	24.5	7.0	6.2	7.2	6.3	7.4	9.1	11.0	7.0	21.2
Ester	25.3	2026.0	1991.9	2713.6	2047.5	2128.3	1870.5	2356.5	2556.5	13.5
	25.9	9.1	10.3	13.8	11.4	8.8	9.6	11.2	12.6	16.1

3.6 Inter trap repeatability

The results up to this point were produced by using only one MCT tube for all of the analyses. The next challenge was to prove reproducibility of results for different MCT sampling tubes.

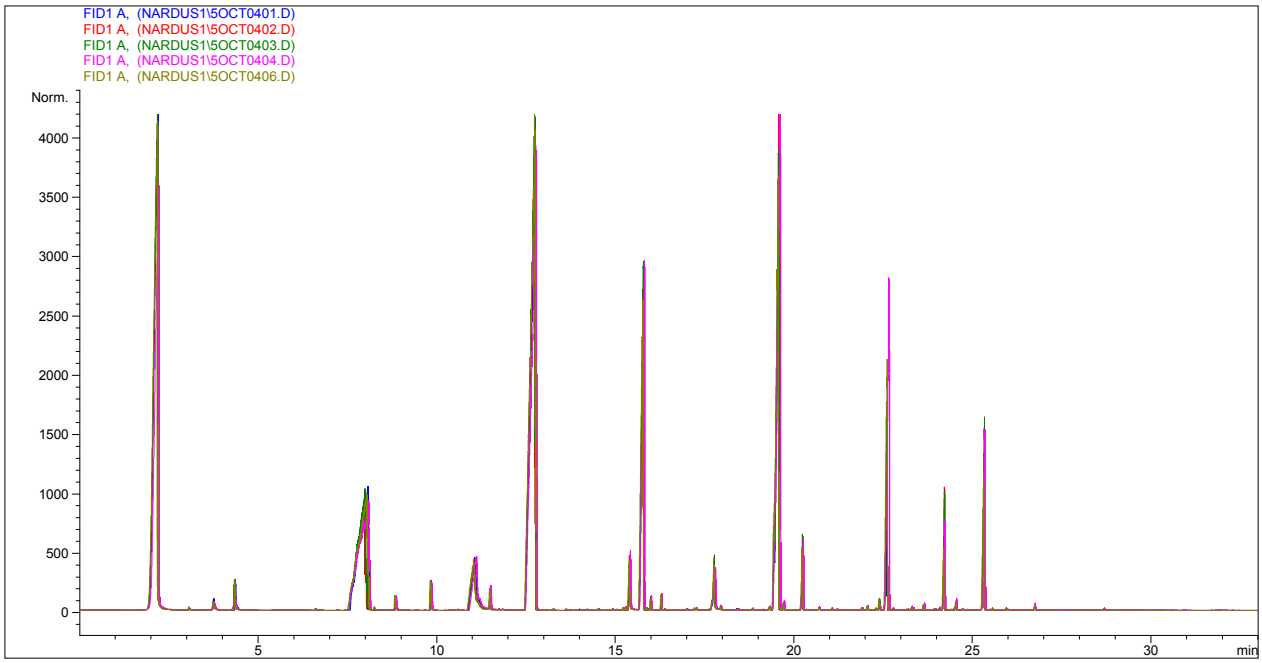


Figure 5.16: One beer brand on five different sampling tubes

Significant variability was observed between samples of the same beer brand sampled onto different MCT tubes. More detail shown in Figure 5.17.

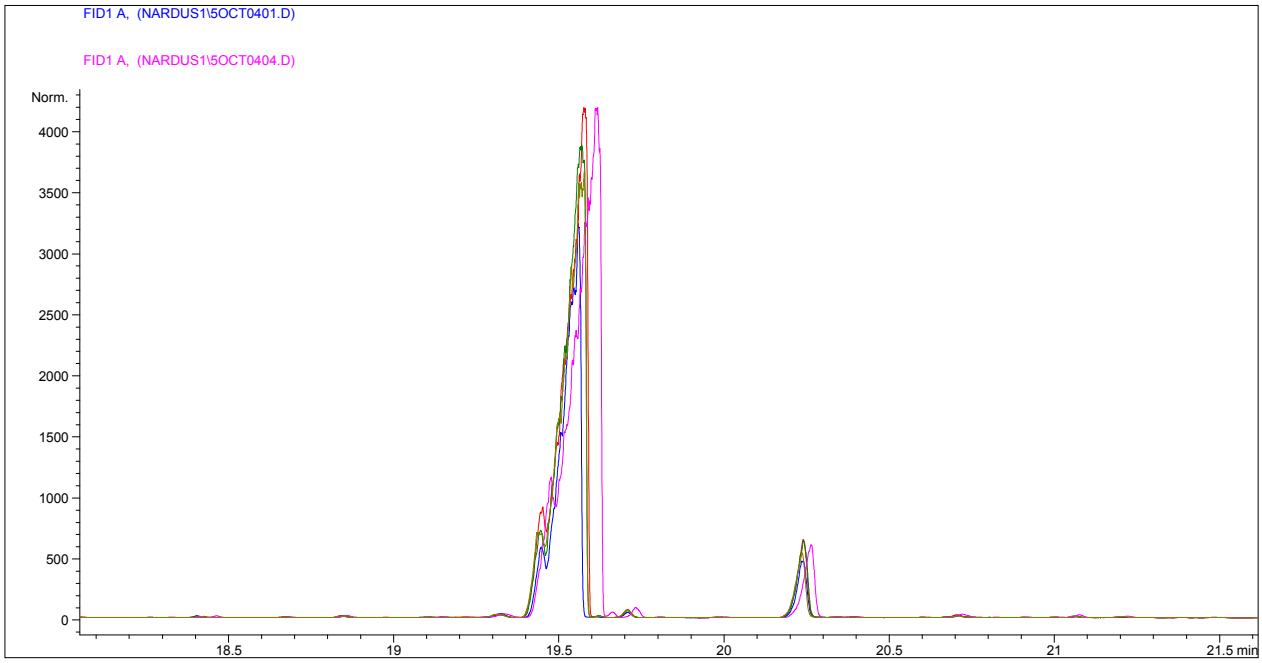


Figure 5.17: Differences in peak areas for samples collected on different MCT traps

Looking closer at two of the peaks from Figure 5.16 clearly shows the variability in peak area.

The general pattern of the flavour profile from these five results proved to be very similar (Figure 5.16), however, closer inspection of the peaks revealed discrepancies in the peak areas of the compounds for each of the analyses (Figure 5.17).

This observation can be explained by the theory provided in Chapter 3 and 4 with regards to dynamic gas phase sampling. The breakthrough volume of a compound is determined by the volatility of the compound and the solubility of the compound in the stationary phase (e.g. silicone rubber) of the collection device used (e.g. MCT trap). It was then realised that the 30-minute sampling time, selected during the optimisation process (Section 3.2.1), now no longer represented a non-breakthrough sampling scenario, but rather one of sampling beyond the point of breakthrough (Figure 5.18).

The initial results used in the selection of 30 minutes as sampling time, was obtained by using an unheated (sometimes even cold) sample and sampling tube. After optimisation, the use of a heated sampling tube (and much warmer sample gas), resulted in compounds accumulating and also breaking through the MCT much faster

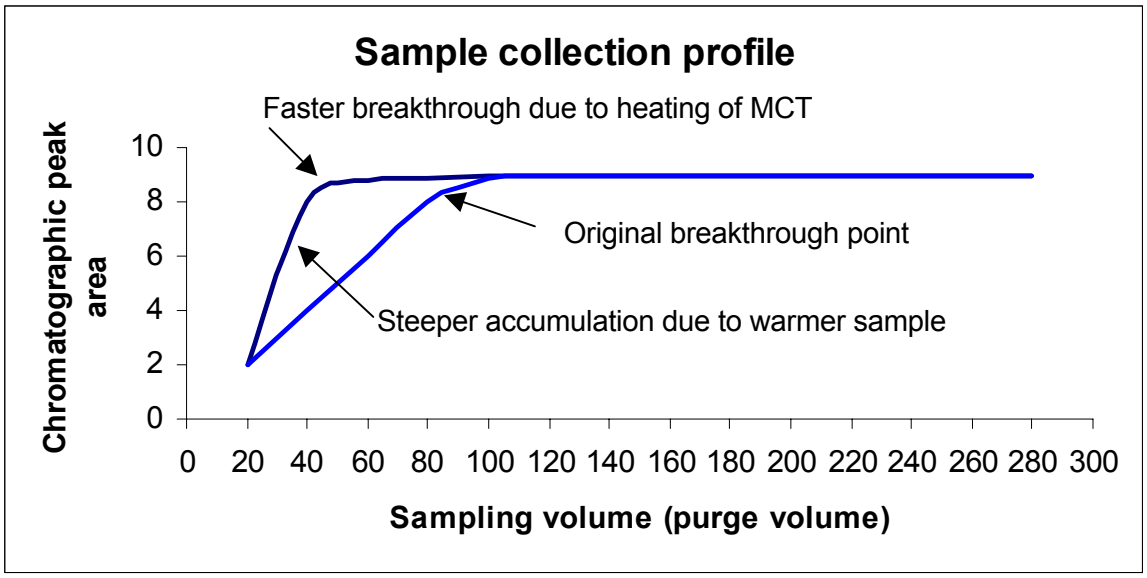


Figure 5.18: Diagram showing difference in sample collection profiles for heated and un-heated MCT and sample

By heating the sampling tube the breakthrough of compounds occur at a lower sampling volume. The higher sample temperature increases the gas phase concentration of the organic compounds with the result that higher total amounts accumulated can be obtained even after onset of breakthrough. The final accumulated (equilibrium) amounts depends on the amount of silicone present in the trap.

Small differences in the amounts of silicone rubber contained in each of the five sampling tubes evaluated, will result in different breakthrough volumes being

observed for identical compounds (Figure 5.18). Small differences in the amount of silicone contained in each trap make it impossible to obtain highly repeatable results under breakthrough sampling conditions. Experiments were consequently undertaken to find the optimum sampling time (below breakthrough volume) for the heated sampling system.

For these experiments two MCT sampling tubes were placed in series. The sampling arrangement was connected, as shown in Figure 5.3, with the front tube heated. The second tube was then connected to the rear of this heated front tube to collect all the compounds that break trough the first tube. The second sampling tube was at room temperature during all experiments due to the absence of a second sampling tube heating device.

Sampling times of 10, 20 and 30 minutes were evaluated.

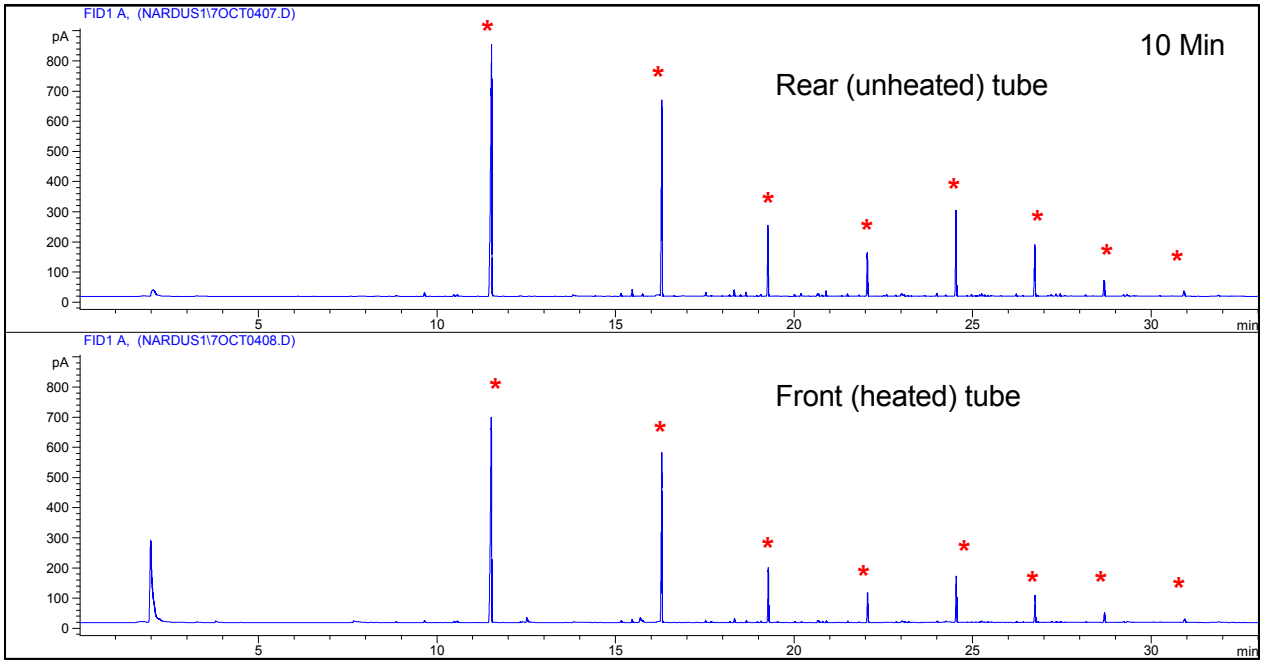


Figure 5.19: Tandem sampling, front and rear tube after 10 minutes of sampling

After 10 minutes of accumulating a beer aroma sample, only silicone peaks (marked with red stars) were observed in the resulting chromatograms.

From the results it is clear that 10 minutes of sampling collected almost nothing (Figure 5.19). Only the silicone peaks from the trap were observed. This was an interesting result that is discussed further in a later section.

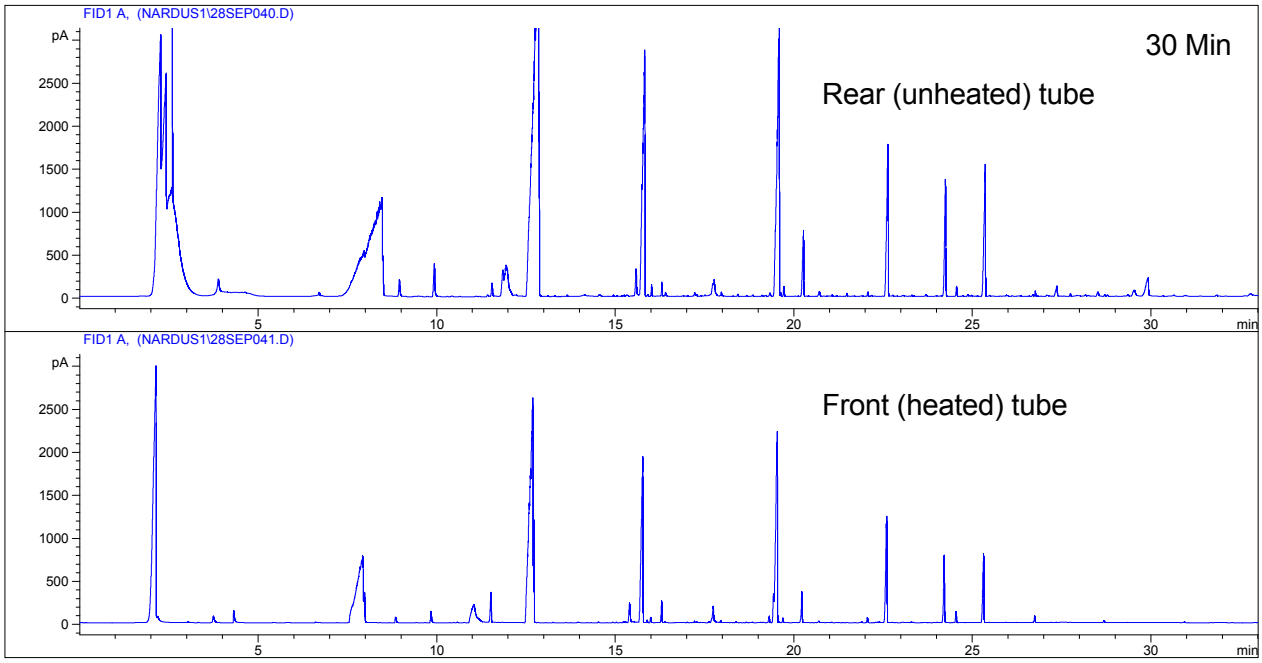


Figure 5.20: Tandem sampling, total breakthrough after 30 minutes of sampling

Notice that the rear tube seems to have accumulated higher amounts of the compounds than the front tube.

30 Minutes of sampling, on the other hand, resulted in total breakthrough (Figure 5.20). (In the absence of a second trap heater, the second trap was at room temperature. The peaks on the second tube were therefore higher and during the analysis the CIS cold trap showed signs of freezing over.) This was a very surprising result especially if one considers the results presented in Figure 4.11 where compounds only showed breakthrough after approximately 8 litres or 5 hours of purging.

The observed phenomenon was, however, not investigated further at the time, as the aim of the experiments was to find the optimum sampling time in order to achieve inter-trap repeatability. For a 20-minute sampling time (Figure 5.21), only the very volatile compounds broke through the first trap.

