The effect of ultraviolet-C treatment on the biochemical composition of beer

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Declaration

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SUMMARY

This study describes:

- Development of analytical tools to investigate the light struck flavour (LSF) in beer by Gas chromatography mass spectrometry (GCMS) and by liquid chromatography mass spectrometry/mass spectrometry (LCMS/MS). Development of a high performance liquid chromatography (HPLC) method to analyse carbohydrates in beer.

- The efficiency a pilot scale ultraviolet (UV-C) system at 254 nm to inactivate spoilage microorganisms spiked in commercial beer. Bacteria test were *Lactobacillus brevis*, *Acetobacter pasteurianus* and *Saccharomyces cerevisiae*

- A pilot scale UV treatment of commercial and non-commercial lager beers at UV dosage of 1000 J/L. Following the UV treatment, the correlation between chemical analyses and sensory tests conducted by consumers' tasters were investigated.

- A pilot scale UV treatment of non-commercial beer brewed with reduced hops iso-α-acids (tetrahydro-iso-α-acids) at UV dosage of 1000 J/L. Sensory changes and chemical properties were investigated.

- The development and optimisation of an UV light emitting diodes (UV-LED) bench scale apparatus. Chemical and microbiological tests were conducted to investigate the effect of UV-LEDs on beer at 250 nm and 275 nm wavelengths.

OPSOMMING

Hierdie studie beskryf:

- Die ontwikkeling van analitiese toerusting om die invloed van lig op die smaakontwikkeling in bier te bestudeer m.b.v gaschromatografie massa spektrometrie (GCMS) en vloeistofchromatografie massa spektrometrie/massa spektrometrie, asook die ontwikkeling van ‘n hoë druk vloeistofchromatografiese metode vir die analise van koolhidrate in bier.

- Die doeltreffendheid van ‘n toetsskaal ultraviolet (UV-C) sisteem om die nadelige mikroorganismes waarmee die bier geïnnokuleer was, by 254 nm te inaktiveer.. Toetse is uitgevoer met die volgende bakterieë, *Lactobacillus brevis*, *Acetobacter pasteuriants* en *Saccharomyces cerevisiae*.

- ‘n Toetsskaal UV behandeling van kommersiële en nie-kommersiële lager biere by ‘n UV dosering van 1000 J/L. Na UV behandeling is die verwantskap tussen chemiese analises en ‘n reeks sensoriese toetse deur vebruikers proeërs ondersoek..

- ‘n Toetsskaal UV behandeling van ‘n nie-kommersiële bier gebrou met verlaagde hops-iso-α-sure (tetrahidro-iso-α -sure) by UV dosering van 1000 J/L. Sensoriese veranderinge asook chemiese eienskappe is ondersoek.

- Die ontwikkeling en optimalisering van ‘n UV-lig emissie diodes bankskaal apparaat. Chemiese en mikrobiologiese toetse is uitgevoer om die effek van UV lig op bier by 250 nm en 275 nm te ondersoek.

To my dear mother **Okev,** and in memory of my sister **Jeanine Abeme Mezui**

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TABLE OF CONTENTS

List of abbreviations..... xv

Chapter 1

INTRODUCTION

1.1 Background..... 1.1

1.2 Objectives..... 1.3

1.3 Goals..... 1.4

1.3.1 Disinfection of beer by UV-C irradiation..... 1.4

1.3.2 Beer trials..... 1.4

1.3.3 Bench scale UV-LEDs..... 1.5

1.4 Layout of dissertation 1.5

1.5 References..... 1.7

Chapter 2

THE PHOTCHEMISTRY OF BEER

2.1 Introduction..... 2.1

2.2 Overview of the brewing process..... 2.1

2.2.1 Beer ingredients..... 2.1

2.2.2 Malting..... 2.2

2.2.3 Brewing 2.4

2.2.4 Fermentation and maturation of wort..... 2.5

2.2.5 Packaging and pasteurisation of beer..... 2.5

2.3 Hop components..... 2.6

2.3.1 Hop essential oils..... 2.8

2.3.2 Hop resins..... 2.10

2.3.3 Iso-alpha acid (Isohumulones)..... 2.11

2.3.4 Beta acids hops..... 2.13

2.3.5 Iso-alpha-acids (isohumulones)..... 2.13

2.4 The light-struck flavour of beer..... 2.14

2.4.1 History of the study of lightstruck flavour of beer..... 2.14

2.4.2 Mechanism of 3-methyl-2-butene-1-thiol (MBT) formation in beer.. 2.16

2.4.2.1 Light source and iso-alpha-acids hops..... 2.18

2.4.2.2 Riboflavin and sulphur source in beer..... 2.18

2.5 Control of lightstruck formation of beer..... 2.21

2.4.1 Packaging technology..... 2.21

2.4.2 Chemically modified hop bitter acids..... 2.22

2.6 Measurement of lightstruck flavour of beer..... 2.24

2.5.1 Properties of 3-methyl-2-butene-1-thiol (MBT)..... 2.24

2.5.2 Instrumental and sensory analysis of MBT..... 2.25

2.7 Concluding remarks..... 2.27

2.8 References..... 2.27

Chapter 3

ULTRAVIOLET IRRADIATION AS A NON-THERMAL DISINFECTION TECHNIQUE

3.1 Introduction..... 3.1

3.2 Nature and source of UV light for sterilisation 3.2

3.3 Effect of UV-C on microbial agents..... 3.3

3.4 Practical applications of UV-C light irradiation..... 3.5

3.4.1 Purification of air, surface and water..... 3.5

3.4.2 UV sterilisation of liquid foods and beverages..... 3.7

3.4.3 Application of UV-C light in the brewing industry..... 3.8

3.5 Reactor designs for UV irradiation of liquid foods and beverages..... 3.8

3.6 Concluding remarks..... 3.11

3.7 References..... 3.11

Chapter 4

THE APPLICATION OF ULTRAVIOLET-C LIGHT FOR BEER STERILISATION USING A LOW PRESSURE MERCURY LAMP: A PILOT SCALE TRIAL

4.1 Introduction..... 4.1

4.2 Materials and methods..... 4.3

4.2.1 Chemical synthesis of MBT..... 4.3

4.2.2 Identification of reaction products by GCMS and NMR..... 4.4

4.2.3 Optimisation of the SEP/GCMS technique for MBT analysis..... 4.5

4.2.4 LCMS of riboflavin..... 4.7

4.2.5 The SurePure® pilot scale turbulent flow UV-C system..... 4.8

4.2.5.1 Description of the pilot-scale unit..... 4.8

4.2.5.2 Energy dosage measurement..... 4.9

4.2.5.3 Cleaning procedure of the UV-C system..... 4.11

4.2.6 UV-C processing of commercial beer..... 4.11

4.2.6.1 Microbiological preparation..... 4.11

4.2.6.2 UV-C irradiation of beer and microbiological analysis..... 4.12

4.3 Results and discussion..... 4.13

4.3.1 Synthesis and analysis of the synthetic MBT..... 4.13

4.3.2 Optimisation of GCMS method for MBT determination using SEP technique..... 4.18

4.3.3 Riboflavin determination using LCMS/MS..... 4.20

4.3.4 UV-C processing of beer samples..... 4.22

4.3.4.1 Microbiological analysis..... 4.22

4.3.3.2 MBT analysis in beer by GCMS..... 4.25

4.3.3.3 Riboflavin analysis in beer by LCMS/MS..... 4.28

4.4 Conclusions..... 4.30

4.5 References 4.31

Chapter 5

SENSORY AND BIOCHEMICAL EVALUATION OF ULTRAVIOLET-C TREATED BEERS

5.1 Introduction 5.1

5.2 Materials and method 5.2

5.2.1 Non-commercial beers 5.2

5.2.2 Commercial beers 5.3

5.2.3 UV-C irradiation of beers 5.3

5.2.4 Sensory analyses 5.4

5.2.4.1 Consumer panel evaluation 5.4

5.2.4.2. Consumer trial 5.5

5.2.4.3 Statistical analysis of data 5.5

5.2.5 Chemical analyses 5.6

5.2.5.1 Riboflavin analysis 5.6

5.2.5.2 Assay for Iso-α-acids by LCMS as an indication of LSF 5.6

5.2.5.3 Carbohydrate analysis in beer by HPLC 5.7

5.3 Results and discussion 5.7

5.3.1 Sensory analyses 5.7

5.3.1.1 Non-commercial beers 5.7

5.3.1.2 Commercial beers 5.10

5.3.2 Riboflavin levels in beer 5.12

5.3.3 Iso-α-acids in beer 5.13

5.3.4 Fermentable sugar 5.17

5.4 Conclusions 5.20

5.5 References 5.21

Chapter 6

THE EFFECT OF UV-C IRRADIATION ON BEER HOPPED WITH REDUCED ISO-α- ACIDS HOPS

6.1 Introduction..... 6.1

6.2 Materials and methods..... 6.3

6.2.1 Microbrewing..... 6.3

6.2.2 Beer treatment..... 6.3

6.2.3 Consumer trial..... 6.3

6.2.4 Statistical analysis of data..... 6.4

6.2.5 Descriptive flavour..... 6.4

6.2.6 Chemical analyses..... 6.5

6.3 Results and discussion..... 6.6

6.3.1 Consumer sensory evaluation..... 6.6

6.3.2 Descriptive flavour analysis..... 6.10

6.3.3 Chemical analyses..... 6.11

6.3.3.1 Riboflavin analysis by LCMS/MS..... 6.11

6.3.3.2 THIA analysis by LCMS..... 6.13

6.3.3.3 GC analysis of beer samples..... 6.14

6.4 Conclusions..... 6.15

6.5 References..... 6.17

Chapter 7

INACTIVATION OF MICROORGANISMS IN BEER WITH ULTRAVIOLET LIGHT-EMITTING-DIODES AT WAVELENGTHS OF 250 AND 275 nm

7.1 Introduction..... 7.1

7.2 Materials and methods..... 7.2

7.2.1 Chemicals..... 7.2

7.2.2 Bench scale UV apparatus..... 7.2

7.2.3 Microorganism cultures and growth conditions..... 7.3

7.2.4 UV-LED irradiation..... 7.4

7.2.5 Determination of the inactivation level of E. coli and L. brevis..... 7.5

7.2.6 Chemical analyses..... 7.6

7.3 Results and discussion..... 7.6

7.3.1 Inactivation of E. coli and L. brevis..... 7.6

7.3.2 Comparison of UV-LEDs emitting at 250 nm and 275 nm..... 7.9

7.3.3 Chemical analyses of riboflavin and hops iso-α-acids by LCMS... 7.10

7.4 Conclusions..... 7.13

7.5 References..... 7.13

Chapter 8

GENERAL DISCUSSION..... 8.1

References..... 8.10

ADDENDUM..... A.1

ABBREVIATIONS

%	percentage
°C	degree Celsius
1D	one-dimensional
1 H-NMR	proton nuclear magnetic resonance
3RF*	triple excited states
13 C-NMR	carbon 13 nuclear magnetic resonance
A. pasteurianus	*Acetobacter pasteurianus*
C	carbon atom
CFU	colonies formed per unit
CFU/mL	colonies formed per unit per millimetre
CIP	cleaning in place
CLB	commercial lager beer
CLB+UV	commercial lager beer UV exposed
cm	centimetre
cm/s	centimetre per second
CPB	commercial pilsner beer
CPB+UV	commercial beer exposed to UV light
D	density
DB	non-commercial beer
DB+UV	non-commercial beer exposed to UV light
DHIA	dihydroiso-alpha-acids

DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
ELSD	evaporative light-scattering detector
ESI+	electrospray ionisation positive
eV	electron volt
FAD	flavin-adenine dinucleotide
FID	flame ionisation detector
Fig.	figure
FNM	flavin mononucleotide
FPD	flame photometry detector
FSOT	fused silica open-tubular
GC	gas chromatography
GCMS	gas chromatography mass spectrometry
H	hydrogen atom
HP-5MS	5% phenyl Methyl Siloxane
HPLC	high performance liquid chromatography
HSSE	headspace sorptive extraction
I	intensity
J/cm2	joule per square centimetre
J/L	joule per litre
kPa	kilo Pascal
KV	kilo volt
L. brevis	*Lactobacillus brevis*

LCMSMS	liquid chromatography with tandem mass spectrometry
LR-EIMS	low-resolution EI mass spectrometry
LSF	light struck flavour
MBT	3-methyl-2-butene-1-thiol
MHZ	mega hertz
mg/L	milligram per litre
mg/mL	milligram per millilitre
mL	millilitre
mM	milli Molar
MRS	Rogosa and Sharpe
MSD	mass spectrometry detector
MS	mass spectrometry
m/z	mass over charge ration
n	number
ng/L	nanogram per litre
nm	nanometre
NMR	nuclear magnetic resonance
O	oxygen atom
OD	optical density
OD	olfactometry detector
Pa	Pascal unit
PLT	non-commercial beer with reduced hops
PLT+UV	non-commercial beer exposed to UV light

PL	non-commercial beer
PL+UV	non-commercial beer exposed to UV light
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
R	*rectus* (stereochemistry)
Refl.	Reflux
RPM	revolution per minute
Rt	retention time
S	*sinister* (stereochemistry)
SAB ltd	South African Breweries within South Africa
SCD	sulphur-specific chemiluminescence detector
S. cerevisiae	*Saccharomyces cerevisiae*
SEP/GCMS	sample enrichment probe gas chromatography mass spectrometry
SIM	selective ion monotoring
SP4	UV apparatus with 4 lamps
T	time
THIA	tetrehydroiso-alpha-acids
TIC	total ion count
TREPR	time-resolved electron paramagnetic resonance
UV	ultraviolet
UV-A	long wave ultraviolet (315-400 nm)
UV-B	medium wave ultraviolet (280-315 nm)

UV-C	short wave ultraviolet (200-280 nm)
UV-LED	ultraviolet light emitting diode
W/m2	watt per square metre

CHAPTER 1

INTRODUCTION

1.1 Background

It is generally accepted that beer constitutes an unfavourable growth medium for many micro-organisms due to the presence of ethanol, hop bitter compounds, high carbon dioxide content, low pH, low oxygen tension and the lack of nutritive substances [1-2]. Beer microbial contaminations have, however, been problematic for hundreds of years worldwide in the brewing industry. A limited number of microorganisms have been reported to spoil beer [3-6], impacting negatively, not only on beer quality, but also on the financial gain of the brewing industry. Among these beer spoilage microorganisms are certain Gram-positive bacteria, such as *Lactobacillus brevis,* and anaerobic Gram-negative bacteria such as *Acetobacter pasteurians* [1, 3, 5, 6-8]. In addition, several wild yeasts from the Saccharomyces species and mould can grow in the brewing medium and spoil the beer. Indeed many microbiological safeguards such as filtration, wort boiling and pasteurisation exist within breweries to prevent the microbial spoilage of beer [9]. Despite these safeguards, a number of microorganisms still manage to grow in beer, affecting the flavour and appearance of the beer and resulting in quality loss of the final product [1, 3, 6, 10, 11].

Microbiological control is very important and indispensable in breweries and should be carried out at various points during the brewing process. The most widely used sterilisation technique, to prevent contaminations of finished beer packaged in cans, bottles and kegs, is the traditional thermal pasteurisation technique. However, it was

reported that certain lactic acid bacteria, including *L. brevis* which is one of the major organisms causing beer spoilage, could survive high external thermal treatment [4, 12]. Moreover, severe thermal processing can produce cooked and biscuity flavours, which are detrimental to the quality of the beer [6, 13]. It was shown that free radical reactions occur in beer during pasteurisation [14] and that off-flavours can develop during pasteurisation of bottled beer [15]. Breweries, like most food industries, should be extremely hygienic and frequent sanitisation is required to avoid microbial contamination. Hence, every effort to minimise microbial spoilage and to achieve the highest standards of purity in beer would be of benefit to consumers and brewers alike.

The use of ultraviolet-C (UV-C) light irradiation, at a wavelength of 254 nm, is gaining increased acceptance within the food and beverage industries as an alternative to thermal disinfection [16]. It is a relatively simple, environmentally friendly, economical and reliable technique, lethal to most types of microorganisms [16-19]. The process of UV-C sterilisation can be employed, either as an alternative to, or in conjunction with other methods of sterilisation, including pasteurisation.

In the brewing industry UV-C applications are mostly used for the disinfection of water, reducing the risk of water-borne biological contamination. It is also used in the treatment of caps, cans, and the disinfection of air in packaging areas. In fact breweries have become major users of UV-C light irradiation to disinfect surfaces, air and water during the early stages of beer production [16].

There are, however, some compounds in beer which prevents the indiscriminate use of UV-C for the sterilisation of beer itself. It is well known in the brewing industry that beer is a light sensitive beverage due to the presence of bitter hops compounds [20-

21]. Exposure of beer to light induces a series of photochemical reactions, which ultimately alters the flavour of the beer by generating the so-called light-struck flavour (LSF). The compound found responsible for LSF is 3-methyl-2-butene-1-thiol (MBT) [20]. MBT has received a great deal of attention and has been the subject of research for many years. It is formed in beer *via* riboflavin as a photosensitiser upon irradiation between 280-500 nm and *via* direct absorption of UV radiation by hops iso-α-acids below 260 nm [20-21]. MBT has an extremely low flavour threshold in beer ranging between 1 – 35 ppt (parts per trillion or ng/L) [22, 23]. Even at such extremely low concentrations, MBT adversely affect the beer quality. The main focus of this study was therefore to investigate the possible use of an UV-C irradiation treatment of beer to remove unwanted microbial contaminants, without impeding the quality of the beverage.

1.2 Objectives

The overall objective of this study was to evaluate a novel sterilisation technique that could provide an alternative to the traditional thermal pasteurisation process. The potential use of germicidal UV-C irradiation technology, as an alternative sterilisation technology, was investigated on different kinds of beer with specific reference to the formation of the LSF.

The technique could help to improve microbiological safeguards within breweries, since certain microbial beer spoilage can survive high thermal treatment. Noting the aforementioned challenges, the specific objectives of this research were therefore:

(i) to verify the potential of a pilot scale UV-C system to reduce spoilage microorganisms in beer;

(ii) to explore the potential use of an UV-C disinfection technique on beer and investigate the adverse effects that the technique could have on beer quality;

(iii) to seek correlation between sensory and chemical analyses;

(iv) to further explore the effect of UV-C irradiation on beer using ultraviolet light at two different wavelengths (250 and 275 nm)

1.3 Goals

In order to meet the specific objectives of this study, a number of tasks were undertaken, as summarised below.

1.3.1 Disinfection of beer by UV-C irradiation

A commercial lager beer was spiked with spoilage microorganisms, which included *L. brevis*, *A. pasteurians and S. cerevisiae*, and exposed to UV-C irradiation at dosage values of 25, 50, 100, 250, 500, 1000, 2000 J/L. The beer samples were analysed for standard colony forming units (CFUs) to determine the survival/inactivation of bacteria after UV exposure.

1.3.2 Beer trials

A number of trials were conducted on different styles of commercial and non-commercial beers to investigate the effect of UV-C with specific focus on the development of LSF. Consumer sensory trials were also conducted on these samples and a trained panel performed a descriptive flavour analysis on these beers. In addition, the

formation of LSF in beer was investigated by the sample enrichment probe technique in conjunction with gas chromatography linked mass spectrometry (SEP/GCMS). This data was verified by an investigation of the riboflavin levels in irradiated beer by liquid chromatography followed by tandem mass spectrometry (LCMS/MS). In addition, the measurements of riboflavin and hops iso-α-acids concentrations in beer were done to indirectly quantify the formation of LSF by LCMS/MS.

1.3.3 Bench scale UV-LEDs

A bench scale UV system was developed and built using germicidal UV-LEDs. *Escherichia coli* and *L. brevis* were used as tests organisms to investigate the ability of UV-LEDs to reduce microorganisms in beer at 250 and 275 nm. The effect of the diodes on beer riboflavin and hops isohumulones was investigated by LCMS.

1.4 Layout of the dissertation

(i) **Chapter 1**. *Introduction.*

(ii) **Chapter 2**. *Photochemistry of beer*. This chapter presents an overview of the current literature on the photochemistry of beer en route to the origin of the light-struck flavour, including the photolysis of bitter acid compounds from hops (*Humulus lupulus* L) and the decomposition of riboflavin as a photosensitiser in beer.

(iii) **Chapter 3**. *Ultraviolet irradiation as a non-thermal disinfection technique.* This chapter presents an overview of the use of UV technology for liquid foods treatment. Commercially available UV light sources and

UV reactor designs used for sterilisation purposes are reviewed.

(iv) **Chapter 4**. *Application of ultraviolet-C (UV-C) light for beer sterilisation using a low pressure mercury lamp in a pilot scale trial.* This chapter outlines the potential use of the UV-C light disinfection technique on beer at a wavelength of 254 nm with UV dosage energies ranging from 25 to 2000 J/L. In addition, the formation of the LSF, specifically MBT, was investigated using GCMS while the decomposition of riboflavin was investigated using LCMS/MS.

(v) **Chapter 5**. *Sensory and biochemical evaluation of UV-C treated beers.* This chapter presents sensory data obtained from consumer trials on commercial and non-commercial beers treated with UV-C light. Chemical analyses were carried out to seek correlation with sensory data.

(vi) **Chapter 6**. *The effect of UV-C irradiation on beer hopped with reduced hops iso-α-acids.* This chapter presents sensory data from a consumer trial of a non-commercial UV-C treated pale lager beer brewed with reduced hops. A panel of trained tasters also established a sensory profile. In addition analytical characterisation of both beer samples were performed by LCMS/MS and headspace sorbent extraction (HSSE) GC.

(vii) **Chapter 7**. *Inactivation of microorganisms in beer with ultraviolet light emitting diodes (UV-LEDs) at 250 and 275 nm wavelengths.* The use of LEDs for sterilisation presents some advantages when compared to the germicidal mercury vapour UV lamp. This chapter discusses the possible use of such a technique to reduce microbial loads in beer. *E. coli* and *L.*

brevis were the test microorganisms used in this study to demonstrate the germicidal efficiency of UV-LEDs in beer at 250 and 275 nm. Additionally, riboflavin and hops iso-α-acids were analysed to determine the extent of LSF formation in the treated beer.

(viii) **Chapter 8**. *General discussion*. This chapter presents and discusses an overview of the results obtained in this study. In addition, future perspectives on scale-up UV systems are discussed.

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CHAPTER 2

PHOTOCHEMISTRY OF BEER

2.1 Introduction

Even though beer is the product of a tightly controlled fermentation process, refined over centuries, the composition of this widely consumed beverage is rather complex. The correct and subtle balance of a range of compounds, which is derived from the ingredients and treatments during the brewing process, determines the flavour and character of any given type of beer. These compounds are sensitive to oxidation-reduction reactions, temperature and light [1-3]. To fully appreciate and understand the effect of UV-light on the chemical composition and flavour characteristics of beer, it is therefore necessary to gain an understanding of the basic steps involved in the brewing process as well as the biochemical origin of potential sensitive compounds involved in the determination of the flavour and character of a beer. In this chapter the photochemistry of beer will be discussed against the background of the basic brewing process and the origin of compounds that can influence beer flavour.

2.2 Overview of the brewing process

2.2.1 Beer ingredients

The following main ingredients are used for brewing beer: water, barley malt, hops, and brewing yeast. Water, which constitutes about 90% of beer, must be free from chlorine or other chemicals and should contain some basic minerals and often has to be

sterilized. Water generally has low concentrations of nitrogen-containing ions and iron, manganese, copper and zinc, which are essential to a healthy fermentation [1].

Barley malt is the most common source of the fermentable sugars in beer and is produced from the barley grain [2]. The barley grain is the main cereal used in brewing today, as it is easily malted in comparison to other cereals such as maize, sorghum, rye, and wheat [2]. Hops (*Humulus lupulus* L.), from the hops female plant, are known to be the spicy and bitter counterpart to the barley malt basis of beer. They are considered as a beer herb, also contributing to a stable foam head and providing a measure of bacteriological stability [3]. Brewing yeast is required to produce alcohol and carbon dioxide with a concomitant decrease in sugar levels. Two unicellular species of yeast are commonly used for brewing, namely *Saccharomyces cerevisiae,* and *Saccharomyces pastorianus* [3]. During the production of beer, six major processes take place. These are malting, brewing, fermentation, maturation, packaging and pasteurisation. Figure 2.1 presents an overview of the brewing process of beer.

2.2.2 Malting

The malting process of barley grains involves steeping, germination and kilning. The raw barley is first collected and visually inspected to assess whether the grain is of uniform size, free of weed seeds, broken corns and rodent droppings [1, 4]. During steeping, barley grains are immersed in water to allow the moisture content of the grains to increase. The steep water is perfused with air to create a perfect aerobic environment for the grains to respire. The steeping process is normally completed in approximately two days with the moisture content of the grains reaching 42%.

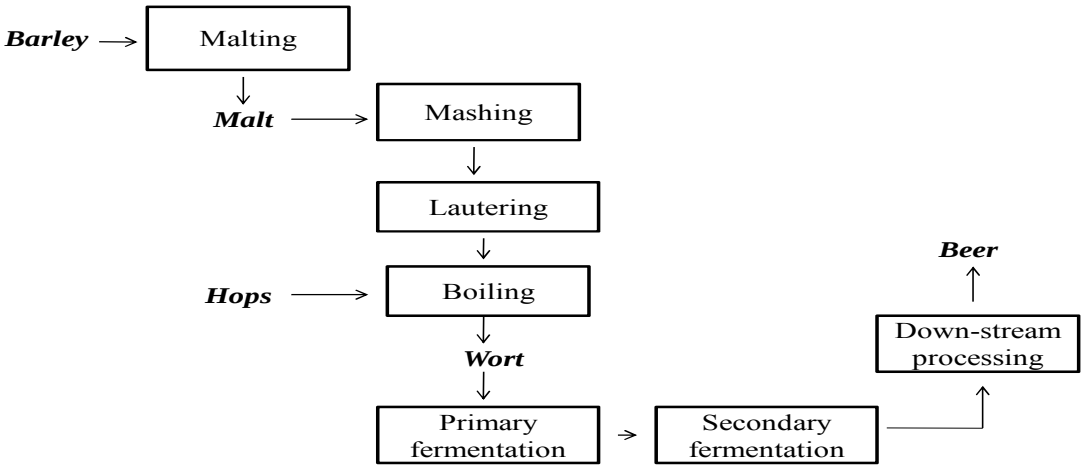


Figure 2.1 Schematic outline of beer brewing process [1].

After steeping, the barley grains go through the germination process for a specific period of time (three to four days), after which it is dried (kiln) for storage [1, 4]. During the germination phase biochemical and physical changes of the grains occur. The germination process is carried out to convert the small and insoluble starch chains from barley grains to water-soluble starches. On the other hand, the vast majority of large starch granules are not solubilised [4]. Hydrolytic enzymes are produced including hemicellulase and the β-glucanases to break down structural polysaccharides found in the cell walls [4]. Moreover protein and fat degradation is also observed throughout the germination process.

The germinated grains are dried out during kilning, a process in which air enters and exits the kiln via heat exchangers to allow drying of the grains. Kilning temperature

and humidity are controlled to prevent inactivation of desired enzymes. After this stage the grains are called malt barley or simply malt.

2.2.3 Brewing of beer

Brewing involves mashing, lautering, boiling, and chilling. First, the process of mashing begins by milling the crushed barley malt and combining it with water to prepare an extract called sweet, green or unboiled wort [1, 5]. During this process, the breakdown of starches, which starts during the malting process, is continued to first produce the disaccharide (maltose) and ultimately to yield monosaccharides (e.g. glucose, fructose, mannose and galactose) [4]. The breakdown of starches is accomplished by several enzymes, which facilitate the extraction of carbohydrates, proteins, amino acids, lipids and polyphenols at specific temperatures in the mash vessel [6]. Second, after mashing follows lautering, a process of separating the sweet wort from the grain fractions of the mash, is started. It is essentially a filtration stage, done in a vessel called a lauter tun. This vessel removes the solids from the liquid unboiled wort. Third, after lautering, the green wort is transferred and boiled in a kettle while hops are added to allow proper mixing and extraction of hop components. The boiling stage is normally required for the following: extracting, isomerising and dissolving the hop α-acids, inactivating enzymes, killing bacteria, fungi and wild yeast and stripping off volatiles [7, 8]. Boiling the wort will also reduce the volume by evaporation and lower the pH of the wort slightly. Finally, after boiling for about 1 hour at 100℃, the wort is cooled down using an immersion or counter-flow system. This process, called chilling, minimises the risk of contamination by *Lactobacillus* or wort-spoilage bacteria [7].

2.2.4 Fermentation and maturation of wort

The cooled wort may undergo a primary fermentation by the yeast in a fermentation vessel. At this point the wort is called immature or green beer. There are factors that need to be taken into consideration for good fermentation performance and beer quality. These include the choice of the yeast strain, the amount of yeast added to the wort, wort condition and pH, the fermentation temperature and pressure, to mention but a few [1, 4]. During the primary fermentation stage, sugars from wort are metabolised into alcohol and carbon dioxide. When the fermentable sugars have been almost completely utilised, the fermentation slows down and the yeast flocculates out at the right time [6]. The maturation process follows the primary fermentation stage.

During the maturation process a secondary fermentation takes place by the remaining yeast. This process, prior to packaging, is critical for the flavour profile of the beer as it is designed to remove some of the more offensive and unwanted flavours (including vicinal diketones) [4]. The maturation process also allows the remaining yeast to settle via the natural yeast flocculation process to the bottom of the fermenter, yielding a clearer product. After the maturation step, the beer is filtered, diluted with water to obtain the correct alcohol concentration and carbonated to specification before packaging.

2.2.5 Packaging and pasteurisation of beer

Packaging involves putting the beer into containers such as bottles, aluminium cans, and kegs. After bottles and cans have been filled with beer and closed, they are pasteurised through tunnel pasteurizers. Beer can also be flash pasteurised before it is packed into kegs [9].

The pasteurisation process is critical for the shelf life of beer as it sterilises the beer from spoilage microorganisms. Beer pasteurisation involves the establishment of minimum times and temperatures required to destroy all expected biological contaminants at the highest concentration that might spoil the beer [1, 4, 10].

2.3 Hop components

Hops are the female cone of the hop plant (*Humulus Lupulus* L) and have been used in brewing since the middle ages [13]. Today hops are an essential ingredient for beer brewing together with water, barley malt and yeast. Most breweries are using hops for its bitter, aromatic, preservative and antiseptic properties [6, 14]. Additionally, hops play an important role in the stability of beer foam and contributes to the microbiological stability of beer [15]. Hop cones are one of the most important commercial ingredients used in brewing. The cones, also called strobilus, consist of stipular bracts and seed-bearing bracteoles attached to a central axis (figure 2.2). The lupulin gland, which contains both the resins and essential oils, is developed at the base of the bracteoles. Hops contain 4-14% polyphenols, mainly phenolic acids, prenylated chalcones, flavonoids, catechins and proanthocyanidins [16, 17]. Hop phenols may represent up to one third of the total phenols in beer. They are present as monomers, dimers, trimers, but also as more complex forms associated with nitrogenous components [11, 18].

Hops can be divided into three groups according to its flavour and aroma characteristics. There are aroma hops, bitter hops, and dual purpose hops. Each of these groups of hops contributes in a different way to the character of the beer. Aroma hops are generally lower in resin content (3.0-7.5%), but contributes desirable flavour and aroma

characteristics. Bitter hops contains about 6.0-13% of resin, but their flavour and aroma characteristics are considered to be less refined (0.5-2.0% of essential oils) [14]. Dual-purpose hops are higher in resin content and contribute desirable flavour and aroma characteristics.

The brewing value of hops is mainly found in its essential oils and resins [10]. The resins and essential oils represent approximately 15 and 0.5% of hops mass, respectively (Table 2.1) [10, 14].

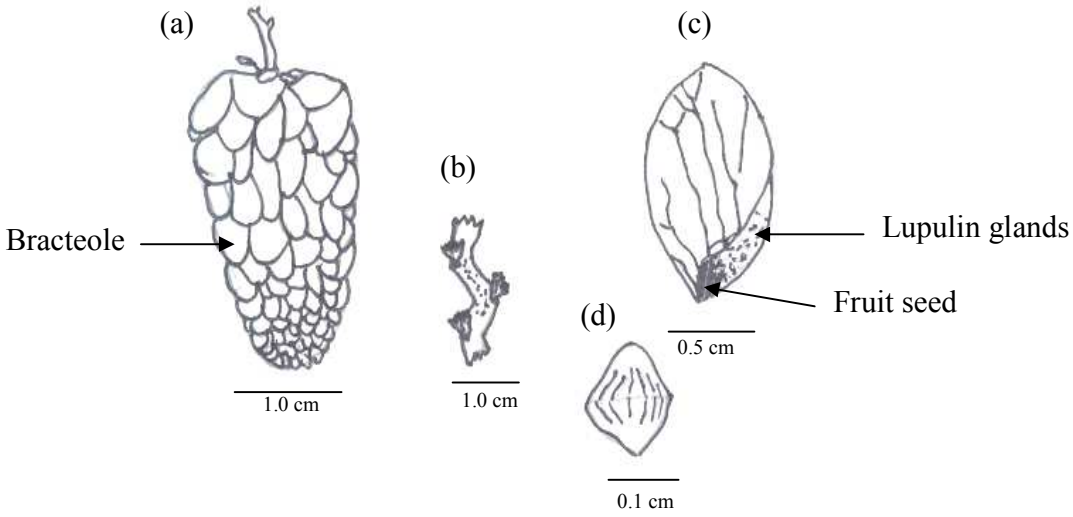


Figure 2.2 Hops plant (*Humulus lupulus* L.) inflorescence. (a) Part of the strig or axis of the female cone, (b) single mature hop cone, (c) bracteole with seed and lupulin glands, and (d) lupulin gland (reproduced from [2 and 5]).

Table 2.1 Chemical compositions of hops (*Humulus lupulus* L). (Collated from [10 and 19])

Hop constituents	**Percent**
Water	10
Total resin	15
Essential oils	0.5
Tannins	4
Monosaccharides	2
Pectin	2
Amino acids	0.1
Proteins	15
Ash	8
Cellulose and other polysaccharides	43.4
Total	**100**

2.3.1 Hop essential oils

The essential oils from hops contribute significantly to beer flavour and aroma although they account for only 0.1-0.5% of the weight of hop cones [1]. These oils are a complex mixture of components widely spread throughout the plant kingdom [20]. More than 250 chemical compounds appearing in beer have been identified and traced in the essential oils of hops [21, 22]. The major classes of essential oils consist of the oxygen-free hydrocarbon fraction (containing only H and C), the oxygenated hydrocarbon (containing H, C, and O), and small amounts of sulphur containing compounds. About

80-90% of the mass of essential oils is typically made up of oxygen free hydrocarbons [23, 24].

The key members of oxygen free hydrocarbons group are terpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$), which are found in largest quantities. Among these key members are three sub-members, which include myrcene (terpenes) humulene and caryophyllene (sequiterpenes). The three sub-members play important roles as key components of hop flavour and aroma [21-25].

Myrcene (figure 2.3) is an aliphatic terpene, which constitutes the largest component of hop essential oils. This compound is frequently characterised as pungent because of its unpleasant and harsh odour which is distinct from that of the essential oils [20, 21]. Depending on the hop variety, myrcene can account for 20 to 65% of the portion of the total essential oils [8]. Myrcene is generally present in lower quantities in aroma hops than in bitter hops.

Humulene and caryophyllene are the two more abundant cyclic compounds of hydrocarbon sequiterpenes and can be easily oxidised in air (figure 2.3) [22]. Unlike myrcene, humulene is found mainly in aroma hops, whereas it is less abundant in bitter hops [21]. Caryophyllene is found in many different plants including cloves *(Caryophyllus aromaticus)* [22]. Humulene and caryophyllene rarely survive in their native form when boiled with the wort [21]. Moreover, they can react with oxygen during storage and boiling to form oxygenated hydrocarbons [21].

One of the most important characteristics of hop essential oils is the ratio of humulene/caryophyllene content. Most hop varieties, used for their aroma in the beer brewery, have high humulene/caryophyllene ratios (H/C) [26].

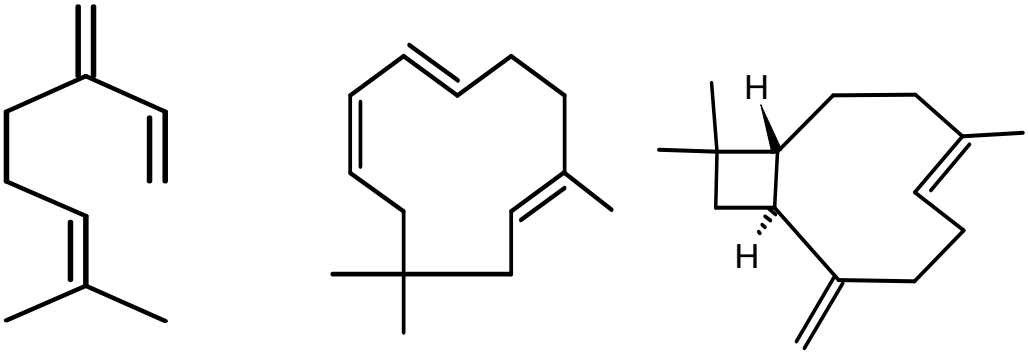


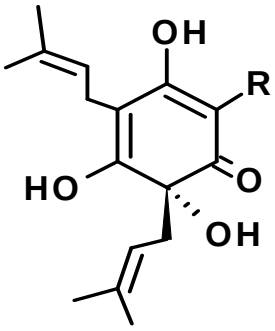
Figure 2.3 Chemical structures of the three most abundant constituents in the essential oils of hops (reproduced from [24]).

