



**Aroma and Bitter Extracts from South African Hops: Characterisation and Extraction
Process Development using Supercritical CO₂**

By

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Thesis submitted in fulfilment of the requirements for the degree

Master of Engineering: Chemical Engineering

In the Faculty of Engineering and the Built Environment

At the Cape Peninsula University of Technology

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ABSTRACT

Hops are cone plants whose flowers are used for their bittering and anti-microbial properties in beer. Due to hops being sensitive to oxidation, they are processed into hop pellets, hop powders and hop extracts. The advantage with hops extracts are that they can provide a more consistent bittering. They provide better hop utilization, residue free, and save on storage and transportation costs. Organic solvent extraction is most commonly used; however, supercritical CO₂ extraction (scCO₂) is gaining more attention industrially, as it produces a solvent-free extract.

Hops are typically grown 45-50° North and South of the equator, South Africa, which lies 34°S of the equator, has successfully adapted and grown hops for the last 50 years. This suggests the possibility that the profile of the South African hop might differ from that of the hops found worldwide. As the South African hop market expands, there is a potential to supply a wider variety of hop products to the world market. Although supercritical fluid extraction (SFE) is well known, there are no commercial plants in South Africa. There is thus a desire to explore the technical feasibility of producing enriched hop extract therefore This work aims to produce South African hop extract fractions using supercritical CO₂, have their characteristics evaluated.

To achieve this aim, the following objectives were set. The conditions for fractionation were estimated using theoretical calculations and literature considerations. The second objective was the experimental extraction and fractionation of hops at a pilot plant scale. These extracts were analysed to compare to their profiles with those known worldwide. The final objective was to compare the simulated products of extract and natural hopping.

Four hop types were used in this study. The results showed that the highest yield obtained was 13.22% at a pressure of 200 bar at 40°C for an extraction time of 2 hours. The Broken-Intact-Cell (BIC) kinetic model showed a good fit with an absolute average relative deviation (AARD%) of 3.5%. The kinetic model revealed that it was possible to maintain the extraction process in the constant rate kinetic zone. This technique can thus ensure overall maximum extraction rates throughout the extraction period spanning three different extraction pressures.

The analysis of the hop extract indicated that the four hop varieties had a similar hop acid profile but differed significantly in the aroma profile. In addition, it was also shown that the South African hop extract contained high amounts of the alpha-humulene, beta-caryophyllene and delta-cadinene, compared to most international varieties.

The three fractions (150, 200 and 250 bar) differed in aroma profiles, with the monoterpene hydrocarbon components generally being more dominant in the extract obtained at the lower pressures. The solubility in scCO₂ alone could not fully predict the fate of each of the but it could be speculated that the kinetics of extraction of components from a solid matrices, together with solubility, played a significant role in the fate of each component.

This study for the first time, provided the components data of South African hop extract in terms of hop aroma and hop acid profiles, and the application of scCO₂ for the extraction and fractionation of the hop into various fractions. The results from this study provides useful reference data that can be used for the development of a commercial scCO₂ plant for hop products.

ACKNOWLEDGEMENTS

I wish to thank:

- My supervisor, Dr T.F.N Madzimbamuto, for the guidance, continuous support, patience and knowledge throughout this research, as this would not be possible without you.
- My co-supervisor, Prof T.V Ojumu, for his advice and encouragement during writing and presentations.
- My research group for their assistance during experimental work and making this journey a memorable one.
- Ms H Small and A Bester always ensured everything needed for experimental work was provided.

DEDICATION

This thesis is dedicated to my parents, Moonsamy Archery and Thiruvassigie Archery. Thank you for encouraging me and allowing me to pursue my goals. Your support, love and patience have played a tremendous role in my success.

Table of Contents

DECLARATION	vi
ABSTRACT	vii
ACKNOWLEDGEMENTS	ix
DEDICATION	x
GLOSSARY	xx
Chapter 1: Introduction	1
1.1 Introduction	1
1.2 South African hops	2
1.3 Hop types and processing	2
1.4 Problem statement	3
1.5 Purpose of the study	3
1.6 Aim and objectives	4
1.7 Hypothesis	4
1.8 Research question	4
1.9 Approach	5
1.9.1 Fractional extraction	5
1.9.2 Maximum extraction	6
1.9.3 Co-solvent	6
1.10 Motivation for study	6
1.11 Significance of the study	7
1.12 Delineation of the study	7
1.13 Current state of knowledge and avenues for further research	7
1.14 Thesis outline	8
Chapter 2: Literature review	9
2.1 Introduction to hops	9
2.1.1 Growing hops	9
2.1.2 Hops uses	10

