DISCRIMINATION BETWEEN BEERS WITH DIFFERENT BEST BEFORE DATES USING GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY

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DISCRIMINATION BETWEEN BEERS WITH DIFFERENT BEST BEFORE DATES USING GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY

by

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Dissertation to be submitted in partial fulfilment of the requirements for the degree of Bachelor of Science (Honours) (Forensic Science)

FEBRUARY 2025

CERTIFICATE

This is to certify that the dissertation entitled

"DISCRIMINATION BETWEEN BEERS WITH DIFFERENT BEST BEFORE DATES USING GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY"

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DECLARATION

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LIST OF ABBREVIATIONS

DCM	Dichloromethane
SPME	Solid-Phase Microextraction
LLE	Liquid-Liquid Extraction
GC-MS	Gas Chromatography-Mass Spectrometry
HS-SPME	Headspace Solid-Phase Microextraction
SBSE	Stir Bar Sorptive Extraction
HSSE	Headspace Sorptive Extraction
SAFE	Solvent-Assisted Flavour Evaporation
VOM	Volatile Organic Metabolite
POF	Phenolic Off-Flavours
VOC	Volatile Organic Compound
PDMS	Polydimethylsiloxane
NMR	Nuclear Magnetic Resonance
PCA	Principal Component Analysis

LIST OF SYMBOLS

℃	Degree Celcius
°F	Degree Fahrenheit
°C min^{-1}	Degree per minute
µL	Microlitre
mL	Mililitre
mL/L	Milligrams per Litre
mL/min	Mililitre per minute
v/v	Volume per Volume
µm	Micrometer
m/z	Mass-to-charge ratio
%	Percent

DISKRIMINASI ANTARA BIR YANG MEMPUNYAI TARIKH LUPUT BERBEZA MENGGUNAKAN KROMATOGRAFI GAS-SPEKTROMETRI JISIM

ABSTRAK

Kualiti bir dipengaruhi oleh pelbagai faktor, termasuk tarikh luput, yang boleh memberi kesan terhadap rasa, aroma, dan komposisi kimianya. Kajian ini bertujuan untuk membezakan sampel bir berdasarkan tarikh luput yang berbeza serta jenama yang berbeza dengan menggunakan pengekstrakan cecair-cecair diikuti oleh kromatografi gas dengan spektrometri jisim (LLE/GC-MS). Dalam kajian ini, sampel bir daripada jenama Carlsberg® telah dipilih untuk mewakili sampel daripada pelbagai tempoh penyimpanan, untuk membandingkan profil GC mereka. Tambahan pula, empat sampel bir yang berasal dari jenama yang berbeza, iaitu Carlsberg®, Asahi®, Heineken® dan Tiger® juga dianalisis dan dibandingkan untuk perbezaan kandungannya. Kaedah GC-MS telah dioptimumkan dari segi pemilihan pelarut, nisbah pelarutan, dan isipadu suntikan bagi meningkatkan ketepatan pengesanan, dan seterusnya analisis sampel bir. Penemuan utama melalui analisis GC-MS menunjukkan bir dengan tarikh luput yang berbeza mempunyai perbezaan ketara dalam profil kimia mereka. Secara keseluruhan, 35 sebatian organik telah dikenal pasti dalam sampel bir Carlsberg® menggunakan kaedah yang dicadangkan. Profil kimia khusus bagi setiap kumpulan sampel bir dan daripada pelbagai jenama juga diperhatikan dan diperlihatkan melalui analisis komponen utama. Kesimpulannya, kajian ini telah berjaya menentukan profil kimia bir dengan tarikh luput yang berbeza dan jenama yang berlainan. Hal ini telah membolehkan pembezaan forensik bagi sampel bir.