2.3.2 Hop resins

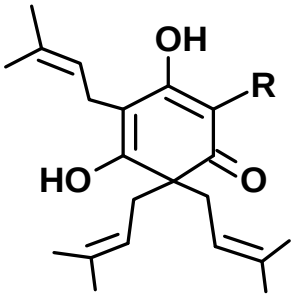
Hop resins are found in the lupulin gland of the hop plant (*humulus lupulus* L.) (refer to figure 2.2). It was shown that the hop resins strongly inhibit the growth of Gram-positive bacteria because of the interference of the prenyl group with the function of the bacterial cell plasma membrane [11]. Therefore, the more prenyl groups present, the stronger the bacteriostatic action. In general hop resins are insoluble in cold wort but soluble in hot wort and their solubility is influenced by pH [2]. Hop resins are mainly subdivided into soft and hard resins. The soft resins are the fraction of the total resins which is soluble in hexane, and the hard resins are the fraction of total resins which is insoluble in hexane [2, 10].

The soft resins can be categorised further as alpha and beta acids. These compounds, also known as hop acids, occur as pale yellowish solids in their pure state [11]. The chemical structures of the alpha and beta acids are presented in table 2.2.

Table 2.2 α-Acids and β-acids of hop with their respective analogues [1, 25].



α-acids



β-acids

Acyl side chain (R)	α-acids	Formula	m.p.	pKa	β-acids	Formula	m.p
COCH2CH(CH3)2 (Isovaleryl)	Humulone	$C_{21}H_{30}O_5$	64.5ºC	5.5	Lupulone	$C_{26}H_{38}O_4$	92ºC
COCH(CH3)2 (Isobutyryl)	Cohumulone	$C_{20}H_{28}O_5$	oil	4.7	Colupulone	$C_{25}H_{36}O_4$	93-94ºC
COCH(CH3)CH2CH3 (2-methylbutyryl)	Adhumulone	$C_{21}H_{30}O_5$	oil	5.7	Adlupulone	$C_{26}H_{38}O_4$	82-83ºC
COCH2CH3 (Propionyl)	Posthumulone	$C_{19}H_{26}O_5$	na	na	Postlupulone	$C_{27}H_{34}O_4$	101ºC
COCH2CH2CH(CH3)2 (4-methylpentanyol)	Prehumulone	$C_{22}H_{32}O_5$	na	na	Prelupulone	$C_{27}H_{40}O_4$	91ºC

2.3.3 Alpha acids

Alpha acids are regarded as the most important class of the hop acids (3 to 15% of total weight) in beer. These compounds impart more bitterness to the beer [11]. The alpha acids are complex mixtures representing a group of acyl-substituted phloroglucinols with an enolic proton in the phloroglucinol ring [27]. They consist of several analogous compounds such as, humulone, cohumulone, adhumulone, prehumulone, and posthumulone (refer to table 2.2) [25].

Although the proportion of each alpha acid analogue in the mixture varies according to the hop variety, humulone is the most easily accessible alpha acid. It can be isolated from the mixture by repeated crystallisation with o-phenylenediamine [25]. Additionally, it was reported that humulone has various biological activities, such as inhibiting angiogenesis and bone resorption [28].

Alpha acids do not have a bitter taste, but the bitterness in beer arises from their isomers. During the boiling step of wort, the alpha acids are isomerised into iso-alpha acids. In fact, the alpha acids are isomerised in an equilibrium mixture of trans-iso-alpha acids (trans-isohumulones, trans-isocohumulone, and trans-isoadhumulone) and cis-iso-alpha acids (cis-isohumulone, cis-isocohumulone, and cis-isoadhumulone). All six isomers are present in beer and do differ significantly with respect to their bitterness [19]. The isomerisation of alpha acids depends on the spatial arrangement of the tertiary alcohol function of the alpha acids at C(4) and its prenyl side chain at C(5) in a ratio of 3:7 [11, 29, 30].

The *cis*-isomers of the alpha acids are much more stable than the trans-isomers and subsequently may affect the *cis:trans* ratio over time [11]. The iso-alpha-acids beer

are intensely bitter with a taste threshold value of about 15 ppm (parts per million or mg/L) in typical American lager beers, 100 ppm in extremely bitter English ale and 6 ppm in water [11].

2.3.4 Beta acids

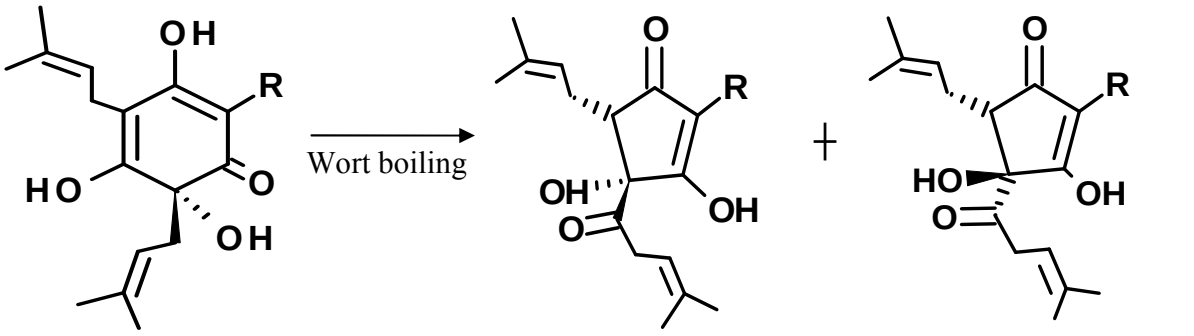
Beta acids form complex mixtures in hops. They consist of five closely related compounds namely lupulone, colupulone, adlupulone, prelupulone and postlupulone (refer to table 2.2). Most hops, grown in Europe, contain about equal amounts of lupulone and colupulone, although the solubility of colupulone is somewhat lower than that of lupulone [25]. Moreover, beta acids are considerably less soluble than alpha acids, and are very sensitive to oxidative decomposition [2, 11].

2.3.5 Iso-alpha-acids (isohumulones)

The most important essential bitter compounds from hops (*Humulus lupulus* L) are iso-alpha-acids [11, 22, 31]. These compounds are extremely bitter-tasting when compared to quinine with a threshold value of 5 ppm [12]. As mentioned earlier, the isomerisation of alpha-acids produces a group of six isomers and homologues including cis-trans epimers (referred to as isohumulones). The *cis*- and *trans*-isohumulone do differ significantly with respect to their bitterness [19, 32]. Based on comparison using impure cis-isohumulone, it seems that the *trans*-isohumulone is less bitter than the *cis* form [25]. It was reported that over a wide pH range, isomerisation of alpha-acids can produce different equilibrium mixtures of *cis*- and *trans*-isohumulone [32]. In aqueous alkaline

isomerisation the *cis:trans* ratio is 7:3 and it is 5:5 via a magnesium catalysed reaction [32].

The absolute configuration of the *cis*-and *trans*-isohumulones were derived by De Keukeleire and Verzele [29, 33]. The *cis*-series has a (4*R*,5*S*)-configuration and the trans-series has a (4*S*,5*S*)-configuration (figure 2.4). The rate of isomerisation of alpha-acids to isohumulones during kettle boiling was found to follow first order kinetics, varying as a function of time and temperature [34]. Beer contains a total concentration of isohumulones ranging from 10 to 100 mg/L [12, 25].



Alpha-acids *cis*-iso-alpha-acids *trans*-iso-alpha-acids

Figure 2.4 Isomerisation reaction of alpha-acids during wort boiling. Reproduced from [11].

2.4 The Light-struck flavour of beer

2.4.1 History of the study of light-struck flavour of beer

The LSF is an unpleasant "skunk-like" aroma formed when beer is exposed to light thus reducing the commercial value of beer and causing complaints from customers. This "off flavour" has been widely studied since it was first mentioned in 1875 by Lintner

[35, 36]. In 1941, it was shown that the light-struck flavour is the result of a photochemical reaction with the production of low molecular weight sulfydryl compounds [37]. Six years later the effective light range for light-struck character formation was given as 420-520 nm by Jacobssen & Hogberg [38]. It was subsequently found that humulone and lupulone, present in hops were involved in the formation of the light-struck flavour.

Obata *et al.* [39] presumed that the light-struck flavour might be a mercaptan compound. They found, by quantitative determination, that a volatile mercaptan was formed on exposing beer to sunlight [39, 40]. Furthermore, they showed that the formation of this mercaptan involved the presence of a prenyl group, therefore leading to a prenyl mercaptan group responsible for the light-struck flavour. Additionally, experiments were carried out to understand the mechanism of prenyl mercaptan formation in beer [39, 40]. The 3-methyl-2-butenyl group of humulones and lupulones was shown to be split off at position 3 by a photochemical reaction [39]. Subsequently, the 3-methyl-2-butenyl radical reacts with sulphur-containing compounds having a thiol group present in the fermented solution to form the light-struck character [39, 40].

According to Kuroiwa & Hasimoto [41, 42], the LSF was also found to be a mercaptan derivative formed photochemically in the absence of humulone, but in the presence of isohumulone degradation products, such as a 3-methyl-2-butenyl radical [39] and hydrogen sulphide. Moreover, they suggested that three factors are essential for the formation of light-struck flavour in beer. Those factors include the presence of isohumulone, fermented wort and visible light between the wavelengths of 400 to 500 nm

[43]. They also found that riboflavin (vitamin B2) might be involved as a photochemical sensitizer in the formation of the LSF [42].

Kuwoira and collaborators [44] showed the presence of 3-methyl-2-butene-1-thiol (MBT) in sun struck beer as the light-struck character by thin layer chromatography. In 1978 Gunst and Verzele [45] confirmed that the LSF is due to the formation of MBT formed by photolysis of iso-alpha-acids in the presence of sulphur-containing amino acids. They used a direct head space gas chromatography analysis and flame photometric detection to identify MBT in beer [45]. An overview of the formation of the LSF of beer is presented in figure 2.5.

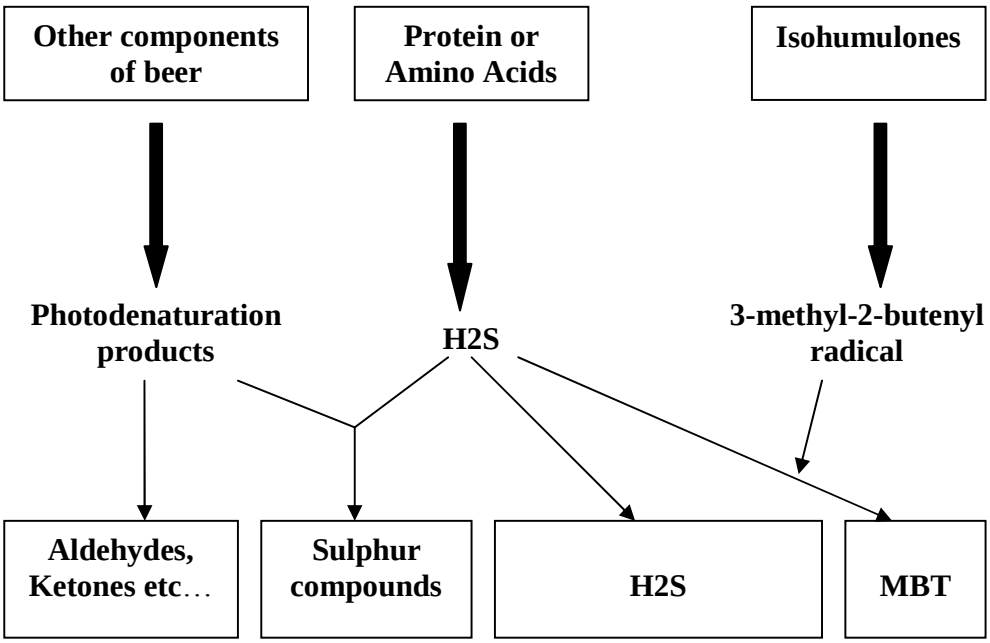


Figure 2.5 Schematic pathways for the formation of LSF in beer. Reproduced from [42]

2.4.2 Mechanism of MBT formation in beer

The mechanism of formation of MBT involves the presence of a light source, hop bitter alpha-acids (isohumulones), flavins (riboflavin) sulphur compounds [36]. These elements have been reported to be pivotal in the formation of light-struck flavour in beer. An overview of the mechanism of formation of 3-methyl-2-butene-1-thiol in the presence of riboflavin, and a sulphur source is presented in figure 2.6.

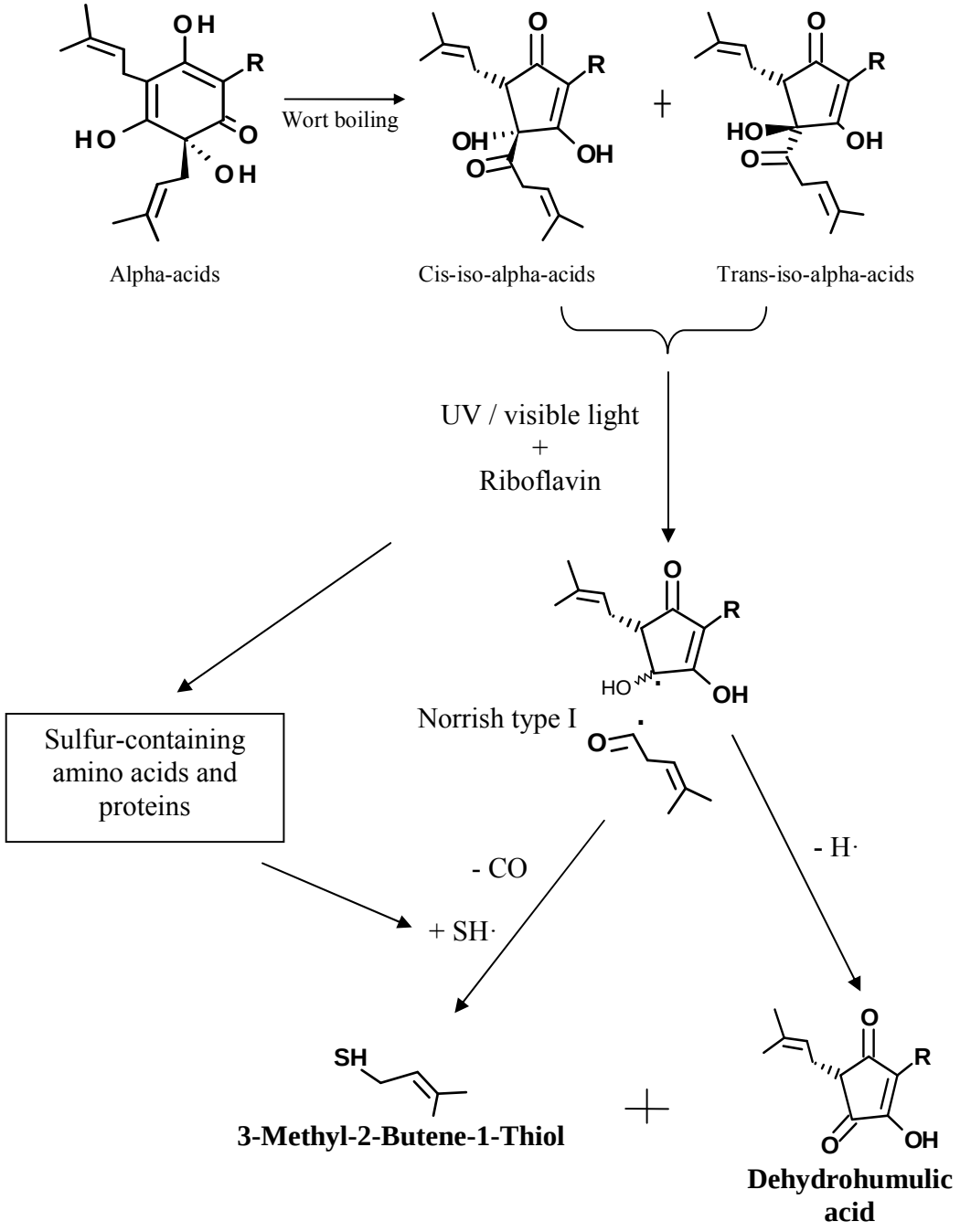


Figure 2.6 Overview of the mechanism of light-struck formation in beer. Reproduced from [11].

2.4.2.1 Light source and iso-alpha-acids hops

It was previously believed that blue, violet and near ultraviolet light was the most effective in causing the LSF [41]. However, Kuroiwa *et al.* [44] showed that the region of light prone to promoting the LSF was in the 350-500 nm region of the spectrum. In fact the light sensitive chromophore in the iso-alpha-acids is the acyloin group composed of the tertiary alcohol function and the carbonyl group of the side chain at C4 [11].

In a recent study, De Kekeuleire *et al.* [46] used time-resolved electron paramagnetic resonance (TREPR) spectroscopy to fully elucidate the mechanism of the LSF formation in beer. They reported that following absorption of UV-B light (280-320 nm), iso-alpha-acids primarily undergo an absorption and formation of the triplet state of its delocalised beta-triketo chromophore, followed by intramolecular energy transfer to the localised alpha-hydroxyketone moiety [46, 47]. This photochemistry leads to the formation of free radicals through a Norrish type I alpha-cleavage, which correlates with Kuroiwa and Hashimoto's postulated mechanism [42, 46]. However, iso-alpha-acids do not absorb light in the most efficient region (350-500 nm) for generating the LSF, which means a photosensitiser must play a role [44, 46-48]. Riboflavin has been identified as a photosensitiser and its presence in beer will subsequently be discussed.

2.4.2.2 Riboflavin and sulphur source in beer

Riboflavin (vitamin B_2) in beer originates from the malt, hop and yeast [49]. It has for a long time been implicated in the formation of the LSF of beer under visible light (350-500 nm) [36, 42, 47, 50-53]. It was suggested that riboflavin may be involved as a photosensitiser in the formation of the LSF in beer (figure 2.7), although the detailed

chemical mechanisms and kinetics are not fully understood [44]. Further mechanistic insights demonstrated that, under irradiation with light of wavelengths from 350 nm up to 500 nm, riboflavin is excited to its triplet state (3RF*) as it exhibits two adsorption bands with maxima at 375 and 446 nm [51]. This activated triplet state can subsequently transfer energy to iso-alpha-acids [51, 52]. However, this mechanism involving energy transfer from riboflavin was shown to be thermodynamically unfeasible since the triplet energy of iso-alpha-acids is higher than that of riboflavin [47].

A laser photolysis experiment indicated that an electron is released from iso-alpha-acids on interaction with 3RF*, resulting in the formation of possible MBT radical precursors [51]. Additionally, spectrophotometric measurement of riboflavin colour loss in light-exposed beer was shown to be a potential tool to determine the extent of LSF formation in beer [54]. Riboflavin and derivatives, including flavin mononucleotide (FNM) and flavin-adenine dinucleotide (FAD), are present in beer in concentrations of few hundreds of ppb and have similar fluorescence properties [49]. Studies have shown that they are indispensable for the formation of MBT from iso-alpha-acids and sulphur-containing amino acids or proteins [55]. Sakuma *et al.* [55] confirmed that, when beer is exposed to light, the formation of MBT was greatly accelerated by riboflavin in the presence of iso-alpha-acids. Figure 2.7 shows a proposed role of riboflavin in aldehyde formation (related to stale flavour) and the LSF formation in presence of oxygen, thiol and iso-alpha-acids [53].

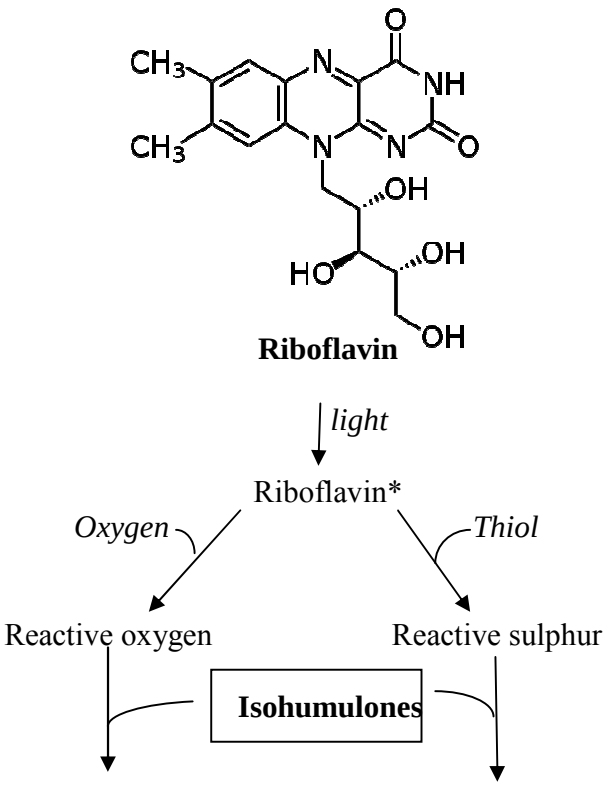


Figure 2.7 Proposed role of riboflavin and hops isohumulone in stale flavour and LSF formation in beer (reproduced from [53]).

A sulphur source is required in the formation of MBT since iso-alpha-acids do not contain sulphur atoms. Riboflavin may also transfer energy to sulphur compounds such as cysteine, cystine and glutathion. Cysteine and cystine have been implicated, by sensory analysis, in the formation of MBT in a model system [44]. Moreover, Sakuma *et al.* [55] used a model system containing iso-alpha-acids and cysteine to show that MBT concentration increased linearly with an increase in riboflavin concentration. However, details on the reaction mechanism were not disclosed until Huevaere *et al.* [52] finally

showed that flavin-induced (riboflavin and derivatives) photoproduction of sulfhydryl radicals are decisive in the formation of the LSF in beer. They reported that sulphur-containing amino acids and proteins are prone to photooxidation in the presence of riboflavin under visible light [52].

2.5 Control of the LSF formation in beer

2.5.1 Packaging technology

The impact of light on beer flavour can be controlled through packaging technology, through the use of chemically modified hop bitter acids, and the use of antioxidants. Packaging technology is essentially a way of preventing light to interact with the beer. This includes the use of brown or dark amber bottles. The technology also includes the use of thicker glass in bottles, bigger labels and paper wrap on bottles, and packaging in cans.

Brown or dark bottles and thicker glass reduce light transmittance. Sakuma *et al.* [55] showed that the formation of MBT increased proportionally with the ability of the bottle to transmit light between 350 and 500 nm. Brown or dark bottles were shown to minimise light ingress in the 350-500 nm region [55, 56]. Beers stored in green or clear bottles have been shown to be more sensitive to light than beer stored in brown or dark bottles [36].

In addition beer makers prefer green bottles or brown bottles since they may be more attractive to consumers [36]. It was also shown that bottles, made of green glass dosed with nickel oxide, may reduce light transmittance since nickel oxide absorbs visible light in 450-500 nm region [36].

Bigger labels and paper wraps on bottles protect a greater area of the bottle and minimise beer exposure to light in the bottle. Packaging in cans, lightproof containers, protect beer from light and prevent the formation of the LSF.

2.5.2 Chemically modified hop bitter iso-alpha-acids

Chemical modification of hop bitter iso-alpha-acids, to prevent MBT formation in beer, aims to render the beer itself lightproof rather than shield it from light. This is currently achieved on an industrial scale in the domain of hop technology aimed at controlling, not only the light stability of beer, but also the degree of bitterness and desired foam features [12]. Iso-alpha-acids hops can be chemically modified by hydrogenating unsaturated side-chains using hydrogen gas in the presence of a palladium catalyst, or using sodium borohydride as a reducing agent [11, 12, 36]. These chemical manipulations of iso-alpha-acids hops produce reduced derivatives, which include dihydro-iso-alpha-acids (DHIA) and tetrahydro-iso-alpha-acids (THIA), the two reduced compounds most prevalent on the market. The chemically modified iso-alpha-acids are shown in figure 2.8.

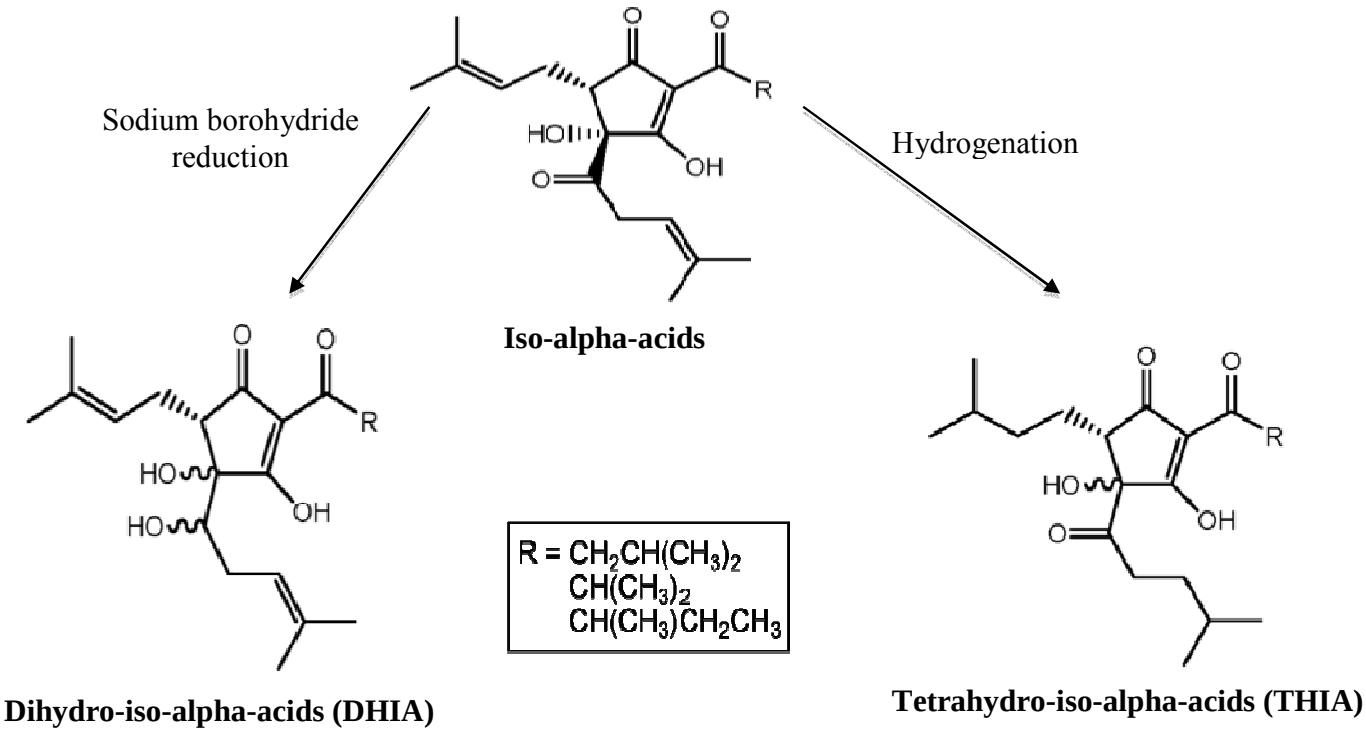


Figure 2.8 Reduction of iso-alpha acids to yield DHIA and THIA (reproduced from [11]).

DHIA hops are obtained by reduction of the carbonyl group in the side chain at C4 of isohumulone. These hops are light stable since the light-sensitive acyloin group has been converted to a diol (figure 2.8) [11]. However, experiments showed that DHIA hops have small residual light sensitivity, hence are susceptible to develop the LSF to some degree [57, 58].

THIA hops are obtained by hydrogenation of the double bonds in the side chain at C4 and C5 of iso-alpha-acids. They can be found commercially as a mixture of six THIAs. It is believed that since the acyloin group is still present in the structure of THIA,

photochemical reactions can occur on light exposure [11]. Yet, beers bittered with THIA do not develop LSF due to the fact there are no double bonds in the side chain to activate radical cleavage and hence promote light-struck formation (figure 2.8) [36].

Overall, these so-called advanced hops products can be used in breweries to bitter beer and to prevent light damage. Additionally, they are believed to enhance beer foam stability relative to their parent hop acids [57], especially the THIA, which has been suggested to give the most stable foam [58]. Moreover, it was shown that THIA are twice as bitter as iso-alpha-acids hops and it has a strong impact on the stability of the head on a glass of beer [12]. These chemically modified hops acids are, however, significantly more expensive than their parent iso-alpha-acids.

Other methods such as the use of antioxidants[36], riboflavin-binding proteins[1] or quenching of the excited triplet state of the iso-alpha-acids and/or riboflavin [12] can be used on an industrial scale to prevent the LSF formation.

2.6 Measurement and significance of the LSF

2.6.1 Properties of the MBT

MBT, also known as prenyl mercaptan, is one of the most powerful taste and flavour active compounds known with a flavour threshold in beer ranging between 1–35 ppt (parts per trillion or ng/L) [11, 36, 50]. When pure and concentrated, it is a liquid with a structure containing a thiol group, an unsaturated carbon-carbon double bond and it is a weak acid (table 2.3).

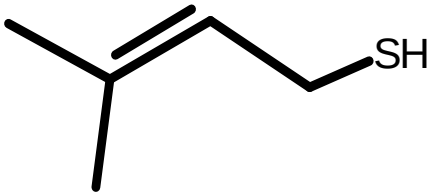
[1] Beer and similar light sensitive beverages with increased flavour stability and process for producing them: European patent application EP0879878

2.5.2 Instrumental and sensory analysis of MBT

The characterisation of components responsible for the LSF formation in beer, including MBT, generally involves two distinct phases [42, 45, 59-61]. The first phase is to assess the presence of the LSF by a sensory test of trained and experimental panellists. However, it was reported that using human assessors has some disadvantages [53, 62-64]. First of all, differences in the use of flavour terminology and in the scoring scale between panels can lead to inconsistent sensory analysis [63].

Table 2.3 Summary of the properties of MBT. The chemical structure of MBT is given on the right (reproduced from [36, 65]).

Property	Description
Concentrated pure liquid:	**Pungent, leek-like**
Diluted (ca 0.1 mg/l)::::	**Foxy, skunky**
Flavour threshold in water	**0.2-0.4 ng/l**
Flavour threshold in beer:	**1-35 ng/l**
Molecular weight	**102.2**
Boiling point:	**123-126 °C**



3-methyl-2-butene-1-thiol (MBT)

Moreover, it was pointed out that panellists can quickly become saturated [62, 64], which reduces the number of assessments that could be made by the same panel. Hughes *et al.* [62] showed that the background levels of other sulphur volatiles in beer, and the formation of varying levels of hydrogen sulphide, can affect the sensory perception of MBT levels.

The second phase is achieved by instrumental analyses. A range of instrumental techniques have been used to measure MBT, or secondary products of the LSF, including riboflavin [54]. Indeed the sensory threshold of MBT in beer was first reported by Kuroiwa and Hashimoto [42] to be within the 50-3200 ppt range. They applied gas-liquid chromatography with a flame ionisation detector (FID) to determine and quantify the presence of MBT in beer. However, as the difference in retention time between alcohol and MBT was too small (one minute), a detection of the small peak of MBT was impossible due to the interference by alcohol [42, 44]. Gunst and Verzele [45] used a direct headspace gas chromatography analysis with flame photometric detection (FPD) to measure MBT by first concentrating the sample solution. In 1993, Goldstein *et al.* [65] developed an analytical procedure to quantitatively determine MBT below its sensory threshold. These authors detected and quantified the LSF in beer at levels below 1 ppt, which was much less than the 50 ppt flavour threshold previously reported [42]. This increase in sensitivity was achieved by optimisation of the purge, trap and desorption technique [59] in which the beer headspace is purged, using an inert gas such as nitrogen. The volatiles are collected, concentrated on the trap packed with an adsorbent material and then adsorbed on to a gas chromatography column. The volatile compounds are then detected with a sulphur-specific chemiluminescence detector (SCD) [62]. Alternatively a mass spectrometric detector (MSD) [66], a flame ionization or photometry detector (FID/ FPD) [58], or olfactometry (OD) [67] can be used for detection.

Additionally, LSF can be explored in beer by the measurement of products involved in the formation of the off flavour (e.g. riboflavin) [49, 52-54]. Duyvis *et al.* [49] developed a method for the quantitative determination of riboflavin levels in beer by

fluorometric apoprotein titration. Poddrik *et al.* [54] investigated the formation of MBT in lager beer by measuring the disappearance of riboflavin using a spectrophotometric method.

2.6 Concluding remarks

Hops are vital to the bitter taste and flavour of beer. However, hops can be photo decomposed by visible and/or ultraviolet (UV) light, furnishing radicals on the route to the formation of the LSF. The LSF development in beer is generally attributed to the presence of MBT, which has an extremely low flavour threshold. In reference to the preceding discussion, it is clear that the presence and properties of MBT could present a considerable challenge in the application of UV-C disinfection technology during the beer making process.

In the following chapter, an overview of UV-C light technology, as a non-thermal disinfection strategy, as well as a potential alternative to the use of traditional thermal pasteurisation in food and beverage industries will be given.

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CHAPTER 3

ULTRAVIOLET IRRADIATION AS A NON-THERMAL DISINFECTION TECHNIQUE

3.1 Introduction

The initial use of ultra violet irradiation (UV-C light) as a sterilisation technique was in the field of water purification [1-3]. At present UV-C disinfection is of growing interest in the food and beverage industry for inactivating food borne pathogenic or spoilage microorganisms [4, 5]. UV-C disinfection technology is performed at low temperatures and is extremely effective in terms of energy consumption. Moreover, it is considered to be environmentally friendly with no chemical input or storage of water needed when compared to the traditional heat treatment pasteurisation technique [6, 7]. It is one of the simplest environmental ways to destroy a broad range of food spoilage microorganisms without forming disinfection by-products [4].

There have been numerous reports showing the efficacy of UV-C irradiation for the reduction of most microorganisms across a wide spectrum of applications including air sanitation in food processing, surface sterilisation, and sterilisation of certain liquid foods such as milk and fruit juices [1, 8-13]. UV-C irradiation can maintain the quality attributes of food products, while ensuring microbiological safety and stability [14-15]. As a non-thermal disinfection technique UV-C irradiation can be used as a primary method for inactivation of microbes, or as a backup for other treatment methods such as carbon filtration, reverse osmosis or pasteurisation.

Although UV-C irradiation is now a well-established and accepted disinfection

technique, the present chapter provides a broad review about the mechanism of UV-C light action and its major practical applications within the food and beverage industry. Consideration will be given to the source of UV radiation, antimicrobial effect, potential applications, and UV reactors suitable for liquid foods processing.

3.2 Nature and source of UV light for sterilisation

UV light forms part of the electromagnetic spectrum from 100-400 nm, which lies between visible light and X-rays (figure 3.1). It is categorised as long wave UV-A (315-400 nm), medium wave UV-B (280-315 nm), short wave UV-C (200-280 nm) and vacuum UV (100-200 nm) [1, 3]. Radiations from UV light is responsible for tanning in human skin (UV-A), causes skin burning and skin cancer (UV-B) and is responsible for the inactivation of most microorganisms (UV-C) [5]. Radiation from UV light is termed "non-ionisation radiation" in contrast to "ionisation radiation", e.g. X-rays. The absorption of UV light radiation leads to electronic excitation of atoms and molecules [5].

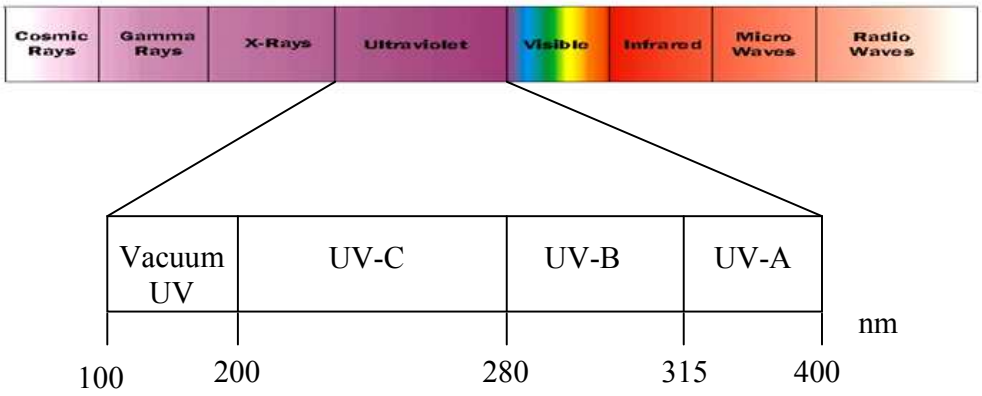

Figure 3.1 Subdivision of the UV light from the electromagnetic spectrum [1].

UV light can be generated by solar as well as artificial sources. The artificial sources of UV light for industrial use include excimer lamps, broadband-pulsed lamps, microwave UV lamps, UV light emitting diodes (UV-LED) and mercury vapour lamps. These sources produce the radiation that creates UV-C light and are commercially available. Mercury vapour lamps are reliable sources and the most widely used for most disinfection treatment. They are designed to generate energy within the germicidal region with an intense maximum energy output at a wavelength of 254 nm and a less intense output at 185 nm. Mercury vapour lamps generate 85-95 % of the total UV light energy at a wavelength of 254 nm [1].

The intensity of UV light radiation can be expressed as a "dose" of UV energy, which corresponds to the amount of UV energy applied to a particular surface over time. UV dose energy can be expressed in watt per square meter (W/m^2) or expressed in joules per litre (J/l). The propagation of UV light through liquid food is influenced by absorption, reflection, refraction, and scattering [5]. These physical phenomena can influence the effectiveness of UV dosage energy reaching microorganisms present in the liquid under treatment.