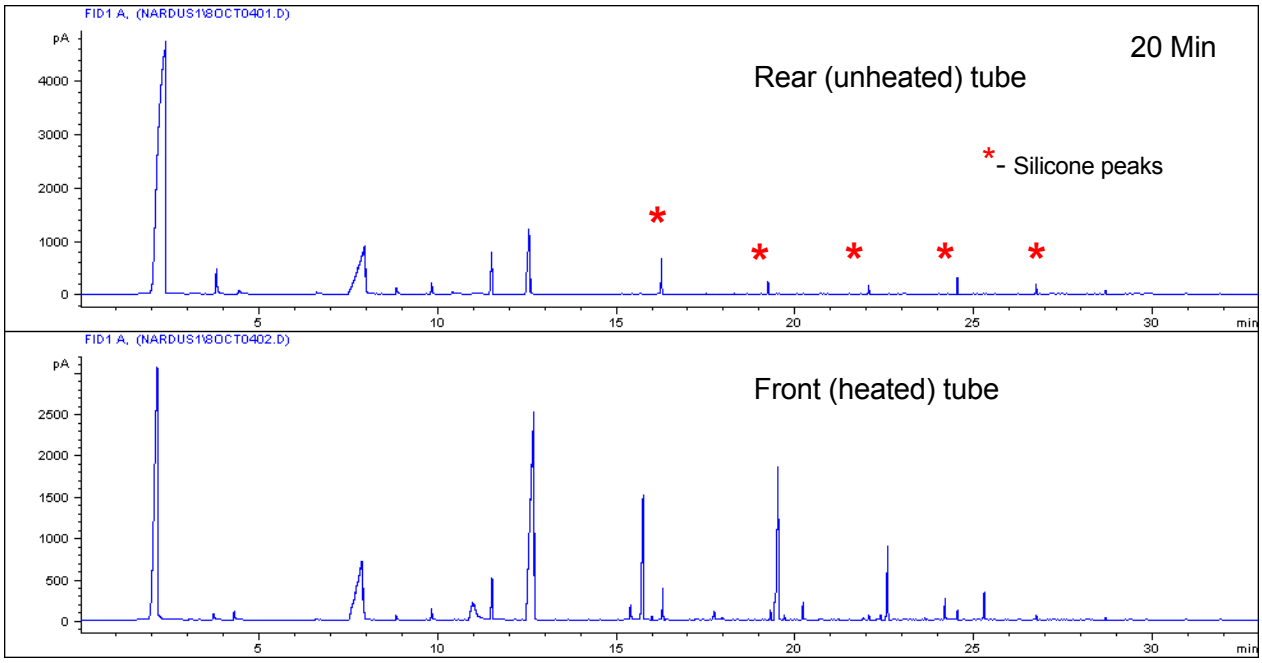


Figure 5.21: Tandem sampling showing breakthrough after 20 minutes of sampling

The less volatile compounds did not break through the first trap after 20 minutes of sampling.

The peaks seen at regular intervals, after 15 minutes on the chromatogram of the rear tube, are silicone peaks. A test was done with one beer to see if a 20-minute sampling showed an improvement in repeatability between two sampling tubes.

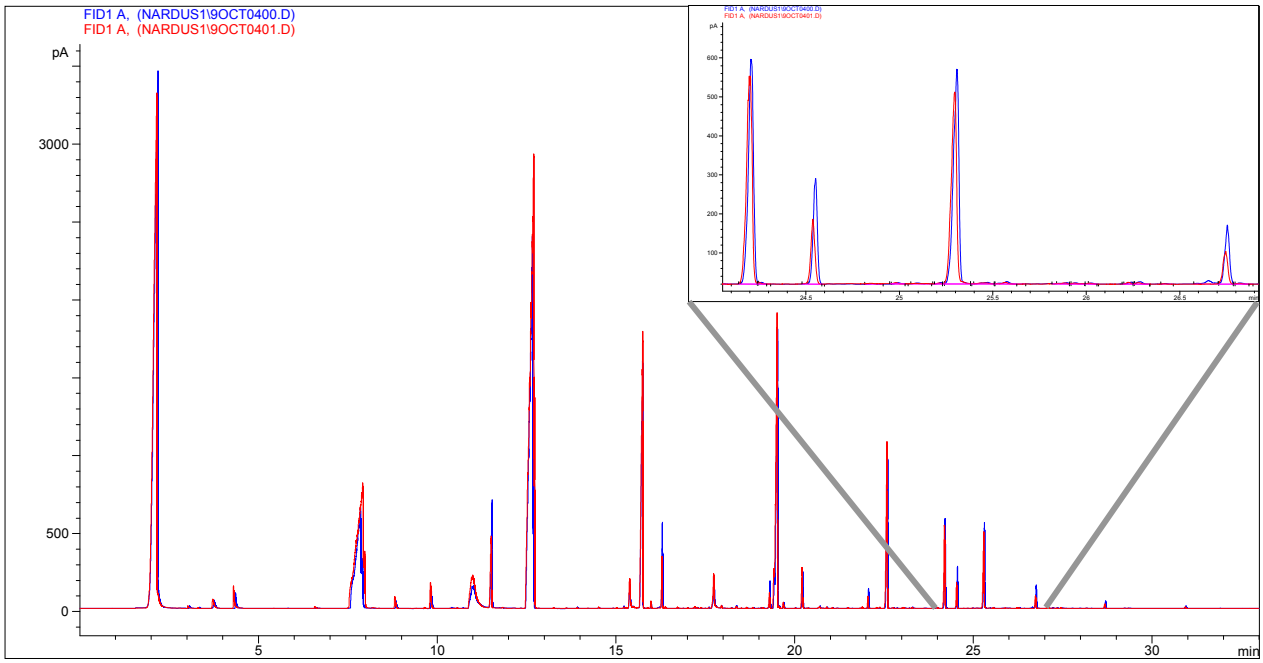


Figure 5.22: Repeatability result after 20 minutes of sampling

Note the variability observed after 20 minutes of sampling.

The result (Figure 5.22) shows that one sampling tube still collects higher quantities of the analytes than the other. The sampling is therefore still being collected beyond the point of breakthrough. In light of this result a sampling time of 15 minutes was selected. The test was repeated on one beer brand with the same two sampling tubes used for the 20-minute sampling time experiment.

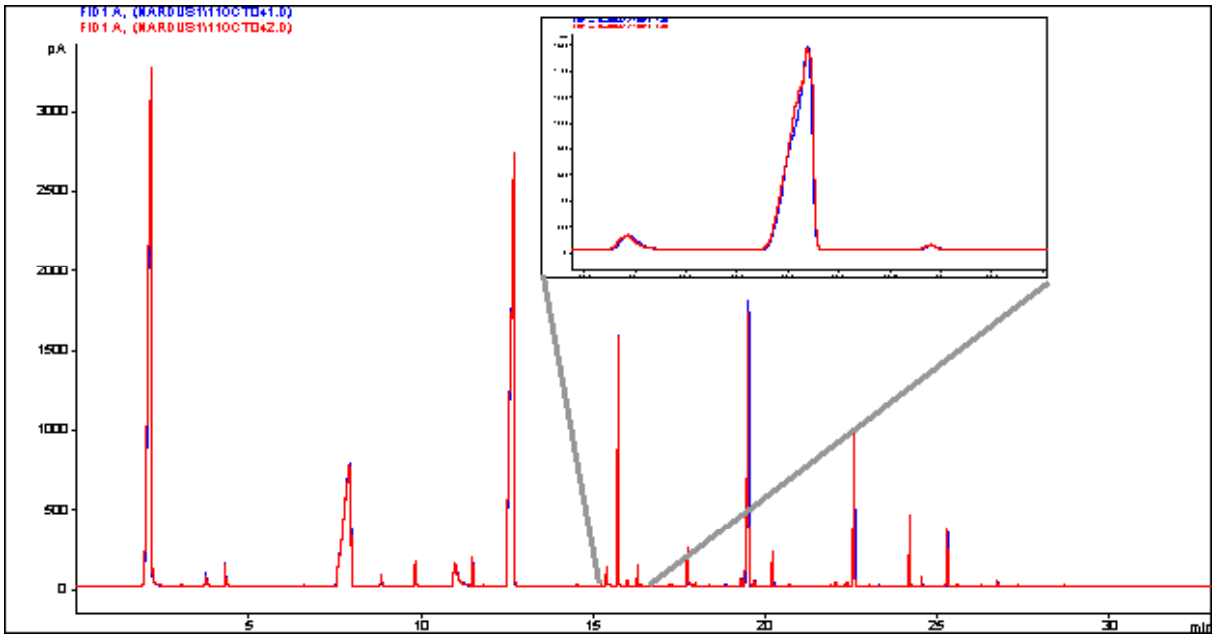


Figure 5.23: Repeatability result after 15 minutes of sampling.

A high degree of repeatability achieved between two different MCT tubes after 15 minutes of sampling.

Figure 5.23 shows that the selected 15 minute sampling time results in a high degree of repeatability between results of two different MCT tubes (see close-up of the peak shown in Figure 5.23).

The interpretation, of the observations made during these experiments (in particular Figure 5.19), is that the air contained in the empty space of the bottle and the sampler has not equilibrated with the beer at the time sampling is started. As described earlier the method currently used for sampling involves dividing one bottle of beer equally into two clean beer bottles and sampling each half separately to compare the results for repeatability. With only half the bottle filled with liquid during sampling, there is a large open volume between the surface of the beer sample and the sampling tube. The bottle used for sampling has a volume of 340 to 350 ml. Half of this is approximately 175 ml. The sampler is made from a 100 ml flask, hence the

open volume between the surface of the beer sample and the sampling tube is estimated at approximately 270 to 280 ml.

The air is displaced by the heavier (CO_2 containing) saturated N_2 purge gas and is pushed out through the sampling tube. Only after the air is displaced does the N_2 with the stripped flavour compounds come into contact with the silicone rubber and the sample is retained. With a purge flow of 25 ml/min. displacing 270 to 280 ml of gas represents a sampling time of between 11 and 12 minutes, and would therefore explain the lack of peaks observed when sampling for a shorter period.

Unfortunately no experiments were performed in order to prove this hypothesis. The explanation presented here for the results shown in Figure 5.19 is therefore speculative and further work would be required to determine the exact cause.

A second less important observation from Figure 5.19 is that the silicone peaks in the “rear tube” chromatogram were slightly higher than that of the “front tube”. A similar observation can be made between chromatograms of blank traps and ones with a beer flavour sample on it.

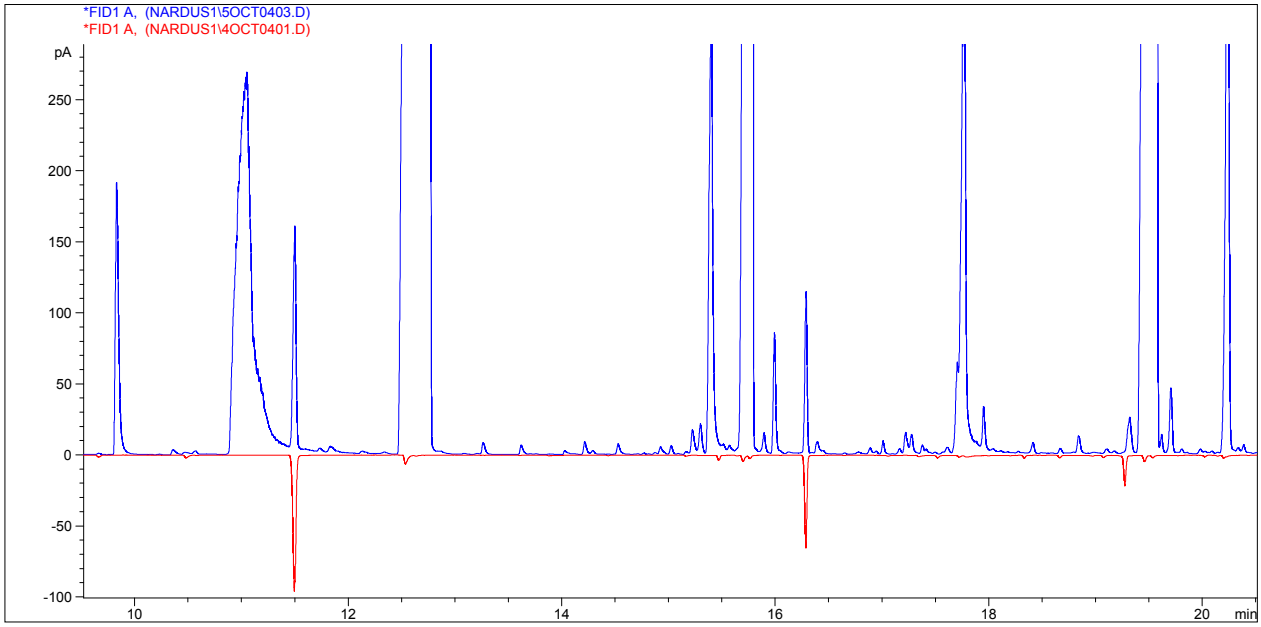


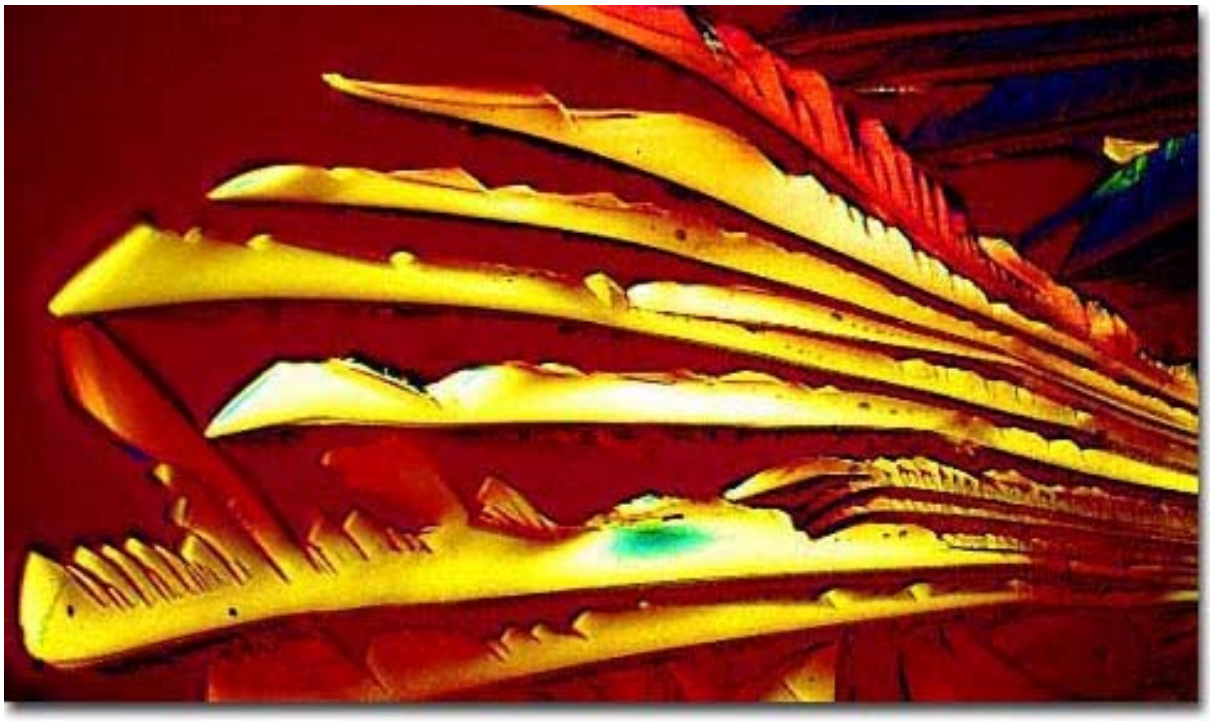
Figure 5.24: Mirror image of a section of beer aroma profile and trap blank showing silicone peak differences

Comparing the height of silicone peaks in a trap blank (bottom chromatogram) shows that the collected sample catalyses the breakdown of the silicone rubber, resulting in higher silicone peaks (top chromatogram).

In Figure 5.24 the silicone peaks from a blank trap desorption (red chromatogram), is compared to the same peaks in a beer aroma profile (the blue chromatogram). Peaks of the silicone compounds desorbed with the beer sample were higher than the ones from the blank trap.

As discussed earlier the breakdown of the silicone rubber is catalysed by water and oxygen. When a beer flavour sample has been collected onto the trap, the observed silicone peaks are higher because of the associated moisture present in the tube. The rear tube (Figure 5.19), which was unheated during the sampling, show higher silicone peaks because more moisture (carried with the CO_2 and purge gas) collected in it than in the heated front tube.

Chapter 6



Miller genuine draft

Text from the bottle label: Miller Genuine Draft - Cold filtered. Brewed and bottled by the Miller Brewing Company, Milwaukee, WI.

Chapter 6

CHARACTERISATION OF BEER AROMA

1. Introduction

The sampling and analysis of beer aroma volatiles with the multichannel silicone rubber trap was successfully optimised and acceptable repeatability of results was achieved. In this chapter the results of the analysis of beer aroma with the optimised method is presented. It will be shown that results can be used for the identification of compounds present in beer aroma and for the visual comparison and recognition of different beer brands. In accordance with an agreement with the South African Breweries which are sponsoring this study, no beer brand is identified or any comments made regarding the quantity and nature of the constituents measured. Only generic compounds present in all lager type beer are identified in order to show the applicability of the evaluated method to the sampling and analysis of beer aroma.

2. Experimental

The final optimised method used for sampling of beer aroma is as follows:

A 340 ml bottle of beer is taken from the fridge where it is stored, opened and divided equally into two washed amber coloured beer bottles. Both the bottles are capped with a washed screw-on crown cap and one is placed into the warm water bath at 40°C for temperature equilibration. After 5 minutes the screw cap is removed from the bottle in the bath and the on-bottle headspace sampling device is placed on the bottle and the multichannel silicone rubber trap (MCT), which is already inside the heating device at 50°C, is connected. The purge gas line is connected to the sampling device and the gas flow is turned on.

The sample is purged at 25 ml/min for a period of 15 minutes during which the headspace is collected on the MCT trap. After the sampling is completed, the purge gas is disconnected and the trap removed from the heating device. The trap is capped and the Gerstel TDS (thermo desorption unit) is prepared for the desorption.

The GC oven is cooled down to 10°C and at the ready signal from the Gerstel the desorption sequence is started first and then the trap is inserted into the TDS. This is done so that the solvent vent split is open before the trap is inserted, thereby ensuring that the excess ethanol and residual water is purged and not transferred into the CIS cold trap.