DISCRIMINATION BETWEEN BEERS WITH DIFFERENT BEST BEFORE DATES USING GAS CHROMATOGRAPHY–MASS SPECTROMETRY

ABSTRACT

The quality of beer is influenced by various factors, including its best-before dates, which may impact its flavour, aroma, and chemical composition. This study aimed to discriminate between beer samples from different best-before dates and different brands using liquid-liquid extraction followed by gas chromatography with mass spectrometry (LLE/GC-MS). In this study, beer samples from the brand Carlsberg® were selected to represent different time points with varying best-before dates to compare for their GC profiles. Additionally, four beer samples originated from different brands, namely Carlsberg®, Asahi®, Heineken® and Tiger® were also analysed and compared for their compositional differences. The GC-MS method was optimised for solvent selection, dissolution ratio, and injection volume to maximize detection sensitivity and accuracy, followed by the analyses of beer samples. Key findings through GC-MS revealed that beers with varying best-before dates exhibited distinct differences in their chemical profiles. Overall, 35 organic compounds were identified from Carlsberg® beer sample using the proposed method. Specific profiles for each beer set and from different brands were observed and visualised through principal component analysis. To conclude, this study has successfully determined the chemical profiles of beers with different best-before dates and of different brands, allowing for forensic discrimination of stale beer samples.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Beer is the most consumed alcoholic beverage worldwide and comes in third place after tea and water. It is an extraordinarily complex beverage as it composes about 3000 distinct constituents, including carbohydrates, proteins, ions, microorganisms, organic acids, and polyphenols, among other. Despite the fact that brewing has been done for quite some time, the complexity of beer makes it difficult to identify and measure the different components that go into it (Anderson et al., 2019). It was said that malted barley is typically used to make beer, other grains like rice, corn or wheat can also be utilised. Ethanol and carbon dioxide are also produced during the brewing process when sugars are fermented from starch. However, the majority of beers now are made in the presence of hops, which serve as a natural stabilising and preservation agent in addition to giving the finished beer its bitterness and aroma (Ruvalcaba & Enrique Duran-Guerrero, 2020). Therefore, understanding these chemical profiles is essential, not only for quality control but also for enhancing consumer experience.

Since beer is a complex alcoholic beverage produced through fermentation techniques, it may possess variation of distinct chemical compositions due to their raw ingredients and manufacturing pathways (Silva et al., 2015). The presence of aromatic compounds was crucial because it may enhance the quality of the finished beer product and the ratio of volatile and non-volatile compounds in a beer is highly related to acceptance and quality of beverage (Siqueira et al., 2008). For instance, esters and diketones, which are responsible for off-flavours, are normally present in beer and higher content of these is possible to assume there is a problem in the fermentation process.

Moreover, the exploration of different best before dates of beers is pertinent in the context of the growing craft beer market, and for some breweries, shelf life of the beer could become crucial concerns where freshness and quality are paramount (Pinho et al., 2006). Consumers are becoming increasingly knowledgeable about the importance of beer freshness and are demanding more premium and high-quality products, yet many may not fully understand the chemical changes that occur over time. Hence, it is important to develop rapid and economical methods to assess beer quality during and after production.

1.2 Problem Statement

Storage makes beer considerably more complicated since it can undergo chemical changes over time, affecting its flavour, aroma, and appearance (Anderson et al., 2019). There was observed to be a lack of research on how the chemical changes in beer past their best before dates that may pose health risks to consumers. This is particularly evident in craft beers, where the lack of regulations and limited emphasis on food safety practices which contribute to the development of off flavours in beers due to the belief that alcoholic beverages are inherently safe from microbial contamination. As a result, some craft brewers avoid pasteurisation, fearing that this process can negatively affect the beer's sensory quality when on the contrary it further increases the risk of contamination in beer (Viejo et al., 2020). Therefore, the chemical profiles of beers near and past their best before dates are compared using gas chromatography-mass spectrometry (GC-MS) to determine if there are any changes in the chemical compositions with time.

1.3 Objectives

1.3.1 General Objective

To investigate the chemical profiles of beers with different best before dates using GC-MS.

1.3.2 Specific Objectives

1. To identify the chemical substances in beer samples.
2. To differentiate between beers of varying best before dates based on substances found in GC-MS profiles.

1.4 Significance of Study

This study has significant implications for food safety, consumer protection, and the beer industry. By identifying the differences between beers with different best before dates, this research can help to prevent the sale of unsafe products and protect consumers from potential health risks. Furthermore, the results of this study can be applied to enhance quality control procedures in the beer sectors and guarantee that customers can obtain high-quality, safe products.

Also, given the significance of aroma quality in consumer perception and the overall enjoyment of beer, there is a critical need for the development of rapid and cost-effective analytical methods to assess beer quality and freshness during and after the brewing process. These tools enable brewers to monitor and manage the chemical composition of beer effectively, ensuring a high-quality product that meets consumer expectations. Therefore, reliable and sensible analytical methodologies are required for the extraction and analysis of a great number of beer volatile compounds.

By focusing on key volatile compounds, we aim to provide valuable insights into the changes in the chemical profiles of beer and their implications for quality. This study not only contributes to the existing body of knowledge regarding beer but also serves as a guide for both brewers and consumers in making informed decisions about beer consumption and preservation because it is such a popular beverage worldwide.

1.5 Scope of Study

This study focused on four brands of beer samples, namely the Carlsberg, Tiger, Heineken and Asahi beer. Each with different best before dates available to the researchers was used for comparison. By comparing these samples, the chemical profiles of beer were compared and potential changes that occur over time were investigated.

CHAPTER 2

LITERATURE REVIEW

2.1 Beer Chemistry

Beer is an alcoholic beverage obtained by fermentation processes and it is a very complex mixture. It contains numerous flavour-active volatile organic metabolites (VOMs) belonging to a diversity of chemical families over a wide range of concentrations, polarities and volatilities (Alves et al., 2020). Therefore, the chemical composition of different beers available varies considerably (Silva et al., 2015).