3.3 Effect of UV-C on microbial agents

UV-C light is considered to be an effective disinfectant due to the fact that it causes permanent inactivation of most micro-organisms such as bacteria, yeasts, moulds, and viruses associated with food and beverage [3]. The most effective wavelengths for direct inactivation of most microorganisms lie in the region of the electromagnetic spectrum between 250 and 270 nm (figure 3.2). The biological effects of such

wavelengths on microorganisms reside at both molecular and cellular levels. Although the maximum germicidal effect is produced at 260 nm, the commercially available UV lamps emitting at a wavelength of 254 nm are lethal to most microorganisms at molecular level.

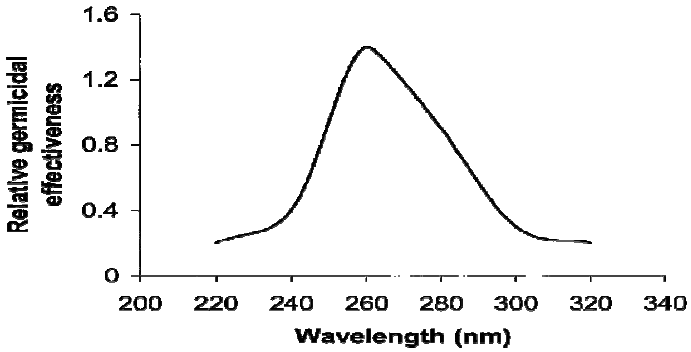

Figure 3.2 Graph of the germicidal region of the UV-C light energy emission at a maximum wavelength of 260 nm (reproduced from [3]).

Microbial DNA absorbs UV-C light maximally at a wavelength of 260 nm due to the presence of pyrimidine and purine bases. This wavelength is close to 254 nm, the maximum energy emission of the mercury UV lamp. The interaction of UV-C light and microbial DNA is complex. There is a series of photochemical reactions that damage specific target molecules of any given microorganism [1]. The primary lethal effect of UV-C light irradiation, at molecular level is, however, a direct alteration of most microbial nuclear DNA through dimerisation of pyrimidine and purine bases, to such an extent that cell division can no longer occur [1]. The main types of photoproduct, i.e. nitrogen base dimmers, in UV-C irradiated DNA contain at least one pyrimidine base. These photoproducts typically include pyrimidine dimers (cyclobutyl-type dimers), pyrimidine adducts (spore photoproducts), and pyrimidine-(6-4)-photoproducts (figure 3.3) [1].

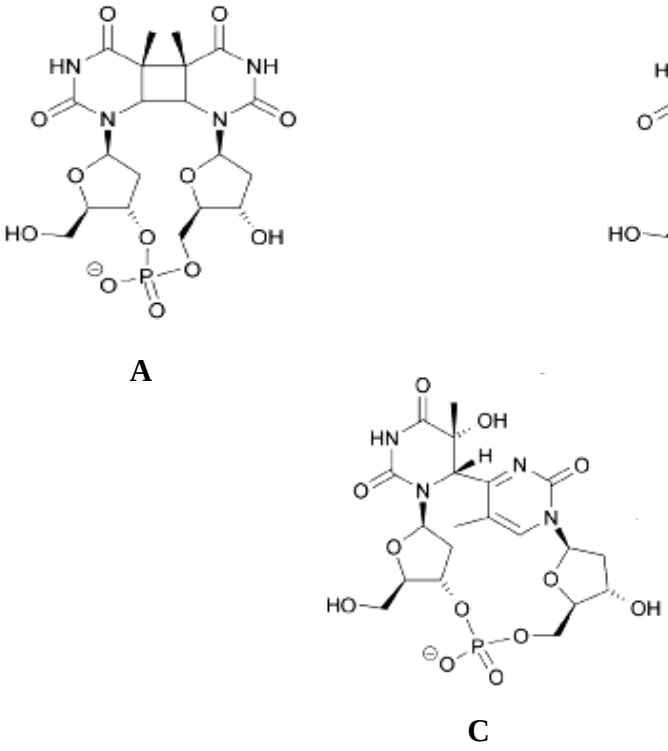

Figure 3.3 Chemical structures of the main UV light induced DNA photoproducts. **A;** cyclobutyl-type dimmers, **B;** pyrimidine adducts and, **C;** pyrimidine-(6-4)-photoproducts.

The efficacy of UV-C light is strongly related to the growth and the physiological state of a microorganism. Factors such as cell wall structure, pigmentation, and stages of bacterial growth cycles, influence the sensitivity of microorganisms to UV-C light irradiation [1, 5, 16]. Even the ability of an organism to repair UV-irradiated DNA can affect the effectiveness of UV-C light irradiation. The UV-C sensitivity of fungi, viruses, yeast, and bacteria is illustrated in figure 3.4. Fungi and viruses appear to be more resistant to UV-C light irradiation than yeast and bacteria. However, in liquid food and beverage processes, viruses may not be of great concern. Spoilage microorganisms, including bacteria, are more susceptible to UV-C light irradiation, although Gram-

positive bacteria are more resistant to UV-C light than Gram-negative bacteria. The conditions under which bacteria are UV irradiated play an important role in the disinfection of liquid food and beverage. The penetration of UV-C into liquid, other than water, is usually low due to the presence of dissolved and suspended particles.

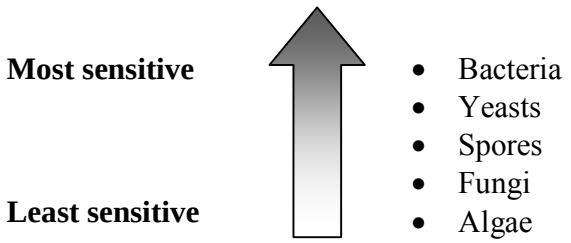

Figure 3.4 UV-C light sensitivity of various microorganisms. Bacteria are more sensitive to UV-C light than most other microorganisms [3].

3.4 Practical applications of UV-C light irradiation

3.4.1 Purification of air, surfaces and water

Disinfection by means of UV-C irradiation is well established, mostly in reducing microbial loads in air, surfaces, clean water and waste water. The use of UV-C light has been successful in reducing the spread of microorganisms in air through buildings including, hospitals, laboratories, food processing facilities, and other locations [1, 3]. For example, in hospitals, UV-C light has been used to decrease the number of air-borne bacteria in operating theatres. Similarly, UV-C treatment of air in the peeling units of fruit and vegetable process plants improves the microbiological quality of air. The concentration of air born bacteria varies directly with the supply of fresh air in a specific enclosed environment. Methods for UV-C irradiation of air are developed according to

the area to be treated. Most of these methods include the use of UV-C lamps to create a curtain or barrier of radiations through which air must pass before reaching a room [3]. In food processing, a system that combines a laminar flow of air and the use of UV-C light has been suggested for the provision of clean sterilised air in the working place [3]. The simplest method for reducing air-borne organisms is by placing germicidal UV lamps on walls slightly above eye level.

UV-C light can also be used to inhibit microorganisms on surfaces in the food industry, including bakeries, fruit and vegetable, and meat plants. However, UV-C light has very low penetrability into solid materials compared to air and water. Yet, UV-C has been used to sterilise packaging materials such as containers, wrappers, bottle caps, bottles, tubes and cartons [16]. Indeed, the efficacy of UV-C on surfaces depends on the material surfaces being clean. The surface must be smooth and free from any dirt, which would absorb the radiations, hence shadowing the efficacy of UV-C on microbes. Alternatively, UV-C light irradiation has the potential to reduce microbial contamination on food surfaces such as meat products and fresh fish [4], vegetables and fruit [18] as well as liquid egg products [14].

UV-C light irradiation is one of the simplest and most environmentally friendly ways of destroying microorganisms in wastewater, drinking water, and swimming pools [19-20]. In fact, the most successful application of UV-C light has been in the field of water purification. The transmittance of UV-C light through high purity water (e.g. drinking water) is high compared to other liquids, which makes water an ideal medium for UV-C irradiation. UV-C light is broadly effective against all waterborne pathogens [13] and can be used to disinfect the water used to rinse or wash process equipment and

work surfaces. It has been reported that UV-C light irradiation has high efficacy against *Cryptosporidium* and *Giardia,* which are the major pathogenic microorganisms in drinking water [13]. The increased information on the formation of hazardous oxidation by-products during chemical disinfection of water has now led to UV-C light irradiation gaining greater acceptance in the field of water purification.

3.4.2 UV sterilisation of liquid foods and beverages

Industrial sterilisation using UV-C light irradiation is finding increased usage in several liquid food and beverage processes. Several studies have shown promising results on the use of UV-C light irradiation for the reduction of microbial contamination in a wide range of liquid food and beverages [4, 12-13, 18, 20, 21]. Unlike water several factors, such as the presence of nutrients, sugar, and vitamins can influence the delivery of the UV-C light dose within liquid food. Moreover, it is worth noting that UV-C light can impact on the physical, chemical and sensory quality of the liquid food treated. Liquid food contains vitamins, some of which are light sensitive (e.g. riboflavin). Several published works showed that, to ensure maximum reduction of microbial load in liquid food and beverages, without affecting their physical, chemical and sensory quality, optimisation of the UV irradiation parameters is essential [11, 22-24]. For example with fruit juices, Caminiti *et al.* [15] showed that an insignificant change in colour and pH was observed in UV-C light treated apple juice at energy dosages ranging from 2.66 to 53.10 J/cm^2. Goat milk, which has been exposed to direct UV-C, sometimes develops off-flavours [23]. It has been suggested that this detrimental effect can be reduced by filtering-out the short UV wavelengths [4]. It is apparent that the correct or optimal

wavelength for a given liquid type can be found within the UV-C spectrum, which would result in maximum bacteriostatic activity and minimum chemical changes.

3.4.3 Application of UV-C light in the brewing industry

UV-C light irradiation technology, as a non-thermal disinfection technique, is gaining increased acceptance within the brewing industry [6]. Its applications are mostly disinfection of the water used in breweries, which reduces the risk of water-borne biological contamination [4]. It is also used in the treatment of caps, cans, and air in packaging areas. To the best of our knowledge, UV-C light irradiation of beer itself has not been achieved on a commercial scale, largely due to the fact that beer is extremely sensitive to light. The optimisation of a non-detrimental UV-C treatment for beer would significantly reduce the energy requirement, and subsequently the carbon footprint, of the beer brewing industry worldwide.

3.5 Reactor designs for UV irradiation of liquid foods and beverages

A number of UV-C reactors, of varying complexity, for disinfection were developed and validated for a variety of liquid foods and beverages. These UV-C reactors can be installed at various points along a process system within liquid food and beverage industries. The principle of most of the designed UV-C reactors is that the liquid flow goes through the annular space between a germicidal UV-C lamp, enclosed in a quartz protection sleeve, and a cylindrical outer jacket. Moreover, opaque liquids with high UV absorbance can be treated by presenting them in a form of a laminar thin film [10]. The degree of UV-C light penetration and the rate of liquid flow through a specific UV-C

light reactor are factors of extreme importance. Indeed, through the use of a correct UV-C reactor design, the interference of high UV-C absorbance and the turbidity, associated with some liquid food and beverage products, can be reduced. Koutchma *et al.* [10] overcame the interference of absorbance and viscosity associated with liquid by using an extremely thin film to decrease the path length of the UV-C light through fresh apple cider. Moreover, Lu *et al.* [25] successfully tested a novel thin-film UV apparatus with quartz optical fibres to inactivate bacteria in beer.

There are two main continuous flow UV-C reactor designs that have been evaluated for use of liquid food and beverage. These reactors are laminar flow reactors and turbulent flow reactors. Tanton *et al.* [22] developed an example of a laminar flow UV-C reactor called "CiderSure®" for the treatment of apple cider. The "CiderSure®" UV reactor design consists of low-pressure mercury lamps mounted within a quartz sleeve running centrally through three individual chambers (figure 3.5). The three individual chambers are connected in tandem with external tubing. It was shown that laminar flow UV reactors produce significant amounts of heat that cause fouling compared to turbulent flow UV reactors. Moreover, Singh and Ghaly [24] showed that the destruction efficiency of thin film laminar UV reactors decreased with an increase in flow rate during cheese whey treatment.

Turbulent flow UV reactors allow all substances in a given liquid to be exposed to UV-C radiation due to better mixing of liquid within the UV reactors. The SurePure® SP4 UV system (Milnerton, South Africa) is an example of a turbulent flow reactor system. The "SurePure®" SP4 UV system typically consists of stainless steel inlet and outlet chambers and 4 corrugated spiral tubes between the chambers (figure 3.5). Treatments

are achieved by passing the liquid between the corrugated spiral tubes and the quartz sleeve. Each spiral tube houses a low-pressure mercury UV lamp protected by a quartz sleeve. In 2007, Keyser *et al.* [11] successfully used the "SurePure®" turbulent UV reactors to treat apple juice, guava-and-pineapple juice, and mango nectar, two different orange and tropical juices. Due to its "turbulator" technology that tumbles liquids over a UV-C light source, the SurePure® UV system can also effectively sterilise turbid liquids [11]. In contrast to laminar flow UV reactors, in turbulent flow UV reactors the higher the flow rate, the higher the inactivation rates [17]. Overall, UV technology has been successfully used to inactivate bacteria in water [12, 13, 20], apple cider [17], liquid egg products [14], fruit juice [11, 15, 17, 26] and milk [9, 27].

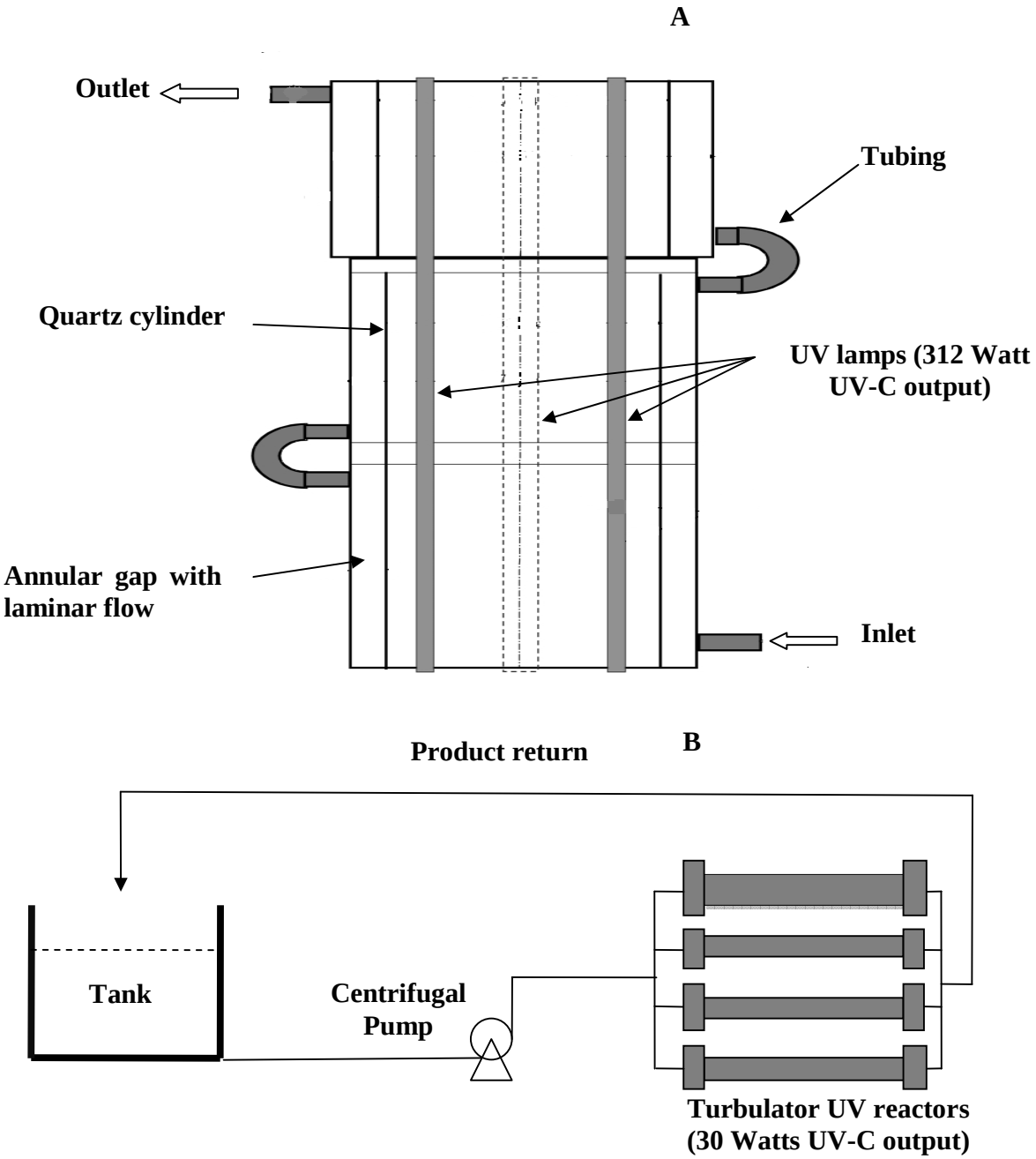

Figure 3.5 Schematic drawing of two reactor designs for UV irradiation of liquid food. **(A)** "CiderSure®" laminar thin film (reproduced from [10]), **(B)** 'SurePure®" turbulent UV system containing 4 germicidal low mercury pressure UV lamps.

3.6 Concluding remarks

UV-C light irradiation has changed the way sterilisation is being performed in food processing. Based on sufficient published data, UV-C light irradiation was shown to be an efficient method for eliminating or reducing the levels of most food borne and spoilage microorganisms. UV-C light irradiation technology could be a valid non-thermal alternative to thermal pasteurisation in liquid foods and beverages. The success of UV-C light sterilisation technology depends on the following factors that are found to consistently affect its efficacy: absorbance changes and UV-C transmittance through the liquid system, the correct UV reactor design, UV lamp, flow pattern and flow rate. The potential use of UV-C light as a non-thermal disinfection method for beer, using a "SurePure®" turbulator UV system, is presented and discussed in the following chapter.

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CHAPTER 4

THE APPLICATION OF ULTRAVIOLET-C LIGHT FOR BEER STERILISATION USING A LOW PRESSURE MERCURY LAMP: A PILOT SCALE TRIAL

4.1 Introduction

The application of UV-C light irradiation, as a non-thermal disinfection technique, is gaining acceptance in the food and beverage industry worldwide [1-4]. This type of irradiation can also be a reliable means for the inactivation of organisms responsible for beer microbial spoilage. The advantage associated with the use of UV-C light irradiation is the low energy consumption during treatment, when compared to thermal pasteurisation, the most popular and best understood disinfection method in the brewing process of beer. Several studies have shown that pasteurisation may affect the quality of beer [5-6] and several beer spoilage bacteria can resist heat treatment [7-8].

Different pasteurisation techniques, such as non-thermal UV-C light irradiation, may represent a viable alternative to the limitations posed by thermal pasteurisation. To the best of our knowledge, no previous studies have been carried out to investigate the microbiological and chemical effects of UV-C light irradiation of beer. Beer is a complex beverage in which it was shown that a number of photochemical reactions could occur when irradiated with light in the UV-spectrum [9-16]. As described in Chapter 3, the most common and well-known photochemical reaction in beer is the photolysis of iso-α-acids hops, which leads to the formation of the LSF, also called the “skunky” flavour, of beer. The LSF is principally attributed to the formation of MBT. The LSF of beer has

been widely studied since it was first highlighted in 1875 [15]. The formation of LSF in beer can be explored directly and indirectly. The direct method entails the detection and quantification of MBT in beer. However, its analysis in beer presents a serious challenge, even to modern instrumentation. This is due to the extreme low flavour threshold of MBT, and the extremely low levels required to adversely affecting the quality of beer [12, 14, 15, 17-21]. Thus, the demand on any instrumental analytical technique to measure MBT accurately is always considerable.

There are reports of the analyses of MBT using gas chromatography (GC) coupled with different detectors such as a sulphur-specific chemiluminescence detector (SCD) [20], a mass spectrometric detector (MSD) [21], a flame ionization or photometry detector (FID/FPD) [17, 19]. The sensitivity of such analytical techniques has been increased by the use of a "purge, trap and desorption technique" (PTD) [17, 19-21]. During the PTD technique, the beer headspace is purged with an inert gas (e.g nitrogen) to concentrate and trap the volatiles on an absorbent material such as tenax TA/GR [17, 21]. The trap, packed with an absorbent material, is then desorbed on to a GC column. Detection and quantification of MBT using such a system has shown that detection limits of 3, 2, and 1 ppt (or ng/L) could be obtained using SCD [20], FPD [17], and MSD [21], respectively. However, the use of such trap requires important adaptation to the GC with thermal desorption equipment [17, 21]. Burger *et al*. [26] have developed a similar purge, trap and desorption method, called the sample enrichment probe (SEP) technique, using a probe coated with polydimethylsiloxane (PDMS) rubber sleeve as an absorbent material, to analyse organic compounds in headspace and aqueous solutions. According to Burger *et al.* [26], using the SEP technique with a GCMS requires only minor inexpensive

adaptations to the GC and does not requires any thermal desorption equipment. The technique is similar to, but differs from, the solid-phase microextraction (SEPM) in that a much larger volume of beer can be sampled [26]. The SEP method has been adapted and optimised for the determination of MBT in beer in this study (see figure 4.1).

The indirect method of exploring LSF in beer entails the measurement of secondary products involved in the formation of LSF (e.g. riboflavin) [22-25]. It is well known that riboflavin plays a pivotal role in the formation of LSF in beer as this natural vitamin acts as a photosensitising agent [13]. The concentration of riboflavin in beer varies according to beer types and brands. MBT is not formed in the absence of riboflavin [22], therefore measurement of riboflavin could potentially be a reliable means of exploring LSF formation in beer.

The objectives of this study were first to optimise analytical tools to explore LSF in irradiated beer, using GCMS and LCMS/MS and second, to determine the potential effectiveness of a pilot scale turbulent UV system against *Saccharomyces cereviciae*, *Lactobacillus brevis* and *Acetobacter pasteurianus* inoculated in commercial lager beer. Formation of LSF in UV treated beer samples was investigated by the analysis of MBT by GCMS and by the measurement of riboflavin concentration by LCMS/MS.

4.2 Materials and Methods

4.2.1 Chemical synthesis of MBT

As MBT is not available commercially, it had to be synthesised. A mixture of prenyl bromide (10g, 67.1 mmol), thiourea (5.1g, 67.1 mmol), (Sigma-Aldrich, Germany) and 34 ml of 95% ethanol (*v/v* in water) was stirred under reflux for three hours in a 250

mL three-neck, round-bottomed flask fitted with a condenser. The condenser was connected to two gas wash bottles connected in series. Both wash bottles contained a bleach solution of sodium hypochlorite 3.5% (*m/v*) (obtained from a local market). A very low argon flow was maintained through the system. After three hours of reflux, the solution was allowed to cool to room temperature and subsequently alkalized by the drop wise addition of 40 mL of a sodium hydroxide solution (0.1 g/mL) (Sigma-Aldrich, Germany) over a period of five minutes. The mixture was stirred under reflux for a further two hours, allowed to cool to room temperature, acidified with 2M sulphuric acid (0.79 ml) (Saarchem, South Africa) and subsequently extracted with hexane (3×10 mL). The combined organic layers, containing the crude thiol as well as other reaction by-products, were washed with water (2×10 ml), dried over sodium sulphate (Saarchem, South Africa), and filtered. The solvent was removed by distillation through a 14/23-cm Vigreux column, and 2.56 g crude thiol was obtained. No further attempt was made to purify the crude thiol due to the extreme odour involved in handling this compound. Stock solutions of the thiol were separately prepared in deionised water, dichloromethane and methanol and stored at 4ºC. The product was stable under these conditions for several weeks.

4.2.2 Identification of reaction products by GCMS and NMR

The crude thiol preparation was analysed by gas chromatography-mass spectrometry (GCMS) and nuclear magnetic resonance spectroscopy (NMR). For GCMS, 1 µL of the thiol solution was analysed with an Agilent 5975 GC mass selective detector (MSD). The inlet injector was maintained at 250 ºC in split mode 20:1, with helium (6.7

mL/min) as a carrier gas. A 5% phenyl methyl siloxane (HP-5MS, 30 m x 0.25 mm x 0.25 µm, film thickness) column was used at a pressure of 21.8 kPa. The initial oven temperature of 40 ºC (held constant for 5 minutes) was programmed to rise to 290 ºC at 10 ºC/minute. The final temperature was then held constant for five minutes for a total run time of 30 minutes. The mass spectrometer was operated in positive electron impact mode, with electron energy of 70 eV. The scanning range was between 35 and 400 *m/z*.

For NMR analyses, a concentration of 20 mg/mL of the thiol sample was prepared in deuterated dichloromethane (CD_2Cl_2) and submitted to a Varian Unity INOVA 600 MHZ NMR spectrometer for analysis. 1D ^{1}H- and ^{13}C- NMR experiments were carried to characterise the synthetic MBT. Data acquisition was done using the VnmrJ 2.1B® software.

4.2.3 Optimisation of the SEP/GCMS technique for MBT analysis

The analysis of MBT at extremely low concentration presents a serious challenge by direct injection without first concentrating the sample. The SEP technique was used for high capacity sample enrichment of MBT in beer. The synthesised MBT standard was used to optimise the SEP/GCMS technique. The conditions for sampling MBT were examined in both water and beer to improve the sensitivity of analysis. The sample volatiles were concentrated and trapped at 0 ºC on a water bath for up to five hours using a 100 mL capped bottle of the type described by Burger *et al.* [26]. After sampling, the SEP was introduced into the split/splitless injector of the gas chromatograph (GC) where the analytes were thermally desorbed for subsequent analysis (figure 4.1). The rounded end of the probe was inserted into a narrow glass tube after each analysis. This was done

to restrict contact of the adsorbent sleeve with laboratory air. After it had cooled down to room temperature the probe was again ready for sample enrichment.

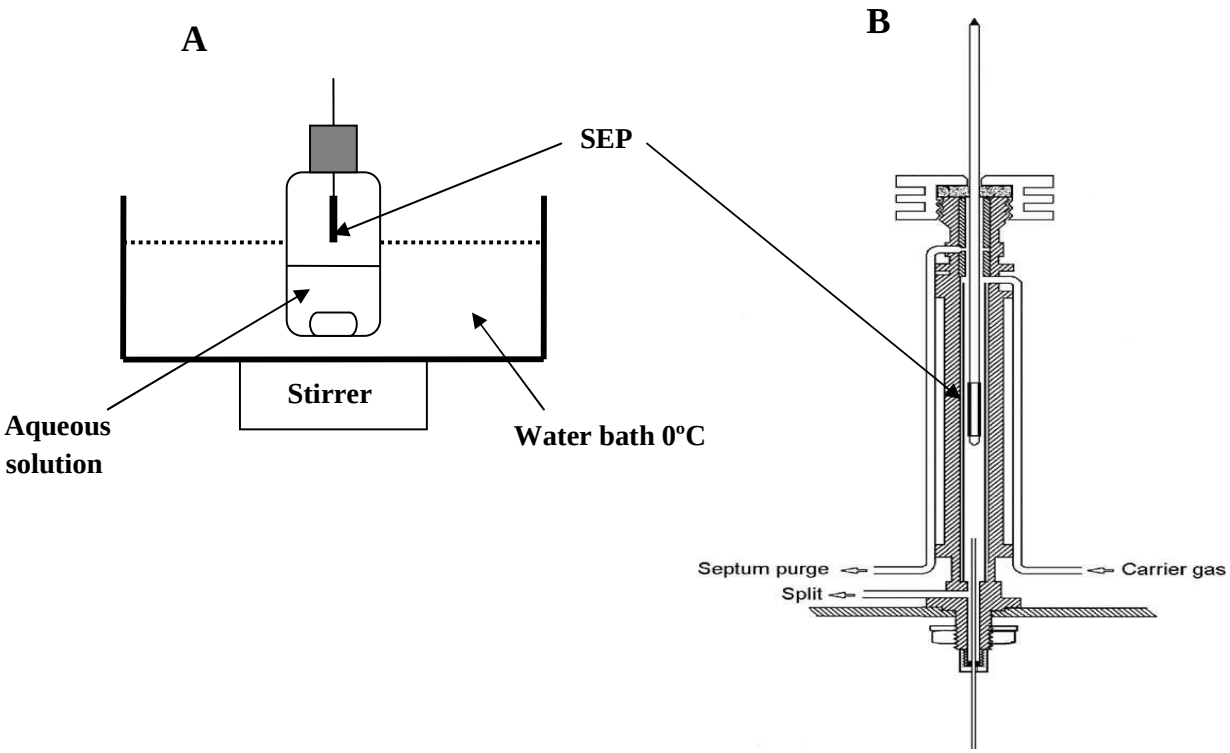

Figure 4.1 Diagrammatic representation of the SEP technique. **(A)** Trapping the MBT from an aqueous solution or beer. **(B)** SEP installed in a standard split/splitless injector of a GC. Reproduced from [26]

Gas chromatography analyses were carried out on a Thermo Finnigan TRACE GC 2000 instrument fitted with a split/splitless injector. The septum-supporting insert of the split/splitless injector of the GC was enlarged to the SEP diameter size using a 2.4 mm drill bit. The SEP was installed in the GC injector directly from the bottle sampling headspace as rapidly as possible with the carrier gas of the GC switched off. This was done to avoid possible evaporation of volatile compounds. Analytes were separated on a

fused silica open-tubular (FSOT) column (33 m x 0.32 mm I.D.), coated with 1.2 um PS-255 (100% polydimethylsiloxane).

The programming rate of the GC was 31ºC (2 minutes) to 250ºC at 4ºC/minute. Analyses were carried out at constant pressure of 40 kPa on the column. Low-resolution electron impact (EI) mass spectrometry (LR-EIMS) was performed on a TRACE GC-MS instrument system using the GC column and conditions specified above. Mass spectra were recorded at 70 eV in scan mode from *m/z* 40 to 120. Helium was used as carrier gas at a linear velocity of 32.25 cm/s at 40ºC. The structure of the MBT was confirmed by means of data obtained from NIST mass spectra search program data reference library.

4.2.4 LCMS of riboflavin

Riboflavin standard (>98% purity) was obtained from Sigma Aldrich (Steinhein, Germany). A standard solution of riboflavin (500ppb or 500µg/L) was freshly prepared in deionised water in the absence of light and was stored in dark glass bottles. The standard solution was used to set up the LCMS instrument. A Waters API Quattro Micro 2695 LCMS with an electrospray ionisation mass spectrometric detector was used to analyse riboflavin in beer. Samples were centrifuged on a Heraeus Biofuge *fresco* at 2000 x g for five minutes at room temperature and filtered through a Millipore (Millex-HV) 0.45 µm pore size filter prior to LCMS/MS analysis. Analyses were carried out on an Xbridge C_{18}, 2.1x 50 mm column with a mobile phase consisting of 0.1% formic acid (solvent A) and acetonitrile (solvent B). A three minute gradient, from 5% to 100% acetonitrile, was used at a flow rate of 0.4 mL/minute. The mass spectrometer was set as follows: capillary

voltage at 3.5 KV, cone voltage 15/25 RF1:40, source 80 ºC, desolvation temperature 350 ºC, desolvation gas: 350 L/h and cone gas 50 L/h.

4.2.5 The SurePure® pilot scale turbulent flow UV-C system

4.2.5.1 Description of the pilot-scale unit

The pilot scale unit was designed and manufactured by Surepure®, Milnerton, South Africa. It consisted of a centrifugal pump with a monitor, a 50 L container and a reactor system equipped with two low-pressure mercury UV lamps in series. The reactor system consisted of stainless steel inlet and outlet chambers with a stainless steel corrugated spiral tube (40 mm in diameter) between the chambers. The spiral tube contained an UV germicidal lamp (15 mm in diameter, 30 W UV-C output), protected by a quartz sleeve (24.5 mm in diameter). According to the manufacturer, the two UV lamps are both 5 mm away from the outer surface of the quartz sleeve with an effective area (As) of UV-C of 661.93 cm². The liquid that required treatment was circulated through the annulus (volume of 0.675 L). The annulus is the area between the corrugated spiral tube and the quartz sleeve (figure 4.2). During each trial, the feed tank was enclosed to avoid contact with external light.

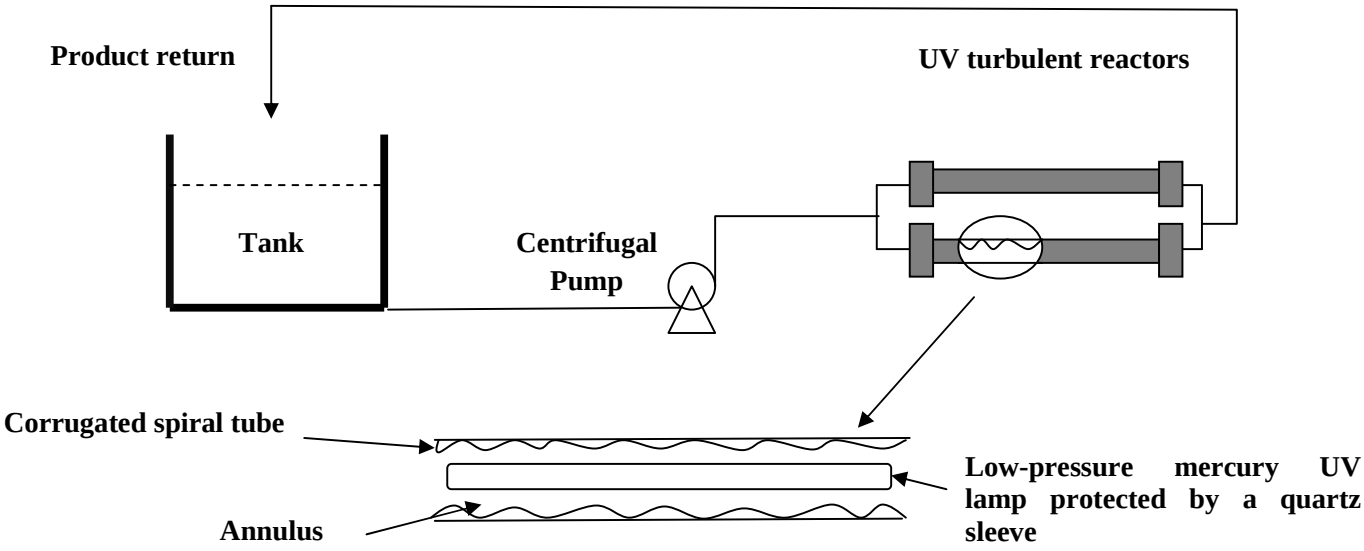

Figure 4.2 Schematic drawing of the Surepure® pilot-scale UV-C turbulent flow system.

4.2.5.2 Energy dosage measurement

The UV dosage (D) can be measured per area or per volume. The dosage (D) per area is the product of the intensity of the light or irradiance (I) and the time of irradiation (T). It is expressed as watts per second per square centimetre ($W.s^{-1}$ cm^{-2}) or joules per square centimetre ($J.cm^{-2}$). The irradiance (I) and time (T) are generally expressed as watts per square centimetre ($W.cm^{-2}$) and seconds (s), respectively. The dosage (D) per area can be calculated by ignoring the volume of the annulus and the type of liquid treated. The total UV energy transmission rate (total UV-C output) to the constant surface of the quartz sleeve (As) from the lamp is 25.5 watts according to the manufacturer (personal communication). This total UV transmission and a flow rate of 4000 L/h are used to calculate the intensity (I) and the retention time (T), respectively as follows:

Intensity (I) = Total UV-C output per unit (W) / Lamp surface area (cm^2)

= 25.5 W / 661.93 cm^2

= 0.039 $W.cm^2$

= 38.5 $mW.cm^2$

Retention time (T) = Volume of annulus (L) / Flow rate (L/h)

= 0.675 L / 4000 L/h

= 0.675 L / 1.111 L/s

= 0.608 s

Hence, the dosage (D) per surface area, at a specified flow rate of 4000 L/h, for one lamp with continuous flow will be:

Dosage (D) = Intensity (I) x Time (T)

= 38.5 $mW.cm^{-2}$ x 0.608 sec

= 23.408 $mW.sec/cm^2$

= 23.408 mJ/cm^2

Unlike the dosage per surface area, the UV dosage per volume is expressed as joules per litres (J/L). It is determined as the total UV output transmission over a specified flow (i.e. 4000 L/h). The UV dosage (D) per litre of liquid treated for one lamp with continuous flow is therefore calculated as follows:

Dosage = Total UV-C output per unit (W) / Flow rate (L/s)

= 25.5 W / 1.11 L/s

= 22.95 W sec/L

= 22.95 J/L

The value of 22.95 J/L corresponds to the UV dosage delivered to a liquid being treated after one pass at a flow rate of 4000 L/h. The time for the same liquid treated to pass through the system once depends on its total volume. For the UV processing of the beer in this study, the dosage was determined in joules per litre.

The run time for each corresponding dosage value was calculated as follows:

Dosage (J/L) = Total UV-C output per unit (W) / Flow rate (L/s)

= [Total UV-C output per unit (W) x Time (s)] / Volume (L)

Thus, the time will be

Time (s) = [Dosage (J/L) x Volume (L)] / Total UV-C output per unit (W)

The value of the total UV-C output per unit is 25.5 Watts. Two units were used to treat the beer and the total UV-C output therefore was 51 Watts. The volume of beer treated was 25 L. The run time for each dosage value is presented below in table 4.1.

Table 4.1 Run time corresponding to each UV dose energy for the treatment of beer at flow rate of 4000 L/h.