The MCT is desorbed and analysed according to the temperature and flow parameters as described in Chapter 4 (Section 2.3). The MCT is left inside the TDS during the whole desorption and GC temperature program. This is to minimize the time that the silicone is exposed to the atmosphere, and prevent contamination. After the GC run is finished the system is made ready for another desorption, with the MCT still inside the TDS. It is then desorbed again as a trap blank, this is to ensure that the MCT is uncontaminated and ready for the next sampling.

After desorption and analysis the MCT is removed from the TDS and capped. It is now ready to be used for sampling.

3. Results and discussion

3.1 Beer aroma profile

Figure 6.1 shows the chromatographic profile of a standard lager beer aroma, as sampled with the MCT.

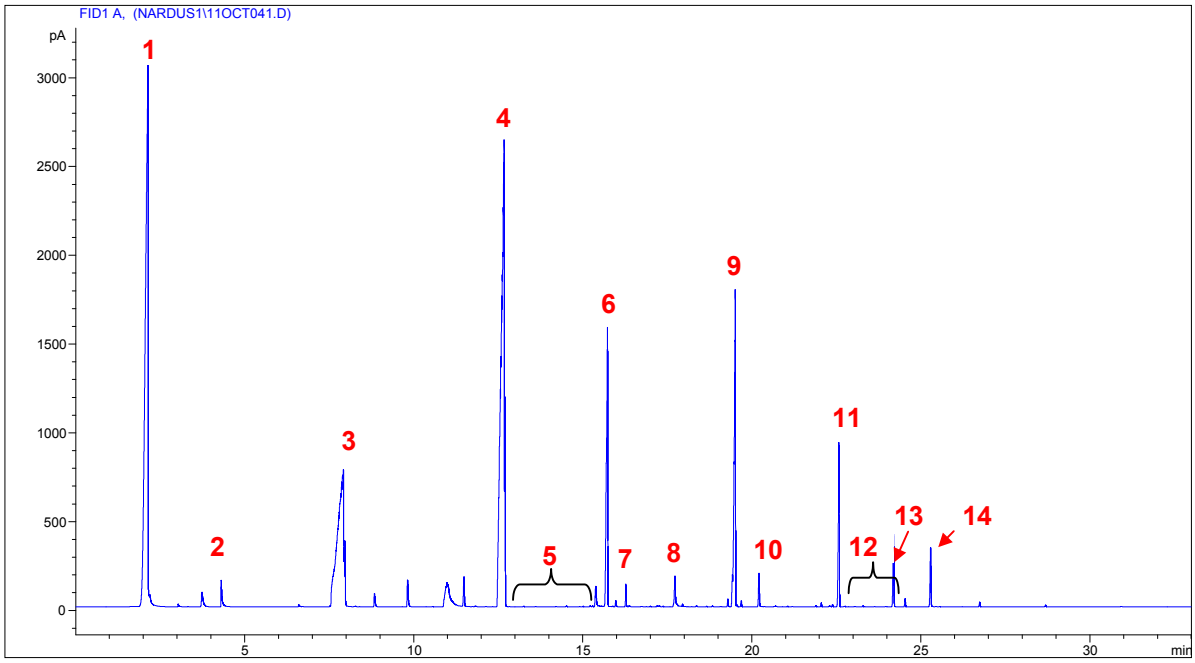


Figure 6.1: Components under discussion in beer aroma profile

From this result it is clear that the compounds have a wide variety of concentrations in the headspace, as mentioned in previous Chapters. A few of the major compounds and their chemical classes are listed in Table 6.1.

Table 6.1: Classes of components in beer aroma profile

Number	Chemical class
1,2,7,13	Alcohol
4,6,8,9,11,14	Ester
10	Aldehyde
3	Carboxilic acid
5	Mono-terpene section
12	Sesqui-terpene section

3.2 Beer brand recognition

As stated in Chapter 1, the aim was to generate results that could be visually compared for off-flavour identification and brand recognition. Of these two applications, brand recognition would be the simplest to attempt first because a number of different brands of beer was easy to obtain. Off-flavours can be either due to extra compounds present in the flavour and breakdown products from degradation of some aroma constituents, or from differences in the relative concentration of some of the components. Brand recognition was therefore selected as a test for the method. Figure 6.2 shows the flavour profiles of two different brands. For these

particular samples (designated A & B) the differences can easily be spotted visually. This is, however, not the case for all of the brands evaluated, some only have very subtle differences in the concentration of a few compounds, resulting in a different aroma.

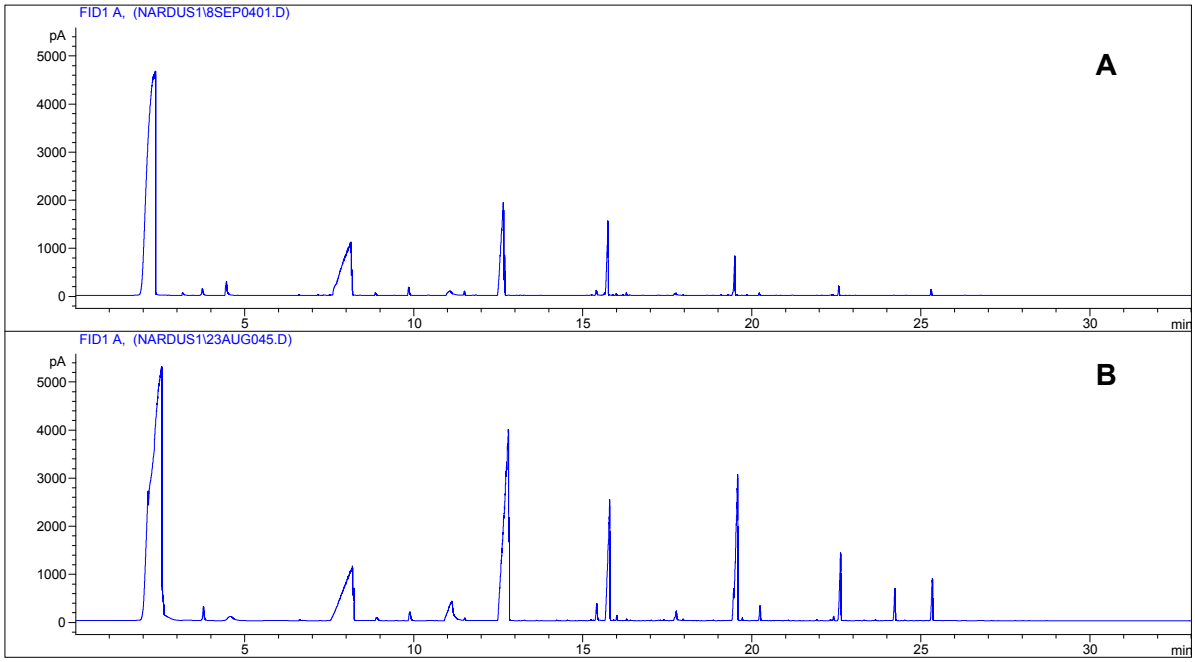


Figure 6.2: Flavour profiles of beer brands A and B

A clear difference can be observed between the profiles of the two beer brands. Note that the ester compounds are present in higher concentrations in the headspace of brand B compared to brand A.

Figure 6.3 shows a section from the aroma profile of two beer brands, closely related in terms of flavour. These two chromatograms, showing the section containing the sesqui-terpenes, seem to be mirror images of one another. All of the peaks (i.e. compounds) present in the one are also present in the other, however, for some components there are slight differences in concentration. Whether these observed differences indeed have any significance to the difference in flavour between the two brands, is outside the scope of the project. These results are merely to demonstrate that small differences such as these can be detected due to the high degree of repeatability achievable with the method.

The very small variation between different brands prompted an idea to use the peak areas of selected compounds in order to characterise the aroma of a particular brand of beer. By plotting the peak areas relative to one another, a scatter plot is generated. The area of selected peaks is plotted, each on one of the two axes.

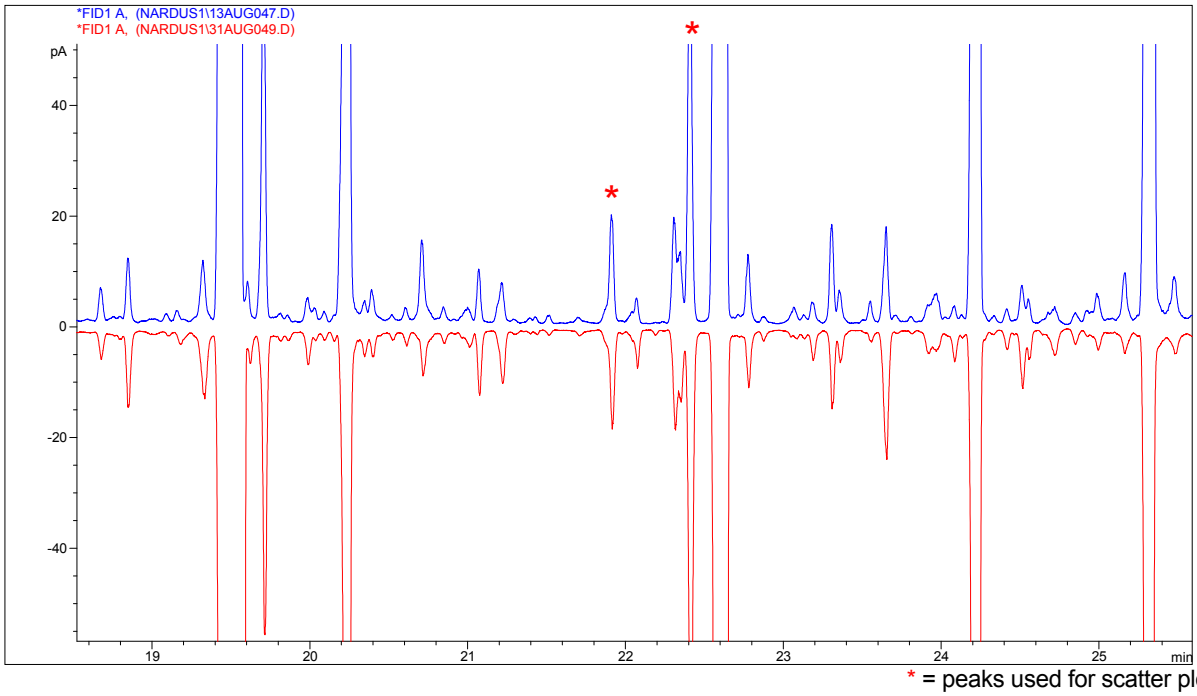


Figure 6.3: Peaks in the sesqui-terpene section of two closely related beer brands

The peaks indicated are confirmed sesqui-terpene compounds, the peak areas of which were used to distinguish different beer brands

The resulting spots group together for a particular brand of beer. If a variation occurs in the area of either of the peaks the ratio changes and the spot moves to a different location.

When this was done for the aroma profiles of three different beer brands (including the two in Figure 6.3), an interesting trend was observed. Multiple analyses of each beer brand were used for the evaluation. The sum of the areas of the first three esters (shown in Figure 6.1) was plotted against the combined area of the last three ester peaks for each of the evaluated chromatograms. From the result of this, shown in Figure 6.4, a clear distinction between the three brands can be seen. For samples with variations in this ratio the spots fall outside the grouping. Such outliers are indicated for brand 1 and 2 in Figure 6.4.

An evaluation of two peaks selected from the sesqui-terpene area (shown in Figure 6.3) revealed a similar trend. Again peak areas from multiple analyses of the three brands generated spots that group together, thereby distinguishing the three brands.

The brand recognition would not have been possible without the required confidence achieved in the determination of peak areas. This is the reward of the lengthy study to optimise sampling reproducibility.

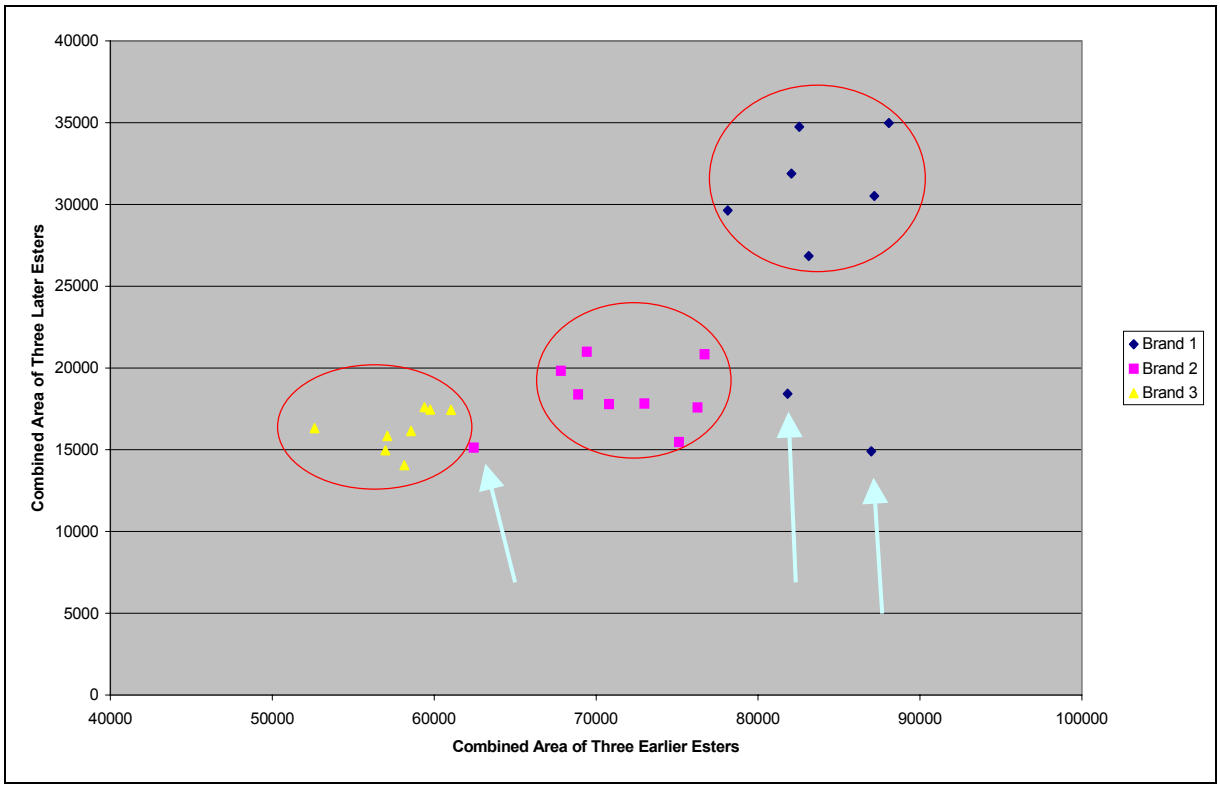


Figure 6.4: **Scatter plot showing combined area of the first three esters vs. that of last three esters**

The scatter plots show that three closely related beer brands has clear differences when areas of peaks (eluting at identical retention times) from multiple chromatographic analyses are plotted. Note the groupings for different brands as well as the outliers.

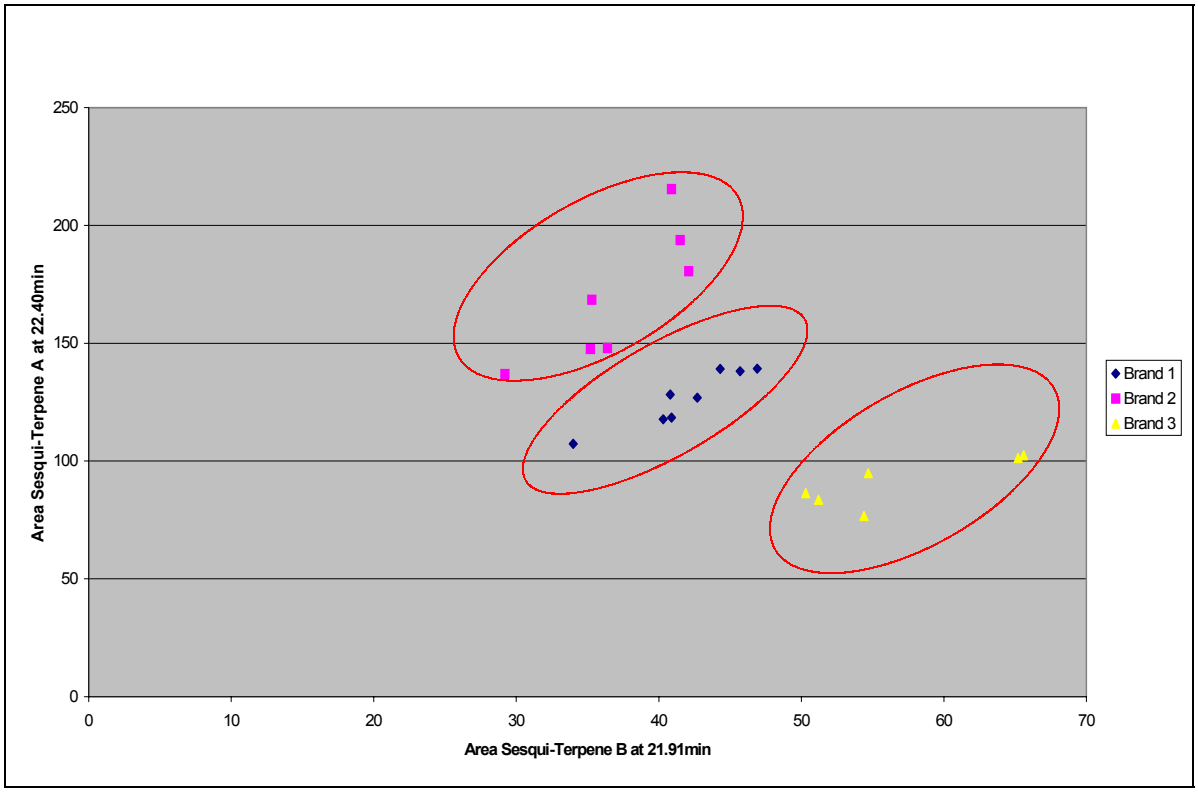


Figure 6.5: **Scatter plot of two sesqui-terpene peak areas**

3.3 Sample stability

Another requirement for the sampling technique is for the concentrated aroma sample to be stable on the sampling device (MCT) for an extended period of time. This would allow aroma samples, in stead of bulky beer samples, to be sent around the world for the comparison of beer aroma. Tests were thus performed by dividing one bottle of beer, sampling one half and analysing it immediately. After analysis of the sample the MCT is conditioned and the second half of the beer is sampled onto it. This sample is then capped and stored for different periods of time under different conditions. After analysis of this sample the two profiles are compared to see whether the concentrated aroma sample remained stable during the time of storage.