2.1.1 Types of Beer

Beer can be categorised into many different types of beers based on the process of fermentation (top/bottom fermentation), colour (dark/light), alcohol content (light/strong), type of additives added, the extracted and the origin. Based on the fermentation process, beer is divided into two types: top fermented beer and bottom fermented beer as shown in Figure 2.1 (Wunderlich & Back, 2009).

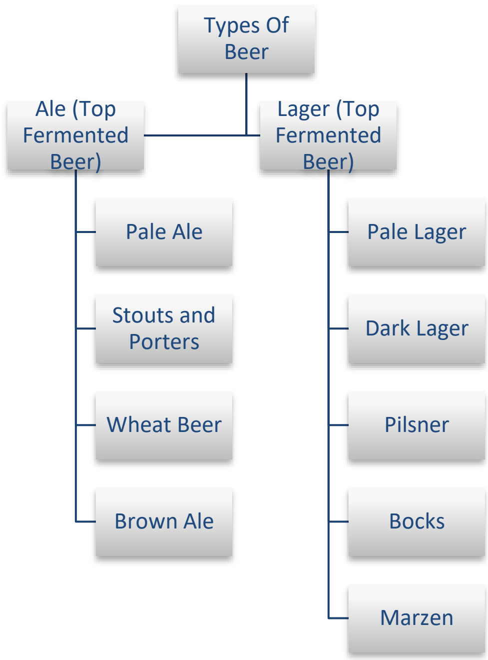

Figure 2.1 Types of Beer (Wunderlich & Back, 2009)

Ale beer is fermented using the best fermented yeasts, primarily *S. cerevisiae*, at a warm temperature (60 to 70 °F) (Granato et al., 2010). This type of beer gathers at the top of the water and floats the tank of the fermenter. These beers ferment in three to five weeks because of their warm temperature and esters. It has a rich taste profile with hints of fruitiness and spice (Polak et al., 2013). There are a few types of ale beer such as pale ale, porter and stouts, wheat beer and brown ale as shown in Figure 2.1.

As for bottom fermented beer, lager is the most popular one that is usually manufactured by ageing in the cold *S. cerevisiae* and *S. pastorianus* and commonly used for fermented beer. The yeast used in lager beer gets settled at the bottom of the liquid vessel after fermentation. The yeast used in bottom fermented beer is more fragile and needs to ferment more slowly and at a lower temperature than the yeast used in the production of ale beer. Pale lager, dark lager, Pilsner, Bocks and Marzen are the few types of lager beer (Archana et al., 2023).

2.1.2 Chemical Composition of Beer

The nature and quality of raw materials, their treatment, storage and finishing operations are the main factors that largely determine the constituents present in a particular beer. Generally, a normal beer includes carbohydrates, peptides or proteins and hop substances and carbon dioxide in major proportions with a small percentage of acetic acid and glycerol. Overall, the finished beer usually contains around 85 – 92 % of water by volume with a pH of 4.1 – 4.5 (Archana et al., 2023).

The components that give beer their scents are crucial since they significantly affect the finished product’s quality. Many volatile substances from a wide range of groups have been found in beer, including alcohols, acids, carbonyl compounds, hydrocarbons, ethers, sulphur compounds, alicyclic compounds, aromatic compounds, heterocyclic compounds, esters and nitrogen compounds. These substances affect the aroma and flavour of beer either individually or in a synergistic or antagonistic way. As a result, certain volatiles could significantly enhance the beer's flavour, while others are only crucial for enhancing the product's background flavour (Pinho et al., 2006).

Aromatic alcohols β-phenylethanol and benzyl alcohol are the major higher alcohols present in alcoholic beverages. These substances may affect flavour and scent in both favourable and bad ways. While appropriate levels of these higher alcohols (>300 mg/L) in beer give attractive qualities, large amounts can result in a harsh, pungent flavour and smell. For instance, 1-hexanol is typically present in a trace amount, but its oily and herbaceous scents have been linked to adverse effects in drinks. The most quantitatively significant flavouring compound in the higher-alcohol group is isoamyl alcohol as it results in a stronger beer taste (Olaniran et al., 2017). Ingestion or inhalation of isoamyl alcohol produces sedative, hypnotic and anticonvulsant effects similar to those of ethanol (Verhagen, 1994).