Dose per volume (J/L)	Run time (min) for 25 L of beer using the Surepure™ turbulator UV system
0	0
25	0.2
50	0.4
100	0.8
250	2
500	4
1000	8
2000	16

4.2.5.3 Cleaning procedure of the UV-C system

A peracetic acid disinfectant (Perasan, Johson Diversey, SA) was used a detergent. The UV system was cleaned before and after each trial. First, the UV apparatus was rinsed with warm water for about 10 minutes followed by the circulation of an alkaline detergent solution (1%) for 15 minutes. The system was subsequently rinsed with warm water for about 5 minutes and then rinsed with cold water for 10 minutes. The cleaning process was carried out with all the UV lamps burning to ensure the effective maximum output of the UV lamps before trials commenced.

4.2.6 UV-C processing of commercial beer

4.2.6.1 Microbiological preparation

A limited number of micro-organisms has been reported to spoil beer [27], impacting negatively not only on beer quality but also on financial gain of the brewing industries. Among these beer spoilage micro-organisms, Gram-positive bacteria *L. brevis* have been shown to be one the most spoilage microorganism in the brewery industry [27]. In addition, wild yeast *S. cerevisiae* can grow and spoil beer. Cultures of *S. cerevisiae* (ATCC 9763), *L. brevis* (ATCC 27305) and *A. pasteurianus* (ATCC 23752) microorganisms were grown up separately in different broth media. Eight to ten granules of a lyophilised *S. cerevisiae* culture were transferred in 250 mL yeast and malt extract broth (0.05% (*m/v*) yeast extract, 0.7 % (*m/v*) malt extract, 0.1% (*m/v*) peptone,) and incubated at 23 ºC in a shaking incubator at 150 RPM for 24 hours before sub-culturing. *L. brevis* cultured from laboratory stocks was sub-cultured in 250 mL of De Man, Rogosa and Sharpe (MRS, Sigma-Aldrich, South Africa) broth (5.1% *m/v*) and incubated at 37 ºC in a shaking incubator at 150 RPM under anaerobic for 24 hours. *A. pasteurianus* cultured from laboratory stocks were sub-cultured in 250 ml MRS broth contain 2% (*v/v*) ethanol and incubated at 37 ºC in a shaking incubator at 150 RPM for 24 hours. All cultures were grown to stationary phase and sub-cultured twice before the experiment. Normal sterile procedures were employed and culture homogeneity (absence of other contaminating organisms) was verified by plating sub-cultured bacteria on agar plates.

4.2.6.2 UV-C irradiation of beer and microbiological analysis

A South African commercial draught lager beer was obtained from a local brewery and subjected to the Surepure® pilot-scale turbulent flow UV system. Each beer was inoculated with stationary phase *S. cerevisiae*, *L. breivs* and *A. pasteurianus*, during different trials to achieve a final concentration of about 10^5 CFU/mL. The beers were then processed at different UV energy dosages (refer to table 4.1). For each experiment, a volume of 25 L of beer was poured into the 50 L container of the pilot-scale UV system. A flow rate of 4000 L/h was produced by the centrifugal pump (Inoxpa) corresponding to a frequency of 62-63 Hz. Samples (50 ml) were taken after each dosage, labelled and wrapped with foil for GCMS analysis. The control sample was run through the UV system with the light switched off.

After irradiation, 1 mL of each UV treated beer sample was aseptically pipette into 9 mL of quarter strength Rangers solution (Merck, Cape Town, South Africa) and vortexed thoroughly. Serial dilutions (10^{-1} - 10^{-3}) were made and beer samples were plated onto nutrient agar in duplicate in order to assess colony forming units (CFU) which would indicate viable microorganism survival. Different nutrient agar plates were used for the different microorganisms namely *S. cerevisiae* inoculated beer samples were plated on potato dextrose agar and incubated at 23- 25 ºC; *L. brevis* inoculated beer samples were plated on MRS agar and incubated at 37 ºC and *A. pasteurianus* inoculated beer samples were plated on MRS agar containing 2% ethanol (*v/v*) and incubated at 37 ºC

All plates were incubated for 24-48 hours and bacteria were manually counted as number of colony forming units per millilitre (CFU/mL). The logarithmic decrease in

CFUs for each microorganism was determined for each exposure time, based on the initial non-irradiated microorganism concentration (N0), using the following equation:

$\log_{10}$ decrease = $\log_{10}(N0/Nt)$

Where Nt is the number of bacteria colonies after irradiation, and N0 is the number of bacteria colonies before irradiation. The inactivation curves were constructed by plotting the $\log_{10}$ decrease (CFU/mL) *versus* UV doses energy (J/L).

4.3 Results and discussion

4.3.1 Synthesis and analysis of the synthetic MBT

The optimisation of the SEP technique for the detection of MBT in beer required standards. Since MBT was not commercially available, it was synthesised. The synthesis of MBT was carried out by a modification of the method of Irwing *et al.* [18] using prenyl bromide as starting material. This synthesis, as shown in figure 4.3, involves the reaction of prenyl bromide with thiourea. The thiolation proceeds through the thiuronium salt, which upon alkine hydrolysis with NaOH, yields MBT [18]. A total yield of 39% MBT was obtained from the chemical synthesis procedure. The synthetic MBT is unstable and very volatile with, as expected, an extremely strong and unpleasant odour, therefore no further attempts were made to purify the MBT.

The synthetic MBT, and impurities, were identified by both GCMS and NMR. Figure 4.4 (A) shows the gas chromatogram of the crude MBT solution. The fraction eluting at 4.8 minutes was identified by MS as MBT. MS of MBT yielded three fragment major ions at *m/z* 41, 69 and 102, corresponding to the structure of MBT (figure 4.4). Two other peak fractions at 4.3 and 8.1 minutes elution time were also observed. These

identified peaks possibly represent some synthetic impurities (by-products and starting materials) from the synthesis reaction or dimeric species. Hughes [12] showed with GC that an apparently pure sample of MBT (>98%) contained a cluster of dimeric species peaks.

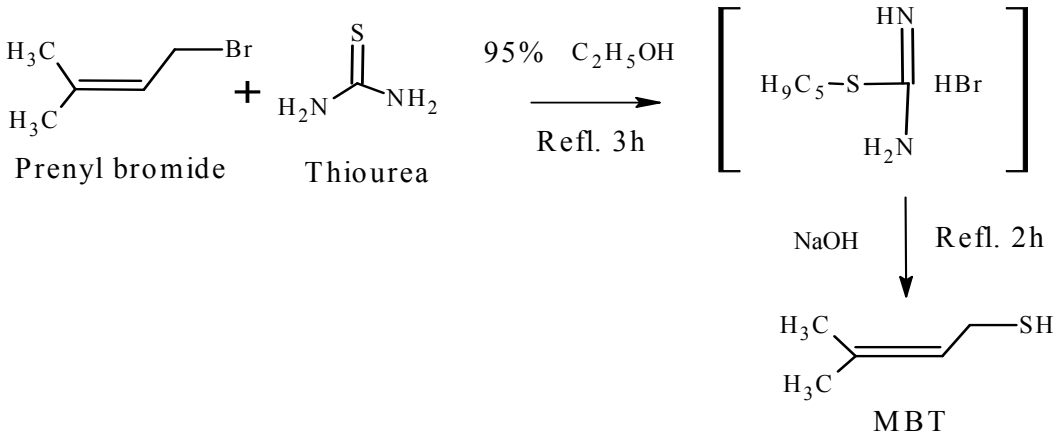

Figure 4.3 synthetic routes for the preparation of MBT from prenyl bromide. Reproduced from [24].

The chemical integrity of synthetic MBT was verified by NMR spectrometry, including ^{1}H and ^{13}C NMR. Figure 4.5 shows the ^{1}H and ^{13}C NMR spectra of the thiol sample with the peaks originating from MBT labelled. All the expected proton and carbon signals could be detected in the NMR spectra. The ^{1}H NMR spectrum of the MBT showed the presence of all the hydrogens at C1, C2, C5 and C6, with chemical shifts of 1.42, 3.15, 1.72, and 1.66 ppm, respectively (figure 4.5A). However, the hydrogen peak at C3 could not be observed. Data on the structure of MBT obtained from the literature reported a chemical shift of the hydrogen at C3 at 5.3 ppm, which corresponded to the solvent peak in the ^{1}H NMR spectrum in figure 4.5A. The detection of the proton chemical shift of the SH functional group suggested that MBT was not in a dimer form.

The other peaks at chemical shifts between 1.2- 0.5 ppm were considered as synthetic impurities although unknown. The ^{1}H NMR spectroscopic data of MBT was in agreement with previous data previously published [28].

The ^{13}C NMR spectrum in figure 4.5B clearly indicated all five carbons of MBT. The carbon C3 and C4 at the double bond of MBT showed the highest chemical shifts of 123.82 and 134.25 ppm, respectively. Carbon C2, C5 and C6 showed chemical shifts at 22.42, 25.61 and 17.45 ppm, respectively. Two small peaks, possibly trace synthetic impurities, were also observed at chemical shifts of 16 and 32 ppm respectively.

Overall, the NMR results confirmed the integrity and structure of the synthetic MBT. The ^{1}H and ^{13}C NMR spectra and assignments data showed that MBT was the major component in the sample. Assignment of H and ^{13}C NMR signals for all the protons and carbons of MBT are summarised in table 4.2.

Table 4.2 ^{1}H and ^{13}C NMR chemical shifts of MBT

	MBT	
Position	^{1}H (ppm)	^{13}C (ppm)
1-SH	1.45	-
2	3.15	22.43
3	-	123.82
4	-	134.25
5	1.72	25.61
6	1.66	17.45

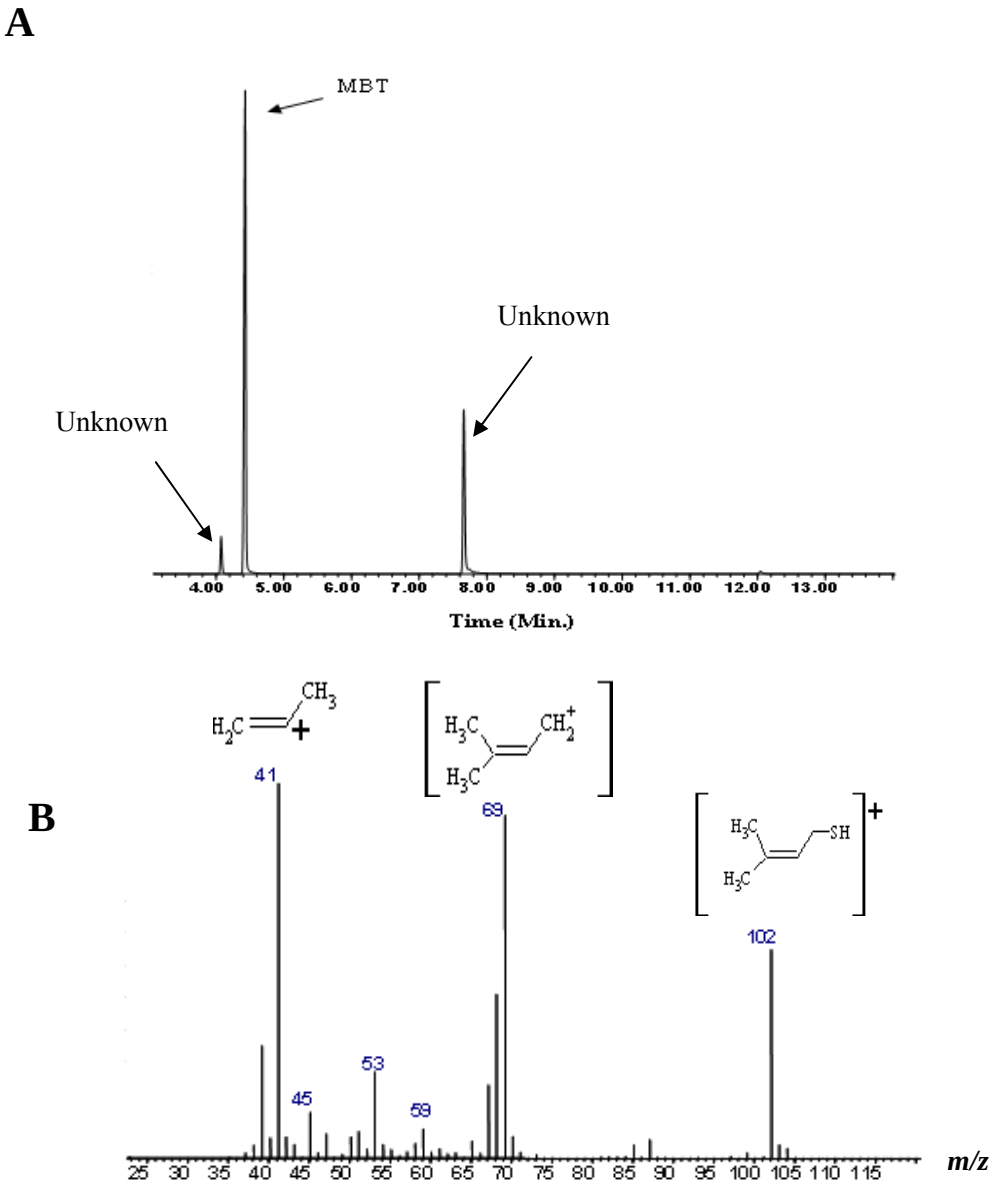

Figure 4.4 GCMS chromatograms of synthesized MBT. **A)** Gas chromatogram showing MBT that eluted at 4.8 minutes. The two other peaks eluted at 4.3 and 8.1 minutes and were identified as impurities from the synthesis. **B)** Electron impact mass spectrum of MBT with the three major ions at *m/z*. 41, 69 and 102. Fragmentation reactions of MBT in MS are also presented [19].

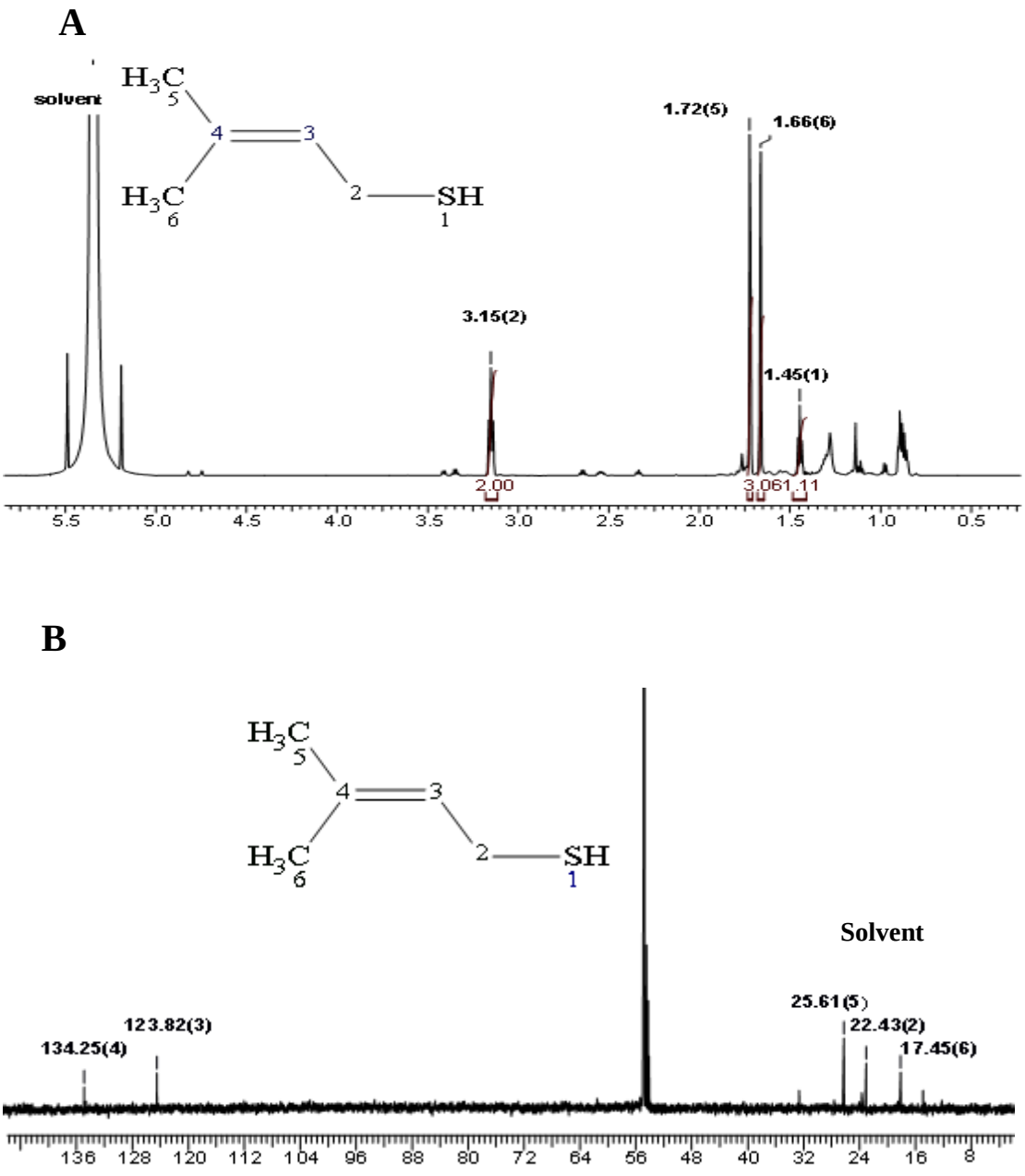

Figure 4.5 NMR plots of the ^{1}H (A) and ^{13}C (B) spectra of MBT**.**

4.3.2 Optimisation of GCMS method for MBT determination using SEP technique

MBT can be detected with relative ease at high concentration (ppb) with direct headspace GC without concentrating the sample solution. However, due to its extremely low flavour threshold in beer, between 1-35 ppt depending on beer [19-22], the SEP technique coupled with a GCMS was used to determine MBT in water and beer in this study. The GCMS instrument and settings used in conjunction with the SEP was different from GCMS settings previously used for characterising the synthetic MBT (refer to paragraphs 4.2.2 and 4.2.3).

A working solution of the synthetic MBT (100 ppt in water) was made and used as an external standard to adapt and optimise the published SEP technique [26]. A solution of deionised water and a fresh pale lager beer were supplemented with the MBT working solution to obtained levels of 1 and 5 ppt of MBT in water and the beer samples, respectively. The conditions of sampling MBT in solution were examined. The length of the sleeve of PDMS rubber (30 mm), water bath temperature (60 and 0℃) and the time required for extraction were major influences on MBT collection efficiency. The conditions of concentrating and trapping MBT on the rubber probe were optimal at a water bath temperature of 0℃ for five hours extraction. These results corroborated previous reports that a water bath temperature of 0℃ was more appropriate than 50 ℃ for MBT extraction, using similar purge and trap techniques [17, 21]. With the SEP GC methodology MBT eluted at around 21 minutes and its identity was confirmed by a typical MBT MS spectrum with fragment ions at *m/z* of 41, 69 and 102. MBT was not the only volatile compound in water and beer, as a number of other peaks were also seen in

the vicinity of the MBT peak. MBT was detected in water and beer, as shown in figure 4.6, at levels of 1 ppt and 5 ppt, respectively.

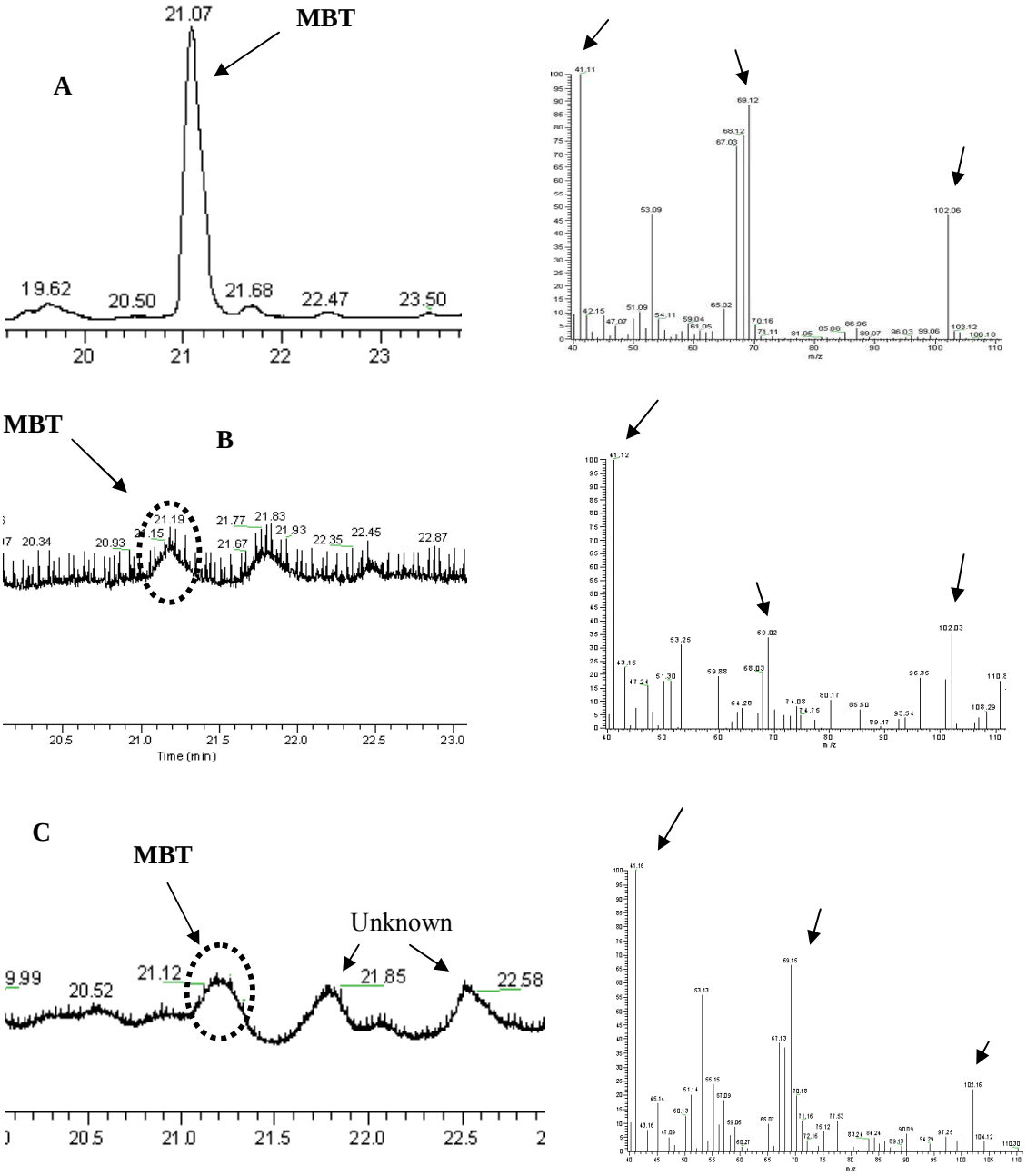

Figure 4.6 GC chromatograms of MBT in water **(A)** and **(B)** and in beer **(C)** obtained using the SEP technique, which eluted at 21.07, 21.15 and 21.12 minutes, respectively. Typical MS spectra of MBT are also shown next to the chromatograms, with its major fragment ions at *m/z* of 41, 69 and 102, highlighted with arrows. The concentrations of MBT were 250 ppb and 1 ppt in water and 5 ppt in beer.

4.3.3 Riboflavin determination using LCMS/MS

Riboflavin is a natural vitamin B_2 present in beer in concentrations of few hundreds of ppb (or µg/L) [29]. It plays an important role as a photosensitiser in the formation of the LSF of beer. An LCMS/MS method was developed to explore LSF on the basis that a decrease in riboflavin concentration in UV treated beer might indirectly reflect the formation of MBT. The purity of the commercially available riboflavin was >98% and it was used as an external standard without further purification. Since beer contains many compounds, including riboflavin, the LCMS/MS method was developed to analyse riboflavin with high selectivity by using its unique MS/MS fragmentation patterns. The chromatogram in figure 4.7A indicates that riboflavin eluted at four minutes using 0.1% (*v/v*) (solvent A) formic acid and acetonitrile (solvent B) as mobile phase.

Standard curves were generated with each separate set of samples analysed for riboflavin and were linear with regression correlation coefficients (R^2) greater than 0.99. The concentrations of riboflavin ranged from 30-500 ppb (or 30-500 µg/L) (figure 4.7B). Standard curves were developed using log-log plots and linearity and repeatability was confirmed over the whole range, although larger errors were observed, as expected, at lower concentrations (Figure 4.7B). From this the lower and higher limits of quantization (LOQ) were taken as the lowest and highest concentrations in the standard range.

Two different collision energies of 25 and 40 eV were used to record the mass spectra for riboflavin. The recorded MS/MS spectra were searched against a library for identification. Figure 4.7C shows daughter scans of riboflavin at different collision energies. At higher collision energy of 40 eV, precursor ion abundance decreases while

fragment ion abundance increases. The MS/MS fragmentation pattern of riboflavin matched the structure available in the library.

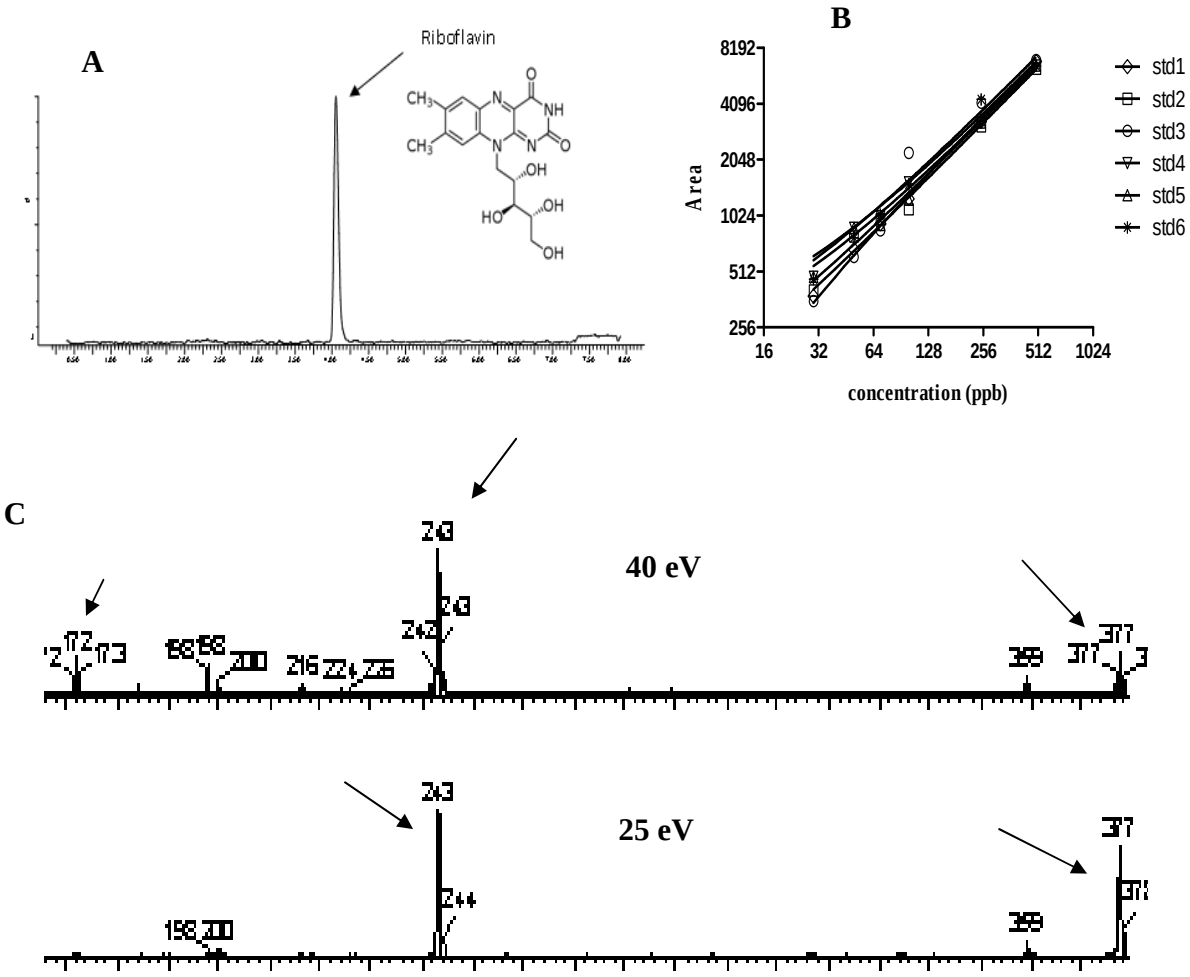

Figure 4.7 LCMS/MS analysis of a standard solution of riboflavin in water. **(A)** Chromatogram of riboflavin eluted at four minutes. **(B)** Overlays of standard curves of riboflavin used in analyses showing good correlation with linearity over the concentrations range from 30-500 ppb. A log-log plot was used in order to assess the standard curve specifically at low concentrations. **(C)** MS/MS fragmentation spectra of riboflavin at different collision energies. Arrows showed precursor ion (*m/z* 377) and typical daughter ions (*m/z* 243 and 172) of riboflavin.

4.3.4 UV-C processing of beer samples

4.3.4.1 Microbiological analysis

Reductions of microorganisms spiked in beer and exposed at different UV doses energy were examined. As shown in figures 4.8 and 4.9, the turbulent flow UV system was found to be successful in reducing microbial load in commercial lager beer. The initial average microbial count before UV processing was 1.07×10^5 CFU/mL *L. brevis*, 1.85×10^5 CFU/mL and 7.3×10^4 CFU/mL *S. cereviciae*. At UV-C dose energy of 500 J/L, the best CFU decrease was achieved with *L. brevis* followed by *A. pasteurianus* and *S. cereviciae* with significant decreases in CFU/ml of 2.5, 2.1, and 1.5 log units, respectively (figure 4.10). The yeast, *S. cereviciae,* was found to be much more resistance to UV than Gram-positive *L. brevis* and Gram-negative *A. pasteurianus* and greater reductions at 5 and 4.8 log units were achieved at UV dose energy at 2000 J/L for *L. brevis* and *A. pasteurianus,* respectively. However, MBT formation was confirmed at 2000 J/L, as shown in figure 4.13.

Lu *et al.* [30] studied the impact of UV-C treatment on microorganisms in Chinese draught beer. They also found that *S. cereviciae* was more resistant to UV than *L. brevis*. Indeed, microorganisms vary in their structure and sensitivity to UV irradiation. The UV sensitivity could be caused by cell size, structure of cell membrane, pigmentation or capacity of repair mechanism as well as stage of growth. Yeast may have shown more resistance to UV because of the fact that the organism has a nucleus, which protects the DNA against the radiations. Even at the highest UV-C dose energy of 2000 J/L, a log 5 reduction could not be achieved for *S. cereviciae*. The efficiency of UV irradiation depends on the resistance of the microorganism to UV light as well as the absorptive

properties of the medium in which the microorganism is suspended [1]. Bacteria were cultured and sub-cultured under stationary phase conditions. This should explain why they showed more resistance to UV-C irradiation and could not reach log 5 decreases at 2000 J/L, except for *L. brevis.*

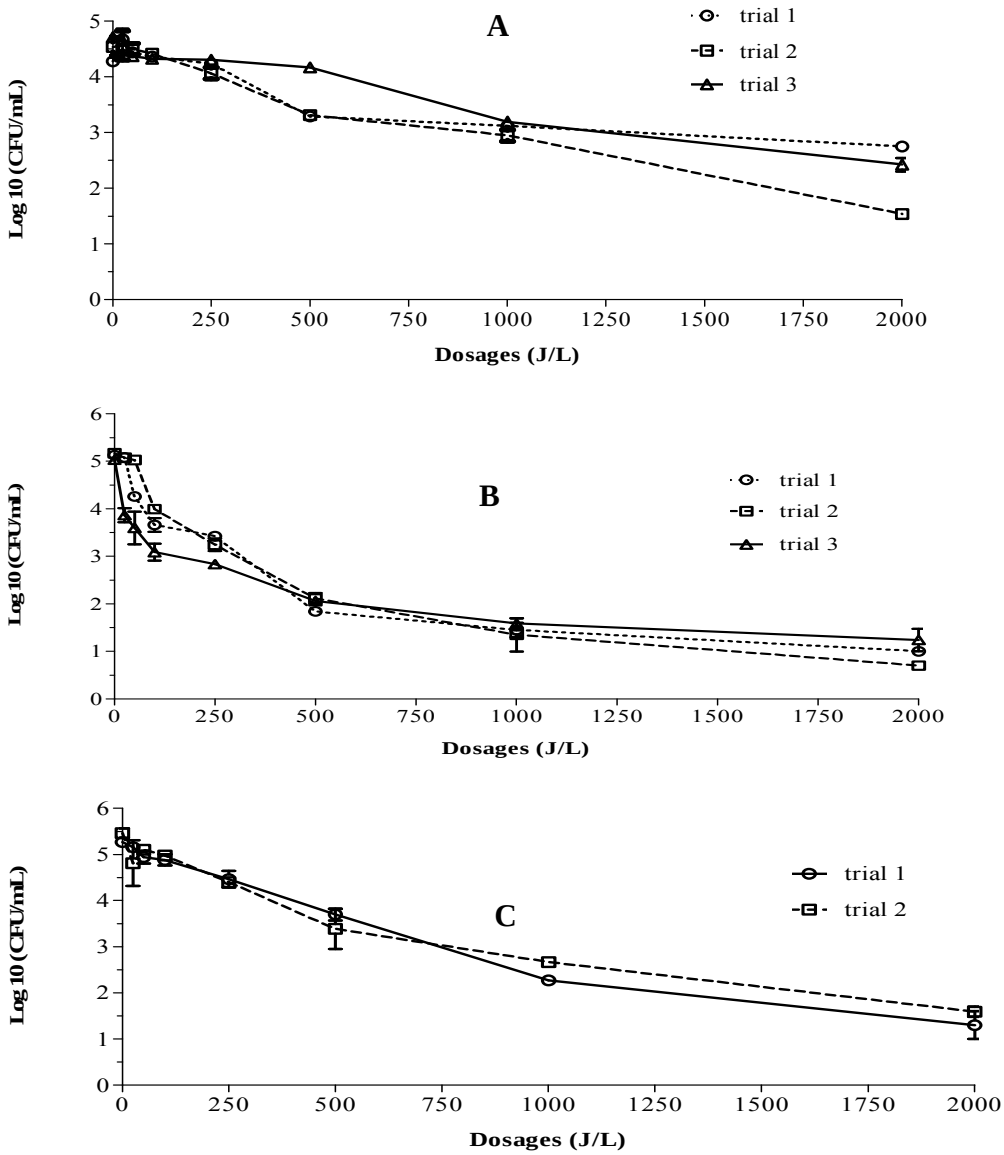

Figure 4.8 Inactivation curves of **(A)** *S. cerevisiae* **(B)** *L. brevis and* **(C)** *A. pasteurianus* in draught beer treated with the SurePure® pilot-scale turbulent flow UV system at different UV dosages (J/L). Error bars represent standard error of the mean (SEM) for each trial (duplicate samples).

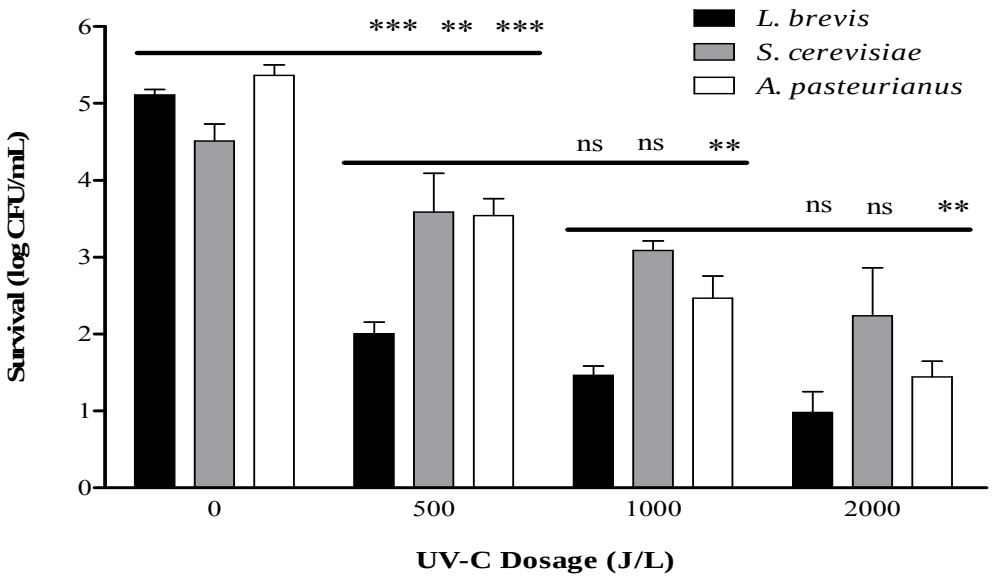

Figure 4.9 Comparison of the UV-C mediated log $_{10}$ decreases of the three spoilage microorganisms treated at 500, 1000 and 2000 J/L. Error bars represent SEM for three trials with duplicate technical repeats for *S. cereviciae* and *L. brevis* and two trials with duplicate technical repeats for *A. pasteurianus*. Statistical analyses were done using Two way ANOVA (Bonferroni's multiple comparison test). Comparisons are indicated with line above groups (***P<0.001, ** P<0.01, ns P<0.05).