The first set of experiments performed was to determine the influence of temperature on a stored sample. Two beer samples were prepared as described above. Both were capped and wrapped in aluminium foil to prevent any exposure to light. One of the samples was stored in a refrigerator and the other on the bench in the laboratory. Both samples were analysed after four days and the resulting chromatograms compared to the result of its respective first half. The results are shown in Figures 6.6 and 6.7 overlain with the first half result.

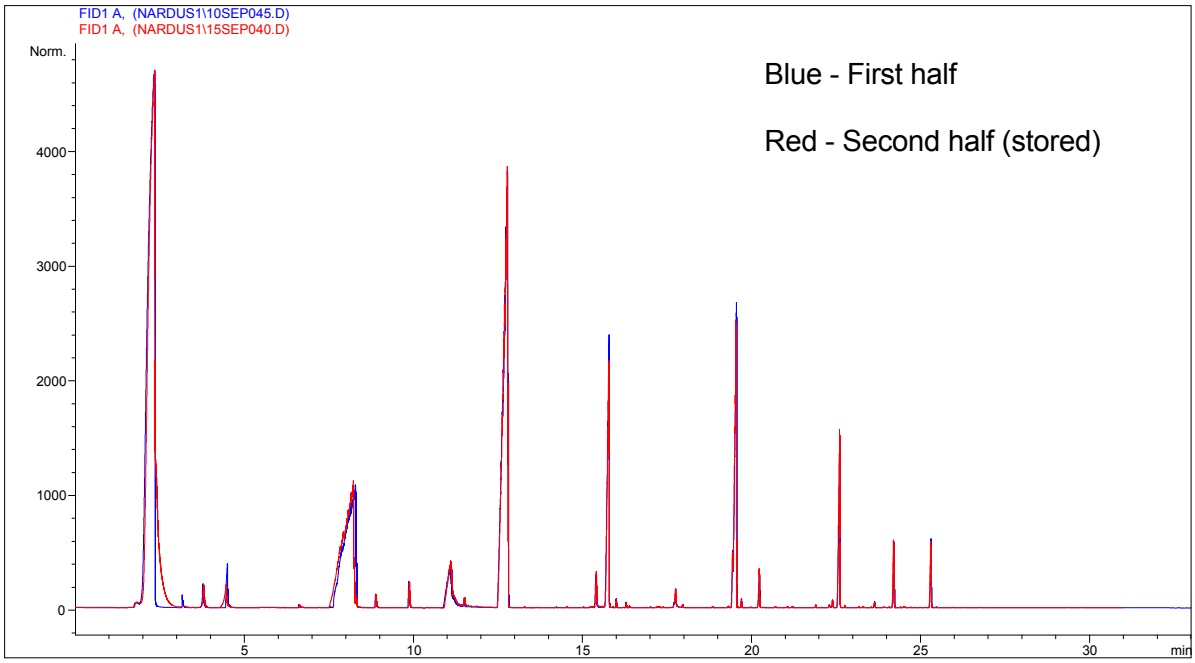


Figure 6.6: Overlay of chromatograms showing stability of peaks after 4 days stored in fridge

The chromatographic profile shows remarkable stability after 4 days of storage, also compare Figures 6.7, 6.8 and 6.9 below.

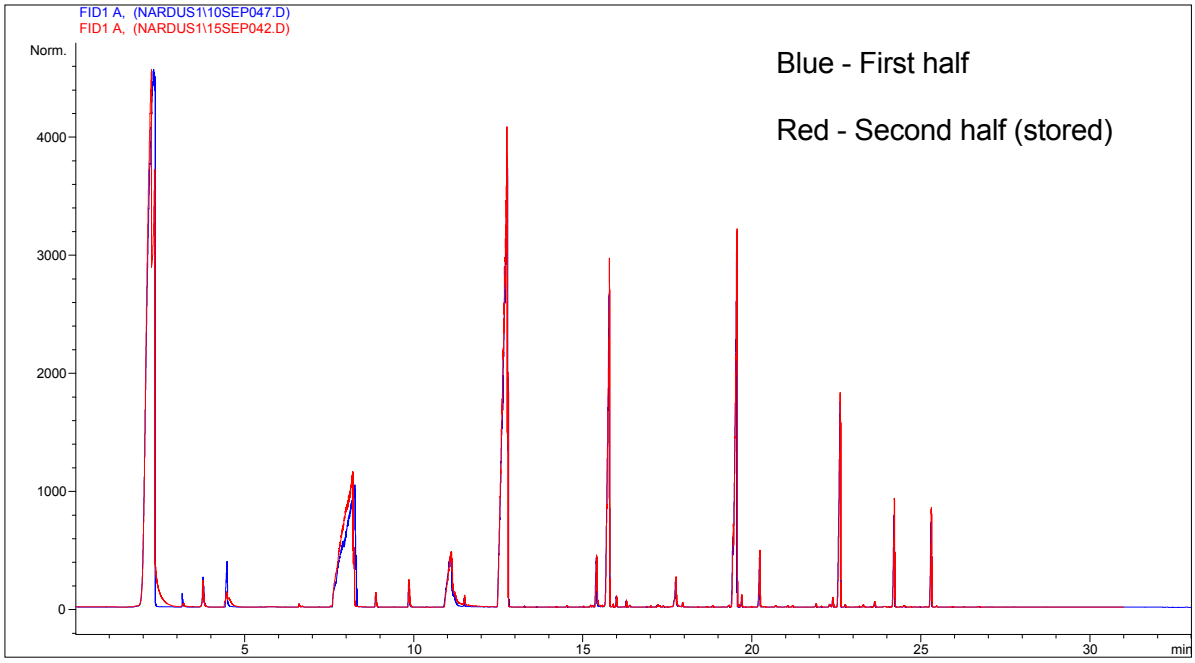


Figure 6.7: **Overlay of chromatograms showing stability of peaks after 4 days stored at room temperature**

The sections of the chromatogram containing the mono- and sesqui-terpene compounds are shown in Figures 6.8 and 6.9 for the sample stored at room temperature in the laboratory.

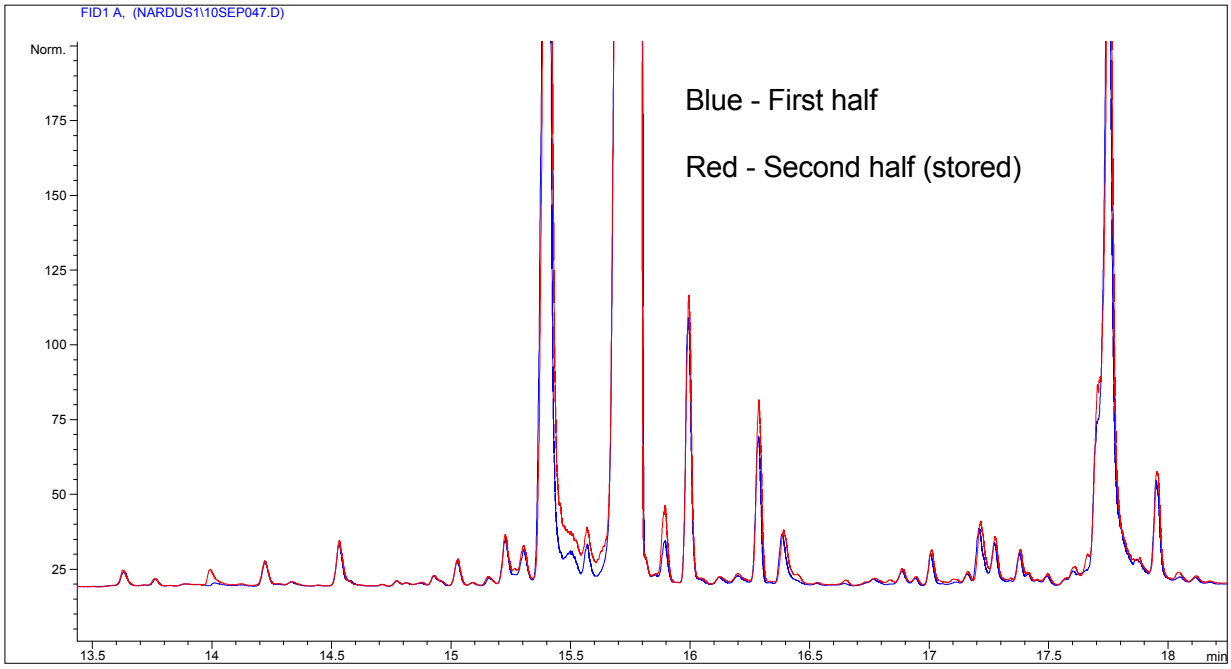


Figure 6.8: **Overlay of chromatograms displaying stability of small peaks (including the mono-terpenes) after 4 days stored at room temperature**

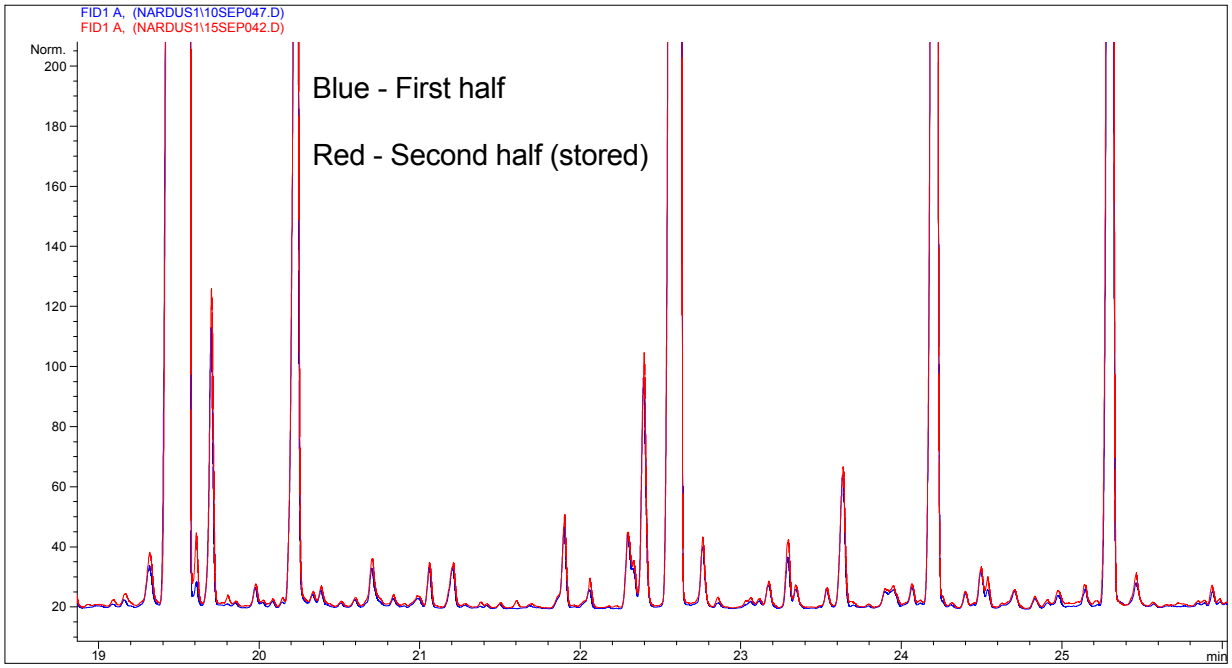


Figure 6.9: **Overlay of chromatograms displaying stability of small peaks (including the sesqui-terpenes) after 4 days stored at room temperature**

Storage for the period of 4 days did not result in any significant visual change to the beer aroma profile. Temperature also seems to have no detrimental effect on sample stability, as results for both conditions of storage showed similar stability over the four-day evaluation period. Even for the small peaks comparison of the profiles show remarkable stability of the sample during storage (see Figures 6.8 and 6.9).

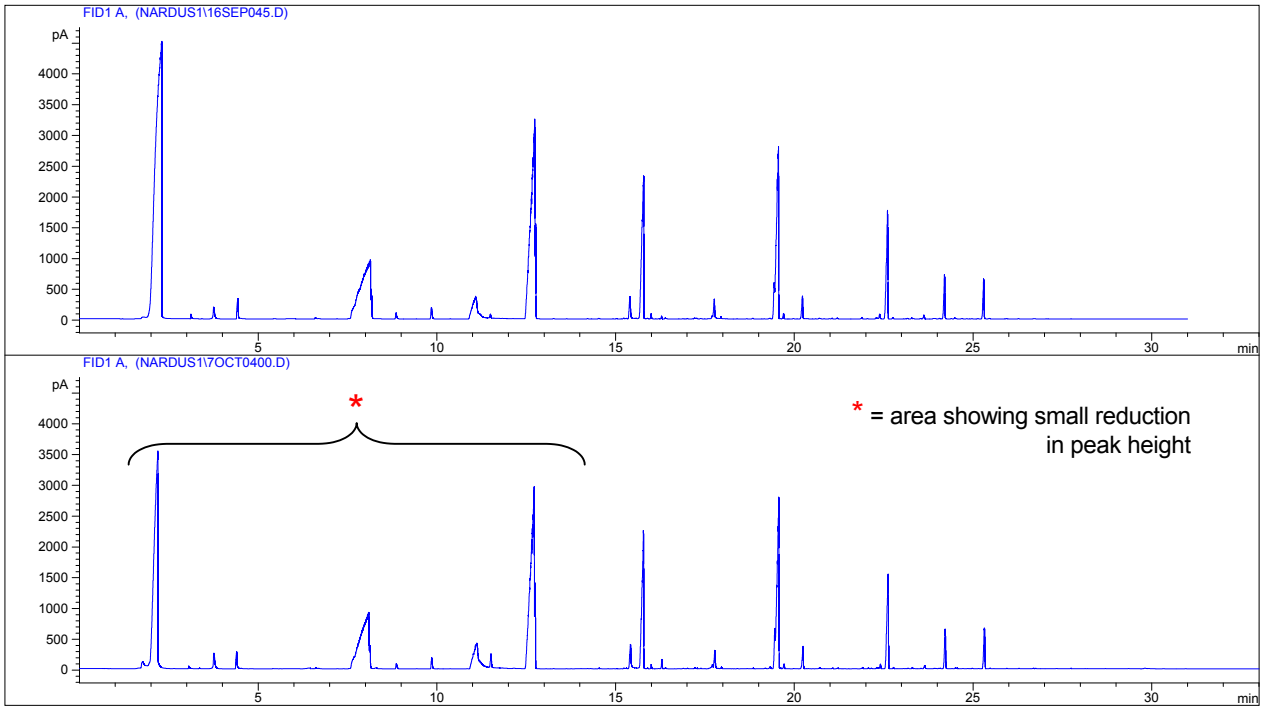


Figure 6.10: **Comparison of chromatograms displaying stability of peaks after 21 days stored at room temperature**

Stability over an extended period of time was evaluated next. A sample was prepared as before, wrapped in aluminium foil and stored in the laboratory (at room temperature) for a period of 21 days before it was analysed.

The results (Figure 6.10) show reasonably good stability for the large peaks eluting later in the chromatogram, however, a reduction in the peak area of the more volatile components, up to the first large ester peak, was observed.

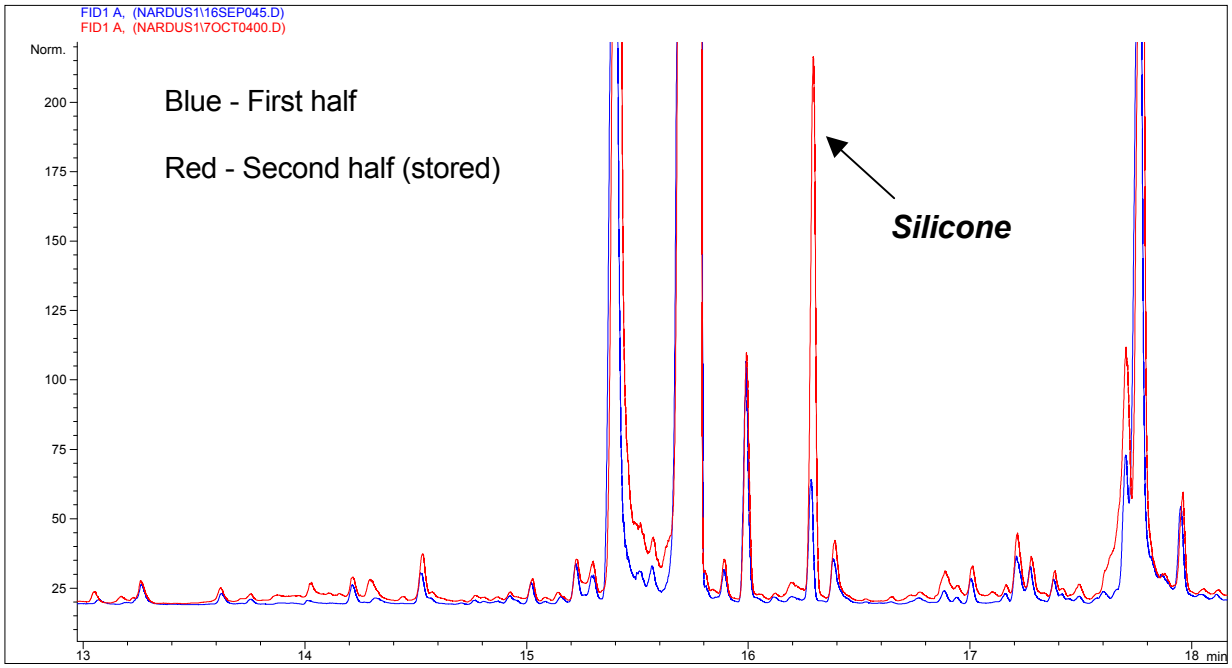


Figure 6.11: Overlay of chromatograms displaying stability of small peaks (including the mono-terpenes) after 21 days stored at room temperature

Note the large increase observed for the silicone peak indicated.