Esters, which are produced by yeast metabolism during alcoholic fermentation, have a big influence on beer aroma. Esters often offer beer a pleasing, full bodied character when used in moderation. However, in excessive, they give beer an excessively fruity flavour which is considered undesirable by most consumers (Olaniran et al., 2017). For instance, ethyl acetate represents approximately one third of all esters in beer and has a threshold concentration of 30 mg/L in beer but for lager-types of beers, the recommended concentration is lower than 5 mg/L (Smogrovicova & Domeny, 1999). If the concentration of ethyl acetate exceeds their threshold, it may give beer an undesirable flavour. Consequently, it is critical to maintain the concentration of volatile esters in the final product below their taste threshold (Olaniran et al., 2017).

Carbonyl compounds are the products of the Maillard reaction, by lipid oxidation during wort production, and as an intermediate product during the conversion pathway of amino acids to alcohols (Paszkot et al., 2023). They are described as grassy, cardboard, papery and so on (Verhagen, 1994). Acetaldehyde is known to be the major aldehyde to consider owing to its importance as an intermediate in the formation of ethanol and acetate. Its levels vary during fermentation and aging and in beers it has a flavour threshold of 10-20 mg/L (Olaniran, et al., 2017). If it exceeds this threshold level, it causes 'green vegetation' or 'vegetable' flavour (Smogrovicova & Domeny, 1999).

2.2 Beer Brewing Process

The process of transforming water, grains and hop to produce beer is known as brewing (Aroh, 2019). Its main purpose is to convert starch source into a sugary liquid which is known as wort and through fermentation process, that wort will be converted into an alcoholic beverage (Archana et al., 2023).

2.2.1 Raw Materials

Beer is typically brewed using four main ingredients which are water, starch sources like malted barley, fermented yeast which converts glucose into alcohol and flavouring agent like hops (Archana et al., 2023). All the ingredients used during brewing contain a wide variety of chemical component which will affect the final production of beer. Thus, it needs to be carefully controlled to ensure the quality of beer and consistency between batches (Anderson et al., 2019).

2.2.1(a) Water

Water is the primary ingredient in beer which comprises of minerals (calcium, magnesium, sulphate, chloride and carbonic acid) and breweries emphasise its purity and suitability for brewing (Wunderlich & Back, 2009). Since brewing is a very water-intensive activity, it uses between five to ten times more water than the amount of beer produced. Some of this water is used for beer production process but the majority of it is used for cleaning and ends up down the drain, unless it is recovered (Anderson et al., 2019).

Water quality is regulated by legislation, requiring it to be potable, free of pathogens and compliant with chemical and microbial standards because water's composition has a significant impact on the beer's flavour and other characteristics (Archana et al., 2023). Additional parameters, such as pH levels also play a crucial role since various brewing stages require specific pH values for optimal performance.

2.2.1(b) Malt

After water, malt is the second most significant ingredient in beer production because it impacts both the brewing process and the quality of the final product. The malting process is as crucial as it increases hydrolytic enzymes, partially breaks down endosperm cell walls and proteins, and modifies grain structure, making starch and protein more accessible for extraction. Malt is important for brewing as it provides the soluble extract needed for fermentation. Fermentable carbohydrates, a major component of malt extract, are needed by yeast for alcoholic fermentation. Malt also provides the starch substrate and amylolytic enzymes for conversion. Modern brewing often replaces malt with other starch or sugar sources, but some malt is still necessary. Yeast also requires specific amino acids, vitamins, and minerals, which malt supplies (Schwarz & Li, 2010).

Malt also significantly influences beer's organic characteristics such as colour and flavour because chemical components like sugars, amino acids, lipids, and phenolics, serve as precursors to colour, flavour, and off-flavour compounds. Beer foam is in large part dependent upon specific proteins derived from the malt. On the negative side, malt may contain factors that adversely impact beer quality, which can result in gushing or premature yeast flocculation (Schwarz & Li, 2010).

2.2.1(c) Yeast

Beer fermentation is caused by yeasts, which are unicellular fungi that multiplied by consuming sugar. Yeast consumes sugar during fermentation, producing carbon dioxide and ethanol. Additionally, yeast adds various aromatic molecules during the fermentation process, giving the beer a distinct flavour (Archana et al., 2023). For high quality brewing, proper yeast propagation and quick fermentation are crucial. Conditions for metabolism must be adjusted to ensure that yeast is in its ideal nutritional state (Wunderlich & Back, 2009). For fermented beer, many yeast varieties are utilised, such as S. *cerevisiae*, which is a top-fermented yeast for ale beer fermentation, and *S. carlsbergenesis*, which are bottom-fermented yeasts for lager beer fermentation (Archana et al., 2023).