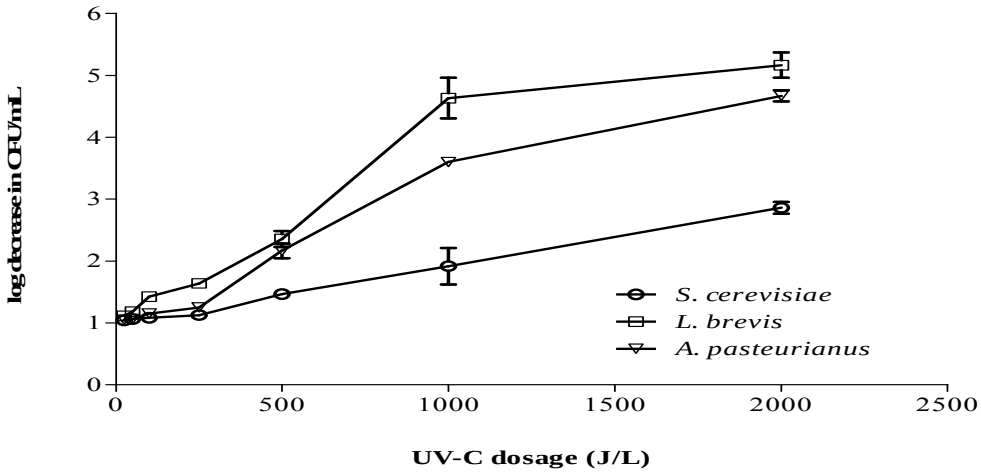

Figure 4.10 UV-C induced decrease of the microorganisms spiked in commercial lager beer and exposed to 254 nm light at different dosages.

Overall, the viability of spoilage bacteria assayed in our commercial lager beers were reduced by greater than log 4 for *L. brevis* and log 3 for *A. pasteurianus* by the Surepure® turbulent UV system at 1000 J/L dosage. However, the same effect could not be obtained for *S. cereviciae* at the same UV dose energy of 1000 J/L. The highest UV dosage level of 2000 J/L resulted in log 5.2, 4.8, and 3 decrease of initial CFU/mL of *L. brevis, A. pasteurianus*, and *S. cerevisiae*, respectively. Despite the fact that the initial microbial count before UV irradiation was high, the results showed promising application of UV irradiation to inactivate microorganisms in beer. Laboratory tests on pasteurised and non-pasteurised beers have indicated that beer spoilage bacteria are found in beer with count from 1 to 100 CFU/mL, depending on brewery [31].

4.3.3.2 MBT analysis in beer by GCMS

Irradiated and non-irradiated beer samples were analysed for MBT using the method described in section 4.2.3. Figure 4.11 represents a chromatogram of the beer control (0 J/L). Beer samples exposed at 500 (figure 4.12) and 2000 J/L (figure 4.13) were submitted to the GCMS for MBT detection. The molecular weight of MBT is 102 and its typical electron impact mass spectra consist of three major ions at *m/z* 102, 69, and 41 (refer to figure 4.4B). However, because of the occurrence of many ions at *m/z* of 41 for other common compounds present in beer, only ions at *m/z* 102 and 69 were chosen for selective ion monitoring (SIM) as shown in figures 4.11, 4.12 and 4.13. Although a peak at a retention time of 21 minutes was observed for the sample irradiated at 500 J/L, the MS of that compound did not indicate MBT (figure 4.12). For the sample

irradiated at 2000 J/L a minor peak at a retention time of 21 minutes was observed. The MS of this compound corresponded to MBT (figure 4.13).

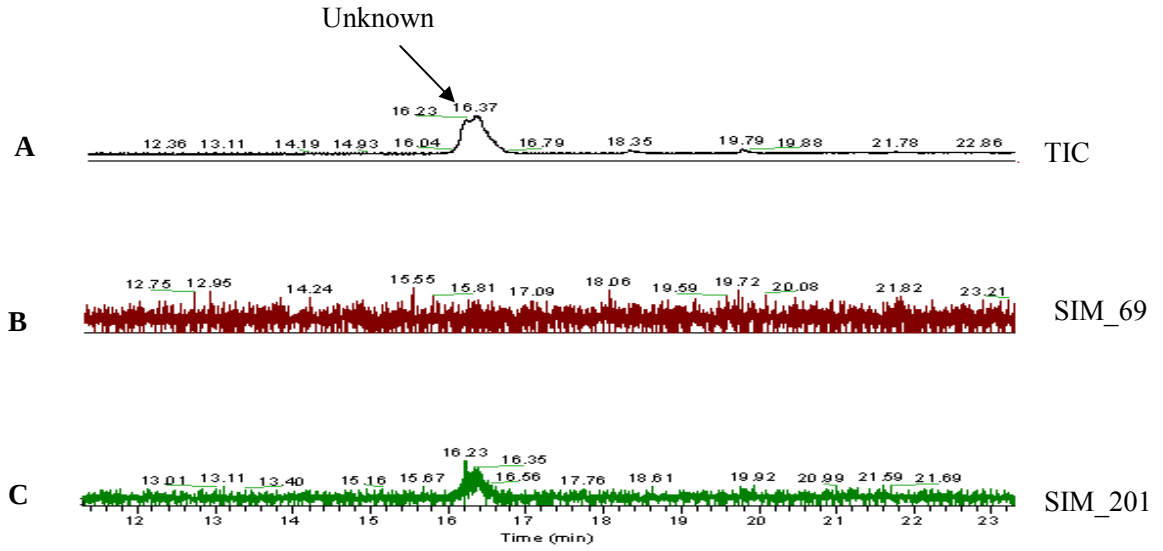

Figure 4.11 Chromatograms of a non- irradiated beer (negative control). Total ion count (TIC) of chromatogram of compounds eluting between 12 to 23 minutes **(A)**; SIM chromatograms of *m/z* 102 **(B)** and 69 **(C).** MBT elutes at 21 minutes under the same SEP/GCMS conditions (refer to figure 4.6A.

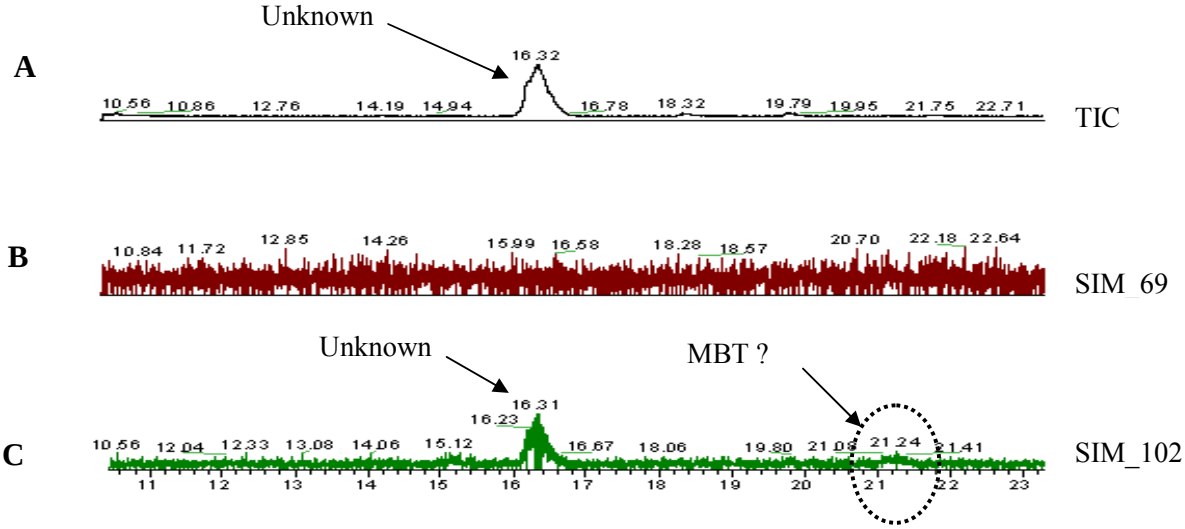

Figure 4.12 Chromatograms of UV treated beer at 500 J/L. TIC chromatogram of compounds eluting between 12 to 22 minutes **(A)**; SIM chromatograms of the ions at *m/z* 102 **(B)** and 69 **(C)**. The small peak at elution time of 21.24 min. corresponds to the MBT elution time. However, the MS spectrum of that peak (not shown) was not the typical spectrum of MBT.

Kuwoiwa *et al.* [9] have shown that light-struck flavour was likely to form when beer was exposed to light at wavelengths from 350-500 nm. In this region of light, riboflavin was suggested to act as a natural photosensitiser. However, Blondeel *et al.* [10] showed that hops isohumulones are prone to light-induced decomposition on direct irradiation without any photosensitiser. Their findings indicated that LSF could be formed on direct irradiation with UV-B light (280-320 nm) [10].

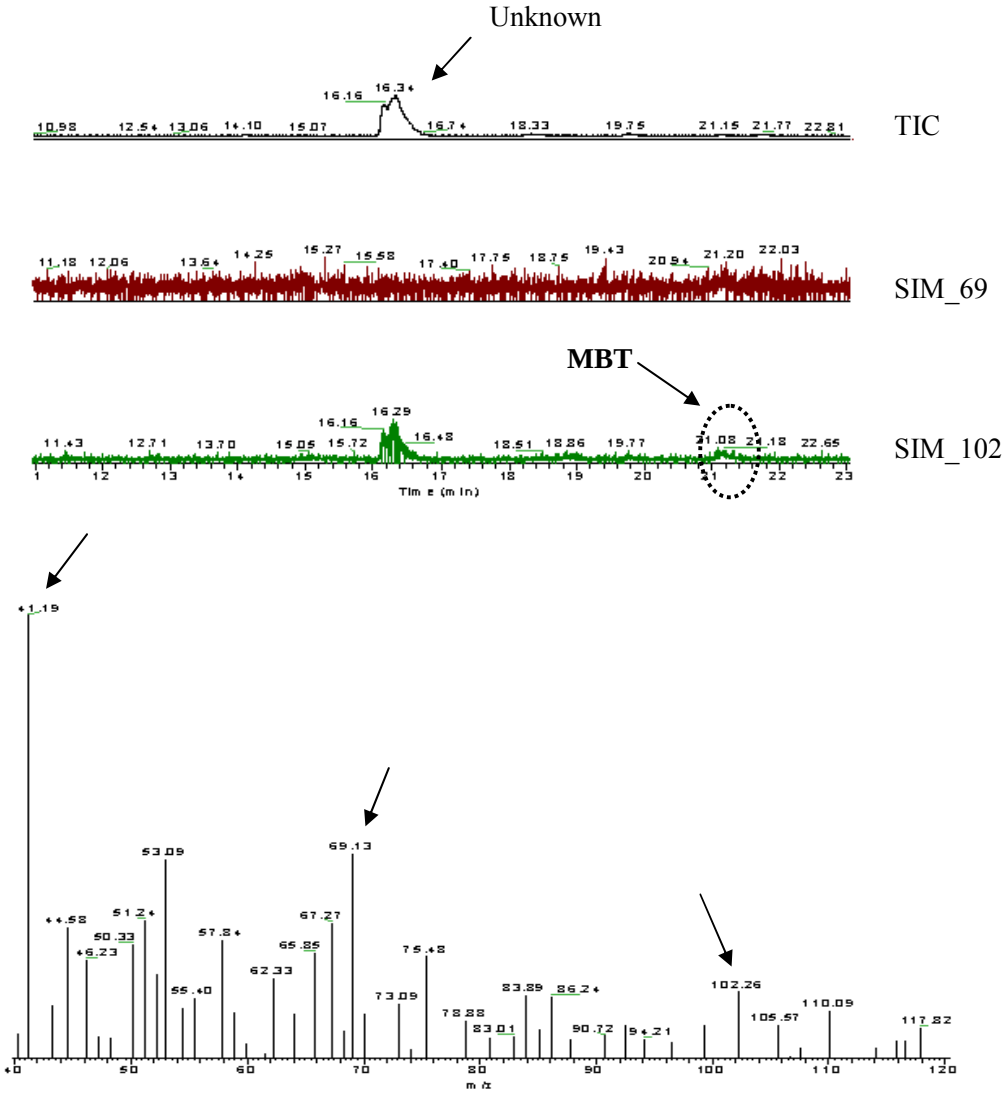

Figure 4.13 Analysis of UV treated beer by SEP/GCMS. **(A)** Gas chromatogram of beer treated at 2000 J/L, with a small peak at 21.18 minutes elution time. **(B)** MS spectrum of the peak representing MBT, with arrows pointed at its three major fragment ions (41, 69 and 102).

4.3.3.3 Riboflavin analysis in beer by LCMS/MS

Riboflavin was analysed in beer using the previously mentioned LCMS/MS method developed. A 500 ppb (*w/v*) concentration of riboflavin solution in deionised water was freshly prepared and used as an external standard. Riboflavin is predominantly in free form in beer and has been shown to contribute to beer flavour deterioration [29]. Its disappearance in beer on exposure to light could be directly linked to LSF formation [22]. However, riboflavin could react with oxygen as well to produce stale compounds. Overall, no significant decrease in riboflavin levels as a function of irradiation time was observed in beer during all trials (Figure 4.14). These findings suggested that UV-C at irradiation at 254 nm does not significantly decompose riboflavin.

On the other hand, beer contains a very wide range of components, including proteins, amino acids, vitamins, and phenolic compounds and some components have been shown to have an inherent ability to prevent riboflavin photodecomposition [32, 33]. Among those components present in beer, tryptophan has been shown to act as a natural quencher of riboflavin in beer, providing some inherent protection against flavour deterioration [31]. Beers were inoculated with microorganisms up to 10^5 or log 5 CFU/mL. There is a possibility that microorganisms could have biosynthesized high amounts of riboflavin, as many microorganisms release riboflavin [33].

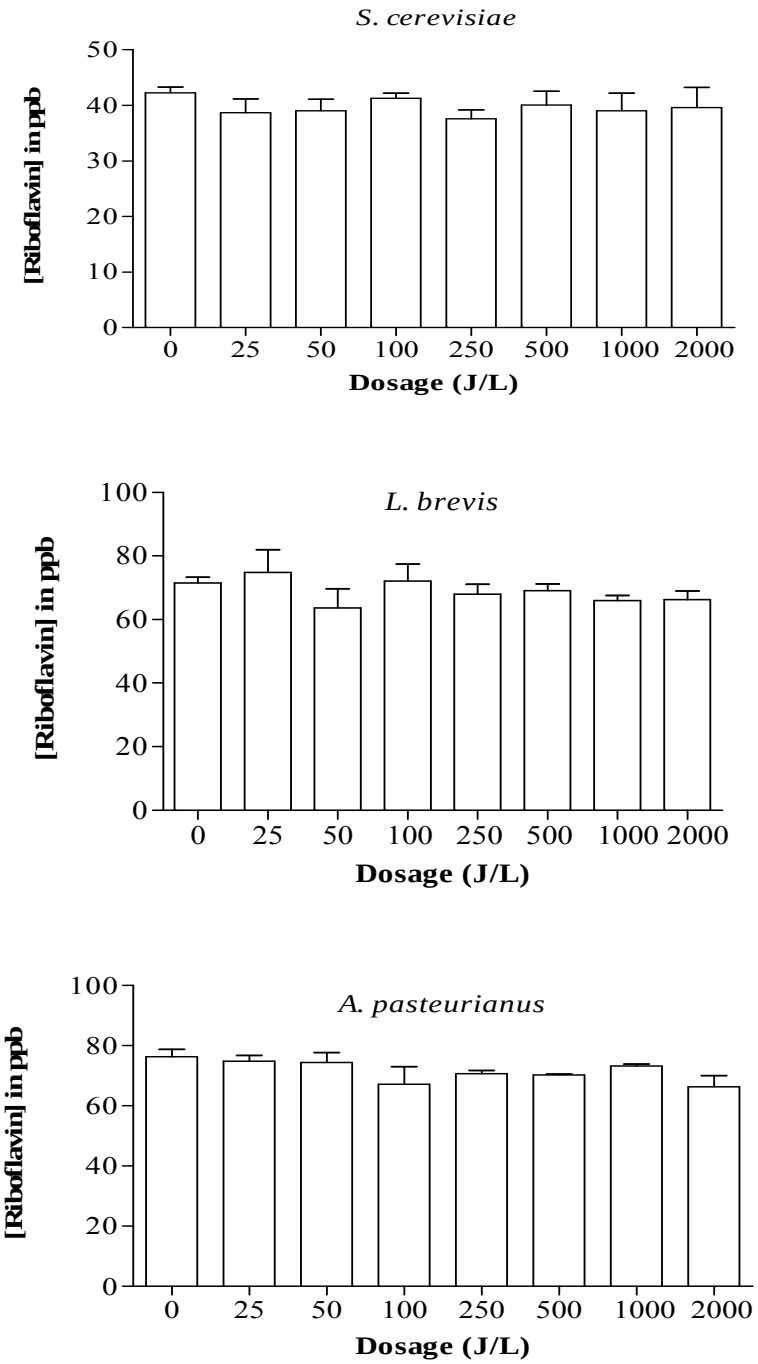

Figure 4.14 Measurement of riboflavin levels by LCMS in beer spiked with microorganisms and treated with the Surepure® UV light system. A statistical one-way ANOVA test was used to verify the average recoveries obtained for each dosage. There was no significant difference in riboflavin concentration in the three batches of beer spiked with microorganisms. Error bars represent means of triplicate determinations with SEM. (No significant differences was found, $P>0.05$, $n=3$).

4.5 Conclusions

This study first showed that MBT was successfully synthesised by adapting a previously published method [18]. A final yield of 39% was obtained; the molecular mass and chemical structure of MBT was identified by GCMS and NMR, respectively. No attempt was made to further purify the MBT due to the strong and unpleasant odour and extreme volatility of the compound. The synthetic MBT was used as an external standard to adapt and optimise the SEP technique coupled with a GCMS. MBT was detected in water and in beer at levels of 1 and 5 ppt, respectively. The SEP technique showed promising results in detecting MBT in beer. Alternatively, the measurement of riboflavin levels in beer by LCMS/MS was a simple and effective method to indirectly explore the formation of MBT. This LCMS/MS method was a reliable and valuable means of investigating the formation of LSF in beer.

The results obtained with the Surepure® pilot scale turbulent UV system indicated that the microbial load in beer could be effectively reduced. This demonstrated a proof of concept for the disinfection of beer using UV-C light at a wavelength of 254 nm. The UV-C sterilisation efficacy was the best for *L. brevis* and *A. pasteurianus*, respectively. From the results obtained it can be deduced that a maximum UV-C dosage of 500 J/L should be sufficient for sterilisation in practice, as the beer used for these experiments was heavily contaminated at 7.3×10^4, 1.07×10^5 and 1.85×10^5 CFU/mL for *S. cerevisiae*, *L. brevis,* and *A. pasteurianus*, respectively, prior to irradiation. However, only at a very high UV dosage of 2000 J/L, a small peak of MBT was detected, yet reduction of riboflavin was statistically insignificant. Therefore, it is uncertain whether a medium to high (500-1000 J/L) dosage 254 nm UV-C light is leading to detrimental

MBT production. The turbulence created by the UV system during trials complicates the sampling of volatiles and therefore may have lead to an underassessment. No sensory tests could be conducted on the beer samples in this study, as the beers were heavily contaminated. The question that remained unanswered was, if the UV-C irradiation at 254 nm caused taste or flavour changes and could such changes be correlated with chemical changes.

Further trials were therefore carried out to investigate any effect UV-C irradiation may have on beer taste and flavour (chapter 5). As volatiles, such as MBT, are difficult to measure, sensory analyses were conducted with consumer panels. Additionally, riboflavin, iso-α-acids and fermentable sugar levels were measured as indicators of chemical change in the irradiated beers. It was also decided to use different types of beers for these trials. Two batches of beers; dark and pale lagers were specially brewed and used in the trial, while two commercial lager beers were included as controls. This work is described in Chapter 5.

4.6 References

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CHAPTER 5

SENSORY AND BIOCHEMICAL EVALUATION OF UTRAVIOLET-C TREATED BEERS

5.1 Introduction

Pilot trials with commercial beers showed that a significant reduction in bacterial count could be achieved by irradiation with UV-C light (Chapter 4). The formation of LSF, and in particular MBT, however, could only be unambiguously indicated at 2000 J/L. In order to obtain more definitive data on off-flavour development in beer irradiated with a commercial UV-sterilisation system, it was decided to attempt to correlate flavour changes in irradiated beer with chemical changes related to MBT formation namely riboflavin and hops iso-α-acids, as well as fermentable carbohydrates. These compounds, chosen for monitoring chemical changes, are also directly related to the general detrimental effects of UV-light on beer [1-4]. In addition, furan, a potential human carcinogen, was reported previously to develop from carbohydrates upon UV treatment of sugar rich beverages [5, 6]. Furan, a cyclic volatile aromatic compound, was found to be present in a large number of processed foods including canned vegetables, fruits, meat, nutrition drinks and coffee [7, 8]. Although furan in food can be formed from many sources, such as ascorbic acid and unsaturated fatty acids [7], its primary source is the thermal degradation of carbohydrates [8]. Glucose, fructose and sucrose contribute the most to furan formation upon thermal and UV-C treatments [5, 6]. A simplified pathway of the formation of furan from sugars is shown in figure 5.1.

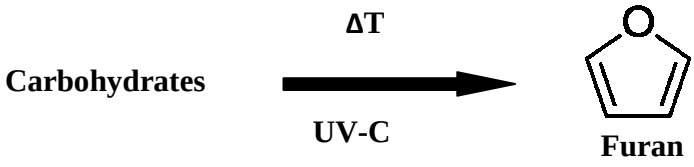


Figure 5.1 Simplified formation of furan from carbohydrates

The objectives of this study were to determine the change in iso-α-acids (as indication of MBT and LSF), riboflavin (as indirect determinant of MBT potential) and fermentable sugars (as indication of furan formation) in UV exposed non-commercial PL (pale lager), DB (dark lager) and commercial CPB (pilsner), CLB (pale lager) beers and to correlate these changes with a consumer taste trial. From the results obtained with the first pilot scale trial (Chapter 4), there was no clear evidence of any chemical changes in irradiated beers up to a dosage of 1000 J/L. However, it was apparent that under normal operating conditions a UV-C dosage of 500 J/L would be sufficient to reduce the microbial load in beer. A single UV dosage of 1000 J/L was therefore chosen for subsequent experiments to ensure that any potential changes that occurred upon irradiation could be detected unambiguously.

5.2 Materials and methods

5.2.1 Non-commercial beers

Raw materials were obtained from SAB-Miller Ltd., (Cape Town, South Africa) and included pale and black malt, aroma and bitter hops (PIH), lactic acid and bottom yeast. Beers were brewed in a 40 L scale pilot mini brewery (Chemical Engineering,

Stellenbosch University) equipped with 50 L fermenter tanks. A dry roller mill was used for malt prior to brewing.

For every batch, malt was mashed at a 3:1 (*v/w*) ratio of water to malt. Lactic acid was used to acidify the mash to pH 5.3 and the brewing was carried out with a mashing program scheme of 64 °C (45 min); 71 °C (20 min) and 78 °C (1 min). The mash was lautered and the wort was transferred to the kettle. The wort was boiled at 101 °C for one hour and immediately cooled to 12 °C. During boiling, hops pellets and liquid adjuncts (maltose) were added for the desired bitterness (15 bitterness unit) and density (13 Plato). Fresh brewery-collected stationary phase yeast was added to the wort. Fermentation was maintained at between 11 and 12 °C for five days until the end of primary fermentation. The primary fermented beer was then chilled to 4 °C and left for three weeks for a secondary fermentation to allow the yeast to settle thoroughly and to inhibit the activity of any microorganisms possibly contaminating the ferment. After three weeks of secondary fermentation, the beer was filtered using a tangential flow filter (0.22 µm pore size, Millipore, USA). The micro-brewed beers were coded and referred to as PL (pale lager) and DB (dark lager).

5.2.2 Commercial beers

Commercial beers were obtained from a local brewery (SAB-Miller Ltd., Cape Town). Two different brands and style of beers were used for experimentation and these brand names are not disclosed in this article for confidential reasons. Therefore, the beers are coded and referred to as CLB (pale lager), and CPB (pale pilsner).

5.2.3 UV-C Irradiation of beers

Beers were UV treated using a pilot scale SP4 UV system (SurePure®, Milnerton, South Africa), which includes an open stainless steel collection tank (~100L), a pump and 4 UV lamps. The UV system was cleaned before and after every beer treatment using a standard 'Cleaning In Place' (CIP) processes. The UV system was rinsed with warm water for about 10 minutes. An alkaline detergent solution (1%) was circulated through the system for 15 minutes at 75 ºC, followed by a warm water rinse for about 5 minutes. Lastly, a final rinse was done three times with cold water. The cleaning process was done with all the UV lamps on to ensure the effective maximum output of the UV lamps during each trial.

Cold beers were decanted from a keg into the tank and re-circulated through the UV apparatus at a flow rate of 4000 L/h. UV dosages were expressed as joules per litres (J/L) and the run time for each corresponding dosage was calculated as follows:

Dosage (J/L)) = Total UV-C output per unit (W) / Flow rate (L/s)

= [Total UV-C output per unit (W) x Time (s)] / Volume (L)

Thus, the run time is calculated as:

Time (s) = [Dosage (J/L) x Volume (L)] / Total UV-C output per unit (W = J/s)

= [1000 J/L x 50 L]/102 J/s

= 490 seconds or 8 minutes

Therefore, in this study, a SurePure® UV-C pilot scale system, producing a total UV-C output of 102 W (four units) was used for the treatment of 50 L batches of beer. The 50 L was circulated for eight minutes to obtain a UV dosage of 1000 J/L. Since oxygen in beer causes stale flavours, which impact on taste and could affect the taste perception during the trials, the control beers (non UV treated) were also re-circulated through the SurePure® UV turbulator system for eight minutes with the lamps turned off.

This was performed to allow both beer samples to have more or less the same exposure to oxygen.

5.2.4 Sensory analyses

5.2.4.1 Consumer panel evaluation

Two consumer tests were carried out in the sensory science laboratory of the Department of Food Science at the University of Stellenbosch (South Africa). The first test was conducted on homemade beers (PL and DB) with a group of 87 consumers that comprised of 32% female and 68% male. The second test was conducted on commercial beers (CLB and CPB) with a group of 113 consumers that comprised of 51% female and 49% male.

During each trial, consumers were asked to indicate which term best described their attitude toward an UV treated and non-UV treated beer from the same batch using a nine point hedonic scale. The scale was presented as follows: 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much and 1= dislike extremely. Consumers were not aware of the UV treatment of beer samples.

5.2.4.2. Consumer trials

The beers PL, DB, CLB and CPB were UV treated at 1000 J/L and kept in the cold at 7°C for 24 h. For each trial, beers were served and presented in glasses coded with a three digit random code. The beer sample size was 25 mL and was served directly from the refrigerator and tasted in a light and temperature controlled room (21 °C). Consumers

were asked to complete a questionnaire to determine their degree of liking using the nine point hedonic scale as the test technique without giving reasons for their preference.

5.2.4.3 Statistical analysis of data

Analysis of variance (ANOVA, SAS®, version 9) was carried out on the data to assess the variability between different beer samples. Consumer's *t*-least significant difference (LSD) was calculated at 5 % significant level to compare treatment means. The Shapiro-Wilk test was used to test for non-normality in the data. Skewness was equal to zero indicating that values were relatively evenly distributed on both side of the mean.

5.2.5 Chemical analyses

5.2.5.1 Riboflavin analysis

The measurement of riboflavin levels in both commercial and non-commercial beers was performed by LCMS/MS as described in Chapter 4 section 4.2.5, as an indication of LSF formation.

5.2.5.2 Assay for Iso-α-acids by LCMS as an indication of LSF.

Iso-α-acids were obtained from Haas Hop Products, UK as an aqueous solution (ca. 30% w/v). The compounds were present as the potassium salts of *trans*-iso-α-acids and *cis*-iso-α-acids and were used as standards. Samples were diluted in deionised water and filtered (Millex-HV 0.45 µm pore size) prior to the LC analyses on a Waters API Quattro Micro 2695 LCMS with electrospray ionisation. A Phenomenex Gemini C_{18} column (2.1 x 50 mm) with a mobile phase consisting of 10 mM ammonium acetate pH

6.0 (solvent A) and acetonitrile (solvent B) was used to separate the different iso-α-acids from beer. A 10 minutes gradient from 20% to 100% acetonitrile was used at a flow rate of 0.4 mL/minute. The ESI source and desolvation temperatures were set to 80 and 400 ℃, respectively, the nitrogen desolvation gas was applied at a flow rate of 400 L/h, the capillary voltage was set to 3.5 kV, and the cone voltage was set to 15 kV and nitrogen cone gas at a flow rate of 50 L/h.

5.2.5.3 Carbohydrate analysis in beer by HPLC

Four carbohydrates, fructose, glucose, maltose and maltotriose, found in beer, were analysed according to a modified method of Noguerira *et al.* [9]. The analysis was performed by HPLC using an evaporative light scattering detector (ELSD). A Prevail© carbohydrate ES column (5 µm, 4.6 x 250 mm) was used. The mobile phase consisted of acetonitrile (solvent A) and analytical grade water (solvent B). A 30 minute gradient from 20% solvent B to 50% solvent B at a flow rate of 1.0 ml/min was employed for optimal separation of the four carbohydrates. Chromeleon© Dionex 6.8 software was used for data processing.

Glucose, maltose, maltotriose and fructose standards were purchased from Saarchem (Gauteng, South Africa). The concentration of standards in the mixture was 5 g/L and was prepared in analytical grade water. Standards were diluted and different concentrations were used to calibrate the chromatographic system for quantification. Calibration curves were constructed for each of the four carbohydrates. For beer analyses, samples were degassed in an ultrasonic bath model and passed through a 0.45 µm pore size Millipore syringe filter prior to injection.

5.3 Results and discussion

5.3.1 Sensory analyses

5.3.1.1 Non-commercial beers

Two styles of beer, a pale and dark lager, were micro-brewed as described previously. The beers coded PL and DB were treated to a UV dosage of 1000 J/L and subjected to a group of 87 consumers. Seventy percent of the panel were between the ages of 20-29, 25% between ages 30-49 and 5% between ages 50-59. Regarding the frequency of consumption of beer, 56% consume beer at least once a week, 30% at least once per month and 14% never. In general consumers demonstrated a dislike for UV exposed beers (Tables 5.1 and 5.2).

Table 5.1 Summary of statistical analysis to determine overall preference for the micro-brewed pale lager beer.

	Mean hedonic value		
	Total group (N=87)	Female consumers (N=28)	Male consumers (N=59)
PL1	6.27a	6.14a	6.33a
PL2 + UV	3.74b	3.4b	3.88b
LSD (P<0.05)	0.61	1.06	0.77

*LSD= Least significant difference at the 5% level of significance. Numbers with different letters in the same column differ significantly at the 5% level of significance. Skewness equal to zero

Table 5.2 Summary of statistical analysis to determine overall preference for the micro-brewed dark lager beer.

	Mean hedonic value		
	Total group (N=87)	Female consumers (N=28)	Male consumers (N=59)
DB1	4.52a	4.42a	4.57a
DB2 + UV	2.68b	2.28b	2.88b
LSD (P<0.05)	0.48	0.79	0.62

*LSD= Least significant difference at the 5% level of significance. Numbers with different letters in the same column differ significantly at the 5% level of significance. Skewness equal to zero

The non-UV and UV-treated beer samples differed significantly ($P<0.05$) with respect to the degree of acceptance exhibited by the consumer panel. Both male and female consumers indicated that they preferred non-UV treated beers for both the pale and dark lagers. In figure 5.2 the acceptability of the in terms of consumer preferences is given.

About 10% of the consumers designated both the non-UV and UV treated pale lager beer samples as "*like slightly*". More than 25% of the consumers placed the non-UV treated pale lager in the "*like very much" category*, while less than 5% placed the UV-treated pale lager in the *"liked very much"* category (figure 5.2A). As shown in figure 5.2B about 25% and 5% of the consumers categorised the non-UV and UV treated dark beer respectively as "*like slightly*". Moreover, the UV treated dark beer showed the highest percentage (35%) of consumer dislike ("*dislike extremely").* Beer colours have been shown to play a pivotal in the formation of LSF [10]. Several studies have shown that dark beers were more susceptible than lighter beers to LSF [10, 14].

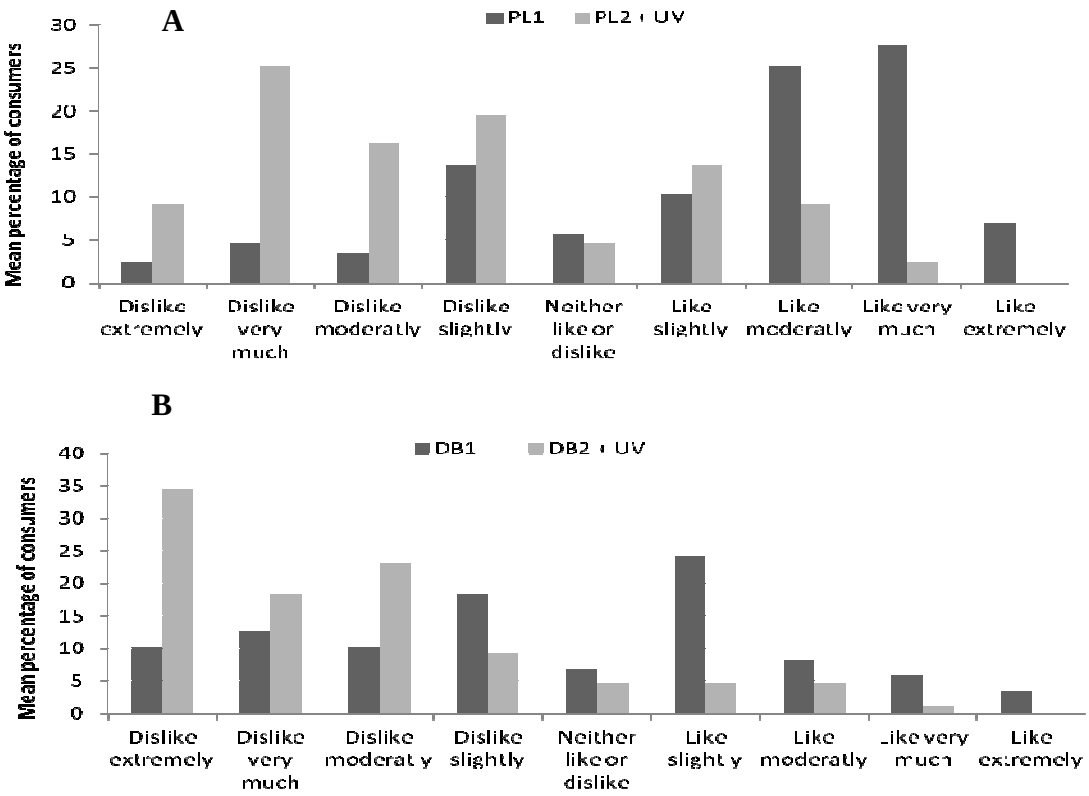


Figure 5.2 Distribution of scores for the total group (N=87) for the non-commercial beers **(A)** pale lager (PL) and **(B)** dark lager (DB).

5.3.1.2 Commercial beers

Two commercial beers were UV treated (1000 J/L) and analysed by a group of 113 consumers. The group consisted of 80 % between the ages of 20-29, 13% between ages 30-49 and 7% between ages 50-60. In terms of beer consumption, 58% consume beer at least once a week, 31% at least once a month, and 11% never. In tables 5.3 and 5.4 it can be seen that there is a significant difference between the preference for non-UV and UV treated beers ($P<0.05$). This result indicates that both commercial and micro-

brewed beer samples treated with UV light have a similar taste profile. This group of consumers showed that they preferred the UV-treated beers significantly less.

Table 5.3 Summary of statistical analysis to determine overall preference for the commercial pale lager beer.

	Mean hedonic value		
Samples	Total group (N=113)	Female consumers (N=58)	Male consumers (N=55)
CLB	6.73a	6.67a	6.78a
CLB + UV	5.39b	5.56b	5.2b
LSD (P<0.05)	0.415	0.582	0.603

*LSD= Least significant difference at the 5% level of significance. Numbers with different letters in the same column differ significantly at the 5% level of significance. Skewness equal to zero

Table 5.4 Summary of statistical analysis to determine overall preference for the commercial Pilsner beer.

	Mean hedonic value		
Samples	Total group (N=113)	Female consumers (N=58)	Male consumers (N=55)
CPB	5.23a	4.96a	5.52a
CPB + UV	3.12b	3.03b	3.21b
LSD (P<0.05)	0.401	0.58	0.567

*LSD= Least significant difference at the 5% level of significance. Numbers with different letters in the same column differ significantly at the 5% level of significance. Skewness equal to zero

The data summarised in figure 5.3 indicate that about 25 % of the total group would put the CLB beer samples (non-UV and UV treated) in the "*like slightly"* category, while 15 % and 35% put the UV treated and the non-UV treated beer in the "*like moderately*" category. Only about 7% described the UV treated CLB beer as "*disliked very much"* compared to 35% for UV treated CPB. It is evident that the CLB (lager) and CPB (pilsner) have distinct taste profiles upon exposure to UV.

Although both the light and dark untreated beers were preferred, there was a marked difference between the preference of UV treated dark and light beer with >70% and <40% showing some dislike in for the dark and light beer, respectively. This was a strong indication that the dark beers are more prone to LSF and off-flavours with UV-C treatment. Our previous studies [15] have shown that UV-C irradiation might accelerate any oxidation reactions in the beer due to the high level of oxygen picked-up in the turbulator during the trial. However, since the same oxidation off-flavours were not detected in the control samples, which were experimentally identically handled, it was unlikely that stale flavours could have played a role during the consumer tasting trials.