A closer look at the results revealed more noticeable variations in some of the smaller peaks of the aroma profile (Figures 6.11 and 6.12). Particularly noticeable are the variations in the silicone peaks, which is most likely due to a catalysed breakdown of silicone rubber similar to that discussed at the end of Section 3.6 (Chapter 5).

The observed reduction in peak height of the more volatile compounds may be because the method used for capping the traps does not seal perfectly or due to permeation through the Teflon tube used for sealing. The more volatile compounds slowly diffuse out of the silicone over time, and escape from the trap.

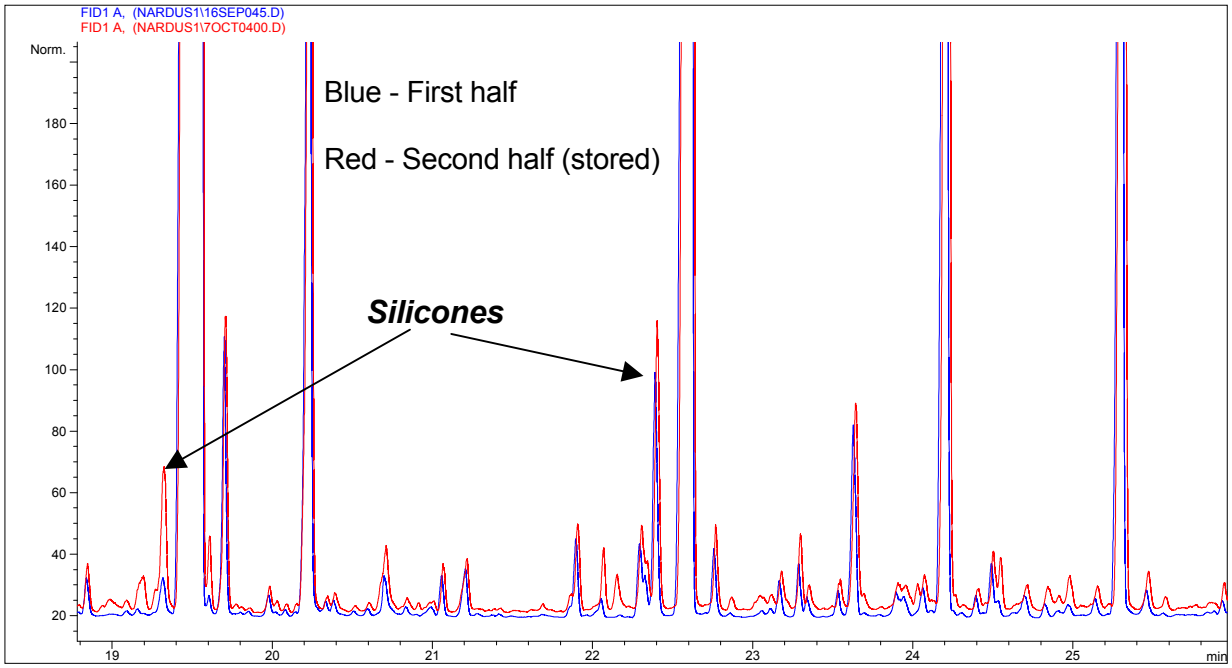


Figure 6.12: Overlay of chromatograms displaying stability of small peaks (including the sesqui-terpenes) after 21 days stored at room temperature

Variations are not observed for all of the peaks indicating that some of the compounds may be stable even for extended periods of storage. Sample stability was, however, not extensively investigated during this study. Duplicate results are therefore not available to confirm these observations. The results, nevertheless, indicate very good sample stability during moderate periods of storage. Factors influencing sample stability will have to be investigated further.

An important point to note, however, is the fact that the small variations (as a result of sample storage) can be observed at all. This is due to the high degree of reproducibility achieved with the sampling method.

A final experiment in the investigation of beer aroma sample stability on the MCT, was to explore the effect of exposure to sunlight. The sample was prepared as before and exposed to direct sunlight for 2 hours. The result of the analysis is shown in Figure 6.13

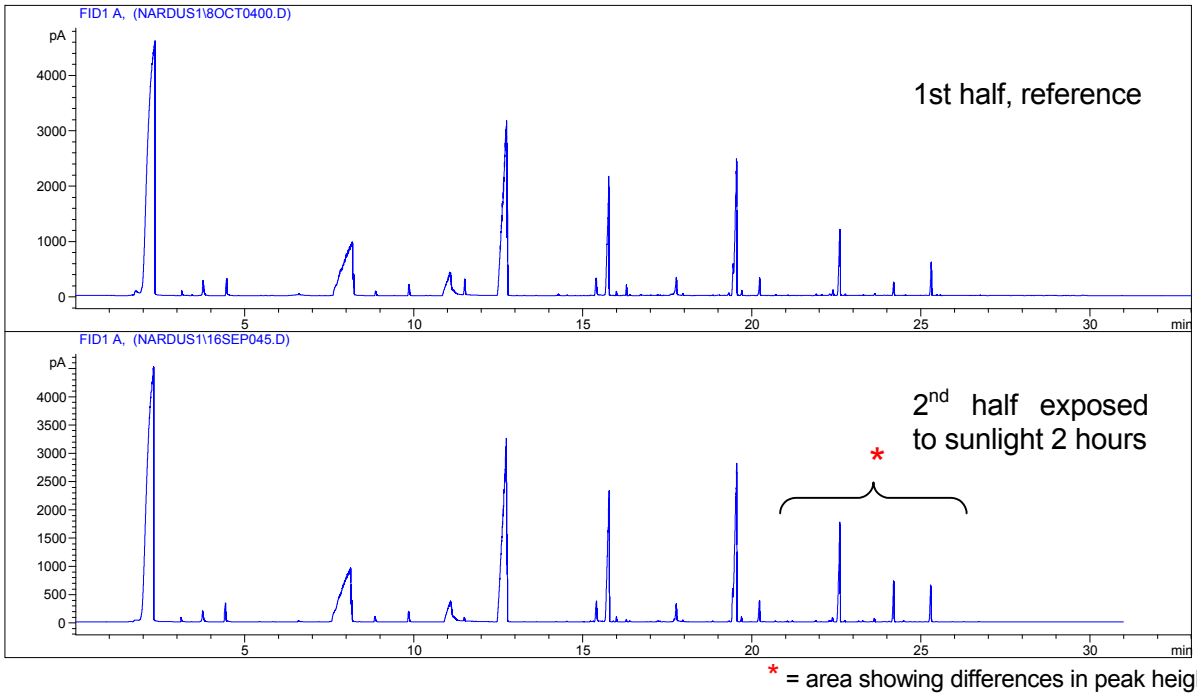


Figure 6.13: Sample stability after exposure to sunlight

The sample showed good stability after exposure to sunlight. The differences observed here were not in the first few peaks as with the previous results, but in the last peaks. No experiments have yet been done to assess the effect of different periods of sun exposure. The cause for the observed increase in peak height for the last few peaks is therefore still unknown. It is, however, advisable not to expose sample laden silicone rubber to sunlight.

3.4 Sensitivity

An experiment was performed in order to establish the detection levels of the sampling method. A standard containing α-pinene in CS_2 was prepared. It was added into a sample of beer at a concentration of 1part per billion (μg/L). The beer sample was sampled and analysed as usual and the result is shown in Figure 6.14. The circled area is the location of the pinene peak.

From figure 6.15 (showing a close-up of this area) it is clear that a high degree of sensitivity is achievable with the sampling method.

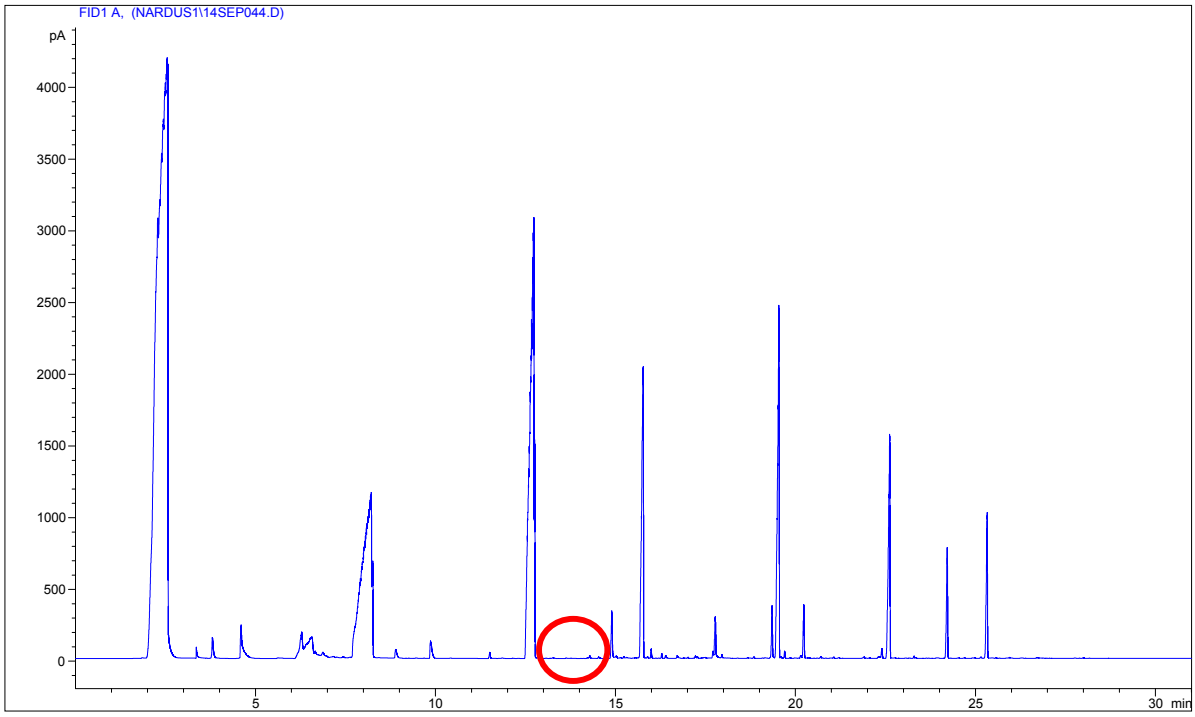


Figure 6.14: Chromatogram of beer sample spiked with 1 ppb α-pinene

Note area where α-pinene elutes. See Figure 6.11 for more detail.

Smaller peaks surrounding the α-pinene indicate that compounds present at concentrations lower than 1 ppb in the beer are still detectable.

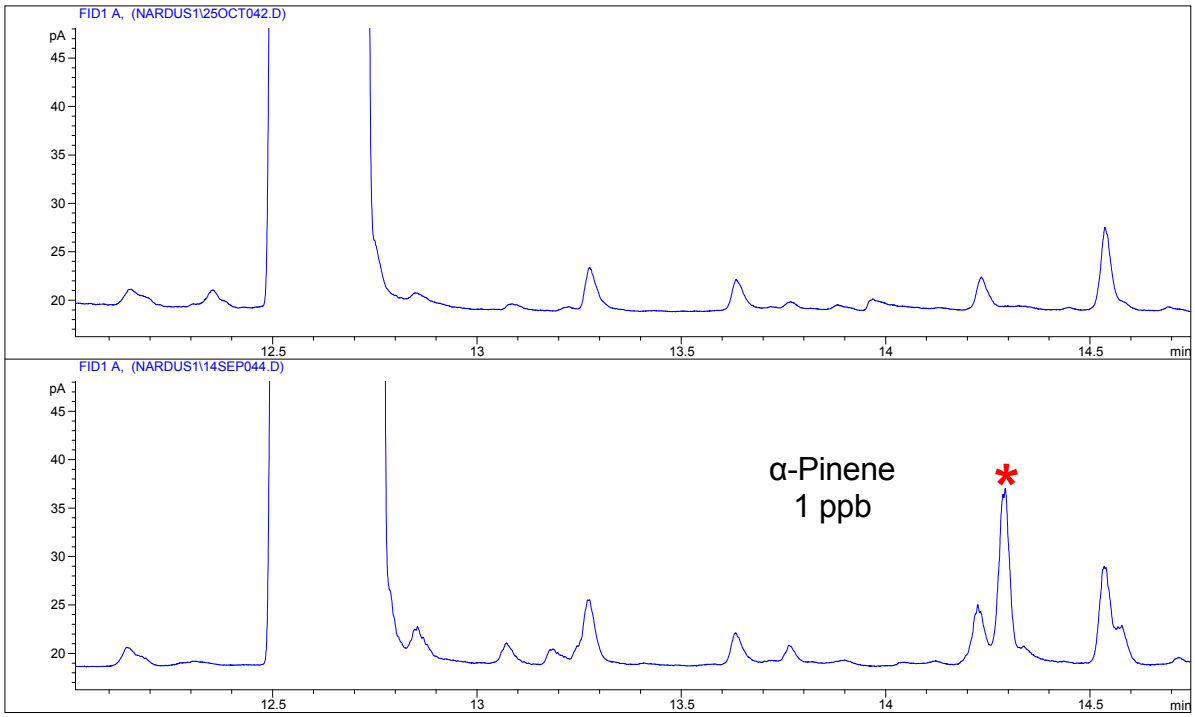


Figure 6.15: Close-up of α-pinene peak

This is an indication that, even with flame ionisation detection, the beer aroma analysis method gives quantitative results for esters and terpenes below the threshold concentration levels of organoleptic flavour perception [55].

Chapter 7

Budweiser

Text from the bottle label: Budweiser, King of Beers. This is the famous Budweiser beer. We know of no brand produced by any other brewer which costs so much to brew and age. Our exclusive Beechwood Aging produces a taste, a smoothness, and a drinkability you will find in no other beer at any price. Brewed by our original all natural process using the choicest Hops, Rice and Best Barley Malt.

Chapter 7

APPLICATION OF THE AROMA SAMPLING TECHNIQUE TO GCxGC AND GC-TOFMS

1. Introduction

One of the challenges presented by the analysis of samples such as beer aroma is the complexity of the mixture of compounds that it contains. Identifying individual compounds can be quite difficult because they often have very similar physical and chemical properties, which complicates their separation by standard chromatographic techniques. It is therefore important to determine whether the optimised sampling and analysis method for beer aroma, utilising the multichannel silicone rubber trap (MCT), is translatable to other more powerful techniques of analysis than GC-FID.

Even though this aspect falls outside the original scope of this study, the potential use of the sampling method with other techniques of analysis was investigated. The optimised sampling method was tested with GCxGC and mass spectrometry techniques. In order to translate the technique, developed with the robust flame ionisation detector (GC-FID), to the different carrier gasses and system pressure conditions required for the operation of the other instruments, a technique similar to retention time locking was used. This technique and its application in the identification of the peaks observed in the complex GCxGC chromatograms will be discussed.

This chapter is intended to show how the sampling technique can be applied to other methods of analysis, and is by no means a complete assessment of the translatability of the sampling method. It presents the results of tests conducted to assess the potential use of the sampling method, with GCxGC and accurate mass time-of-flight mass spectrometry (GC-TOF), for the identification of different chemical classes and compounds in beer aroma.

2. Analysis by multidimensional chromatography

Beer aroma samples, which were sampled onto multichannel silicone rubber traps, were desorbed and analysed by means of GCxGC. As expected, results of the

GCxGC analysis (Figure 7.1) showed many more peaks than can be obtained on one-dimensional GC.