2.2.1(d) Hops (*Humulus lupulus*)

Beer gets its typical bitterness and hop aroma from hops. While the aroma of beer is obtained from a variety of volatile chemicals derived from the hop essential oils, the bitterness is produced by isomerising alpha acids (humulones) into a more soluble acid during the boiling of the wort (Anderson et al., 2019). Hops can be added at different stages of boiling process to change the beer’s bitterness and foamability. The volatile fragrance chemicals tend to evaporate and are lost when introduced at the beginning of the boil. Therefore, to prevent this, hops can be added in the final 30 minutes of boiling, a technique known as “late hopping” which retains more volatile ingredients in the wort so they can be included in the final beer. A “dry hopping” method which involves adding the hops right before packaging is to allow some of the original ingredients to be carried straight into the drink to give beer a distinct hoppy character (Anderson et al., 2019).

Hops are also added throughout the brewing process because they have preservation properties such as antifungal and antibacterial qualities which can positively impact the stability of beer foam (Anderson et al., 2019). Further, hop also contains pharmacologically active substances, for instance it is soporific, or to induce sleep (Wunderlich & Back, 2009).

2.2.2 Stages of Brewing

The brewing process begins by mashing the barley in warm water to create wort, which is a clear, sweet, brown liquid. The carbohydrate in the wort is required in the late fermentation process. Hops, which give beer its bitterness and aroma, are added to the wort after it has been heated to a boil. Important bittering compounds are removed from the hops during boiling. The complex blend of terpenes found in hops gives beer its fragrances, which are typically characterised as fruity, citrussy, floral, spicy, and herbal. As for the fermentation step, yeast is introduced during this process to produce carbon dioxide, ethanol, and volatile phenolic compounds which could result in “phenolic off-flavours” (POF) in the beer. Following a fermentation period ranging from a few days to several weeks, the yeast is extracted, and the beer is allowed to mature to acquire further flavour before being filtered and packaged for retail sale (Anderson et al., 2019).

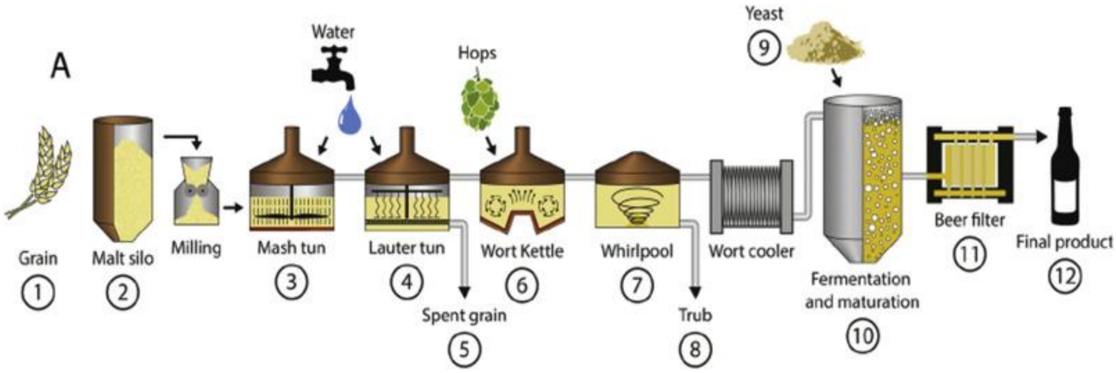

Figure 2.2 Stages of brewing process, including when specific ingredients are added. (Anderson, et al., 2019)

2.3 Extraction Studies

Prior to instrumental analysis, an extraction technique must be used for the analysis of volatile components in beers. The reason is that beer contains non-volatile chemicals including non-fermentable carbohydrates and proteins and injecting the sample directly into the gas chromatograph can contaminate and clog the device, thus requiring frequent maintenance of the instrument to obtain accurate results (Ng, 2002).

2.3.1 Solid Phase Microextraction

Solid phase micro-extraction (SPME) technique is method that offers several advantages, including the elimination of toxic organic solvents, high sensitivity allowing for the quantification of a wide range of volatile compounds at low detection limits (ppb and ppm), and achieving good linearity (Snow & Slack, 2002). SPME is also characterised by its simplicity, requiring minimal sample preparation and offering rapid analysis time and relatively inexpensive compared to other extraction methods (Lancas, 2003). Furthermore, its high sensitivity enables the use of smaller sample volumes, protecting the separation column from excessive exposure to water or solvents (Ferreira & Guido, 2023).

SPME also utilises a coated fused silica fibre to concentrate analytes present in the headspace (Snow & Slack, 2002). During headspace-SPME extraction, equilibrium is established between the liquid sample, the headspace, and the fibre before analyte extraction occurs. This technique is also valuable for analysing samples where minimising matrix interference is crucial. The extraction of volatile compounds from the headspace (HS) is preferred over direct injection because beer's highly volatile compounds, such as acetaldehyde, are not easy to detect in the liquid sample (Snow & Slack, 2002).