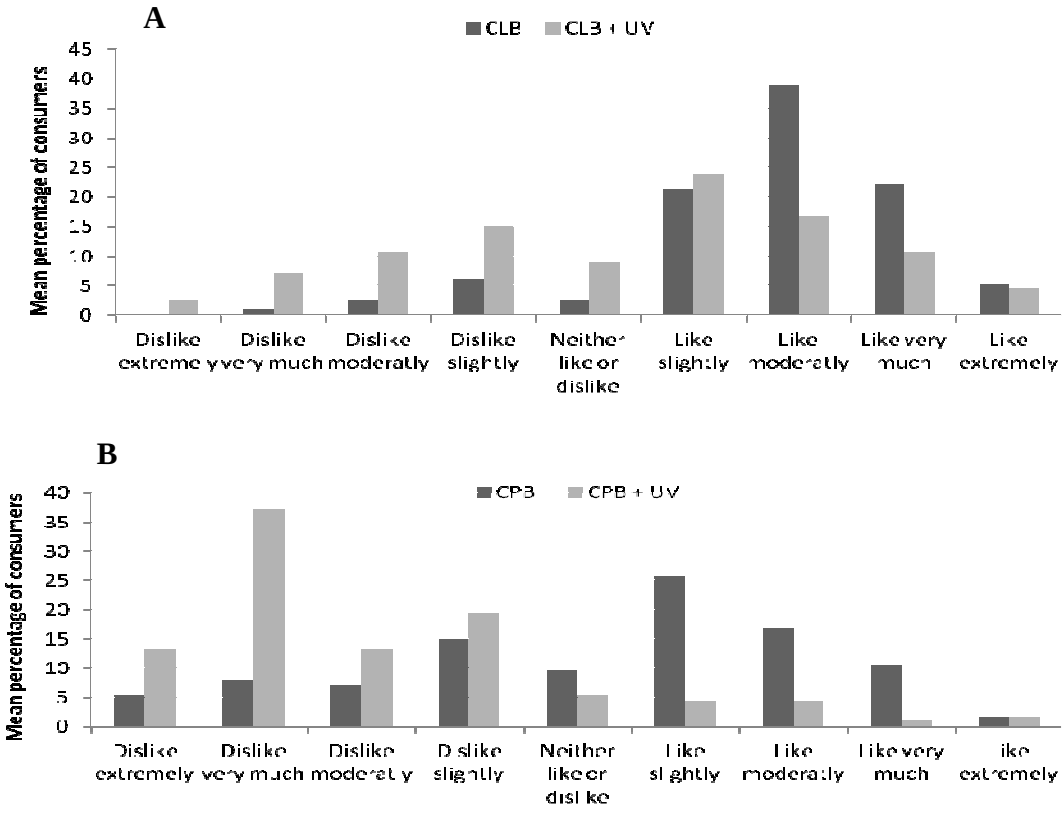


Figure 5.3 Distribution of scores for the total consumer group (N=113) for the commercial beers, **(A) (**CLB) and **(B) (**CPB).

5.3.2 Riboflavin levels in beer

Riboflavin levels were measured by LCMS/MS in beer in order to explore the potential of LSF formation upon irradiation at 254 nm. In this study, beers DB and CPB showed a statistically significant decrease in riboflavin concentrations (figure 5.4). However, no significant decrease in riboflavin was observed among the UV treated beers CLB and PL. The concentration of riboflavin depends on the type of beer since it is a natural vitamin. It is evident that the extent of riboflavin photo-degradation might differ amongst different beer types. DB and CPB were dark and pilsner lager beers, respectively. A study has reported that dark beers are more susceptible to flavour deterioration than pale yellow beers [10]. The UV treated DB and CPB beers were most disliked during consumer tests (refer to figures 5.2 and 5.3). CLB and PL, with no decrease in riboflavin levels, were both pale yellow lager beers. The beers that had no decrease in riboflavin were more preferred during consumer tests. These results correlated with the fact that photo decomposition of riboflavin, in irradiated beers, may contribute to any detrimental effect on beer quality. There is a well established relationship between riboflavin loss and beer flavour deterioration [2, 3].

Photo-reduced riboflavin can be oxidized back to its original structure or to a derivative riboflavin when beer is bubbled with oxygen [2]. A significant amount of oxygen was introduced into the beer samples during and after the UV-C light treatment. This phenomenon may explain why there was no significant decrease detected in riboflavin levels of some of the UV treated beers, since consumers preferred all the non-UV treated beers. Yet, there was a correlation between the chemical and sensory data collected in this trial.

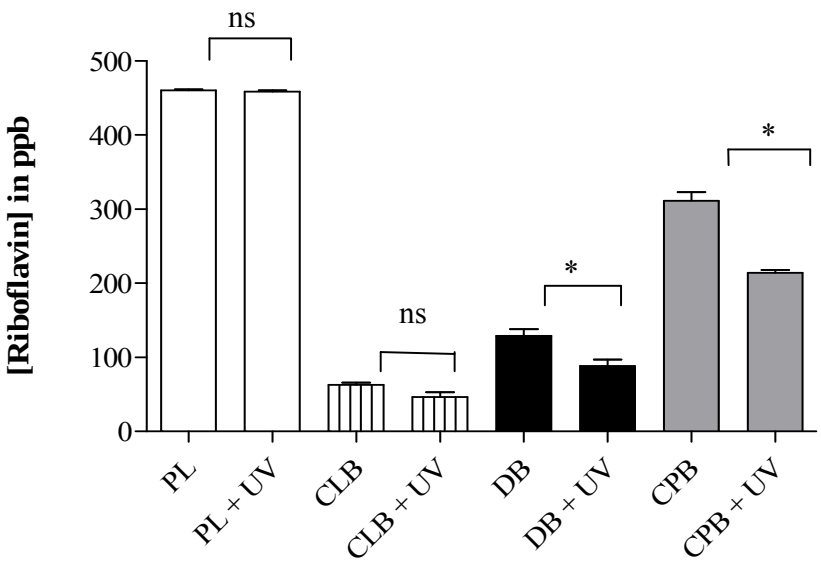
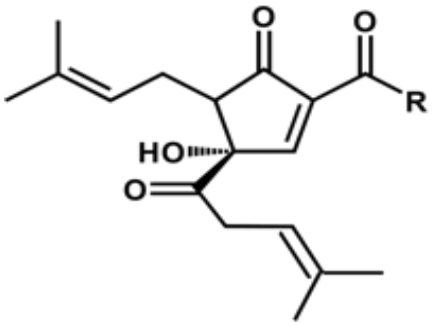


Figure 5.4 Riboflavin concentration measurements by LCMS of both commercial and non-commercial beers. Results were compared independently using an unpaired t test and data are expressed as a mean of duplicate repeats with SEM. (*P< 0.05; non significant (ns) P> 0.05).

5.3.3 Iso-α-acids in beer

A modified LCMS method of Vanhoenacker *et al*. [11] was utilised for the analysis of hop bitter acids in beers by direct injection. Hop bitter acids (Iso-α-acids) are the primary bittering compounds in beer and consist of three pairs of cis-trans isomers. The three pairs are isohumulones, isocohumulones and isoadhumulones and were obtained in a mixture as an aqueous solution (ca. 30% w/v) of potassium salts. The structures of the iso-α-acids are shown in figure 5.5



Isocohumulone: R= C_4OH_7

Isohumulone: R= C_5OH_9 (isovaleryl)

Isoadhumulone: R= C_5OH_9 (2-methylbutyryl)

Figure 5.5 Structures of bitter hops iso-α-acids.

The chromatograms in figure 5.6 show major peaks for isocohumulone, eluting first at 4.31 minutes and isohumulone and isoadhumulone co-eluting at 4.70 minutes. Ammonium acetate/acetonitrile, 10 mM at pH 6.0 was found to be a good elution buffer for the separation of isocohumulone and isohumulone/isoadhumulone. However, isohumulone and isoadhumulone could not be separated. These mobile phases were compatible with MS detection with the use of an electrospray ionisation (ESI) in negative mode and further optimisation using other mobile phases was complicated by interference with detection. The identification of iso-α-acids hops was obtained from their molecular ions at *m/z* 347 and 361 respectively (Figure 5.6 C and D). Differentiation of isohumulone and isoadhumulone by their mass spectra was not possible using this method since they have identical molecular masses. In order to quantify the hops iso-α-acids standard curves were constructed with each separate set of samples analysed for hops iso-α-acids (figure 5.7).

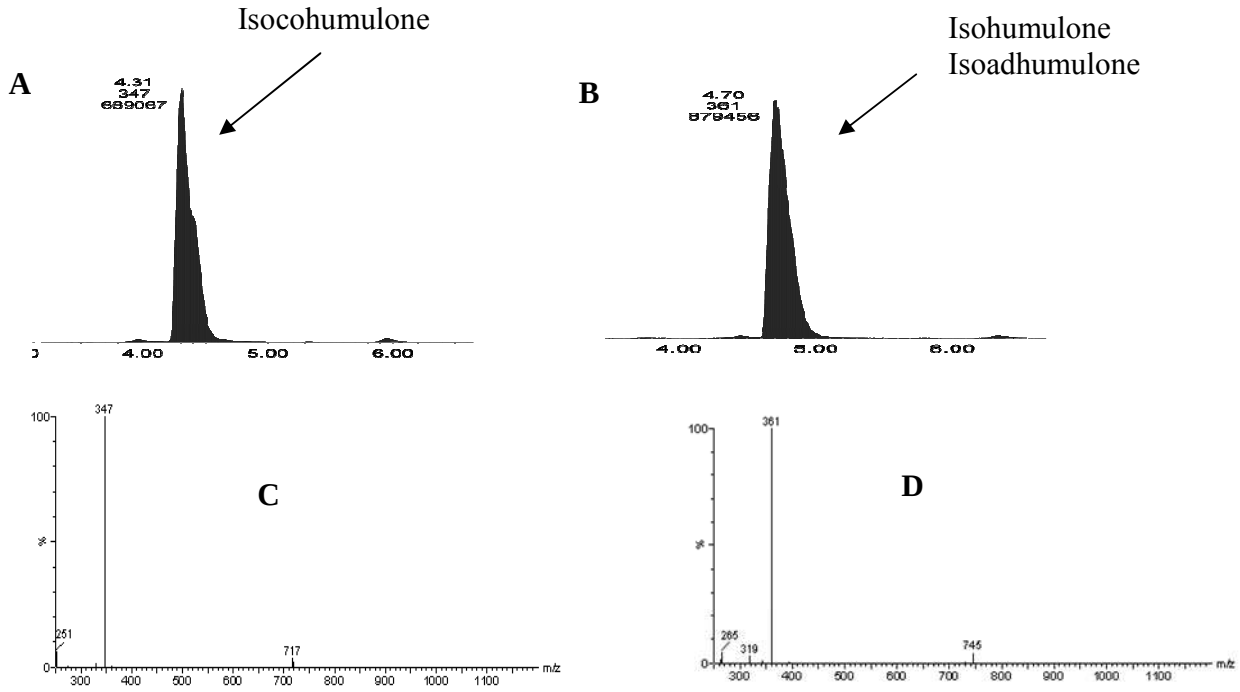


Figure 5.6 LCMS chromatogram of mixture of the tree pairs of iso-alpha-acids standards. **(A)** isocohumulones peaks (Rt 4.31 minutes), **(B)** isohumulone and isoadhumulones eluted together (Rt 4.7 minutes). MS spectra of iso-alpha-acids showing the molecular ions of isocohumulone at *m/z* 347 (**C)** and molecular ion of isohumulone and isoadhumulone at *m/z* 361 **(D)**.

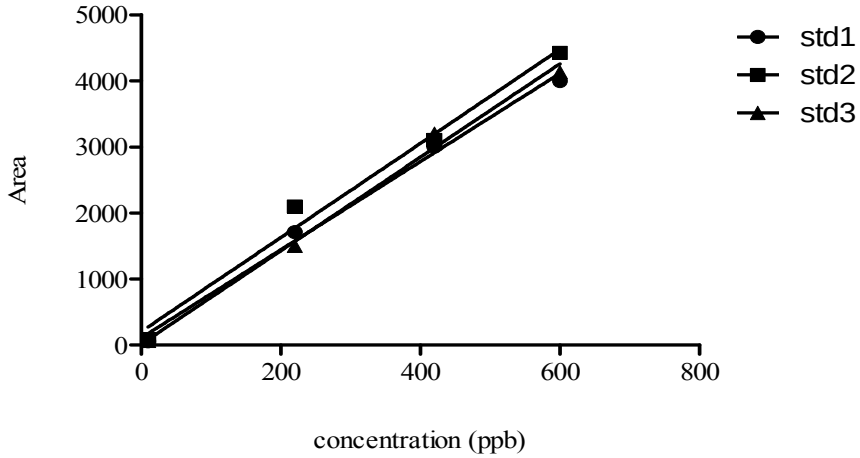


Figure 5.7 Representative standard curves of hops iso-α-acids, as obtained with LCMS, used to quantify bitter compounds in UV treated beer. The R^2 values calculated for the linear fits of all the standard curves used in quantification were >0.99.

Using the above quantitative LCMS method, the iso-α-acid concentrations were determined in UV and non-UV treated beers. The quantification was carried out using calibration curves constructed between 10 to 600 ppb. A comparison of the concentrations of hops isohumulones in both commercial and non-commercial beers shows that the concentration of hops iso-α-acids present in beer depends on the type of beer analyzed (figure 5.8). Beer contains between 10 to 100 ppm of iso-α-acids [11]. However, the extremely low levels of iso-α-acids (between 5-380 ppb) in both beer samples (before and after UV treatment) suggested that a large amount of iso-α-acids might have deteriorated during the trials. Studies have shown that iso-α-acids degrade in the presence of oxygen, resulting in formation of volatile oxidised derivatives [16]. Nonetheless, the commercial pilsner beer had the highest iso-α-acids levels and these levels were significantly reduced upon exposure to UV light (figure 5.8A). Non-commercial UV-treated PL and DB beers also showed significant decreases in iso-α-acids concentrations (figure 5.8B).

Iso-α-acid decomposition was shown to be essential for the development of LSF [1]. The significant differences in concentration in beers CLB, CPB and PL after exposure to UV light indicates that the hops iso-α-acids underwent a Norrish Type I α-cleavage [12, 13], which eventually leads to the formation of a 3-methylbut-2-enyl radical (refer to Chapter 2, section 2.4.2). Several studies [12-14] have shown that visible and UV light (280 - 500 nm) induce iso-α-acids hops decomposition in the presence and/or absence of riboflavin. In this study significant decreases in isohumulones concentrations were also observed in beer irradiated at 254 nm (UV-C). These results

indicate that iso-α-acids hops can readily undergo decomposition on direct irradiation with relative high dosages of UV-C light and subsequently adversely affect beer quality.

CLB pale lager beer showed no significant decrease in iso-α-acids upon UV irradiation. This finding strongly correlated with riboflavin analysis as well as the sensory evaluation (refer to figures 5.3, 5.4, and 5.8). However, it was noted that less than one molecule in a million of iso-α-acids can be converted to MBT to produce LSF [17]. Such a minute change could escape any instrumental detection. The formation of MBT results from iso-α-acids, to which riboflavin transfers energy from its photodecomposition. The more riboflavin radicals formed, the higher the potential of decomposition of iso-α-acids.

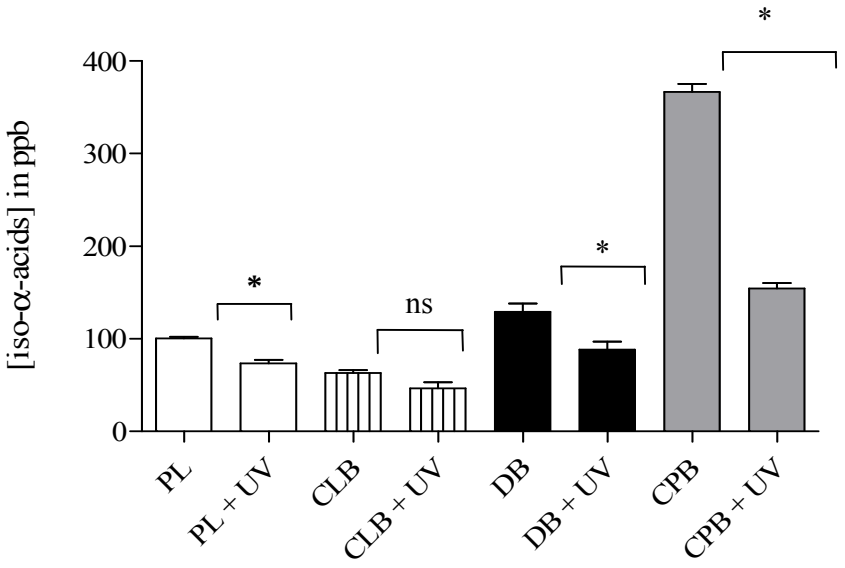


Figure 5.8 Concentration of hops iso-α-acids in both UV and non-UV treated beers are compared. Results were compared independently using an unpaired t test and data are expressed as a mean of duplicate determinations with SEM. (*P< 0.05, non-significant (ns) P> 0.05).

5.3.4 Fermentable sugar

The effect of UV-C irradiation on fermentable sugars leading to unwanted furan formation was investigated by measuring their concentration in irradiated beer. Separation and quantification of the sugars were performed using an HPLC equipped with an ELSD detector [9]. A typical separation obtained in this study is shown in figure 5.9 with elution times of 8.48 (fructose), 10.40 (glucose), 14.22 (maltose) and 17.76 minutes (maltotriose) and correlated well with other studies [9]. Calibration curves were constructed with each separate set of samples analysed for each of the four carbohydrates (table 5.6). Concentrations were ranging from 0.15 to 3.63 g/L with regression correlation coefficient (R^2) always greater than 0.99.

Table 5.5 Representative parameters of calibration curves determined for the four carbohydrate standards.

Carbohydrates	Concentration range (g/L)	Points[1]	Slope	Coefficient of correlation
Fructose	0.15-3.63	5	1.5449	0.9948
Glucose	0.15-4.34	5	1.5339	0.9968
Maltose	0.15-4.46	5	1.5267	0.9986
Maltotriose	0.15-4.43	5	1.5325	0.9993

[1] Number of points considered for the regression.

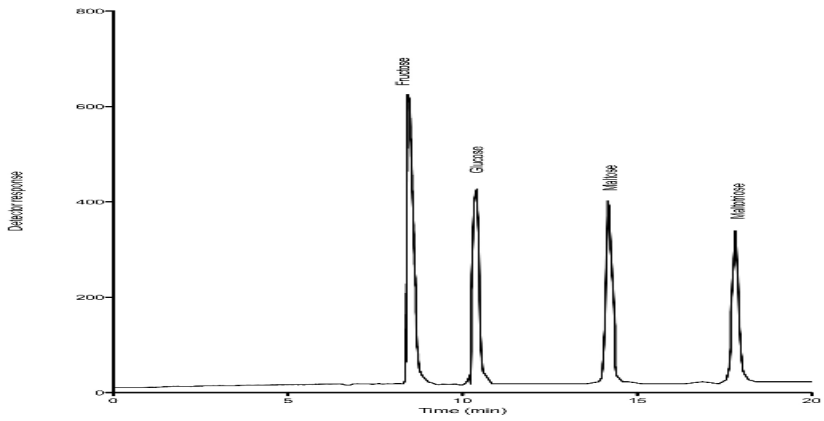


Figure 5.9 Typical HPLC/ELSD chromatogram for the separation of fructose, glucose, maltose and maltotriose in a mixture solution.

The preliminary HPLC/ELSD method showed the concentration levels of fructose, glucose, maltose and maltotriose in beer samples in the range of 0.1 to 1.3 g/L (table 5.7). The higher sugar content may presumably contribute to a higher amount of furan. However, the levels of furan in beer are typically between 1 to 5 ppb (µg/L) [18]. This indicates that any reduction in sugar levels, as an indicator of potential for furan formation in beer, would most probably be undetectable.

Table 5.6 Concentrations of four main fermentable sugars found in beer.

Beer samples	Fructose	Glucose	Maltose	Maltotriose
PL	0.4	0.87	0.32	1.12
DB	0.41	0.1	-	1.29
CPB	0.26	0.5	0.31	0.67
CLB	0.31	0.8	1.08	1.12

Concentrations are expressed in g of carbohydrate/ L (g/L)

5.4 Conclusions

Four different types of beer (commercial and non-commercial) were UV irradiated at 254 nm. Sensory data were obtained from consumer tests and correlated with relevant analytical data using LCMS/MS. The results presented showed that LSF was likely formed in UV treated beers at a dosage of 1000 J/L, with a clear correlation between consumer approval and photo-degradation of riboflavin and iso-α-acids. Overall, consumers significantly disliked all the UV-C irradiated beers ($P<0.05$). It can be assumed that no bias was displayed during consumer trials, as consumers were not aware of the origin of the beers tasted (UV treated or not). Thus, consumers declared mainly

their preference base on the sensory characteristics. Amongst all the UV-C treated beers, only the commercial pale lager beer CLB showed no significant reduction in both riboflavin and iso-α-acids concentrations. UV-C treated beer CLB was more liked than UV-C beers CPB, DB, and PL. The high sugar content and the extremely low concentration of furan found in beer presumably suggested that measurement of sugar reduction by HPLC/ELSD might be a very less sensitive approach for furan formation. Therefore a method to measure furan formation in beer with a high sensitivity and good selectivity should be developed.

Compounds responsible for LSF from UV-C irradiation are most likely originating from iso-α-acids in the presence of riboflavin as a photosensitiser. From these results, it is apparent that irradiation at a UV dosage of 1000 J/L of beers containing iso-α-acids might have given rise to LSF. Photodecomposition of iso-α-acids has been shown to be a key step on the route to LSF formation in beer [1, 14]. Therefore, degradation of iso-α-acids is likely to give rise to LSF upon UV-C exposure, irreversibly affecting the quality of the beer. Also, the formation of LSF may depend on the type of beer UV treated with dark beer being more sensitive than light beers. This latter result may be directly related to the higher content of iso-α-acids. Another attempt to investigate effect of UV-C irradiation at 1000 J/L was carried out with a non-commercial beer hopped with reduced iso-α-acids (THIA). The study is presented and discussed in chapter 6.

5.5 References

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CHAPTER 6

THE EFFECT OF UV-C IRRADIATION ON BEER HOPPED WITH REDUCED ISO-α-ACIDS HOPS

6.1 Introduction

Hops iso-α-acids are responsible for the pronounced sensitivity of beer to light [1-4]. It has been shown that UV-C irradiation at certain UV dosage, of beers hopped with hops iso-α-acids, could adversely affect the quality of the beer (refer to Chapter 5). Hops iso-α-acids can, however, be chemically modified to reduce or eliminate their ability to contribute to LSF formation in beer [5].

The chemically modified variants of hops iso-α-acids, also known as reduced iso-α-acids, have been shown to contribute significantly to both the light and the foam stability of beer [5-7]. Their use in the brewing process can provide a suitable way to reduce or eliminate LSF. The reduced hops compounds include dihydroiso-α-acid (DHIA), also known as rho-isohumulones, and tetrahydroiso-α-acids (THIA). Currently, much attention is given to THIA since they impart bitterness and do not create the LSF (MBT) upon exposure to light [8]. The use of such reduced hops has become very popular in the brewing of so-called “lightproof” beers with improved foam characteristics [7-9]. However, De Keukeleire *et al*. [1] showed that THIA can undergo a photodecomposition.

Double bonds in the side chains at C_4 and C_5 of iso-alpha-acids can be hydrogenated, using hydrogen gas in the presence of a palladium catalyst, to produce

THIA [1]. The chemical reaction produces three pairs of THIA, tetrahydroiso-co-α-acids (THI-co-A), tetrahydroiso-ad-α-acids (THI-ad-A), and tetrahydroiso-n-α-acids (THI-n-A). Each pair exists as *cis* and *trans* isomeric forms, totalling six stereoisomers. THIA has no unsaturated side chains to activate radical cleavage that lead to LSF formation [1, 7]. Structures for THIA are shown in figure 6.1.

The objective of the study, described in this chapter, was to examine the sensory and chemical effects a UV dosage of 1000 J/L could have on a non-commercial beer hopped with THIA. It must again be stressed that this dosage exceeds the dosage needed for UV sterilisation of beer (refer to Chapter 4), but it was used to ensure that any chemical changes that occur were large enough to be measured accurately. For sensory analyses, the consumer acceptance of the UV treated beer was assessed, as described in Chapter 5, using a nine-point hedonic scale. To evaluate the consumer preferences a panel of experienced tasters established a flavour profile of the beer. The chemical analyses entailed analyses of riboflavin and THIA hops by LCMSMS and headspace sorptive extraction (HSSE) followed by gas chromatography (GC) to examine and to compare volatiles arising from non-UV treated and UV treated beers hopped with THIA.

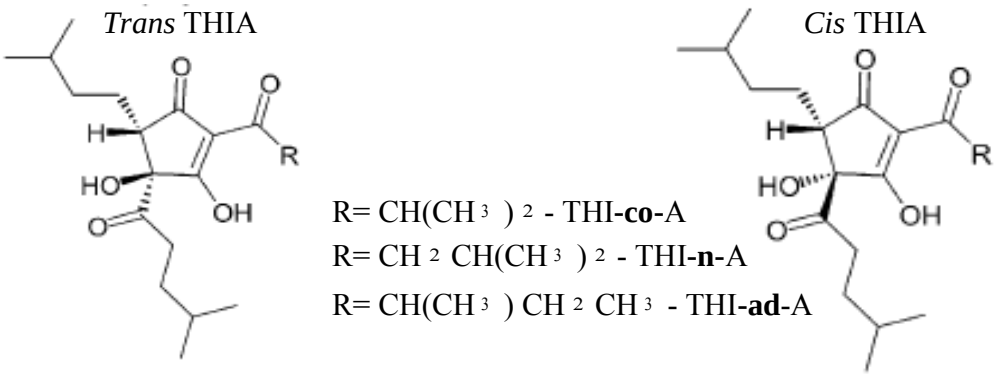

Figure 6.1 Chemical structures of reduced iso-α-acids THIA.

6.2 Materials and methods

6.2.1 Microbrewing

Pale lager beer was brewed in a 40 L scale pilot mini brewery (Chemical Engineering, Stellenbosch University) equipped with 50 L fermenter tanks as previously described in Chapter 5. The beer was hopped with reduced THIA hops purchased from SAB-Miller Ltd. (Alrode, South Africa) to obtain a level of bitterness of 17. Pale malt, lactic acid and fresh yeast were obtained from SAB-Miller Ltd, (Cape Town, South Africa).

6.2.2 Beer treatment

Beer was exposed to UV-C irradiation using the SurePure® pilot scale UV apparatus (see chapter 5). The UV system consisted of 4 UV low-mercury lamps (30 UV-C Watts, 90% 254 nm and 90% emittance) housed in quartz sleeve. A volume of 30 litres of beer was treated at dosage level of 1000 J/L and was immediately collected following UV-C exposure. Samples were collected in aseptic 750 ml brown quartz bottles and

stored at 4° C for 2 weeks before sensory evaluations. The control was identical beer passed through the SurePure® UV turbulator system with the lamps turned off.

6.2.3 Consumer trial

Consumer tests were carried out in the sensory science laboratory of the Department of Food Science at the University of Stellenbosch (South Africa). The PLT beer (treated and control) was served and presented in glasses coded with a three digit random code. The beer sample size was 25 mL and was served directly from the refrigerator and was tasted in a light and temperature controlled room (21°C).

Prior to the testing, the consumer filled out a brief questionnaire, which was used to obtain gender and age information, and frequency of beer consumption. During the taste test, consumers indicated which term best described their attitude towards a given beer sample from the same batch using the nine point hedonic scale [10], without giving reasons for their preference. The scale was presented as follows: 9= like extremely; 8= like very much; 7= like moderately; 6= like slightly; 5= neither like nor dislike; 4= dislike slightly; 3= dislike moderately; dislike very much and 1= dislike extremely.

6.2.4 Statistical analysis of data

The Shapiro-Wilk test was used to test for normality. Skewness was equal to zero indicating that values were relatively evenly distributed on both side of the mean. Analysis of variance ANOVA (SAS®, version 9, SAS® Institute Inc, Cary, USA) was carried out to assess the variability between different beer samples, followed by the least

significant difference post-hoc test (LSD). The level of significance was taken as $P < 0.05$.

6.2.5 Descriptive flavour

Trained sensory tasters at the local brewery performed a descriptive flavour analysis. A panel of six professional tasters evaluated both beer samples (UV and non-UV treated) using a short format comprising an appropriate selection of flavour descriptors. The panel was asked to evaluate the intensity of the major flavour and aroma attributes in both beer samples. The intensity of each attribute was rated using a scale from 0-9. The mean intensity value of each score was reported on a spider radar graph.

6.2.6 Chemical analyses

Riboflavin and THIA hops were analysed by LCMS/MS and LCMS, respectively, as previously described in Chapters 4 and 5. Samples were centrifuged (Biofuge fresco by Heraeus) at 2500 rpm for 5 minutes and filtered through 0.45 µm pore size Millipore (Millex-HV) filter prior to injection. Calibration curves were constructed using external standards in order to quantify riboflavin and tetrahydroiso-α-acids hops in treated and untreated PLT beer.

Headspace sorptive extraction (HSSE) analyses were performed on a 6890 GCT (Agilent Technologies) equipped with a CTC CombiPAL Autosampler. Extraction of volatiles from beer was performed with 20 mL headspace sample vials sealed with magnet caps for 10 minutes at 35 ℃ using a PDMS fibre coating. Components were

separated on a 5% phenyl methyl siloxane (HP-5MS, 30 m x 0.25 mm x 0.25 µm, film thickness) column using helium as a carrier gas. The inlet injector was maintained at 260 ºC in splitless mode. The oven temperature was held at 30 ºC for 5 minutes and subsequently raised to 260 ºC at 5 ºC/min, followed by an increase to 320 ºC at 50 ºC/minute. The final temperature was maintained for an additional 5 minutes.

6.3 Results and discussion

6.3.1 Consumer sensory evaluation

The panel group consisted of 108 consumers, comprising 53 % females and 47 % males. Sixty four percent of the consumer panel were between the ages of 18 and 29, 21% between the ages of 30 and 39, and 15% between the ages of 40 and 59. Regarding the frequency of consumption of beer, 47% indicated that they consumed beer at least once a week, 37% at least once per month and 16% have never consumed beer.

Results, shown in table 1 indicate significant differences between the consumer preference for UV-C and non UV-C exposed beer at 1000 J/L. As found previously (Chapter 5) the non UV-C treated beer was preferred over the treated beer, indicating that certain chemical changes had indeed been affected. Figure 6.2 shows the distribution of scores over the nine classes of the hedonic scale, indicating which part of the scale most consumers preferred. The nine classes of hedonic scale were divided in three parties. *Dislike extremely, dislike very much, dislike moderately* and *dislike slightly* are

considered as one class of *dislike*. *Like extremely, like very much, like moderately,* and *like slightly* are a class of *like,* whereas the third class was *neither like nor dislike.*

Regarding the total consumer group, the non UV-C treated beer was described as *like* by more than 70% of the consumers. Approximately 9% described the non UV-C treated beer as *neither like* or *dislike*, while 21% *disliked* the beer. Only 30% of the panel indicated the UV-C treated beer as *like*, 12% neither *liked nor disliked*, while 58% *disliked* the UV-C treated beer. Both genders displayed a similar acceptability pattern toward the UV-C treated beer sample. As illustrated in figures 6.2, 51% of female consumer and 56 % of male consumer *disliked* the UV-C treated beer, respectively. Only 36 % female and 34% male *liked* the beer exposed to UV-C light. The UV-C treated beer consumers' appreciation is mainly influenced by the sensory characteristics of the product. Hence, from the trial results it was evident that both beer samples had a distinct flavour and taste.

There are contradicting reports indicating that beers hopped with THIA do not develop light-struck flavour [1, 11, 12]. However, UV-C light at 1000 J/L may have an impact on beer flavour and taste, which explains the consumer preference for the non-treated over the UV-treated beer. Similar results were obtained for UV-treated beer hopped with non-reduced hops (iso-α-acids) in our previous studies [Chapter 5], where consumers displayed a significant preference for a non UV-C treated beer over a UV-C sample at the same UV dosage levels of 1000 J/L.

It is quite obvious that irradiation of beer by UV-C light at high UV dosage impacts on the quality of beer. It must be stressed, however, that at relatively low UV

dosages, UV disinfection could be a viable non-thermal alternative in beer brewing and beer could receive more acceptability from consumers. In this regard, Stephenson [6], for instance, has shown that consumers can prefer a beer with a tone of LSF character. Furthermore, it must be kept in mind that consumer opinion for beer tasting can differ from those of experts [13].

Table 6.1 Overall preference for the UV and non-UV treated non-commercial beer enriched with reduced hops.

	Arithmetic mean hedonic value		
	Total group (N=108)	Female consumers (N=57)	Male consumers (N=51)
PLT	6.03 [a]	6.1 [a]	6.33 a
PLT + UV	4.48 [b]	4.45 [b]	4.5 b
LSD ($p<0.05$)	0.51	0.75	0.71

LSD= Least significant difference at the 5% level of significance. [a&b] differ significantly at the 5% level of significance

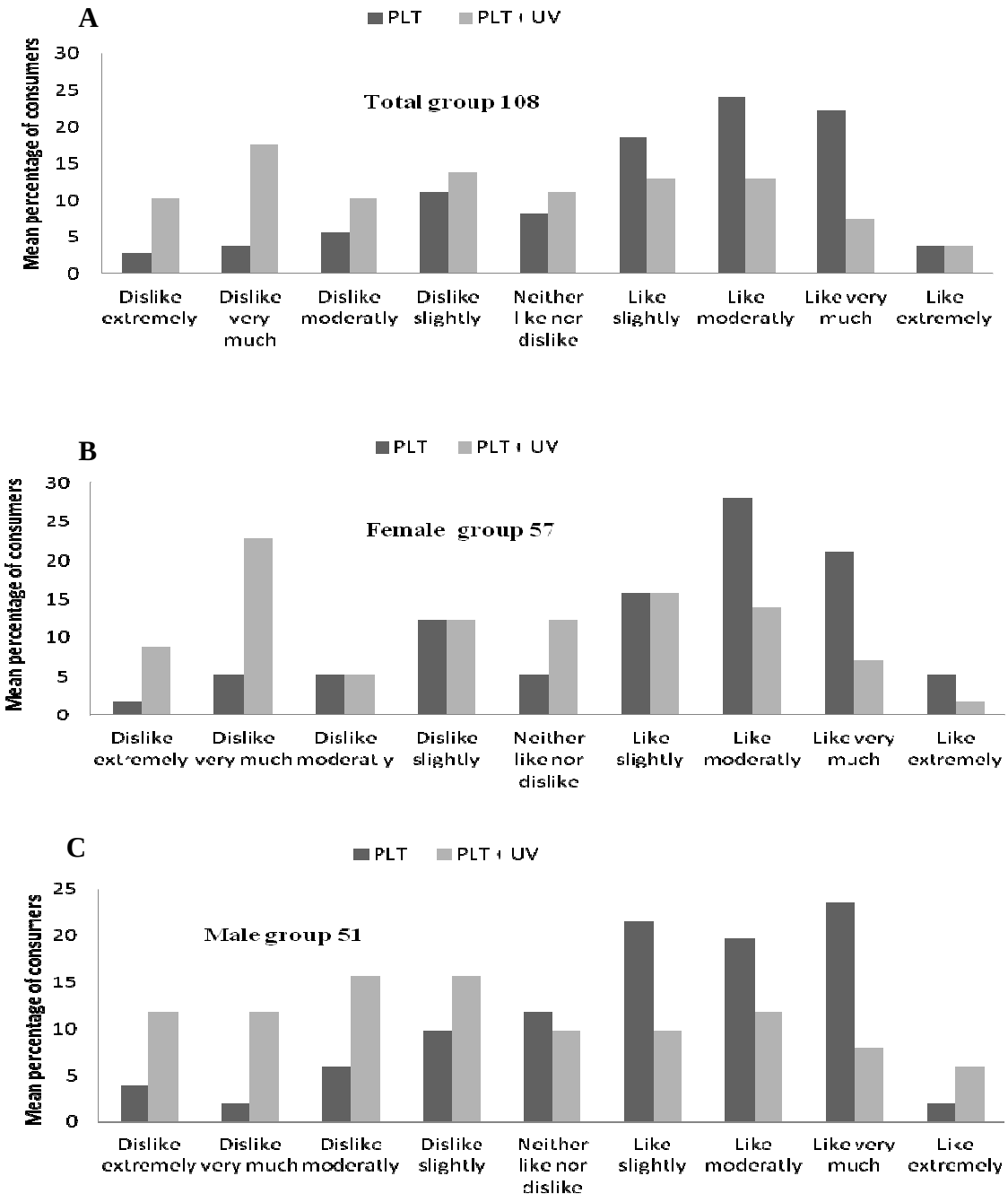

Figure 6.2 Distribution of scores for consumer beer trial. **(A)** Preference for the total group of consumer (N=108), **(B)** female group consumer (N=57) and **(C)** male group consumer (N=51).

6.3.2 Descriptive flavour analysis

A panel of six experienced tasters from SAB-Miller Ltd (Cape Town, South Africa) conducted a sensory evaluation on both UV-C and non UV-C treated beers. The beers assessed by the trained panel were the same as those tested by the consumer panel. Figure 6.3 displays a radar of descriptive flavour characteristics in both beers with the flavour intensity score from 0 to 9. During the tasting, the assessors' attention was specifically directed to the formation of LSF and other off-flavours. According to the results in figure 6.3, no light-struck flavour was detected in either beer samples at UV dosage of 1000 J/L. However, the panel detected a non-specific burnt flavour at an intensity score of 4 and 1 for UV-C and non UV-C exposed beers, respectively. Moreover, an average score of 2 was given for the presence of a smoky flavour in UV-C treated beer, while the flavour was absent in non UV-C treated beer. Burnt, smoky, light-struck and spicy flavours are all considered to be off-flavours in beer. The burnt and smoky off-flavours might form from an over-roasting of some beer constituents by UV-C irradiation. The assessment of the beer body by the trained panel was relevant as it showed that both the non-UV (control) and UV treated (1000 J/L) did not differ. The professional tasters gave the "body" of the beer a high intensity score of six, which can be classified as medium body. The body of the control and the UV treated beer had the same score. This indicated that the body of the beer did not influence the panel and that taste was the only difference between the control and the UV treated beer (figure 6.3). The UV-C treatment did not affect the taste e.g. sweetness, bitterness, of the beer tested, except for the spiciness that was found in the control, but not in the irradiated beer.