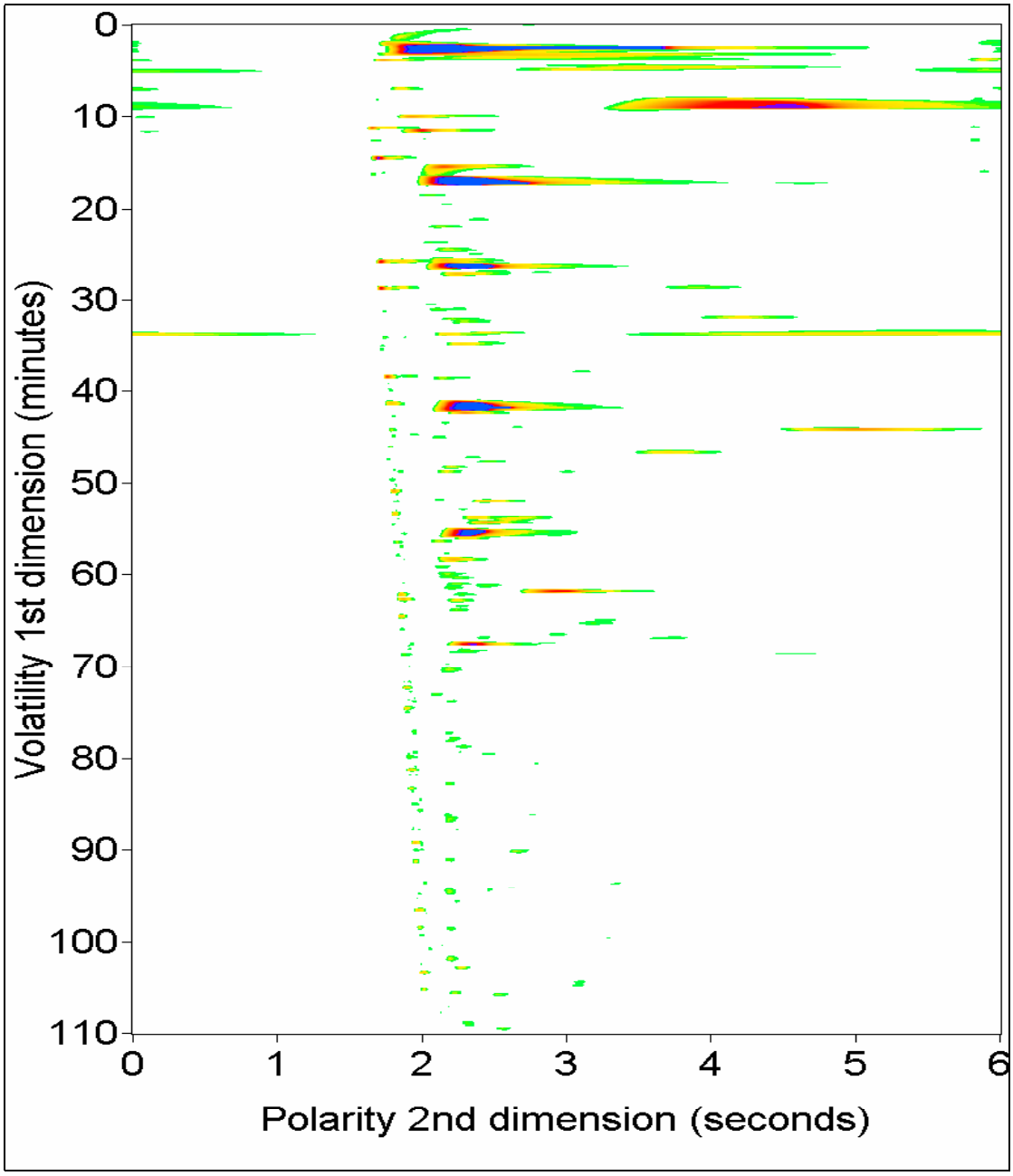


Figure 7.1: Two dimensional representation of GCxGC analysis of beer aroma

The series of scattered 'blobs' are the peaks observed in a traditional GC chromatogram now separated on a second dimension, in this case utilising compound polarity. Compounds with similar chemical properties (i.e. a homologous series) exhibit similar behaviour on the two-dimensional plane. Groups of chemical compounds with similar polarities are therefore separated in bands across the second dimension. In Figure 7.2, it is clear how the first dimension volatility

separation (along the vertical axis) is matched with the peaks in a standard one-dimensional chromatogram. Along the second dimension (horizontal axis), the compounds identified as esters elute in a line, while the compound identified as an alcohol (Alcohol 2) elutes slightly later, indicating that it has a different polarity to the esters and therefore is not of the same functionality.

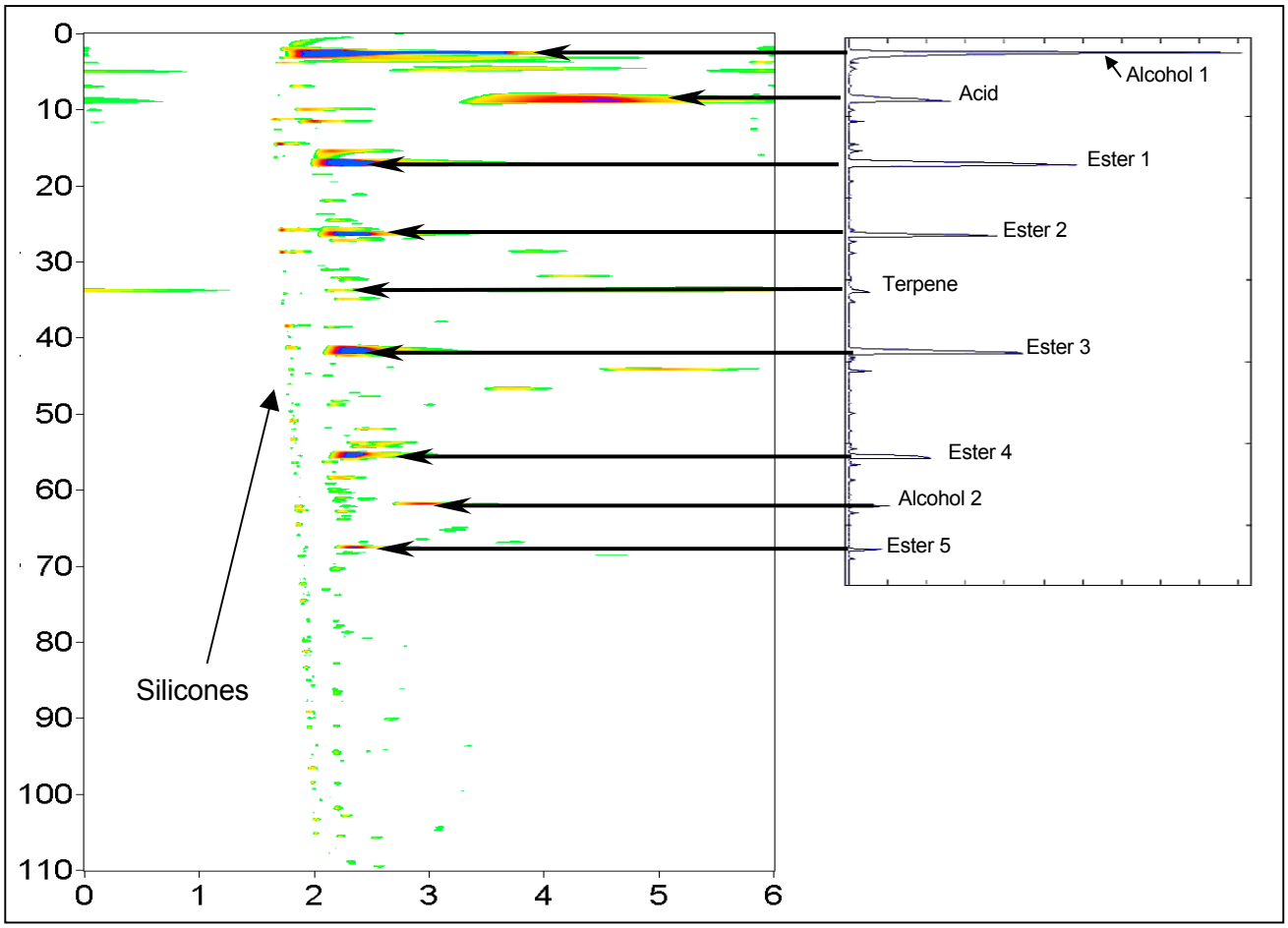


Figure 7.2: Corresponding peaks between GCxGC and traditional chromatograms.

Similarly, the compound identified as a carboxylic acid is more polar than most other and is retained longer on the second dimension. This demonstrates how two compounds with similar volatility, which would appear as one peak on a one-dimensional chromatogram based on volatility separation, can be separated by means of GCxGC.

Interestingly, the row of smaller peaks, indicated in Figure 7.2, elute slightly earlier than the rest of the compounds. These are the silicone compounds that are released from the MCT during desorption. These silicone compounds are not as well retained by the polar stationary phase in the second chromatographic column (at the time and

temperature corresponding to their elution from the dimethylsiloxane first dimension column) and therefore elute earlier than the more polar beer aroma compounds.

As demonstrated in Chapter 4, the silicone background peaks are stable, appearing in the same position and ratio in all the beer aroma chromatograms. These background peaks, for which retention indices are known, therefore have the potential to be used for the identification of compounds.

The technique of multi-dimensional gas chromatography shows promise in the field of quality control. The more detailed GCxGC results can easily be compared visually so any additional compounds, which may be the cause of off-flavours, can be spotted, even if these are present at very low concentrations. The second dimension separation also allows compounds that may interfere with the identification of off-flavour compounds to be clearly separated from the analytes under investigation.

The results presented were produced using flame ionisation as method of detection. The compounds shown can unfortunately neither be quantified nor the peaks identified without the use of standards for each compound. A solution to this problem is to use fast scan time-of flight mass spectrometry (TOFMS) as detector to a GCxGC instrument. The separation power of the GCxGC coupled to the mass spectral identification of the TOFMS is ideal for separating and identifying compounds in complex samples. Such an instrument was, however, not available to this project.

The separating power of a GCxGC instrument alone can, however, still be utilised in a similar manner for complex mixture analysis, by making use of a technique called retention time locking. With this technique the retention times of the multi-dimensionally separated compounds can be correlated with those of compounds identified on a GC combined with high-resolution mass detection (Micromass®, GCT™). The Micromass® GCT™ generates high quality, full mass spectra with superior resolution. Superior resolution reduces the chance of mass interference. Also, the precise linear relationship between ion arrival time and the square root of its mass permits good mass measurement accuracy using only a single internal reference mass. This accurate mass measurement can provide elemental composition of unknowns as well as confirming the identification of compounds [56].

With the technique of retention time locking the GCT™ can be used to identify peaks in the GCxGC(FID) chromatogram, thereby presenting an alternative solution to complex analysis.

3. Retention time locking

Retention time locking was developed for use with one-dimensional gas chromatographic methods. With this technique the chromatographic retention times of one instrument is very closely matched to that of another instrument using the same nominal column [57]. It allows one to generate similar peak elution patterns from instruments utilising columns of different dimensions, different carrier gasses and different outlet pressures, as required by different detectors [58,59,60]. For this technique to work, both instruments have to apply constant pressure throughout the chromatographic temperature program and use columns with the same stationary phase and phase ratio [60].

To lock two instruments, the method used to obtain a set of retention times on a GC-FID, for instance, is translated for use on a GC-MS (with vacuum at the column outlet) so that the retention times match as closely as possible. Mass spectra at the matching retention times can then be used to determine the identity of peaks observed in the GC chromatogram [59].

Method translation is a calculation technique developed at Agilent Technologies [58,59,61,62,63] for the translation of a capillary column GC method to different chromatographic conditions. The changes in inlet pressure, oven ramp rates and hold times, required to maintain the peak elution order of a specific reference method, is calculated. These calculated values will result in a close, but not exact, match to the desired scaled retention times. To match the times precisely the new method must be retention time locked. Retention time locking (RTL^{TM}), a technique also developed at Agilent Technologies [58,59,61,62,63], is the procedure of determining the exact inlet pressure (from a calibration curve of inlet pressure versus retention time) needed to achieve the required retention times.

Retention time locking, as performed with the aid of the RTL™ and method translation software supplied by Agilent Technologies, was shown to be very successful for

numerous applications utilising one-dimensional GC using selective detectors locked with GC-MS [61,58,63].

The advantage with these systems is that the outlet pressure of the system, required for the calculations of the method translation software, is known. In GC-MS the outlet pressure of the column is vacuum, while for most other GC detectors the outlet pressure is at or near atmospheric pressure [59].

This is, however, not the case with an instrument such as a GCxGC, where the outlet pressure of the first column is elevated above atmospheric pressure due to the smaller inner diameter of the second column [57].

Other authors have shown that two comprehensively coupled two-dimensional gas chromatographs could be retention time matched successfully with the aid of precise pressure control at the junction of the first and second columns [64]. A similar technique showed how to translate retention times in two dimensions for a GCxGC-FID and GCxGC-TOFMS by adjusting the second column outlet pressure of the latter. This provides a closer pressure match between the atmospheric pressure outlet of the FID and vacuum of the TOFMS, which allows easier correlation of the total flow [65].

The aim of this investigation was to find a simpler method to retention time match a one-dimensional instrument with the first dimension of a comprehensive two-dimensional instrument, without the use of additional pressure control.

For this, standard methods of retention time locking and method translation were to be used. By estimating the elevated outlet pressure of the GCxGC first dimension, and using it for the calculations of the method translation, unrealistic values for the new method was suggested by the method translation software.

From this it was clear that a different strategy for locking these instruments had to be employed. The following section describes the experimental procedures used to achieve matched retention times between the two instruments.

4. Experimental

4.1 Instrumentation

All analysis were performed on Agilent 6890A GC systems using:

- Electronic pneumatic control (EPC) operated in constant pressure mode
- Gerstel CIS 4 Thermal desorption systems for sample introduction

The GC temperature program used was 10°C to 220°C at 2°C/min (110 min analysis time).

4.1.1 GCxGC-FID

For the GCxGC, the temperature program used was as described above (10°C to 220°C at 2°C/min). The second column was constantly maintained at 30°C higher than the first and ramped, at the same rate of 2°C/min, until reaching 250°C. Both ovens were held for 5 min at maximum temperature.

The modulation system used in the GCxGC was the KT-2001 cryogenic two stage jet modulation system, supplied by Zoex Corporation. The GCxGC system used as first dimension column a 30 m x 0.25 mm id x 0.25 µm HP-1 column (part number. 19091Z-433) and the second dimension used was a 1 m cut piece of 0.1 mm id x 0.1 µm Carbowax 20M (part number: 007-CW-25NB-0.1F) supplied by the Quadrex Corporation. The GCxGC system used a FID heated to 300°C as detector. The carrier gas for the GCxGC was hydrogen at a head pressure of 110 kPa.

4.1.2 GC-TOFMS

Retention time locked; accurate mass GC-TOFMS was performed using a Micromass® GCT™ coupled to an Agilent 6890A GC. The column used was a J&W Scientific DB1MS 30 m x 0.25 mm id x 0.25 µm (part number: 122-0132). The carrier gas was helium 5.0 with a head pressure of 65 kPa (velocity 43 cm/s). The transfer line temperature was 250°C. The mass range scanned was 10-600 amu, at 0.9 scans/s.

4.1.3 Sample and sample introduction

A seven-component test mixture was used for all the matching experiments. This mixture contained C9 to C15 *n*-alkanes prepared in dichloromethane. The injected volume in all experiments was 1 µl, injected onto an empty glass tube and desorbed onto the column for analysis.

The desorption conditions used were as described in Chapter 4 (Section 2.3).

4.2 Optimisation of GCxGC retention times

The strategy for locking the two systems was to first get repeatable first dimension retention times for the *n*-alkane test mixture on the GCxGC system. Once this could be achieved, matching of these retention times on the GC-TOFMS system was attempted.

The alkane standard mixture was desorbed from an empty glass tube and run on the GCxGC system, as described in the experimental section. The results were contrary to what was expected for such a hydrocarbon mixture run at a linear temperature ramp. Instead of the expected constant time spacing between the compounds, the retention time differences between the earlier eluting compounds were higher than that measured for the later eluting compounds (Table 7.1).

Table 7.1: Differences in retention time between components of the *n*-alkane test mixture

***n*-Alkane**	**Retention times (min)**	**Difference**
C9	20.92	
C10	27.82	6.9 (C9 – C10)
C11	35.24	7.4 (C10 – C11)
C12	42.57	7.3 (C11 – C12)
C13	49.53	7.0 (C12 – C13)
C14	56.26	6.7 (C13 – C14)
C15	62.53	6.2 (C14 – C15)

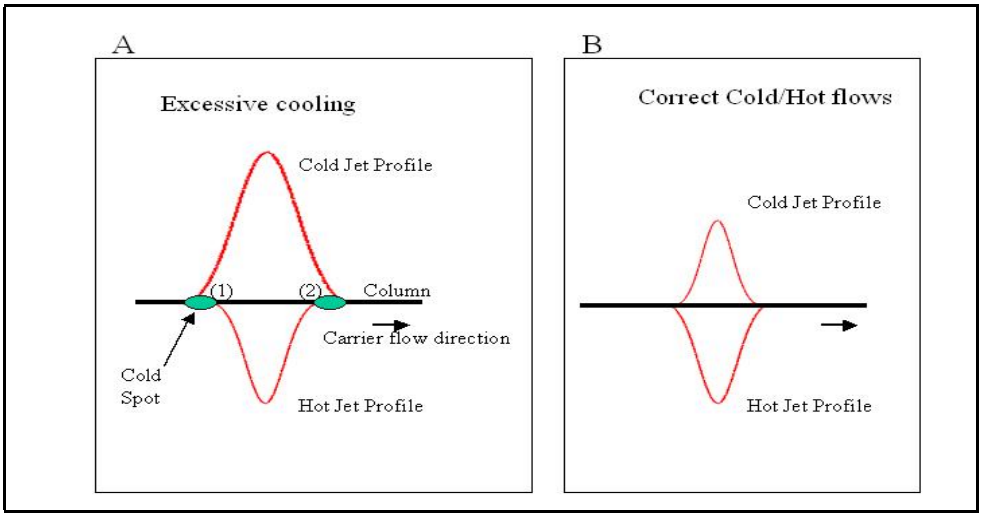
It was thought that the modulation was introducing this variation into the retention times. However, at that stage the exact contribution of the modulation to the observed retention time was unknown. To determine this contribution, the alkane mixture was run on the 2-column GCxGC system without modulation. Compared to the modulated retention times, this experiment produced a surprising result.

From Table 7.2 one notices that the retention times of the modulated peaks show larger differences for the C9 and C10 *n*-alkanes compared with the C12 to C15 *n*-alkanes.

Table 7.2: Comparison of GCxGC 'modulated' and 'un-modulated' retention times

***n*-Alkane**	**Retention times (min)**		**Difference**
	Modulated	Un-Modulated	
C9	20.92	19.37	1.55
C10	27.82	27.33	0.49
C11	35.24	35.26	-0.02
C12	42.57	42.79	-0.22
C13	49.53	49.92	-0.39
C14	56.26	56.59	-0.33
C15	62.53	62.93	-0.40

The reason for the longer retention times, observed early in the modulated run, could be attributed to the formation of a cold spot due to a too large cooling-gas flow in the modulator, as demonstrated by Zellelow [66] (Figure 7.3).



[From 66]

Figure 7.3: Formation of the cold spot due to large cold gas flow in modulator

A: Excessive cooling-gas flow volume results in a larger area of the column being cooled than the following hot gas flow can warm. The result is cold spots forming where the gas flow profiles do not overlap. B: Reducing the cooling gas flow solves the problem.