In a recent study that evaluates the effectiveness of four extraction techniques which is headspace solid-phase microextraction (HS-SPME), stir bar sorptive extraction (SBSE), headspace sorptive extraction (HSSE) and solvent-assisted flavour extraction (SAFE) for isolating hop-derived volatile organic compounds (VOCs) in beer brewed with Nelson Sauvin hops revealed that HS-SPME was the least sensitive method out of all because it extracts approximately 40% fewer compounds compared to other techniques. The author concludes that HSSE was identified as the most suitable method for extracting a broad range of hop-derived aroma compounds, closely followed by SBSE. This paper also highlighted the importance of choosing the right extraction method because it could significantly influence the analysis of beer aroma compounds (Richer et al., 2017).

Previous research on beer volatile analysis also studied the optimised parameters of HS-SPME such as sample volume, extraction time, temperature and salt addition to enhance the extraction efficiency of volatile compounds. Under the optimised conditions, nine key volatile compounds in beer, including esters, alcohols, and acids were identified and quantified (Charry-Parra et al., 2011). The paper that studied the analysis of lambic beer also utilised SPME/GC-MS as an analytical method. It focused on using SPME to extract volatile and semi-volatile compounds found in the aging gauze sample and there was a total of 41 compounds identified using a combination of retention index and mass spectral matching against library standards. GC-MS on the other hand was utilised to characterise all volatile and semi-volatile compounds fund within the aging samples in the study. The author revealed that there was a significant change in the concentration and composition of these 41 compounds as the beer aged, which highlights how aging influences the sensory characteristics of Lambic beer (Witrick et al., 2020).

2.3.2 Liquid-Liquid Extraction

Liquid-liquid extraction (LLE) is acknowledged as the traditional sample preparation methods. It is known to be one of the most common sample pre-treatments used extensively to increase sensitivity by concentrating the analyte from a large sample volume or to improve selectivity by separating the analyte from the matrix. The separation is based on the partition equilibrium of a species between the organic and aqueous phases, represented by the partition coefficient.

This extraction methods have been extensively employed in sample preparation for the analysis of wine and beer aromas, (Costa et al., 2004) utilised LLE in combination with GC-MS analysis to monitor the levels of methional and phenylacetaldehyde compounds associated with the aged aromas of lager-type beers. The paper highlighted the identification of four key compounds (methional, phenylacetaldehyde, β-damascenone and 3-hydroxy-4,5-dimethyl-2(5H)-furanone) in beer using GC-MS and chemical standards, which significantly contribute to the beer's aroma profile, particularly in aged samples. Trans-2-nonenal compound which is a well-known marker for oxidation related off-flavours in beer was not detected, but Strecker aldehydes (methional and phenylacetaldehyde) were identified which proves the consistent role of these two compounds in defining the aged beer aroma.

On the other hand, Tsenang et al. (2023) also utilised LLE with GC-FID for quantitative analysis of ethanol in different types of home-brewed alcoholic beverages of Botswana. The study identified ethyl acetate as the most effective solvent for ethanol extraction with a sample to solvent ratio of 3:1 yielding the highest recovery (93.48%), making it suitable for routine analysis. This paper also revealed the ethanol concentrations ranging from 2.56% to 36% v/v in the 26 samples, with significant variation attributed to differences in brewing methods, raw materials, and additives used by brewers.

2.4 Analysis of Beer Samples

The analysis of beer samples is a critical step in evaluating the chemical composition and sensory attributes that define beer quality. This section highlights the identification of important volatile compounds in beer using advanced analytical techniques like GC-MS.

2.4.1 Principle of Gas Chromatography-Mass Spectrometry

GC-MS is an analytical technique that allows the separation and identification of variety of compounds in a single sample. This technique is suitable for gaseous samples, liquid solutions, and volatile solids (Al-Bukhaiti & Al-Farga, 2017).

The basis of GC principle is that after the sample is injected into a carrier gas stream, separation of the analytes in the mixture occurs based on the differences in the mode of partitioning between mobile and stationary phase (Rudi, 2020). In GC, the more volatile a substance is, the longer its molecules will travel through the carrier gas and the faster they will exit the column. In this way, each substance in the column is separated and is directed to the MS section for further analysis, leading to compound identification using the MS library (Maji et al., 2023).

2.4.2 Benefits and Usage of GC-MS

GC is a powerful method employed for separating and analysing complex mixtures of compound. It works especially well for substances with boiling points below 200°C. It is widely applied in environmental analysis, fire investigation, pharmaceutical drug analysis, food analysis and more (Maji et al., 2023). Its common uses in food and drink involve analysing food contents quantitatively and/or qualitatively, specifically identifying components of flavour and scent.