The irradiation of beer at high UV dosage level could impact negatively on the beer flavour even when beer is hopped with hops that do not develop MBT. Hughes [2] indicated that LSF in beer is not solely due to the formation of MBT and that other off-flavours might develop in beer on exposure to UV-C light. Other studies have shown that after prolonged UV-C irradiation, beer developed methanethiol and hydrogen sulphide, which can negatively affect the beer quality [2, 6]. Huvaere and De Keukeleire [14] demonstrated that when beer, hopped with THIA, was exposed to UV-B (280-320nm) light, an obnoxious off-flavour, which was not MBT, formed.

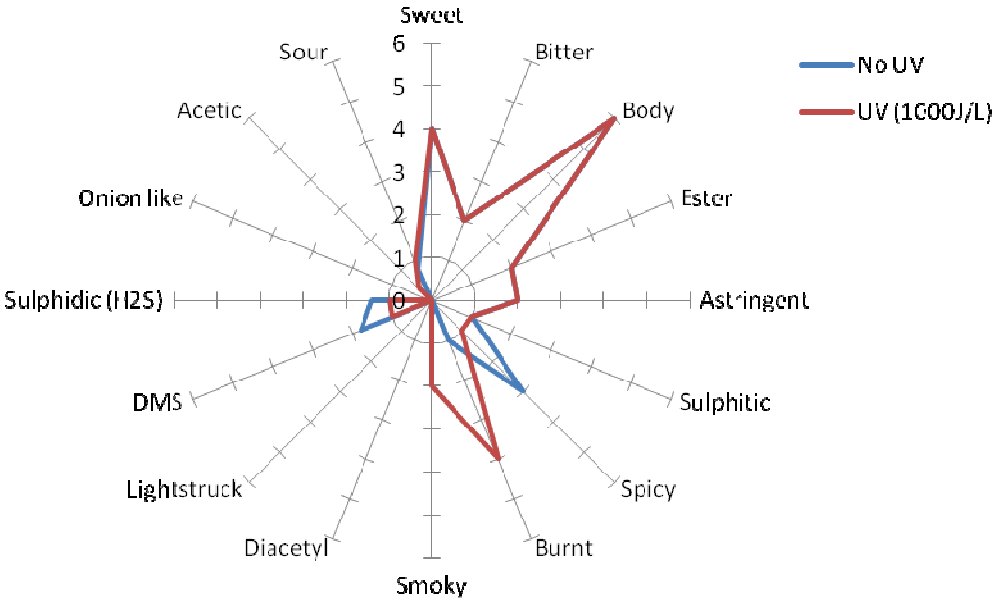

Figure 6.3 Descriptive analysis of core flavours in beer. A comparative flavour profile was done between the UV treated beer at 1000J/L and the control (non-UV treated). The intensity scale for scoring was from 0 (absent) to 9 (extreme).

6.3.3 Chemical analyses

6.3.3.1 Riboflavin analysis by LCMS/MS

Riboflavin was analysed in the control and in the UV irradiated beers by LCMS/MS as previously described (refer to Chapters 4 and 5). The riboflavin concentrations in control beer samples did not significantly differ (P>0.05) from beer treated at a UV dosage of 1000 J/L (figure 6.4). The trained panel also did not detect any LSF associated with MBT in the UV irradiated beer suggesting that MBT was not produced in detectable levels in the UV-C treated beer. Interestingly, about 30% of the total consumer group liked the UV treated beer, probably due to the presence of other off-flavours, e.g. smoky and burnt (refer to figures 6.2 and 6.3). There is no evidence that these off-flavours were triggered by riboflavin. The decrease in riboflavin levels in beer upon irradiation is due to photochemical reduction of riboflavin [15], subsequently leading to MBT formation.

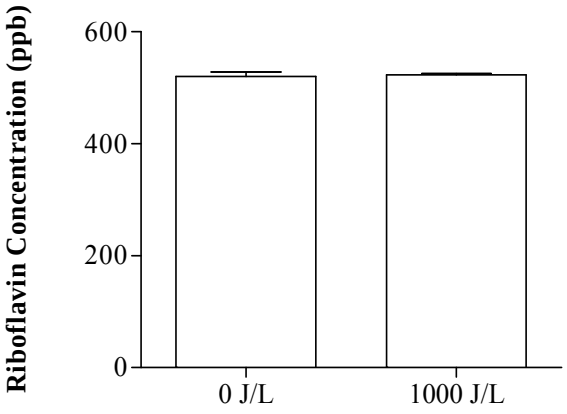

Figure 6.4 Riboflavin concentration in beer before (0 J/L) and after (1000 J/L) UV irradiation. Bars represent means with standard error (SEM) of the mean of duplicate determinations. Differences were statistically insignificant (P>0.05).

6.3.3.2 THIA analysis by LCMS

The THIA was analysed in beer by LCMS as described in chapter 5. The THIA was obtained from SAB-Miller Ltd (Alrode, South Africa) and was used as external standard for calibration. Our LCMS method optimised for unmodified hops-α-acid separation did resolve some of THIAs, however, the two pairs of THI-n-A and THI-ad-A, co-eluted and could not be separated (figure 6.5A). In the beer samples the THIA levels were not significantly different between the control and the overexposed UV beers (figure 6.5B). However, the THIA concentration was slightly lower in the UV treated beer suggesting the possible formation of photodecomposition products from THIA hops. This result is in agreement with several previous studies that revealed that, although THIA hops do not develop LSF upon UV treatment, the hops still could undergo photodecomposition reactions leading to radicals that could develop off-flavours [1, 2, 4].

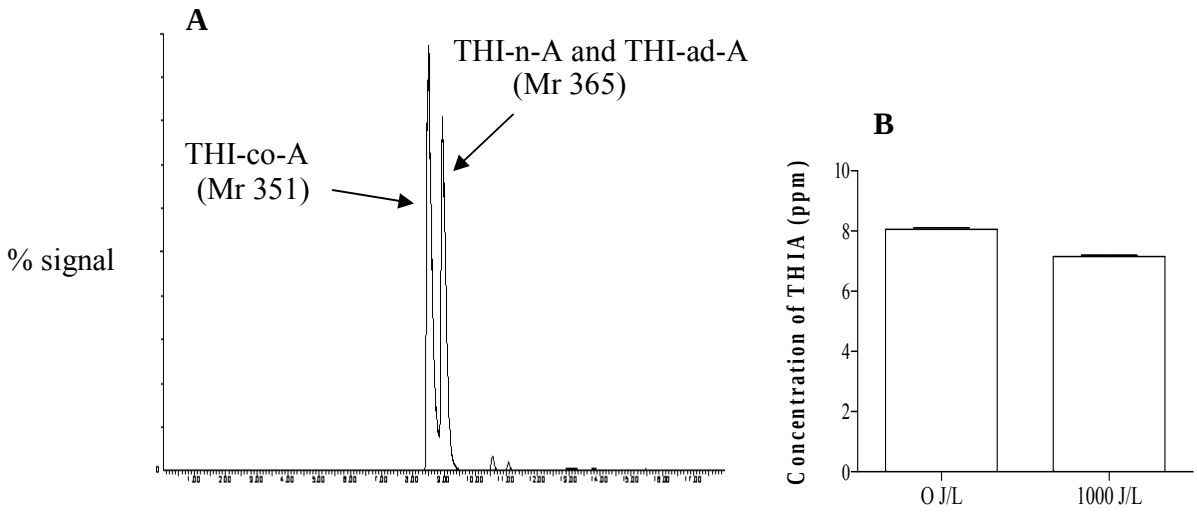

Figure 6.5 Analysis of THIA in beer by LCMSMS. (A) Representative chromatogram of the THIA standard used for calibration. **(B)** THIA hops concentration in beer before (0 J/L) and after (1000 J/L) UV irradiation. Bars represent means of duplicate analyses with SEM. Differences were statistically insignificant (P>0.05).

6.3.3.3 GC analysis of beer samples

Potential volatiles arising from control and UV irradiated beers were analysed by headspace GC. Figure 6.6 shows TIC chromatograms of both the control (0 J/L) and the UV exposed (1000 J/L) beer samples analysed by HSSE/GC. The two headspace chromatographic profiles differed significantly with respect to the intensity and ratio of the peaks (indicated with arrow in figure 6.6B). Three peak fractions, which eluted at 10.04, 13.33 and 15.50 minutes, significantly increased in the UV treated beer. These increases probably contributed to the flavour changes between the control and UV irradiated beer, described by the trained assessors (refer to figure 6.4). Two small peaks, which eluted at 14.45 and 15.12 minutes, indicating the formation of possible new volatile compounds, were observed in the chromatogram of the UV exposed beer (encircled in figure 6.6B). These small peaks were not observed in the control beer, thus may also be implicated in flavour changes and defects found in the UV exposed beer. Since THIA apparently do not give rise to MBT, possible off-flavours other than MBT were therefore observed by HSSE/GC. A more detailed investigation, which will include the identification of compound in these peaks, will form a part of future studies.

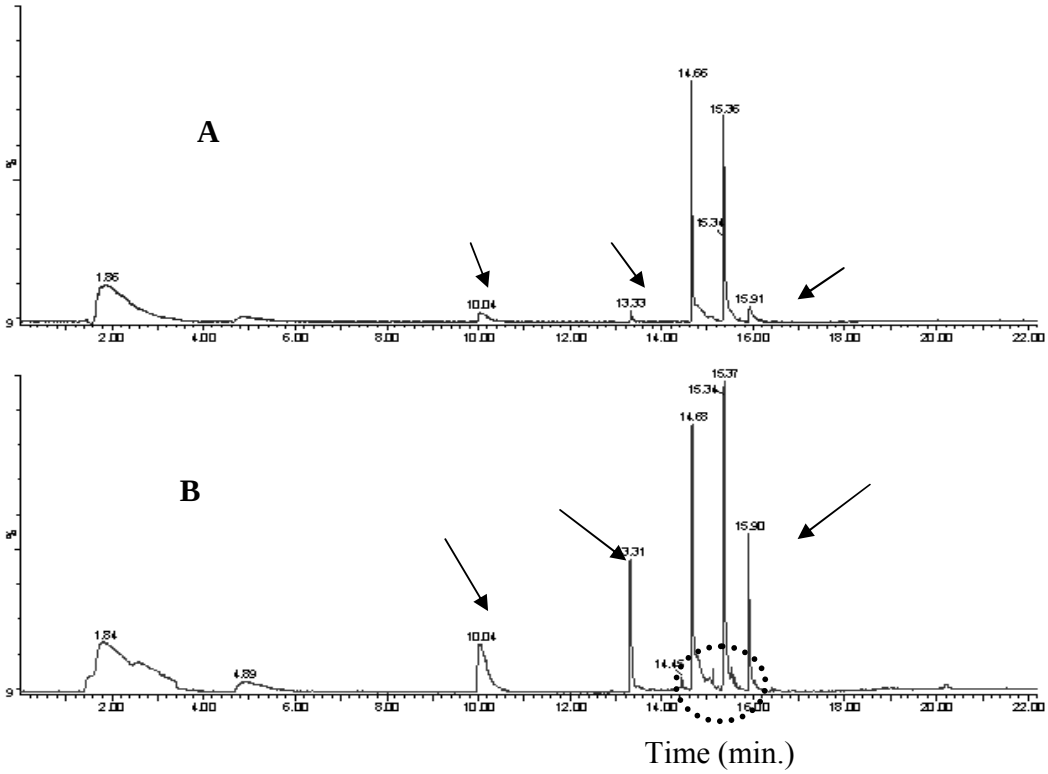

Figure 6.6 TIC chromatograms of non-UV treated **(A)** (O J/L) and UV treated beers **(B)** (1000 J/L). Arrows indicate the peaks of possible flavour/aroma compounds detected in the headspace.

6.4 Conclusions

The data from this chapter indicate that UV-C applied at a high dosage level to beer has a detrimental effect on the flavour and aroma of beer. The use of THIA in brewing has been claimed to produce beer that are not sensitive to light [12]. However, unidentified burnt and smoky off-flavours developed in a non-commercial beer hopped with THIA and exposed to UV light at dosage of 1000 J/L. There was a clear correlation between the consumer trial data and the descriptive flavour from a panel of professional

tasters. No significant change in both riboflavin and THIA concentrations was observed. However, off-flavours, e.g. smoky and burnt, were pointed out in the beer exposed to UV-C by sensory analyses. This finding correlated with the significant difference observed between the intensities and ratios in the peaks of compounds in the headspace as analysed by GC. Overall, the results in this study were in good agreement with previous findings [2, 14] that THIA do not produce MBT (as indicated by our expert taste panel) upon UV exposure, but could furnish other obnoxious off-flavours. Given the complexity of beer, it was apparent that other off-flavours formed on irradiation with UV-C light.

From the results obtained in this study it is apparent that MBT is not the major component of LSF generated in beer containing THIA by UV-C irradiation. In contrast, it is well established that the LSF induced by UV and visible light, between the wavelengths of 280 to 500 nm, can be mainly attributed to MBT [1-3, 11]. The composition or chemicals, producing LSF in beer therefore depends on the wavelength of the UV-light the beer is exposed to. To investigate the influence of UV light at different wavelengths, a smaller bench scale UV system was needed. A system consisting of UV emitting diodes (UV-LEDs) to sterilise beer, was thus developed. The design and data obtained with this system are presented and discussed in Chapter 7.

6.5 References

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CHAPTER 7

INACTIVATION OF MICROORGANISMS IN BEER WITH ULTRAVIOLET LIGHT-EMITTING-DIODES AT WAVELENGTHS OF 250 AND 275 nm

7.1 Introduction

The uses of commercial germicidal lamps that are enclosed in a quartz protection sleeve have mostly been available for generating UV light. Low-pressure (10^2 to 10^3 Pa) mercury lamps designed to produce energy in the germicidal region of 254 nm are the most practical for industrial and laboratory disinfection. However, mercury lamps cause a number of environmental concerns revolving around the use of toxic mercury to generate UV radiation [1]. In addition, sterilising beer using germicidal low-pressure mercury UV lamps provide UV light with a too high radiation intensity, which could affect adversely beer flavour (see chapter 5 and 6).

UV light emitting diodes (UV-LEDs) can be an excellent replacement for mercury lamps due to their compact size and low voltage operation. UV-LEDs constitute an exceptionally stable light source and do not contain glass, filament or mercury [2-5]. They are semiconductor based light sources, which emit focussed radiation in the UV spectrum and can be operated by direct or alternating currents. Although research into the use of UV-LEDs in disinfection technology is still in its infancy, the use of UV-LEDs has received a considerable amount of attention in water disinfection [6-9].

The aim of this study was to evaluate the use of UV-LEDs at output wavelengths of 250 and 275 nm for the treatment of beer through the following objectives: first, investigating the potential inactivation of *L. brevis* inoculated in beer as test organism and

second, investigating the photo degradation effect of UV-LEDs on riboflavin and hops iso-α-acids present in beer *en route* to the development of light struck flavour. To evaluate the effects of the UV-LEDs on beer, a small bench-scale UV-LEDs system was designed and constructed to process beer at room temperature. This system can handle a small volume of beer with low power requirements. Moreover, the laboratory scale device offers the possibility to use UV-LEDs that emit light at different output wavelengths. The demonstration of the potential application of the device will provide a new approach regarding the treatment of beer by UV light.

7.2 Materials and methods

7.2.1 Chemicals

Tryptone, glucose, yeast extract and De Man, Rogosa and Sharpe (MRS) broth were obtained from Sigma-Aldrich (South Africa). Tween 80 (polyoxyethylene sorbitan monooleate) was obtained from Merck (South Africa). Tryptone, yeast extract and Agar were obtained from Fluka (Spain).

7.2.2 Bench scale UV-LEDs apparatus

A novel small bench scale UV diode system was designed and manufactured at the University of Stellenbosch. The system configuration consisted of a 6 mm diameter by 150 mm long quartz tube fixed vertically in an enclosed container as shown in Figure 7.1. The UV diode (AlGan/Gan LED chip/chip array) was encapsulated in a metal-glass with a UV-transparent optical lens and was fixed inside the box 48 mm (the focal point of the lens) from the quartz tube. The UV-LEDs at 250 and 275 nm produce an optical power

(radiant flux) of 300 and 500 µW, respectively, at a manufacturer recommended current value of 20 mA. The radiant flux was not measured during the experiment; therefore UV dose energy could not be calculated. Beer was pumped through the system from a feed 50 mL glass bottle using a small PERISTA pump (Chromatograph ATTA, Japan).

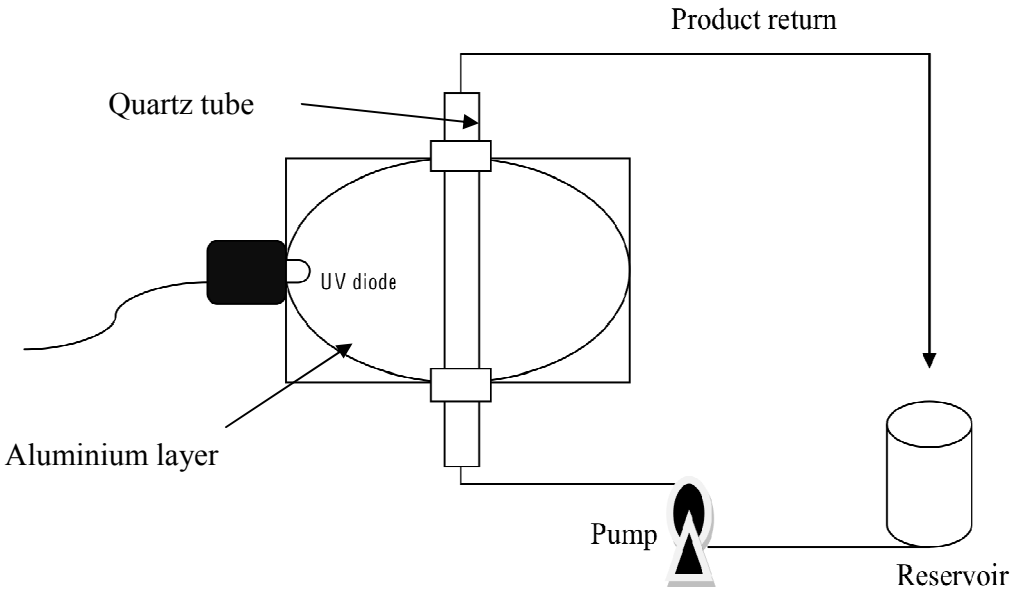

Figure 7.1 Flow diagram of the bench scale UV-LED apparatus.

7.2.3 Microorganism cultures and growth conditions

Escherichia coli (GM 109) and *Lactobacillus brevis* (ATCC 27305) cultures were obtained from the University of Stellenbosch as frozen culture stocks. *E. coli* and *L. brevis* were inoculated direction from frozen stocks onto LB and MRS agar plates,

respectively, and incubated for 48 hours at 37 °C before each experiment. Three to four colonies of bacteria population were removed from the agar plate and incubated under optimal conditions in nutrient broth at 37 °C overnight in rotary shaker (150 RPM). Overnight cultures were diluted into fresh medium and sub-cultured before the commencement of the experiments. The optical density (OD) of sub-cultured bacteria was measured at 600 nm with a DU 650 spectrophotometer (Beckman, USA). Sub-cultured *bacteria* were added to a commercial lager beer to achieve a final concentration of 10^4 to 10^5 CFU/mL.

7.2.4 UV-LED irradiation

The UV-LED irradiation was performed at room temperature. Commercial lager beer were spiked with bacterial cultures and exposed to UV-LEDs at 250 and 275 nm wavelengths. The spiked beer was pumped and circulated through the bench scale UV-LED system. The beer was exposed for 2.5 hours for *E. coli* with samples taken every 30 minutes, while for *L. brevis*, the beer was exposed up to 5 hours and samples taken every 60 minutes. To ensure penetration of the UV light, a low flow rate of 2 min/mL was utilised in the system. Controls were identical beer samples spiked with bacterial cultures and circulated with the UV-LED light turned off over the same period of time. Bacterial counts were determined on controls before and after experiments to ensure that bacteria were not inadvertently inactivated by procedures during the test, but by the UV-LED intensity. All the UV-LED irradiation experiments were performed in the dark at room temperature.

The system was cleaned using standard "Cleaning In Place" (CIP) principles after each run. First, the device was washed by cold analytical grade water (MilliQ Waters, Massachusetts) for 30 minutes and cleaned by 0.1 % NaOH for 30 minutes. The NaOH was removed by 0.5% paracetic acid for 30 min and finally the system was cleaned by warm analytical grade water for 45 minutes. The "dead volume" after cleaning was microbiologically analysed to ensure sterility.

7.2.5 Determination of the inactivation level of E. coli and L. brevis

UV irradiated beer samples were analysed for microbial survival by standard plate count. A series of dilutions were made with respective culture broth medium and plated in duplicate on plastic Petri dishes. Plates were incubated at 37°C for 48 hours and the number of colonies which appeared on the plate was manually counted (figure. 7.2) for calculation of colony forming units per mL (CFU/mL). The log reduction of the bacteria was determined for each exposure time, based on the initial non-irradiated bacteria concentration (N0), using the following equation: **log reduction = log (N0/Nt),** where Nt is the number of bacteria colonies after irradiation, and N0 is the number of bacteria colonies before irradiation. The log reduction was plotted against the exposure time in hours.

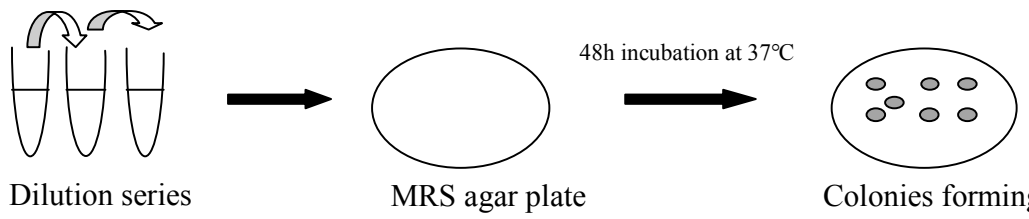

Figure 7.2 Standard plate count assay for determining bacterial survival in beer samples irradiated with UV-LED.

7.2.6 Chemical analysis

Riboflavin and hops iso-α-acids concentrations were estimated in the beer exposed to UV-LED at 250 and 275 nm by LCMS/MS as previously described (see Chapter 4 and 5). Beer samples were centrifuged (Biofuge fresco by Heraeus) at 2500 RPM for 5 minutes at room temperature and filtered through a Millipore Millex-HV 0.45 µm pore size filter before submission to LCMS analyses.

7.3 Results and Discussion

7.3.1 Inactivation of E. coli and L. brevis

The possibility of using commercially available UV-LED systems for water sterilisation has been illustrated [5-7] and these studies reported significant reductions of *E. coli* in water [5, 6]. The potential ability of our UV-LED system to inactivate *E. coli* in beer at 250 and 275 nm was first examined (figure 7.3) using time trials. The beer was exposed for two hours and 30 minutes, and samples were taken every 30 minutes to determine the bacterial count/survival. The time trial results showed that the UV-LED system can inactivate more than 99% of the *E. coli* in beer within two hours, with irradiation at 250 nm more efficient than 275 nm. After two hours exposure, a log 2.3 reduction was obtained at 250 nm while at 275 nm a log 1.5 reduction was observed.

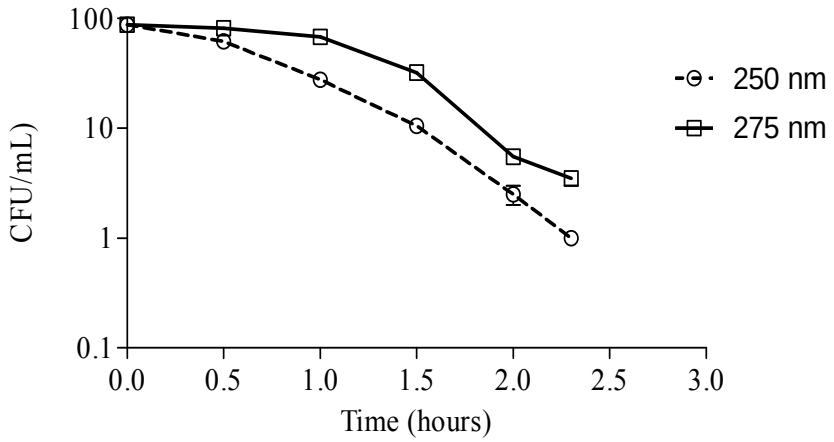

Figure 7.3 Inactivation of *E. coli* in beer by UV-LED at 250 nm and 275 nm wavelengths. Data points represent mean of duplicate repeats with SEM.

L. brevis is a Gram-positive bacterium which constitutes a serious concern to the brewing industry [10]. The inactivation of *L. brevis* was also determined by a colony-forming assay. Log reductions were measured at mid-exponential (OD_{600} = 0.38) and stationary (OD_{600}= 0.59) phases of *L. brevis*. The inactivation of *L. brevis* at different cell concentrations was compared at both 250 and 275nm wavelength exposure. Figure 7.4A shows UV-LED inactivation of *L. brevis* at 250 nm. The highest microbial reduction was observed after five hours exposure with more than 99% of *L. brevis* inactivated. These bacteria at mid-exponential phase showed more sensitivity toward the UV-LED irradiation at 250 nm with a log 3.3 reduction after five hours treatment. Beer samples spiked with cultures in the beginning of the stationary phase (OD_{600}= 0.59) of *L. brevis*, cells showed more resistance to UV-LED irradiation with only a log 1.1 reduction. Figure 7.4 shows the inactivation rate at an output wavelength of 250 nm. A similar, but less efficient, inactivation pattern was obtained as with the irradiation at 275 nm (Figure 7.4). The mid-exponential growth phase of *L. brevis* (OD_{600}= 0.38) showed more sensitivity

toward lethal UV-LED radiation than the stationary phase (OD_{600}=0.58). A log 2 reduction was obtained after five hours exposure for cells at log phase (OD_{600}= 0.38), while at stationary phase, the log reduction was only 0.4. The log reduction was insignificant during the first hour of exposure, but increased with an increase in exposure time indicating an inability of the damaged bacteria to recover after a certain period of exposure.

The sensitivity of the test organism is essential for any disinfection technique. Several studies have shown that the physiological state of the microorganism, such as the growth phase, can affect its sensitivity toward UV light [11, 12]. Our study showed that the UV sensitivity of *L. brevis* is related to its growth phase; with the highest sensitivity towards UV radiation in the mid log phase and a lower sensitivity in the stationary phase.

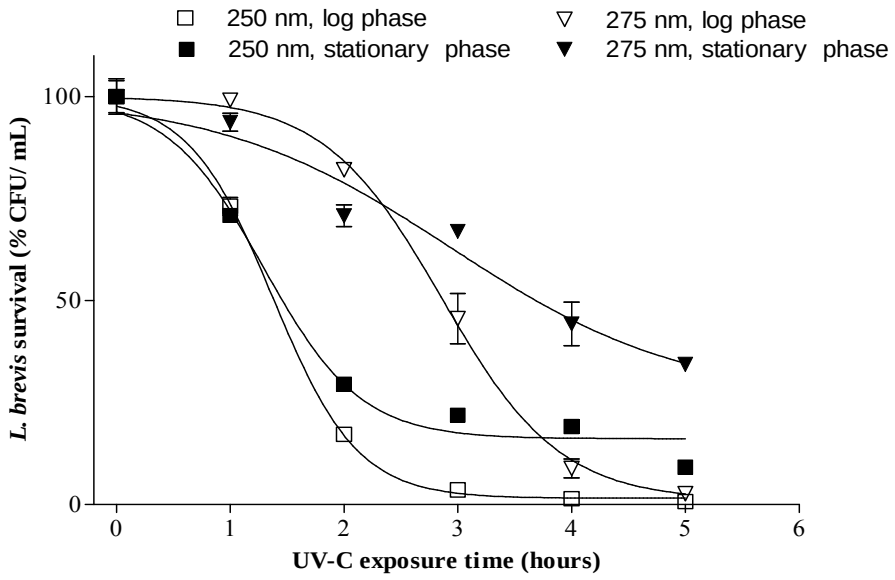

Figure 7.4 Inactivation of *L. brevis* in beer by UV-LED at 250 and 275 nm. Bacteria were exposed at two different growth phases (log and stationary phases). Data points represent means of duplicate repeats with SEM.

7.3.2 Comparison of UV-LEDs emitting at 250 nm and 275 nm

The 250 and 275 nm UV-LEDs have different power outputs, which may impact the UV efficiency on the microorganism. The disinfection efficiency at 250 and 275 nm were compared with *L. brevis* at mid exponential growth phase (OD= 0.38) (figure 7.5). This was expressed by plotting log reduction (Nt/N0) against the exposure time. These results shows that the lower wavelength at 250 nm was more efficient than the higher wavelength at 275 nm after two hours exposure to UV light (figure 7.5). After a longer exposure of five hours the UV-LED at 250 nm gave approximately a log 2 reduction with 99% inactivation of *L. brevis*. The 275 nm wavelength gave approximately a log 1.8 reduction with more that 90% inactivation of the bacterium with the same period of exposure time (five hours).

Disinfection at 250 nm wavelengths is preferable for the overall performance of the UV-LEDs sterilisation of *L. brevis* in beer. Bowker *et al.* [13] showed that UV-LEDs that produce much lower power output result in lower incident irradiance values in the inactivation of *E. coli*. They compared 255 and 275 nm UV-LEDs to inactivate *E. coli* in water, and found that 275 nm produced the higher inactivation rate. However, from our study the 250 nm UV-LED produced the higher *E. coli* and *L. brevis* inactivation than the 275 nm UV-LED. Latter could be due to the fact that the absorption of *L. brevis* at a wavelength 250 nm is greater than at 275 nm [14, 15]. Comparing 250 nm and 275 nm UV-LEDs was based on the same input power and time, resulting obviously in different dosage energies. However, UV dosages energy was not determined in this study.

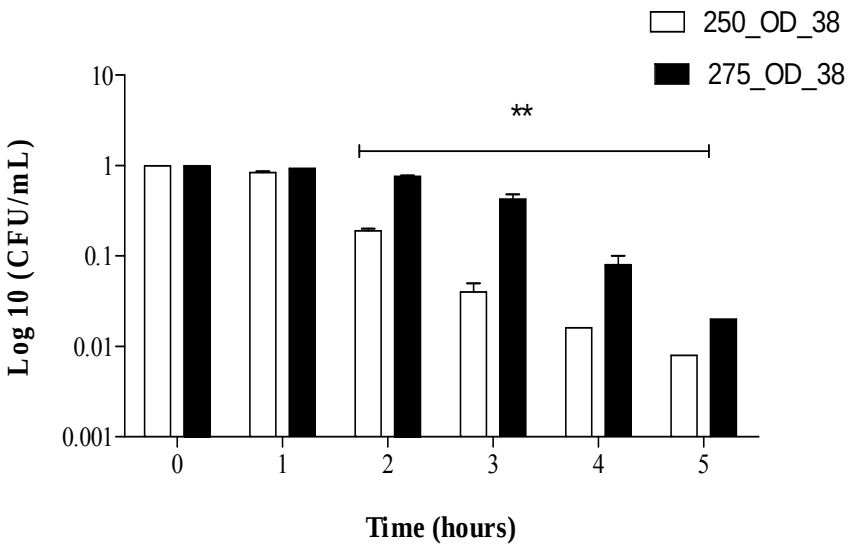

Figure 7.5 Reduction rates of *L. brevis* at 250 and 275 nm wavelengths of UV-LED irradiation. Data points represent means of duplicate repeats with SEM. ** $P<0.05$ for 250 nm treatment compared to 275 nm treatment.

7.3.3 Chemical analyses of riboflavin and hops iso-α-acids by LCMS

Riboflavin and hops iso-α-acids were analysed as previously described (Chapters 4 and 5) in the UV-LED irradiated beer samples. Overall, riboflavin showed a significant decrease for both output wavelengths of 250 and 275 nm, after 5 hours exposure to UV-LED, as shown in figure 7.6 ($P<0.001$). Since the intensity produced by UV-LED at 250 and 275 nm is small compared to the intensity from a low pressure mercury UV lamp [16], a significant decrease of riboflavin was not expected. Ahmad *et al.* [17] demonstrated that the rate and magnitude of the photo-degradation reaction of riboflavin in phosphate buffer depends on light intensity, as well as the output wavelengths of irradiation. The long exposure time (five hours) may have contributed to the photo-degradation of riboflavin in UV-LED treated beer. However, the concentration of

riboflavin started to significantly decrease after two hours exposure at 250 nm (P<0.01). Riboflavin has absorption maxima at 254, 313, 366 and 445 nm [17], which may make it more susceptible to absorb light at 250 nm than 275 nm. Moreover, 250 nm showed to be significantly more efficient than 275 nm in reducing microbial load in beer (refer to figure 7.5). The statistically significant decrease of riboflavin level in irradiated beer at 250 and 275 nm suggested that LSF is liable to form on prolonged exposure.

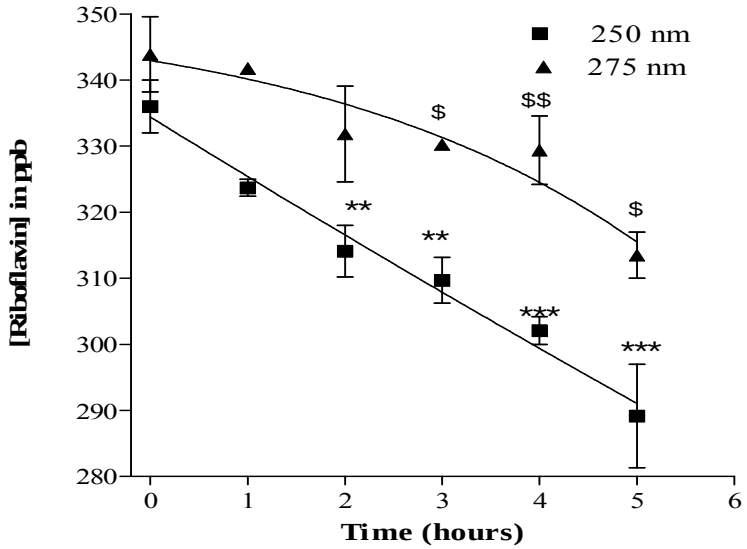

Figure 7.6 Riboflavin concentrations in beer irradiated with UV-LED at 250 and 275 nm. Error bars represent means of two repeats with SEM. $$ P<0.01; $ P<0.05 for 275 nm compared to 250 nm treatment. *** P<0.001; **P<0.01 for treatment to control (0 hours).

The photolysis of hops iso-α-acids in beer is part of the formation of light-struck flavour. Liquid chromatography was applied to measured hops iso-α-acids in lager beer exposed to UV-LEDs at 250 and 275 nm. No significant decrease of iso-α-acids was observed at 250 nm after five hours exposure time, as shown in figure 7.7. However, significant decreases began after four hours at 275 nm (P<0.05). These findings were not

expected as iso-α-acids have absorption bands with a maximum around 250-255 nm [18]. Nevertheless, there was a decrease at 250 nm but not statistically different to the control beer.

Like riboflavin, the photo-degradation of hops iso-α-acids by UV-C light is an indication of the formation of LSF [18-20]. Depletion of iso-α-acids upon UV-LED irradiation correlated with riboflavin decrease at both wavelengths (250 and 275 nm). These findings suggested long exposure time using UV-LED at 250 and 275 nm may have affected chemical and sensory qualities of the beer tested. However, these results require further investigation, which includes measurement of MBT in beer by an optimised SEP/GCMS, as well as conducting sensory tasting trials.

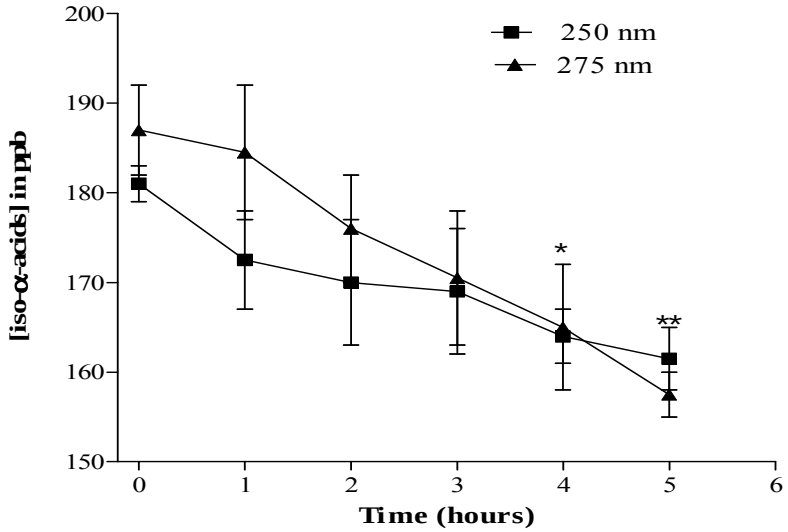

Figure 7.7 Iso-α-acids concentration in beer irradiated with UV-LEDs at 250 nm **(A)** and 275 nm **(B).** Data points represent means of two repeats with SEM. **P<0.01; * P<0.05 for treatment at 275 nm compared to control (0 hours).