As the oven temperature rises during the run, the temperature of the interface also increases to such an extent that cold spot is not so readily formed. The larger differences in retention time are therefore observed for the more volatile compounds, which elute during cooler oven temperatures. This explanation (illustrated graphically in Figure 7.3) was proved by adjusting the cooling-gas flow volumes. The problem

was easily corrected by a small reduction in the cooling-gas flow from 15 l/min to 11 l/min for the duration of the run. The cold spot (1) has the effect of slowing down peak migration, before modulation and injection, into the second column. The cold spot (2) is not regarded as important to the observed effect, as the warm carrier gas should effectively heat this cold spot during the hot jet heating cycle of the modulator.

The presence of such a cold spot (1) is not noticeable by the shape of the eluting peak because it doesn't trap the eluting compound but only slows down the elution slightly. It can only be observed through a retention time comparison such as the one performed here.

A second comparison of retention times observed for correctly modulated *n*-alkane peaks with those from an un-modulated run, revealed that un-modulated peaks consistently show larger retention times than observed for the modulated run (Table 7.3).

Table 7.3: Comparison of un-modulated retention times and correctly modulated retention times (reduced modulator cooling gas flow)

***n*-Alkane**	**Retention times (min)**		**Difference**
	Modulated	Un-Modulated	
C9	19.16	19.37	-0.21
C10	27.02	27.33	-0.31
C11	34.83	35.26	-0.43
C12	42.41	42.79	-0.38
C13	49.45	49.92	-0.47
C14	56.22	56.59	-0.37
C15	62.55	62.93	-0.38

This was a surprising result but was found to be due to another cold spot caused by the modulator block through which the second column is threaded. During the un-modulated runs the modulator heating power was switched off. As a result the modulator block does not heat up as fast as the rest of the oven and forms a cold spot at the tensioning ferrule, retaining the eluting compounds for a slightly longer period. The effect of this is clearly observed by comparing the chromatograms of un-modulated and correctly modulated runs (Figure 7.4).

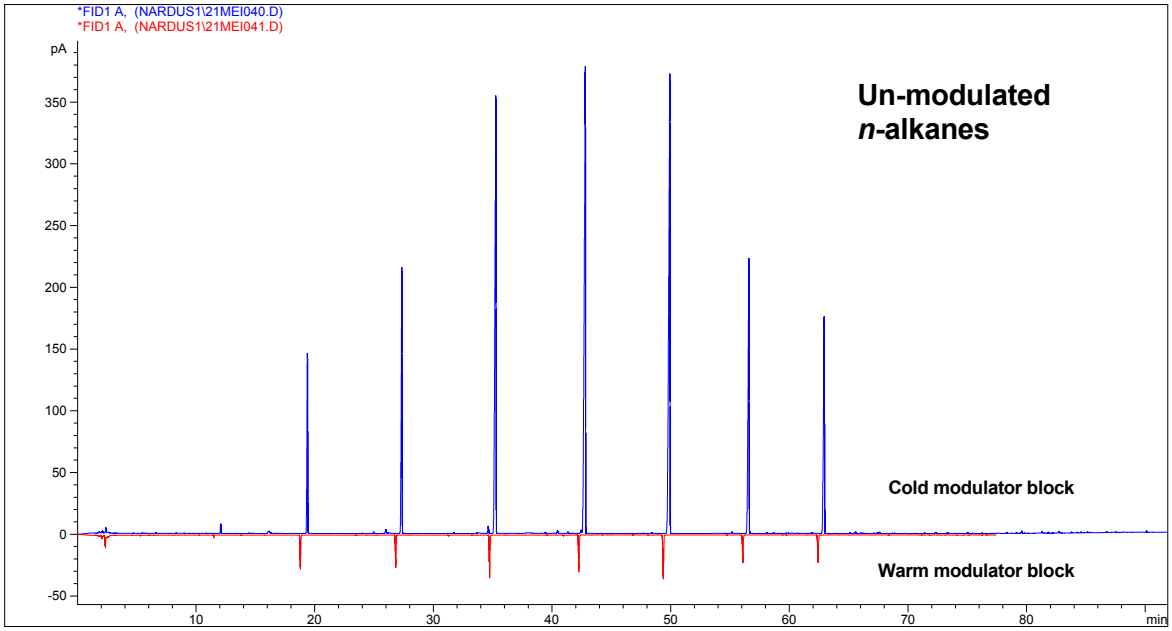


Figure 7.4: **Difference in the retention of *n*-alkane peaks resulting from an un-heated modulator block**

Notice the clear retention time difference between the alkane peaks from a run with an unheated modulator block compared to a heated block.

Heating the modulator block during un-modulated runs, produces retention times with a small, reasonably constant difference from correctly modulated retention times, as shown in Table 7.4.

Table 7.4: **Comparison of un-modulated GCxGC retention times with correctly modulated retention times (modulator block heated during un-modulated run)**

n-Alkane	Retention times (min)		Difference
	Modulated	Un modulated	
C9	19.03	19.16	-0.13
C10	26.93	27.02	-0.09
C11	34.77	34.83	-0.06
C12	42.28	42.41	-0.13
C13	49.36	49.45	-0.09
C14	56.08	56.22	-0.14
C15	62.41	62.55	-0.14

The retention times achieved with the heated modulator block were found to be about 0.1 minute or 6 seconds faster than those achieved under correctly modulated conditions. With the accurate retention time contribution of the modulation known, and the modulation now working correctly for repeatable retention times, matching of the GCxGC and GC-TOFMS instruments could be attempted.

When reproducing GCxGC (first dimension) retention times in a GC-MS, the primary complication is the large differences in these retention times observed between the

two systems. This problem is the result of the higher pressure at the interface between the first and second column of the GCxGC and the large differences in outlet pressures between the two systems [65].

In an attempt to calculate the inlet pressure for use on the GC-TOFMS, the first column outlet pressure of the GCxGC system was estimated and used. The method translation calculation unfortunately a suggested negative inlet pressure value, and was obviously not applicable to this situation.

In order to deduce an approximate inlet pressure a pressure calibration curve was used. For this the *n*-alkane mixture was run at five different inlet pressures on the GC-TOFMS and a curve of pressure vs. retention time was drawn. From this curve the inlet pressures required for the desired retention times were extracted for all of the *n*-alkane peaks. The average value of these calculated pressures was used. This average value (65 kPa) matched the retention times very well. No further optimisation efforts were undertaken and this pressure was used as locking pressure for the rest of the experiments.

Table 7.5 shows the GCxGC retention times for the alkanes as recorded with the optimised modulator. These retention times closely match GC-TOFMS retention times, for the same set of compounds, with differences of less than 0.1 minute.

Table 7.5: Comparison of GCxGC adjusted retention times with GC-TOFMS retention times

Retention times (min)			
***n*-Alkane**	**GCxGC Modulated**	**GC-TOFMS**	**Difference: Modulated vs. GC-MS**
C9	19.16	19.08	0.08
C10	27.02	26.97	0.05
C11	34.83	34.82	0.01
C12	42.41	42.32	0.09
C13	49.45	49.44	0.01
C14	56.22	56.16	0.06
C15	62.55	62.49	0.06

The slow temperature program used (2°C/min), results in chromatographic peaks that are anywhere between 18 and 24 seconds wide at their base. A retention time difference of less than 6 seconds means that retention times for the two instruments are excellently matched and can be used for correlation of results.

4.3 Analysis of a beer aroma and identification of peaks by matching retention times

In order to show that the optimised sampling technique can be used for the identification of components in a beer aroma sample the retention time matching technique, as described above, was used. For the experiment, the headspace of a beer sample was collected onto a multichannel silicone rubber trap. Onto this trap 1 µl of the 7-component *n*-alkane mixture was placed, in order to confirm that retention times match those of the optimised experiments.

The trap was desorbed and analysed by GCxGC-FID. The contour plot of this GCxGC-FID chromatogram is given in Figure 7.6. The seven *n*-alkane peaks indicated are clearly visible and the first dimension retention times of these peaks were extracted and were found to match those given in Table 7.5 exactly. In addition, the *n*-alkane peaks can be used to calculate a retention index of peaks observed in the GCxGC contour plot, for identification of the chemical compound. The chemical classes shown in Figure 7.2 were identified my means of their relative positions in the second dimension (and to the *n*-alkane peaks) of the GCxGC chromatogram.

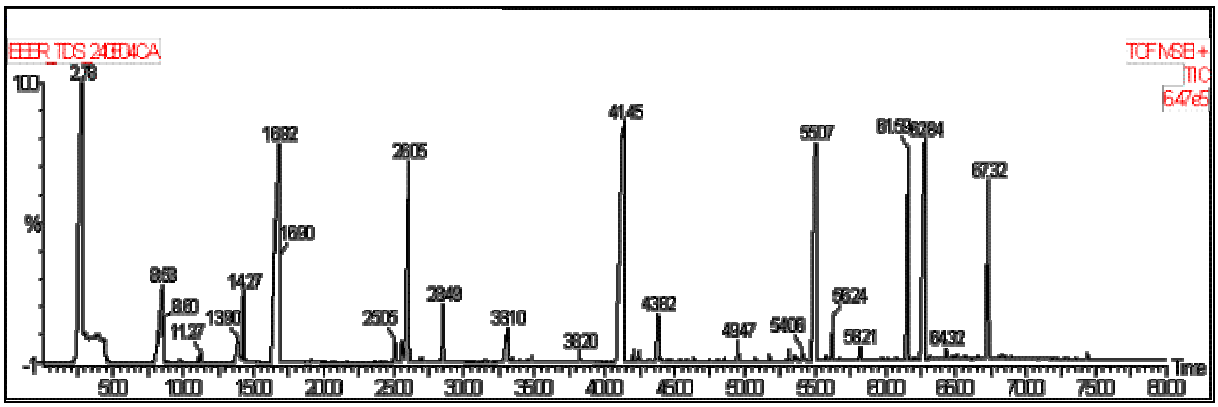


Figure 7.5: The total ion chromatogram of beer aroma

The total ion chromatogram of beer aroma from the retention time locked GC-TOFMS

Interesting chemical classes, such as the Terpenes indicated in Figure 7.6, as well as low concentration and co-eluting components were selected from the GCxGC contour plot for identification on the retention time matched GC-TOFMS.

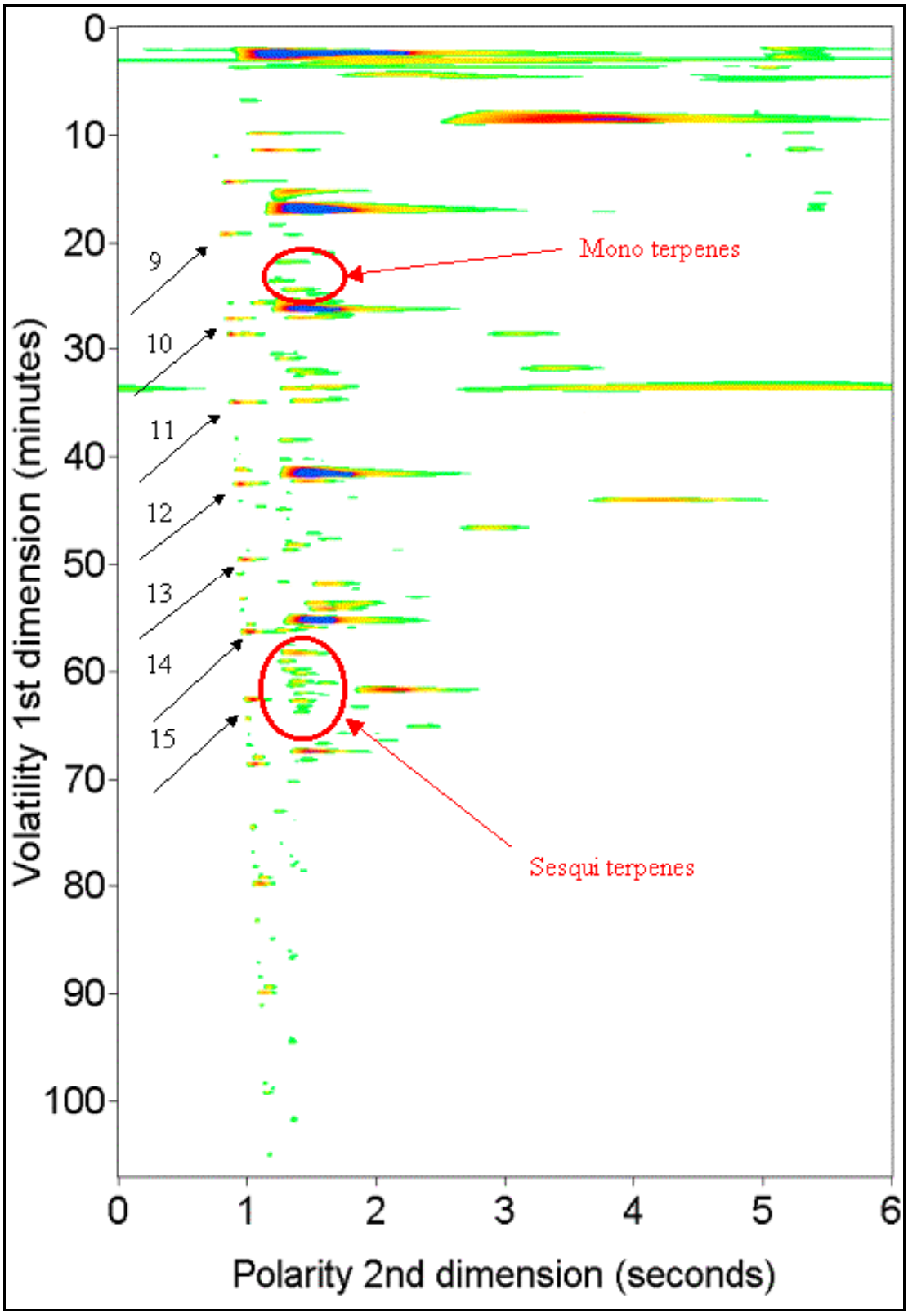


Figure 7.6: **Contour plot of beer aroma GCxGC-FID chromatogram**

Indicated are the 7 *n*-alkane peaks from C9 to C15. Notice how the alkanes elute unretained in the second dimension as is the case for the silicone peaks (from the silicone rubber in the trap). Either of the series could be used for a retention index scale.

The GC-TOFMS total ion chromatogram (TIC) of a beer aroma sample, with the 7-component *n*-alkane mixture, is shown in Figure 7.5. Four peaks from the GCxGC chromatogram were selected and identified from the TIC for demonstration purposes. These four identified peaks are shown in Figures 7.7 and 7.8.

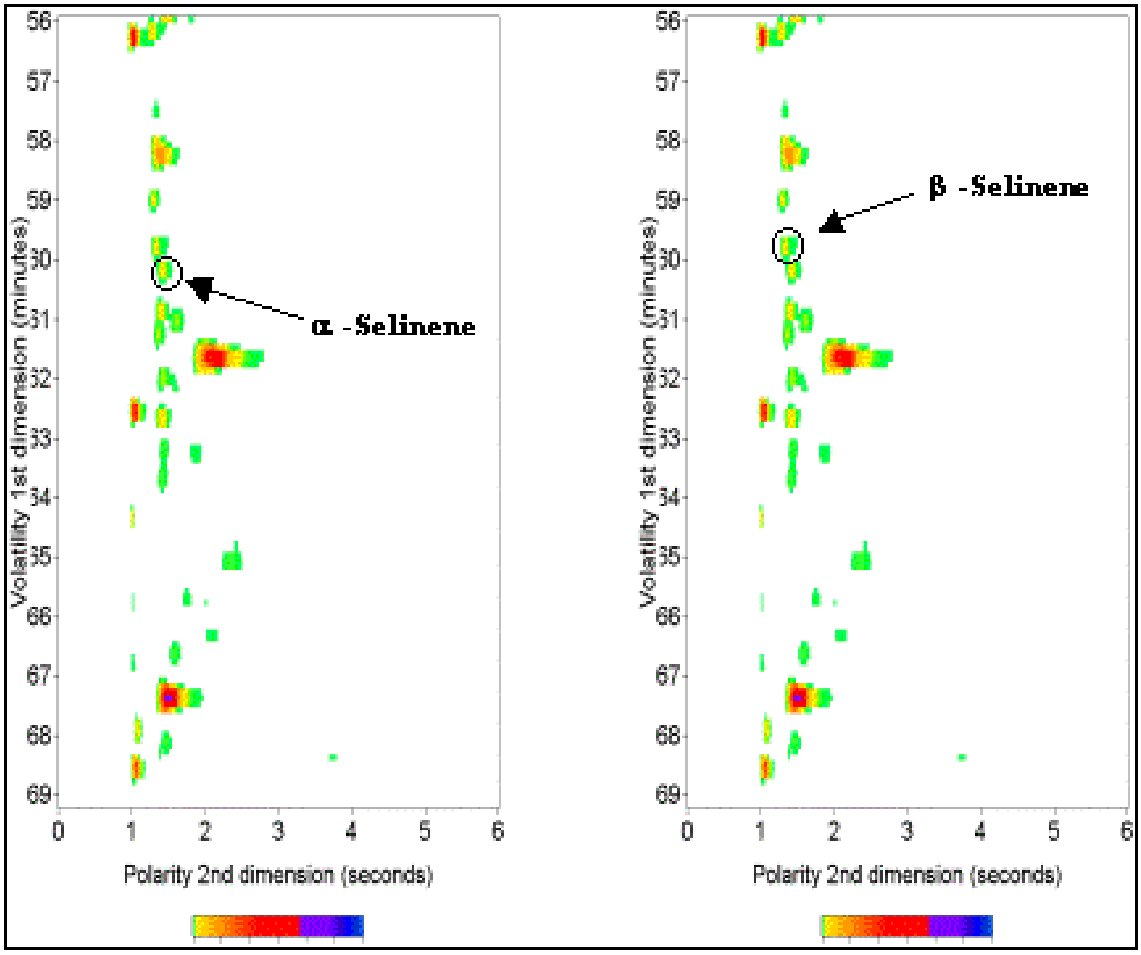


Figure 7.7: Two sesqui-terpene compounds identified

These two closely related sesqui-terpene compounds on the GCxGC chromatogram identified on the GC-TOFMS.