Good resolution, high repeatability and reproducibility of retention times, and high precision and accuracy in quantification based on peak area measurements are the three main benefits of GC-MS analysis. GC-MS also requires minimal sample for identification but result in sample destruction during analysis (Maji et al., 2023). The concentration of volatile in beer could be measured using GC-MS which allows for the simultaneous measurement of a variety of substances, including ethers, esters, acids, aldehydes, ketones, alcohols, and sulphur compounds (Ferreira, 2021).

Previous study using GC-MS method for analysing volatile compounds in alcoholic beverages utilises ethanol as an internal standard and applied it to 36 samples which includes commercially available spirits from 18 countries and homemade distillates. This research found that the suggested method is proven to be precise and reliable when employed on GC-MS instrument and can be used in routine analysis for quality control of alcoholic products (Korban et al., 2021). In a recent study that utilized HS-SPME combined with GC-MS was used to establish volatile fingerprints of beer and 43 volatile metabolites were identified using mass spectrometry. These metabolites were grouped into six chemical classes mainly esters, aldehydes, alcohols, furanic compounds, acids and terpenes based on their LRI values. The paper also highlighted how storage time and temperature influence the chemical composition and the sensory properties of beer (Ferreira, 2021). Another research study examining volatile compounds in beer through SPME combined with GC-MS successfully assessed various SPME fibres and extraction conditions to identify the most effective parameters for extraction. This research revealed that a 100 µm polydimethylsiloxane (PDMS) fibre offered the highest extraction efficiency, with optimal conditions achieved at 40 °C within a 30-minute extraction time and the addition of salt to the sample. By employing this optimised SPME-GC-MS method, this study managed to identify a diverse range of volatile compounds in beer, including alcohols, esters, aldehydes, ketones, and acids which are key contributors to the sensory properties of beer (Kleinova & Klejdus, 2014).

2.5 Beer Analysis in Forensic Science

According to a research paper by (Ng, 2002) which explores the application of gas chromatography-mass spectrometry (GC-MS) in detecting and quantifying fatty acids and esters in alcoholic beverages, including beer shows the importance of beer analysis particularly in cases involving beverage residues at crime scenes, counterfeit alcohol investigations, and toxicological analysis. The technique used in this study can detect trace amounts of fatty acids and esters in spilled or dried beverage samples which can help to reconstruct crime scene, verify alcohol authenticity and further support legal investigations.

A study from (Lima, et al., 2023) also explores how beer's aroma profile changes over time depending on its packaging and has significant forensic applications, particularly in determining storage conditions and timelines. By analysing volatile compounds in aged beer samples, forensic experts can estimate how long a beer has been stored and under what conditions, which can be useful in legal cases where beverage consumption history is relevant. Additionally, the use of multivariate analysis from this research paper further enables forensic scientists to compare beer samples from different sources, assisting in investigations involving tampered or disputed beverage and establishing connections or discrepancies relevant to the investigations. Overall, this research paper provides valuable analytical tools for forensic science to support the assessment of storage history, authenticity and sample identification in legal contexts.

The findings of a research paper that examines how the aroma profile of lambic beer evolves over time is useful in determining the age of beer samples as analysing specific volatile compounds can help forensic experts to estimate storage duration and consumption timelines. Additionally, this paper also provides an insight into the fermentation activity of beer which can assist forensic investigations in cases involving contamination or product tampering (Witrick, et al., 2020)

CHAPTER 3

METHODOLOGY

3.1 Materials and Reagents

Materials and reagents used in this study were listed in Table 3.1.

Table 3.1 Chemicals used in this study.

Chemical	Brand	Production Company	Country of Origin
Methanol	Merck KGaA	EMD Millipore Corporation	Germany
Dichloromethane	Merck KGaA	EMD Millipore Corporation	Germany
Ethyl Acetate	Merck KGaA	EMD Millipore Corporation	Germany
Anhydrous sodium sulfate	Merck KGaA	EMD Millipore Corporation	Germany
Beer Sample	Carlsberg®	Carlsberg Brewery Malaysia Berhad	Malaysia
	Heineken®	Heineken Malaysia Berhad	Holland
	Asahi®	Carlsberg Brewery Malaysia Berhad	Japan
	Tiger®	Asia Pacific Brewery	Singapore
Glass wool			

3.2 Apparatus

Apparatus used in this study were listed as follows:

i. GC vials (Agilent Technologies, USA)
ii. Plastic test tube
iii. Test tube rack
iv. Glass pipette (1 mL)
v. Forceps
vi. Pipette sucker
vii. Beakers
viii. GC inserts
ix. Glass dropper

3.3 Instruments

Instruments used in this study were listed as follows:

i. 7890B Series Gas Chromatography – Mass Spectrometry (GC-MS) equipped with 7693 Series Autosampler (Agilent Technology, Santa Clara, CA, USA)
ii. Computer model (HP, Palo Alto, CA, USA) using MassHunter software (Agilent Technologies, Santa Clara, CA, USA)

3.4 Method Optimisation

Optimisation was performed to determine the optimum GC methods for the detection of organic substances within the beer samples. It was performed using the beer sample from Carlsberg, serving as the representation sample in this study.