7.4 Conclusions

We illustrated with our novel bench scale UV-LED system that UV-LEDs at 250 and 275 nm wavelengths can inactivate bacteria in beer. The reduction of *E. coli* was more efficient than *L. brevis*. The highest log reduction was obtained at the mid-exponential growth phase of the test organism for both wavelengths. UV-C exposure at 250 nm was more efficient than an exposure at 275 nm for the same exposure time. Previous reports cited in the literature have shown the ability of UV-LEDs to inactivate *E. coli* in water at 365, 282, 269 and 265 nm. This is an indication that a wide range of germicidal wavelengths can be applied to sterilise beer. Moreover a UV-LED sterilisation device can be developed as an alternative UV-source to low-pressure mercury lamps.

Riboflavin and iso-α-acids in irradiated beer were significantly affected at both wavelengths of 250 and 275 nm due to prolonged exposures. However, the effect of UV-LED on physical, chemical, and sensory properties of beer requires a more detailed investigation. The UV-LED application is still limited by long exposure times, although this novel technology can provide a promising approach for disinfection of beer.

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CHAPTER 8

GENERAL DISCUSSION

The use of UV-C disinfection is gaining acceptance as a non-thermal process in the beverage and food industry [1-4]. In the brewery industry, the applications of UV-C irradiation are mostly disinfection of the water used in breweries, also in the treatment of caps, cans, and air in packaging areas. It has been known for quite some time that beer is a light sensitive beverage [5-7]. Thus, beer must be stored, either in cans, or in brown bottles to prevent any deterioration from light. On exposure to visible and UV lights, beer develops an unpleasant flavour, the so- called LSF. The compound responsible for LSF was identified to be MBT, a mercaptan with an extremely low flavour threshold between 1-35 ppt [8]. MBT is produced when iso-α-acids, hop-derived bitter agents, react with sulphur-containing compounds in the presence of light and riboflavin [9-10]. Iso-α-acids hops can be chemically modified to prevent MBT formation. These chemically modified hops, also called reduced hops, such as THIA, have been used to control the impact of light on beer flavour [10]. THIA do not develop MBT; therefore provide an effective means of controlling LSF sensitivity.

The aim of this PhD was to investigate the effect of a UV-C disinfection strategy on the chemical composition of beer and to correlate these changes with potential flavour changes that might occur upon UV-C treatment. The specific goals were to examine the ability of pilot scale and bench scale UV-C systems to reduced spoilage microorganisms in beer, as well as investigating chemical properties and sensory changes with a specific attention on the development of LSF. To the best of our knowledge, very little is known on the potential applications of UV-C disinfection of beer and no previous studies have taken an integrated approach to investigating the microbiological, chemical and sensory quality effects

of UV-C irradiation of beer. In order to assess any possible formation of LSF in UV treated beer we first developed analytical tools for the analysis of chemical changes with respect to compounds that are associated with the development of LSF in beer. A range of analytical techniques have previously been used to study LSF in beer [8, 10-15]. These techniques included a direct assessment of LSF by the analysis of MBT in beer [8, 11, 13] and an indirect assessment by the analyses of iso-α-acids hops and riboflavin [14-17] as the role of riboflavin in the formation of MBT in beer is well documented [9, 10]. In this study GCMS and LCMS techniques were developed and customized to analyse these LSF related chemical changes in beer upon UV-C treatment.

The analysis of MBT presents a serious challenge by any analytical instrumentation due to its extreme volatility, low flavour threshold and concentration in beer. There are reports of the detection and quantification of MBT by the use of a purge, trap and desorption technique with a trap adsorbent material such as tenax GR/TA [11, 13]. However, the use of such trap requires important adaptation to the GC with thermal desorption equipment [11, 13]. A similar purge, trap and desorption method, using a probe coated with polydimethylsiloxane (PDMS) rubber sleeve as an absorbent material, called the SEP [18] was optimised to detect MBT in beer in this study. Detection as low as 1 ppt of MBT in water and 5 ppt of MBT in beer was possible with the use of the SEP technique. The SEP technique showed promising results for the detection of MBT in beer and required only minor inexpensive adaptations to the GC and did not require any thermal desorption equipment. To detect MBT at ≤ 1ppt in beer more optimisation, which may include a different absorbent material and more sensitive instrument, is necessary to incorporate this method into routine analyses of irradiated beer.

The analysis of photo-induced degradation of riboflavin and iso-α-acids hops were performed by LCMS/MS as an indirect means to investigate the development of LSF in beer

(see Chapter 4 & 5). The susceptibility of beer to develop LSF upon exposure to UV-C light can be determined by measuring riboflavin concentration before and after UV irradiation. Riboflavin has been intensively studied for his role as a photosensitiser in the formation of LSF [14, 15]. Thus, a simple and effective method was developed for the determination of riboflavin concentrations in beer using LCMS/MS. This direct LCMS/MS method can be used for the routine analysis of riboflavin in beer. However, the usefulness of this type of analysis is not unequivocal, as we did not observe significant changes in any of our trials of UV-C irradiated beer.

The formation of photo-degradation products from iso-α-acids hops in UV irradiated beer is essential for the development of LSF [9]. Any decrease in hops iso-α-acids concentration upon UV-C exposure could lead to 3-methylbut-2-enyl radical formation on route to LSF. LCMS has been shown to be an efficient tool for the analysis of hops iso-α-acids in beer [16]. Vanhoenacker *et al*. [16] successfully developed an LCMS method for quantification of hop-derived bitter compounds in beer. Thus, a modified LCMS method of Vanhoenacker *et al*. [16] was developed to measure iso-α-acids hops in beer (Chapter 5). The measurement of hops iso-α-acids concentration in beer by the LCMS method was simple and reliable. Significant changes in hops iso-α-acids in our trials of UV-C irradiated beer were found and confirming that this direct LCMS method can be used for the routine analysis of hops iso-α-acids in beer, both in quality control and to assess the LSF potential.

To assess the impact of UV on microbial contamination in beer a series of pilot scale UV treatments of beers were conducted with a turbulent flow UV-C system from SurePure® at the Department of Food Sciences (University of Stellenbosch). The first trial (Chapter 4) was to evaluate the efficacy of the UV treatment system at 254 nm to reduce bacterial load in beer with *L. brevis* and *A. pasteurianus* as test bacterial strains. The impact of UV irradiation

of yeast was tested using *S. cerevisiae* as test organism. A range of different UV dosages energy of 25, 50, 100, 250, 500, 1000, and 2000 J/L were applied to a commercial draught pale lager beer. An estimate log 10 reductions (CFU/mL) of 2.3, 4.7, and 3.8 for *S. cereviciae, L. brevis*, and *A. pasteurianus*, respectively, was achieved at 1000 J/L. The pilot scale turbulent UV system successfully reduced the microbial load in the beer treated, although *S. cereviciae* was more resistant to UV than *L. brevis* and *A. pasteurianus*. Keyser *et al.* [19] successfully applied the same turbulent UV technology to reduce bacteria in fruit juices and nectar. From a microbiological point of view, the use of such UV system showed to give promising results for improving microbial safety in beer. Results obtained from this trial indicated that the riboflavin concentration did not differ significantly between non-UV and UV treated beer samples for all UV dosage levels applied. However, MBT was detected in samples irradiated at a UV dosage of 2000 J/L. De Keukelereire *et al.* [9] showed that iso-α-acids hops could decompose following direct absorption of UV-B light (280-320 nm) without any intervention of riboflavin. UV-C dosage of 500 J/L yielded an adequate reduction in bacterial count, but no MBT at ≤ 5 ppt was detected nor changes in riboflavin concentration in the beer sterilised at this dosage. Thus, our results suggested that MBT could be formed on direct exposure to UV-C (254 nm) at high dosage level without the intervention of riboflavin. The ability of the pilot scale UV system to reduce microorganisms in beer, without compromising detectable chemical integrity, was promising and subsequent investigations therefore focussed on sensory and chemical properties of irradiated beer. It was therefore decided to overexpose the beer in subsequent experiments at 1000 J/L to bring about measurable changes in off flavour producing compounds in order to better understand the potential side effects of treating beer with UV-C irradiation for sterilisation.

The second beer trial (Chapter 5) was carried out on non-commercial and commercial beers exposed at an UV dosage of 1000 J/L and included a series of sensory tests conducted by consumers' tasters. The objective was to first investigate any sensory changes and to subsequently obtain a correlation with chemical analyses. The consumer tasting trial showed significant difference in preference between non-UV treated and UV treated beers. Consumers' choice was less in favour of both the commercial and non-commercial UV exposed beers at 1000 J/L. Stephenson and Bamforth [20] conducted a consumer study on the impact of LSF and stale character in beers. They found that there is a significant probability that consumers may prefer beer with a measure of LSF character. This finding suggests that consumers could perceive LSF as a positive attribute to specific beers although brewers regard LSF as a deficient flavour. At a level below its flavour threshold, MBT might therefore play a positive role in beer flavour as it possibly does in other beverages [10]. Riboflavin levels did not significantly change although there was some decrease, but a significant decrease in hops iso-α-acids concentration were observed in UV treated beers in this study. These findings correlated to the consumer sensory evaluation in which a general a dislike of UV-C was indicated. Heyerick *et al.* [14] noted that LSF was clearly detectable by sensory analyses without noticeable degradation of iso-α-acids hops. In fact, only one iso-α-acids molecule in one million needs to be degraded by light to give LSF [21]. The development of LSF appeared to depend on the type of beers. The dark lager beer showed to be more susceptible than pale yellow lager to flavour deterioration by UV irradiation, which is in agreement with previous studies [10, 21]. Moreover, the beer with the highest concentration of iso-α-acids, i.e. the pilsner beer used in this study, also was more susceptible than the other beers to LSF formation and elicited a more negative sensory evaluation.

The third trial (Chapter 6) was conducted with a non-commercial lager beer hopped with modified (reduced) iso-acids such as THIA at UV dosage of 1000 J/L. THIA impart bitterness, but do not degrade upon exposure to UV-C light forming LSF [24-26]. However, in the absence of overt potential to form LSF, consumers again displayed significant preference for the non-UV treated beer compared to UV treated beer. A panel of professional tasters did, however, not detect LSF in both beer samples (UV and non-UV). The flavour profile analysis revealed the presence of burnt and smoky off-flavours in the UV treated beer, which may point to the degradation of other abundant compounds such as amino acids, peptides, proteins [10, 15] and carbohydrates (other than the sugars that we analysed in this study) and other sulphur compounds. These results clearly indicate that other off-flavours, which are not MBT related, may develop in beer upon exposure to UV-C at 1000 J/L. Huvaere and De Keukeleire [27] also reported that UV irradiation (280- 320 nm) of beers, hopped with THIA, did not produce MBT, but furnished an obnoxious off-flavour that has to date not been identified. Both trials described in Chapters 5 and 6 confirmed that UV-C treatment, at a dosage of 1000 J/L, could change the sensory and chemical properties of the beers tested. It also clearly showed that UV-C treatment did not induce MBT formation but that the concentration of existing volatile flavour components was significantly increased. It is evident that future studies will have to focus on the identification of these compounds in order to determine the exact effect UV-C treatment will have on the chemical composition of beer.

The composition or chemicals, producing LSF in beer depends on the wavelength of the UV-C light the beer is exposed to [9]. The final goal was therefore to perform a series of bench scale tests to sterilise beer at wavelengths 250 and 275 nm. A bench scale UV-LED system was designed and developed to inactivate microorganisms in beer (see Chapter 7). UV-LEDs offer the possibility to use different wavelengths and require low power and

voltages compared to low pressure mercury UV lamp [28-31]. *E. coli* and *L. brevis* was spiked in beer and exposed to UV-LEDs at output wavelengths of 250 and 275 nm. The results from the study showed that UV-LEDs were efficient in reducing *E. coli* and *L. brevis* in beer. The lower wavelength at 250 nm was significantly more efficient in inactivating the bacteria than the higher wavelength at 275 nm. Although UV-LED technology is still in its infancy and has only been tested for water disinfection [28-31], our results demonstrated the functionality of the developed UV-LED disinfection device. Thus, the application of this novel device provides a promising approach to sterilise beer using UV-LEDs. However, significant changes in both riboflavin and iso-α-acids concentrations were observed in beer treated by UV-LED. These chemical changes may have been occurring due to a prolonged exposure time of beer to UV-LEDs. Further investigation is needed in order to elucidate the effects of UV-LED on sensory and chemical properties of beer and to consider UV-LED as an alternative to UV mercury lamps. LEDs also provide the opportunity to use combinations of wavelengths in a single treatment to optimize disinfection and minimize chemical and flavour changes in beer.

In conclusion, this study showed that UV-C irradiation could significantly reduce microbial load in beer at 254 nm using turbulent flow UV system with mercury lamps. There have been no studies to date on the efficiency of UV-C on microorganisms in beer using turbulent flow UV system. Lu *et al.* [32] investigated the impact of UV-C on microorganisms in beer using thin-film UV apparatus. Moreover, we showed that UV-LEDs have the ability to inactivate microorganisms in beer at 250 and 275 nm wavelengths. This disinfection system could be a promising approach as an alternative to low-pressure (10^2 to 10^3 Pa) mercury UV lamps. The development of LSF is by far the most important process in UV irradiated beers [9, 10]. The application of a UV dosage level of 1000 J/L at 254 nm, which

exceeds the dosage needed to sterilise beer, confirmed that formation of LSF was the major issue in irradiated beers. LSF should not solely be attributed to MBT formation, as clearly there were other off-flavours that developed during our trials. However, UV-C irradiation of beer at 250 and 275 nm did not produce chemical degradations associated with LSF. This suggests that the sensitivity of beer to light depends on the wavelength applied and possibly incident radiation which is less with LED than mercury lamps [9, 29].

Overall, this study has resulted in clear insights into the application of UV-C light as a non-thermal disinfection technique. The measurement of changes in riboflavin concentration was a useful means in the assessment of LSF in beer as it correlated well with sensory evaluations. However, the quantification of one of the most prevalent compounds associated with LSF namely MBT requires further optimisation. A more detailed study on LSF, not associated with MBT and hops iso-acids is still needed and must include sensory qualities of UV irradiated beers. In addition, furan, a potential human carcinogen, was reported previously to develop from carbohydrates upon UV treatment of sugar rich beverages [22, 23]. Glucose, fructose and sucrose contribute the most to furan formation upon thermal and UV-C treatments [5, 6]. Therefore, a sensitive method should be developed to measure furan in beer upon UV treatment. These studies, combined with optimisation of UV-C light irradiation of beer, in particular using LED's, will be important for the validation and future application of UV-C in beer treatment. Our findings that UV-C irradiation can sterilise beer at low dosage energy while having marginal effects on chemical and sensory properties will hopefully further stimulate the growing interest in this viable non-thermal technology.

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ADDENDUM

The initial work on the following paper, focusing on the effect of UV-C irradiation on the sensory and aromatic properties of beer, was published in the *Journal of the Institute of Brewing*, 2010, **116**, 348-353.

Effect of UV-C Disinfection of Beer – Sensory Analyses and Consumer Ranking

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ABSTRACT

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Ultraviolet (UV-C) light irradiation is gaining rapid acceptance within the food and beverage industry as a non-thermal disinfection technique. A series of trials, using a pilot scale UV-C treatment system, were conducted to investigate the effect of UV-C on beer with specific attention to lightstruck flavour formation. Both commercial and micro-brewed beers were treated with UV-C light at 254 nm. Samples were analysed by consumer and trained panels. Sensory analyses revealed that at a low UV-C level, lightstruck flavour was apparent and this increasingly gave way to a more intense burnt rubber off-flavour as the UV-C exposure was increased. A sample enrichment probe technique coupled with a gas chromatography-mass spectrometry (SEP/GCMS) revealed the presence of lightstruck flavour in all the treated beers.

Key words: 3-methyl-2-buten-1-thiol, lightstruck flavour, sensory analyses, SEP/GCMS, UV-C disinfection.

INTRODUCTION

Sterilisation by means of UV-C light irradiation is considered as a non-thermal technique with very little energy consumption. It is also considered to be environmentally friendly with no chemical input or storage of water when compared to the traditional heat treatment-pasteurisation techniques[10,14,16,17]. Today, UV-C light irradiation is finding increased usage in several food and beverage processes for industrial sterilisation. It was shown that it could be a reliable means for disinfection of raw or pasteurised milk, juice, brine, whey, and natural water[10,16,17].

Beer is a complex fermented beverage, in which it can be advanced that a number of chemical reactions are occurring. It has been well established that visible and UV light have detrimental effects on beer causing the so-called lightstruck flavour[2–9,11,12]. The formation of 3-methyl-2-buten-1-thiol (MBT) was shown to be responsible for the lightstruck flavour. De Keukeleire et al.[2–4], have shown that the formation of lightstruck flavour in beer is likely to occur at wavelengths between 500–280 nm (visible and UV-A and UV-B). However, whether UV irradiation at the bactericidal wavelength of 254 nm (UV-C) has a detrimental effect on the quality of beer has not been well established. In this paper we investigated the effect of a non-thermal disinfection technique (UV irradiation) on the sensory and aromatic properties of beer using both a consumer and a trained panel ranking test for evaluation.

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MATERIALS AND METHODS

Micro-brewed beers

Raw materials were obtained from SAB Ltd (Cape Town, South Africa) and included pale and black malt, aroma and bitter hops (PIH), lactic acid and bottom yeast. Beers were brewed in a 40 L scale pilot mini brewery (Chemical Engineering, Stellenbosch University) equipped with 50 L fermenter tanks. A dry roller mill was used for the malt prior to brewing.

For every batch, malt was mashed at a 3:1 (v/w) ratio of water to malt. Lactic acid was used to acidify the mash to pH 5.3 and the brewing was carried out with a mashing program scheme of 64°C (45 min), 71°C (20 min), and 78°C (1 min). The mash was lautered and the wort was transferred to the kettle. The wort was boiled at 101°C for 1 h and immediately cooled to 12°C. During boiling, hops pellets and liquid adjuncts (maltose) were added for the desired bitterness and density. Fresh brewery-collected stationary phase yeast was added to the wort. Fermentation was maintained at between 11 and 12°C for 5 days until the end of primary fermentation. The primary fermented beer was then chilled to 4°C and left for 3 weeks for a secondary fermentation to allow the yeast to settle thoroughly and to inhibit the activity of any microorganisms possibly contaminating the ferment. After 3 weeks of secondary fermentation, the beer was filtered using a tangential flow filter (0.22 µm pore size, Millipore, USA). The micro-brewed beers were coded and referred to as PL (pale lager) and DB (dark lager).

Commercial beers

Commercial beers were obtained from a local brewery (SAB ltd, Cape Town). Three different brands and styles of beers were used for experimentation and the brand names have not disclosed in this article for confidential reasons. The beers were coded and referred to as BL and CL (pale lagers), and HP (Pilsner).

UV-C equipment

Beers were UV treated using a pilot scale SP4 UV system (SurePure, Milnerton, South Africa), which includes

an open stainless steel collection tank (~100 L), a pump and four germicidal low mercury UV lamps (Fig. 1). The UV system was cleaned before and after every beer treatment using a standard 'Cleaning In Place' (CIP) process. The cleaning process was performed with the UV lamps on to ensure the effective maximum output of the UV lamps during each trial.

Cold beers were decanted from a keg into the tank and re-circulated through the UV apparatus at a flow rate of 4,000 L/h. UV dosages were expressed as joules per litres ($J.L^{-1}$) and the running time for each corresponding dosage was calculated as following:

Dosage ($J.L^{-1}$) = Total UV-C output per unit (W) / Flow rate ($L.s^{-1}$)

= [Total UV-C output per unit (W) × Time (s)] / Volume (L)

Thus, the running time will be:

Time (s) = [Dosage ($J.L^{-1}$) × Volume (L)] / Total UV-C output per unit (W = J/s)

= [1,000 J/L × 50 L] / 102 J/s

= 490 seconds

= 8 min

- Total UV-C output for 4 units = 102 W
- Volume of beer being treated = 50 L
- Time for running the beer = 8 min

Consumer panel evaluation

Two consumer tests were carried out in the sensory science laboratory of the Department of Food Science at the University of Stellenbosch (South Africa). The first test was conducted on the non-commercial beers (PL and DB) with a group of 87 consumers, with 32% female and 68% male tasters. The second test was conducted on the commercial beers (BL and HP) with a group of 113 consumers, with 51% female and 49% male tasters.

During each trial, consumers were asked to indicate which term best described their attitude toward an UV treated and non-UV treated beer from the same batch, using a nine point hedonic scale. The scale was presented as follows: 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much and 1= dislike extremely. Consumers were not aware of the UV treatment of the beer samples.

Consumer trials

The beers PL, DB, BL and HP were UV treated at 1,000 J/L and kept in the cold at 7°C for 24 h. For each trial, beers were served and presented in glasses coded with a three digit random code. The beer sample size was 25 mL and was served directly from the refrigerator and tasted in a light and temperature controlled room (21°C). Consumers were asked to complete a questionnaire to determine their degree of liking, using the nine point hedonic scale as the test technique[13], without giving reasons for their preference.

Statistical analysis of data

Analysis of variance (ANOVA, SAS, version 9) was carried out on the data to assess the variability between different beer samples. Consumer's *t*-least significant difference (LSD) was calculated at a 5% significant level to compare treatment means. The Shapiro-Wilk test was used to test for non-normality in the data. Skewness was equal to zero, indicating that values were relatively evenly distributed on both sides of the mean.

Triangle panel evaluation

A triangle taste testing was carried out with a panel of 10 tasters (a mix of 8 trained and 2 untrained) on UV-C treated beer. Ultraviolet dosages of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1,000 J/L were applied to a commercial lager beer (CL). The control was the same beer passed through the UV unit (Fig. 1) with the lamps switched off.

Chemical analysis

Beer samples were analysed by a sampling enrichment probe (SEP) technique[1] (Fig. 2) coupled to a gas chromatograph. Gas chromatography (GC) analysis was carried out on a Thermo Finnigan TRACE GC 2000 instrument fitted with a split/splitless injector. The septum-supporting insert of the split/splitless injector of the GC was enlarged using a 2.4 mm drill bit. Analytes were separated on a fused silica open-tubular (FSOT) column (33 m × 0.32 mm I.D.), coated with 1.2 um PS-255 (100% polydimethylsiloxane). The programming rate of the GC was 31°C (2 min) to 250°C at 4°C/min. Analyses were carried out at constant head pressure of 40 kPa of the column.

Low-resolution EI mass spectrometry (LR-EIMS) was performed on a TRACE GC-MS instrument system using the GC column and conditions specified above. Mass spectra were recorded at 70 eV in scan mode from m/z 40 to 120. Helium was used as carrier gas at a linear velocity of 32.25 cm/s at 40°C. The structure of the MBT was confirmed by means of data obtained from the reference library NIST mass spectra search program data.

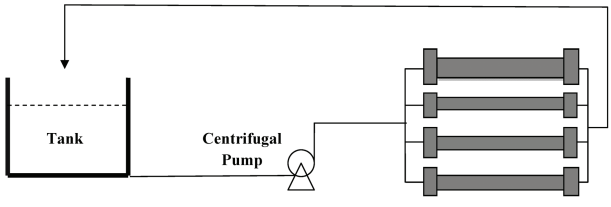

Fig. 1. Schematic drawing of a pilot-scale SP4 UV system (SurePure, Milnerton South Africa).

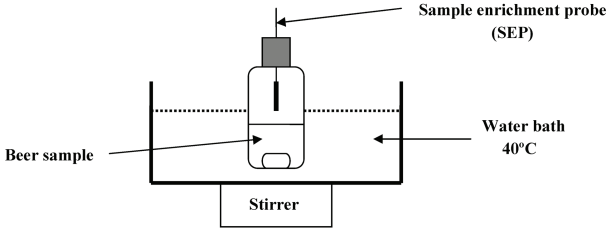

Fig. 2. The sample enrichment probe (SEP) technique used to trap the MBT from the aqueous solution.

RESULTS AND DISCUSSION

Consumer ranking for micro-brewed beers

Two styles of beer, a pale and dark lager, were micro-brewed as described previously. The beers coded PL and DB were treated to a UV dosage of 1,000 J/L and subjected to a group of 87 consumers. Of the panel 70% was between 20–29 years of age, 25% between 30–49 years of age, and 5% between 50–59 years of age. Regarding the frequency of consumption of beer, 56% consumed beer at least once a week, 30% at least once per month and 14% never.

In general consumers demonstrated a lower degree of acceptance for UV exposed beers (Tables I and II). The non-UV and UV-treated beer samples differed significantly ($p = 0.05$) with respect to the degree of acceptance exhibited by the panel. Both male and female consumers indicated that they preferred non-UV treated beers for both the pale and dark lagers. In Figs. 3 and 4 the acceptability of the beers in terms of consumer preference is given. About 10% of the consumers designated both the non-UV and UV treated pale lager beer samples as "*like slightly*". More than 25% of the consumers placed the non-UV treated pale lager in the "*like very much*" category, while less than 5% found the UV-treated pale lager totally acceptable and in the "*liked very much*" category. As shown in Fig. 4 about 25% and 5% of the consumers designated the non-UV and UV treated dark beer respectively as "*like slightly*". Moreover, the UV treated dark beer showed the highest percentage (35%) of consumer dislike ("*dislike extremely*").

Consumer ranking for commercial beers

Two commercial beers were UV treated (1,000 J/L) and analysed by a group of 113 consumers. The group consisted of 80% between the ages of 20–29, 13% between the ages of 30–49 and 7% between the ages of 50–60. In terms of beer consumption, 58% consumed beer at least once a week, 31% at least once a month, and 11% never. In Tables III and IV it can be seen that there is a significant difference between the preference for non-UV and UV treated beers ($P = 0.005$). This indicates that both commercial and micro-brewed beer samples treated with UV light had a similar taste profile. This group of consumers showed that there was less preference for the UV-treated beers. The data summarised in Fig. 5 indicate that about 25 % of the total group would put the BL beer samples (non-UV and UV treated) in the "*like slightly*" category, while 15 % and 35% put the UV treated and the non-UV treated beer in the "*like moderately*" category. Only about 7% described the UV treated BL beer as *disliked very much*" compared to 35% for UV treated HP (Fig. 6). It is evident that the BL (lager) and HP (Pilsner) had a distinct taste profile upon exposure to UV.

Table I. Overall preference for the micro-brewed pale lager beer (PL).[a]

	Mean hedonic value		
Samples	**Total group (N = 87)**	**Female consumers (N = 28)**	**Male consumers (N = 59)**
PL1	6.27a	6.14a	6.33a
PL2 + UV	3.74b	3.4b	3.88b
LSD (p = 0.05)	0.61	1.06	0.77

[a] LSD = Least significant difference at the 5% level of significance. Number with different letters in the same column differ significantly at the 5% level of significance. Skewness equal to zero.

Table II. Overall preference for the micro-brewed dark lager beer (DB).[a]

	Mean hedonic value		
Samples	**Total group (N = 87)**	**Female consumers (N = 28)**	**Male consumers (N = 59)**
DB1	4.52a	4.42a	4.57a
DB2 + UV	2.68b	2.28b	2.88b
LSD (p = 0.05)	0.48	0.79	0.62

[a] LSD = Least significant difference at the 5% level of significance. Number with different letters in the same column differ significantly at the 5% level of significance. Skewness equal to zero.

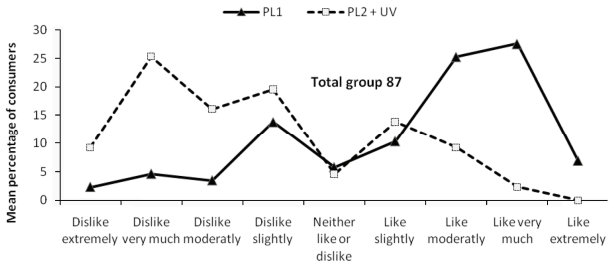

Fig. 3. Distribution of scores for the total group (N = 87) for the micro-brewed pale lager (PL).

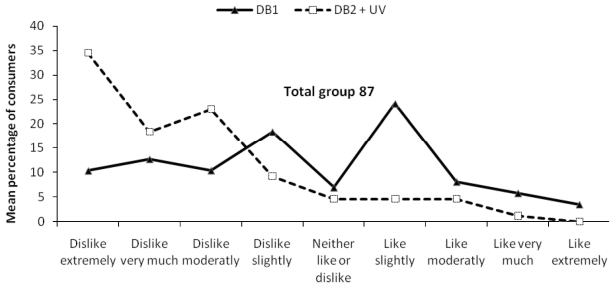

Fig. 4. Distribution of scores for the total group (N = 87) for the micro-brewed dark lager (DB).

Triangle taste

A commercial lager beer (coded CL) was exposed to UV at dosages of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1,000 J/L. The beer samples were tasted on a triangle taste testing 24 h after the treatment by a panel of 8 trained and 2 untrained consumers. According to Table V, 100% of the panel showed correct recognition of the odd glass for 100, 300 and 400 J/L exposure, while 90% showed correct recognition of the odd sample at the 200 Joule level. For all the UV dosage levels tasted (100–400 J/L), a difference in the product was evident to the panel. It was deemed unnecessary to proceed to taste the higher exposure levels, since at the lowest UV dosages the differences in the beer samples was already obvious to all the tasters. Among the trained and advanced tasters, it was apparent that the typical off flavours of beer were present, including lightstruck, burnt rubber, catty and oxidation. The general observation was that at the low UV dosage, only the lightstruck flavour was present, but that this increasingly gave way to a more intense burnt rubber off flavour as the UV exposure increased. It is interesting to note that a high amount of dissolved oxygen would have been accumulated during the trial run. However, no oxidation off-flavours were detected in the control samples, which were passed through the UV system in an identical manner, but with the UV lamps switched off. Since oxidation was apparent in all UV treated beer samples, it was suggested that the UV treatment most likely accelerated any oxidation reactions of dissolved oxygen present in the beer.

Table III. Overall preference for the commercial pale lager beer (BL).[a]

	Mean hedonic value		
Samples	**Total group (N = 113)**	**Female consumers (N = 58)**	**Male consumers (N = 55)**
BL1	6.73a	6.67a	6.78a
BL2 + UV	5.39b	5.56b	5.2b
LSD (p = 0.05)	0.415	0.582	0.603

[a] LSD = Least significant difference at the 5% level of significance. Number with different letters in the same column differ significantly at the 5% level of significance. Skewness equal to zero.

Table IV. Overall preference for the commercial Pilsner beer (HP).[a]

	Mean hedonic value		
Samples	**Total group (N = 113)**	**Female consumers (N = 58)**	**Male consumers (N = 55)**
HP1	5.23a	4.96a	5.52a
HP2 + UV	3.12b	3.03b	3.21b
LSD (p = 0.05)	0.401	0.58	0.567

[a] LSD = Least significant difference at the 5% level of significance. Number with different letters in the same column differ significantly at the 5% level of significance. Skewness equal to zero.

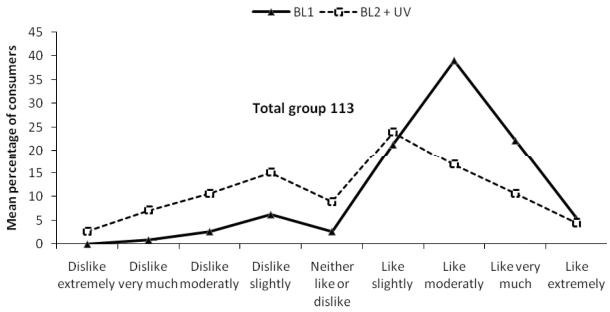

Fig. 5. Distribution of scores for the total consumer group (N = 113) for the commercial pale lager (BL).

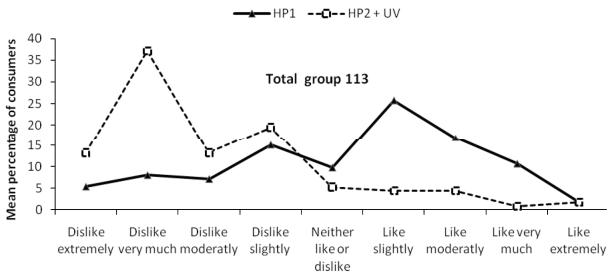

Fig. 6. Distribution of scores for the total consumer group (N = 113) for the commercial Pilsner (HP).

Table V. Triangle taste testing conducted on a commercial pale lager beer (CL).

Doses (J/L)	Beer A	Beer B	Beer D	Taster 1	Taster 2	Taster 3	Taster 4	Taster 5	Taster 6	Taster 7	Taster 8	Taster 9	Taster 10	%
0	T[a]	T	C[b]	B	D	B	B	B	A	D	B	D	D	
100	T	C	C	A	A	A	A	A	A	A	A	A	A	100%
200	T	C	T	B	B	B	B	B	B	B	B	B	A	90%
300	C	C	T	D	D	D	D	D	D	D	D	D	D	100%
400	C	T	T	A	A	A	A	A	A	A	A	A	A	100%

[a] UV treated beer.
[b] Control.

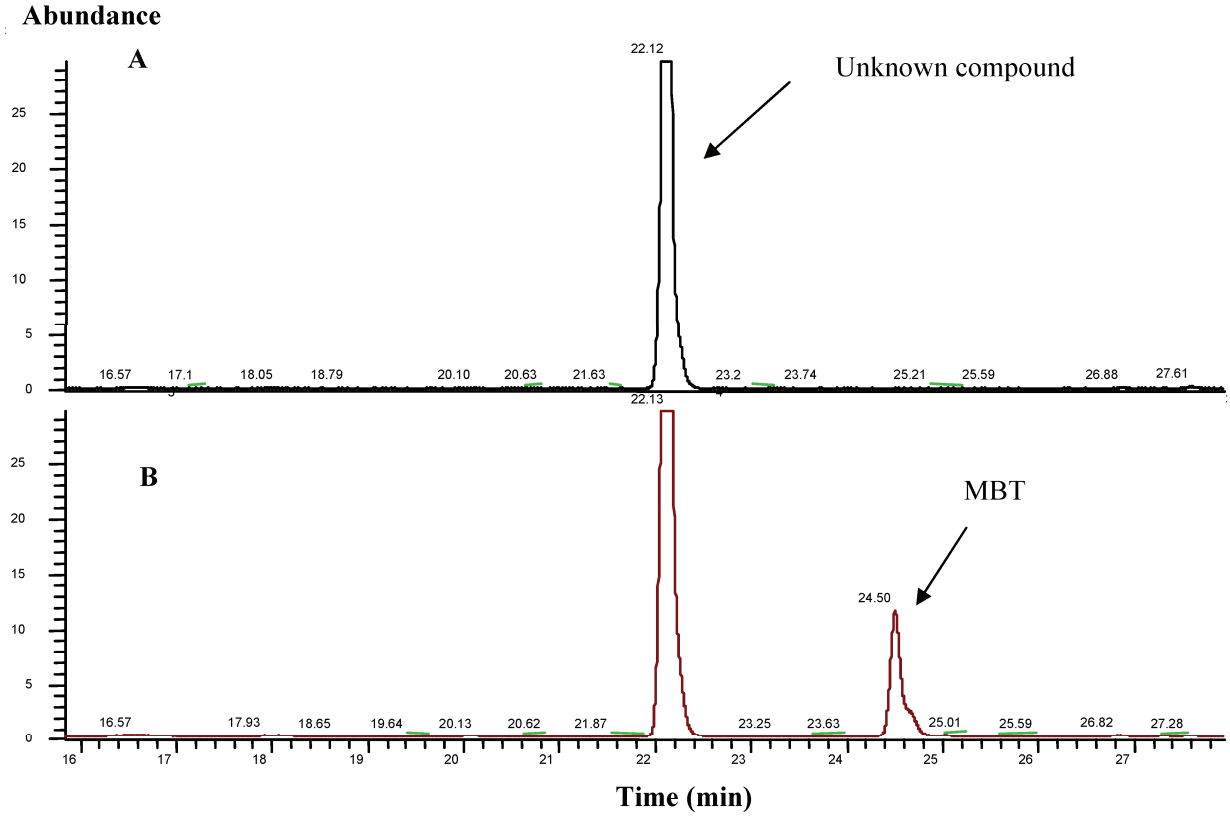

Fig. 7. Chromatogram graphs of non UV (A) and UV treated (B) commercial pale lager beer at 400 J/L displaying a peak of 3-methyl-2-buten-1-thiol (MBT) at an elution time of 24.5 min.

Chemical analysis

Beer samples were analysed by SEP/GCMS[1] to determine the level of MBT and seek correlation between sensory and analytical data. It was observed during the triangle taste testing that lightstruck flavour was apparent in all UV treated beer samples, even at the lowest dose level of 100 J/L. A clear peak of MBT eluted (at retention time Rt = 24.5) and was identified on the chromatogram of a beer treated at 400 J/L obtained using the SEP/GCMS (Fig. 7). Beers treated at 400 J/L and the control were analysed on selected ion monitoring (SIM) at m/z 102 and on total ion count (TIC). The mass spectrum of the MBT was compared with the reference library (not shown). However, MBT in the beer could not be properly quantified, as a standard was not available. MBT is very unstable and has a short half-life in a diluted solution[9]. An unknown peak was also observed in both beer samples (UV and non-UV exposed) at 22.12 minutes on the chromatograph.

CONCLUSIONS

Five different brands of beers were exposed to UV-C radiation at a 254 nm wavelength and analysed for lightstruck formation. Four beers were analysed by a consumer panel and one by a panel in a triangle taste test. Sensory and analytical data revealed that lightstruck flavour formation appeared to be the main problem in beer regarding UV disinfection at 254 nm. The UV treated beers were less liked, when compared to the non-treated beers. More UV trials will be conducted on beer hopped with reduced hops, which are claimed to be "lightproof".

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