Figure 7.7 shows extracts of the GCxGC contour plot between the C14 n-alkane and the last of the ester compounds observed in beer aroma. This section contains the sesqui-terpene compounds as indicated in Figure 7.6. Two closely related sesqui-terpene compounds, indicated as α- and β-selinene in Figure 7.7, were identified.

The retention time of β selinene on the GC-MSTOFMS was 60.16 minutes and the calculated retention index 1453. On the GCxGC-FID the retention time of the peak was 59.97 minutes and the calculated retention index 1450. For α-selinene the values are: retention time GC-TOFMS 60.82 minutes, retention index 1462; retention

time GCxGC-FID 61.00 minutes, retention index 1453. The compounds identified from the GC-TOFMS can therefore be correlated with the peaks observed in the GCxGC plot to a high degree of certainty.

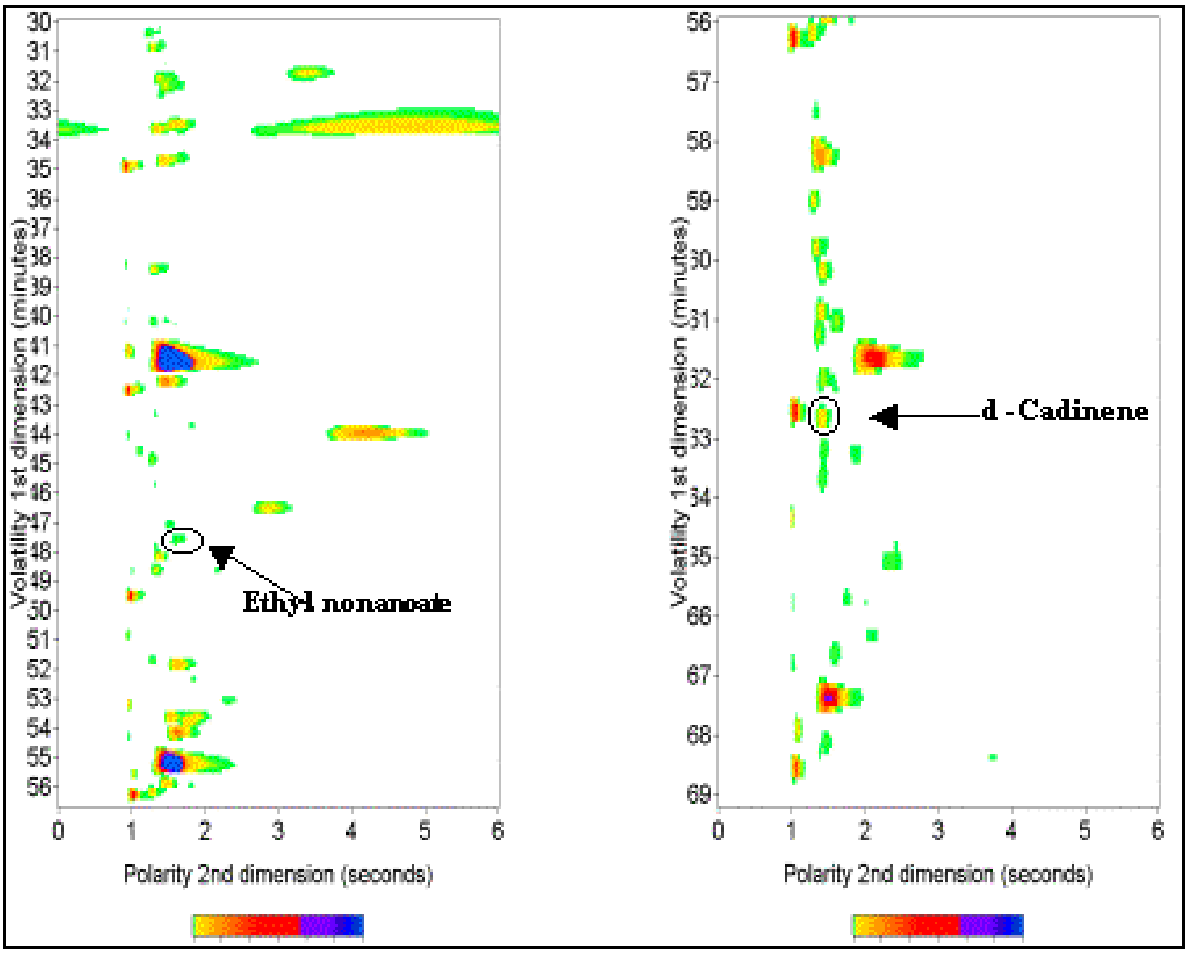


Figure 7.8: **Two further compounds identified in the GCxGC contour plot**

Notice the delta-cadinene co-elutes with another compound in the first dimension. From a chromatogram produced on a traditional one column chromatograph this information would not be available for pattern recognition.

In the section shown in on the left in Figure 7.8, ethyl nonanoate, which is a small but known constituent of beer aroma, was positively identified between the ethyl octanoate and ethyl decanoate peaks (shaded blue in the contour plot). This compound is present at a very low concentration in the beer aroma and is represented by a very small peak in the chromatogram. In a traditional chromatogram such small peaks may be lost among the background peaks. In a GCxGC contour plot the aroma compounds are separated from the background (silicone) peaks so small aroma peaks are clearly visible.

The advantage of the second dimension separation of GCxGC is further demonstrated on the right in Figure 7.8. Delta-cadinene is a sesqui-terpene compound that co-elutes with with another compound in the first dimension. Its slightly higer retention on the second column in the GCxGC made it visible on the contour plot. It was sucessfully identified from the GC-TOFMS mass spectrum because of the accurate mass determination capabilities of the Micromass® GCT™.

5. Conclusion

The results presented show that the sampling technique can be successfully used with techniques of analysis other than GC-FID, on which it was developed. The retention time matching technique was successfully applied to the identification of beer aroma compounds. The high degree of repeatability and sensitivity of the sampling technique made it possible to demonstrate the advantage of multi-dimensional analysis for the identification of small and co-eluting peaks in beer aroma.

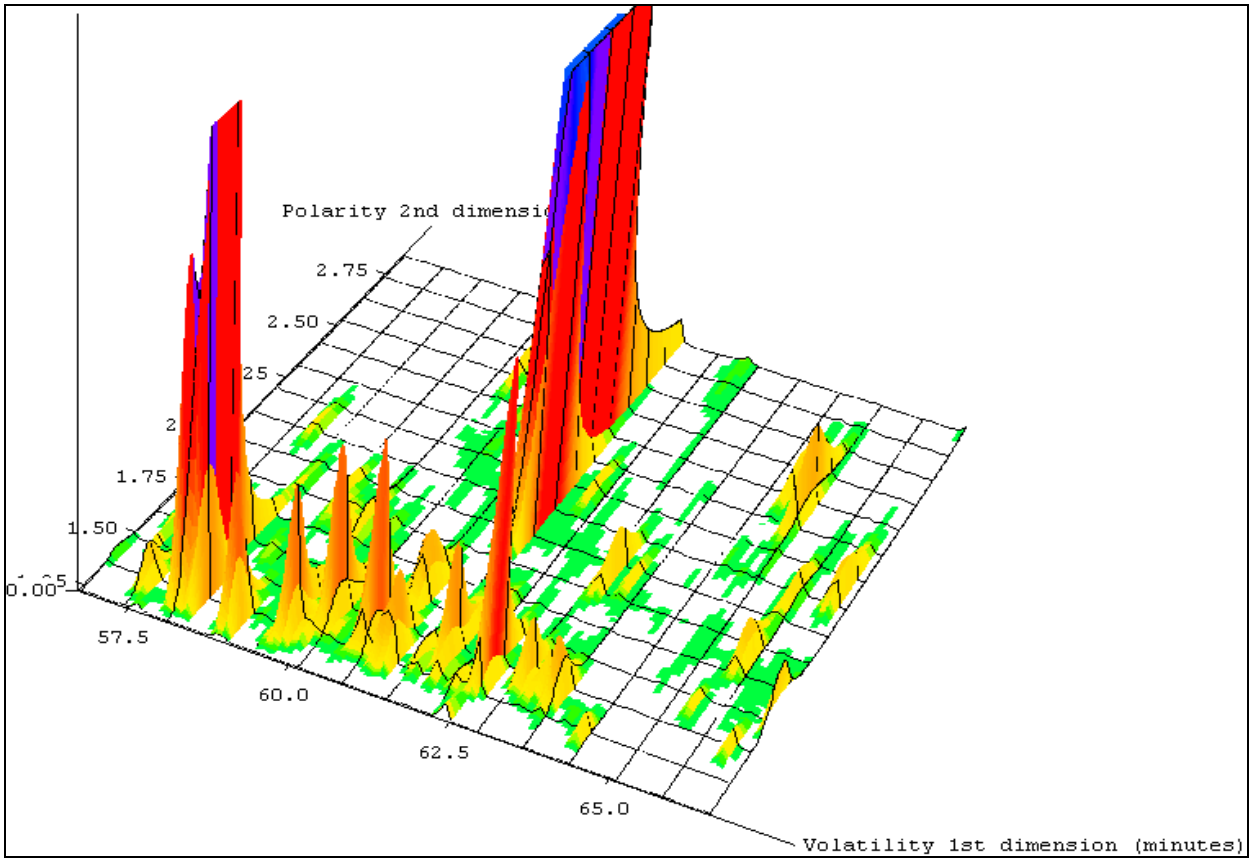
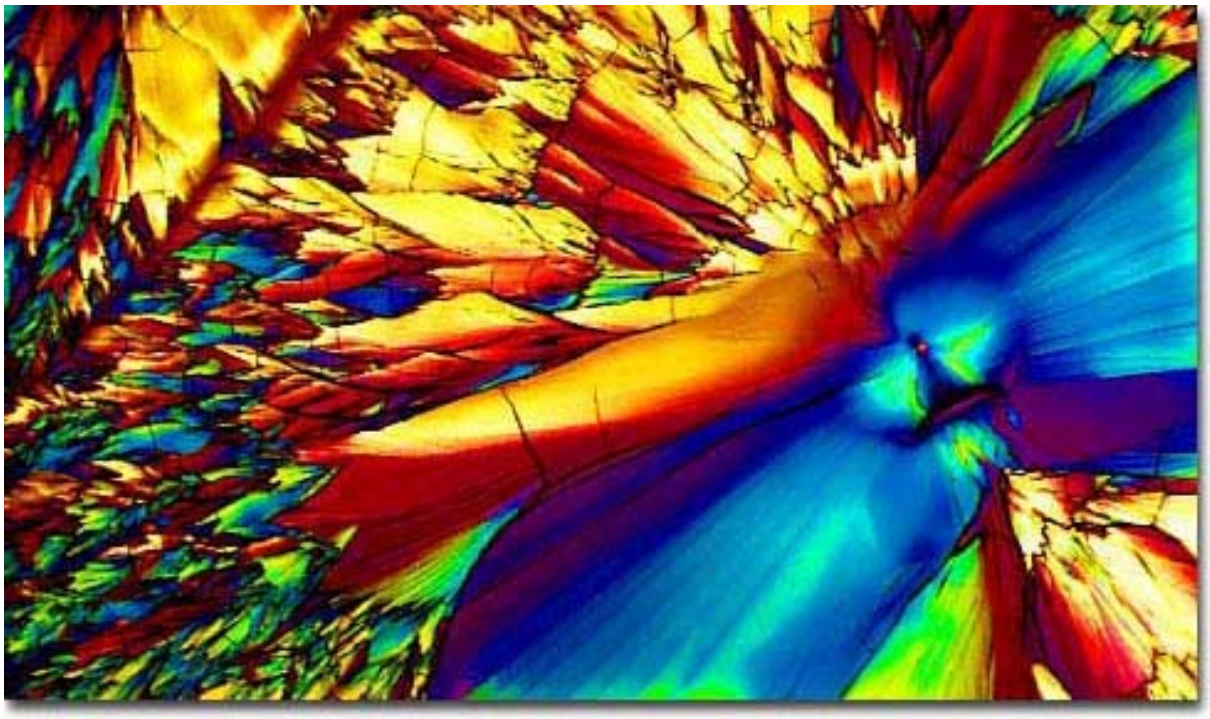


Figure 7.9: A section of sesqui-terpenes.

An expanded three dimensional plot of the sesqui terpene area indicated in Figure 7.5

The terpene compounds (a three dimensional plot of the sesqui-terpene area is shown in Figure 7.9) is a very interesting and characteristic part of beer aroma that could be studied further with the aid of the silicone rubber trap as sampling device.

Chapter 8



Pilsner Urquell

Text from the bottle label: Pilsner Urquell - The only genuine Pilsner. Brewed and bottled by Pilsner Urquell-Plzen/Pilsen/Czech Republic.

Chapter 8

CONCLUSION

1. Summary

The results presented in the foregoing chapters serves as proof that the multichannel silicone rubber trap (MCT) can successfully be used to sample volatile compounds which constitute beer aroma. It was demonstrated that by using the MCT as part of a purge-and-trap sampling arrangement, a wide range of beer aroma compounds could be successfully collected, stored and analysed to produce repeatable results that could be directly, visually, compared and used for the purpose of quality control.

2. Problems addressed during the study

Difficulties encountered during the research were primarily related to the collection characteristics of the multichannel silicone rubber trap. These problems were successfully resolved and the resulting sampling method was demonstrated to be repeatable and robust enough to fulfil the requirements set out for the project. The problems addressed were:

2.1 The accumulation of excess water

Water accumulating in the concentration device (silicone rubber trap) during sampling caused difficulties during the chromatographic analysis of the aroma sample. This problem was solved through a combination of optimisation of the thermal desorption technique (used for releasing the aroma sample from the MCT) and controlling the temperature of the sampling tube relative to that of the sample in order to prevent the condensation of water.

2.2 Collecting the full range of beer aroma compounds

The differences in the volatility of the beer aroma compounds (and subsequent differences in breakthrough volumes of these compounds on the MCT) complicated the simultaneous collection of the full range of compounds. By careful optimisation of the sampling time (i.e. purge volume) and sample temperature, a series of compounds believed to represent the characteristic aroma of a beer could successfully be collected.

2.3 Repeatability

One of the principle aims of the project was to produce chromatographic aroma profiles accurate enough to distinguish between beer brands and to recognise foreign peaks when searching for compounds responsible for off-flavours.

At first, it proved extremely difficult to obtain repeatable results with the optimised sampling method. Numerous attempts at solving the problem through further optimisation of the sampling method proved unsuccessful. It was eventually found that the variability was not only a result of the sampling method, but was partly due to inconsistencies in the analysis. This was easily rectified and the resulting improvement in the repeatability enabled direct visual comparison of results.

3. Achievements

The repeatability of the results was proved for both intra-trap and inter-trap sampling scenarios. Over 80 visually reproducible peaks, which could positively be distinguished from the chromatographic background, were identified. Statistical peak area reproducibility was confirmed on a subset of 64 of these peaks, selected from the automated peak area listing, based on clearly matching peaks in the consecutive chromatograms evaluated. The stability of the sample on the MCT was preliminary tested for extended periods of storage and for various temperature and exposure conditions. The consistent performance of the MCT under these conditions established the value of the technique for sampling and transporting samples from distant breweries to a central quality control laboratory.

The high degree of reproducibility was achieved, not only for the large-area peaks in the chromatographic profiles, but also for the very small peaks representing trace aroma compounds, such as the terpenes. The method was shown to have a high degree of sensitivity and compounds present at concentrations below one part per billion (1 μg/litre) in beer could be detected. Compared to the odour threshold concentrations of terpene compounds in water, which is typically well above 1 ppb [55], this level of sensitivity seems adequate for quality control purposes.

The combination of good sensitivity and excellent reproducibility is required for successful aroma profiling. The capability of the sampling method was demonstrated

by readily distinguishing between three very closely related beer brands on the basis of differences in relative peak intensities as observed in the chromatographic aroma profiles of each.

To my knowledge this is the first attempt at sampling and gas chromatographic analysis of beer aroma for the purpose of producing highly reproducible profiles for quality control purposes. The literature found on the chromatographic analysis of beer mainly discusses the identification and quantification of target compounds. Earlier work on beer aroma, notably the contributions of Morten Meilgaard [16] focused on the identification of a wide range of compounds in beer and beer aroma. No other reference discussing the sampling and transport of aroma samples or the reproducibility of beer aroma profiles could be found in the recent chromatographic literature.

The sampling and desorption technique had to be developed and studied first with GC-FID, because of the robustness and stability required for optimising repeatability. However, in order for this sampling and analysis technique to be useful for identifying off-flavour compounds, the application of the technique to more powerful methods of analysis had to be investigated. Although this aspect fell outside the original scope of this project, it was shown that the sampling method could successfully be translated to other methods of analysis. This was demonstrated by analysing a sample of beer aroma volatiles with multi-dimensional gas chromatography (GCxGC), and identifying some of the peaks in the two-dimensional scatter plots with accurate mass time-of-flight mass spectrometry.

4. Future prospects

The GCxGC analysis of beer aroma has the potential of producing much more detailed patterns for quality control purposes. The combination of these more complex patterns with the ability of GC-TOFMS to determine accurate masses is ideal for the identification of compounds responsible for off-flavours in beer. Of course, use of the technique with commercial GCxGC-TOFMS (low resolution) is a further possibility to be investigated.

The results achieved during this study holds promise for the future application of the sampling technique in other areas of quality control and for the study of aroma. The

technique shows potential for selective accumulation of compounds from complex aroma samples and for studying the contribution of compounds to characteristic aromas.

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