3.4.1 Optimisation of Solvent

In this study, three solvents (methanol, dichloromethane and ethyl acetate) used to dissolve the beer samples were investigated. Each beer sample was mixed with the selected solvent based on sample to solvent ratio as described in section 3.4.2. The mixture was vortexed for 1 minute to allow uniform mixing and passed through drying agent (anhydrous sodium sulphate). All the solution was then transferred into clean GC vial for the analysis.

3.4.2 Optimisation of Dissolution Ratio

As for the optimisation of dissolution ratio, three different solutions were prepared using sample to solvent ratios of 1:1, 1:4 and 1:9. For 1:1 ratio, an equal amount of solvent and the beer sample was mixed while for the 1:4 ratio, 1 ml of solvent was mixed with 4 mL of beer sample. Similarly, for the 1:9 ratio, 1 mL of solvent was mixed with a 9 mL of the beer sample.

3.4.3 Optimisation of Injection Volume

To enhance the recovery of the targeted sample, the injection volume was also adjusted to 0.2 µL and 0.5 µL in addition to 1 µL as the default setting. In other words, the same sample was injected with different volume by the autosampler, and the generated chromatograms were compared.

3.5 Analysis of Beer Sample

For the experimental design, the repeatability study and the recovery study, a can of Carlsberg was used because it is commercially available. Then, three samples of the beer from the same can were analysed according to the optimised LLE/GC-MS method.

To investigate the differences between different best before dates of beer from same brand as well as different brand, beer samples were categorised into three groups: (1) Beer samples nearing best before date, (2) beer samples past their best before dates from the same brand, and (3) beer samples from different brands past their best before dates. All the analyses were carried out in duplicate.

3.6 Liquid-Liquid Extraction

The LLE extraction technique was adopted from Tsenang et al. (2023) used to determine the best extraction solvent to analyse homemade alcoholic beverages prior to GC injection. Extraction of volatile compounds was initially performed by dissolving 1 mL of a home-brewed alcoholic beverage sample in 1 mL of each of the four solvents. Following optimisation, the solvent with the most recovery was chosen as the working solvent. For the optimised method, 1 mL of the beer sample was dissolved in 9 mL of solvent. Following this, the mixture was vortexed for 1 minute to ensure uniform mixing. The solution was then passed through anhydrous sodium sulphate for the drying process before being transferred into clean GC vials for analysis.

3.7 GC-MS Analysis

GC-MS was used to aid in the identification of the substances in beer samples. A 7890B Series Gas Chromatography – Mass Spectrometry (GC-MS) with 7693 Series Autosampler and a HP-5MS column composed of 5% diphenyl-95% dimethylpolysiloxane equipped with Mass Hunter software and the NIST 14 library were used.

The GC-MS method was adapted from (Tsenang et al., 2023) and (Ferreira, 2021). Table 3.2 shows the optimised conditions of GC-MS instrument.

Table 3.2 Optimised conditions of GC-MS instrument

Parameters	Conditions	
Column (HP-5MS)	Purified helium gas	
Mobile flow rate	1 mL/min	
Injection volume	0.5 µL	
Mode of injection	Splitless	
Oven	Initial Temperature	40 °C
	Rate	4 °C min^{-1}
	Final Temperature	240 °C
	Acquisition delay	15 minutes
Mass Spectrometry	Temperature of transfer line of MS	240 °C and 220 °C
	Scanning range of MS	30 to 300 m/z
Total analysis time	70 minutes	

3.8 Data Processing

The GC-MS analysis produced GC chromatogram from all samples run by the system. The data produced were initially pre-processed to create the data matrix for suitable data analysis. The data processing was started with the integration of peaks from chromatogram using Agilent Mass Hunter. Chromatograms with sharp peaks and 80% quality were identified and searched in the library because the quality score indicated that the compound is likely present. Then, the selected peak was compared to the NIST library and potential matches of compound were displayed. The peak area of each peak was then recorded and transferred to Microsoft Excel® Spreadsheet for further data analysis.

3.9 Data Analysis

To prevent bias in data selection, the dataset was normalised using Microsoft Excel® Spreadsheet. Normalisation was conducted where each peak area was divided by the total peak area of the sample, ensuring that the sum of all peaks equals to 1. This method makes peak intensities comparable across different samples. All the normalised data were subjected to principal component analysis (PCA) for more specific and reliable characteristics of the beer samples using Minitab® 22 software (State College, Pennsylvania, USA). This software was used to study the clustering of beer brands based on their chemical composition and examine how the chemical profiles vary across different best before dates within each brand.