2.2	South African hop varieties	10
2.3	Thermodynamic modelling	11
2.3.1	Equations of state (EOS)	11
2.3.2	Data regression	13
2.3.3	Phase equilibrium calculation	13
2.4	Composition and yield of extracts worldwide	13
2.5	Hop analysis found worldwide	14
2.6	Hops around the world	15
2.6.1	China	15
2.6.2	USA	15
2.6.3	Europe	15
2.6.4	Australia	15
2.7	Hop forms and products	16
2.7.1	Hop cones	16
2.7.2	Hop pellets	16
2.7.3	Hop extracts	16
2.8	Hops composition and structures	16
2.8.1	Hop essential Oils	17
2.8.2	Total Resin	17
2.9	Extraction methods for hops	19
2.9.1	Organic solvent extraction	19
2.9.2	Supercritical fluid extraction	20
2.10	Process modelling	23
2.10.1	Mass Transfer	23
2.11	Brewing with hop cones vs hop pellets vs hop extract	26
2.12	Chapter outcomes	26
Chapter 3:	Method and Methodology	27
3.1	Introduction	27

3.2	Materials	28
3.3	Equipment	29
3.4	Methods	30
3.4.1	Estimation of conditions	30
3.4.2	Pilot plant extraction	36
3.4.3	Control experiment	37
3.4.4	Analysis	39
3.5	Chapter outcomes	40
Chapter 4:	Results	41
4.1	Introduction	41
4.2	Estimation of conditions	41
4.2.1	Theoretical estimation: Use of VLE thermodynamic modelling	41
4.3	Experimental validation	43
4.3.1	Summary of conditions used	44
4.4	Experimental work	44
4.4.1	Summary of experimental runs	44
4.4.2	Fractional extraction: Material balance and yield	46
4.5	Kinetic models	48
4.5.1	Broken-Intact-Cells model	48
4.5.2	Martinez model	49
4.6	Analysis	52
4.6.1	HPLC	52
4.6.2	GC-MS	55
4.7	Control experimental	56
4.8	Chapter outcomes	57
Chapter 5:	Discussion	58
5.1	Introduction	58
5.2	Estimation of conditions	58

5.3	Experimental work	62
5.3.1	Fractional extraction: Material balance	62
5.3.2	Fractional yield	64
5.4	Kinetic models discussion	71
5.4.1	Broken-Intact-Cells model	71
5.4.2	Martinez Model	73
5.5	Analysis data	75
5.5.1	HPLC	75
5.5.2	GC-MS	78
5.6	Comparison of SA extract to worldwide extract	85
5.7	Control experiment	86
5.8	Chapter outcomes	87
Chapter 6:	Summary and conclusion	89
6.1	Summary	89
6.2	Conclusion	90
6.3	Project outcomes	90
6.4	Recommendations	91
References		91
Appendix A:	P&ID of pilot plant	97
Appendix B:	Control Experiment	98
Appendix C:	Estimation of CO ₂ flow rate	99
Appendix D:	Kinetic modelling	102
Appendix E:	Lab journal	101

LIST OF FIGURES

Figure 1.1: Production volume of hop worldwide in 2018, by country in metric tons (Shahbandeh, 2021).	2
Figure 1.2: Approach to experimental work	5
Figure 2.1: Hops anatomy (Olsovska et al., 2016)	17
Figure 2.2: Hop Composition (Almaguer et al., 2014) and (Palamand & Aldenhoff, 1973)	18
Figure 3.1: Overview of the method	27
Figure 3.2: Feed preparation	29
Figure 3.3: Schematic diagram of pilot plant	30
Figure 3.4: Effect of pressure on yield with an increase in temperature (del Valle 2003).	34
Figure 3.5: Effect of pressure at a constant temperature on yield (Kupski 2017).	34
Figure 3.6: Effect of low and high pressure on yield (Zekovic 2006).	35
Figure 3.7: CO ₂ flow-rate estimation	36
Figure 3.8: Set-up for the control experiment	39
Figure 4.1: Yield of the validation experiment	44
Figure 4.2: Constant extraction for experimental run 1	48
Figure 4.3: Constant extraction for experimental run 5	49
Figure 4.4: BIC model for experimental run 1	49
Figure 4.5: BIC model for experimental run 5	49
Figure 4.6: Martinez model for experimental run 1	50
Figure 4.7: Martinez model for experimental run 2	50
Figure 4.8: Martinez model for experimental run 3	51
Figure 4.9: Martinez model for experimental run 4	51
Figure 4.10: Martinez model for experimental run 5	52
Figure 4.11: Martinez model for experimental run 6	52
Figure 4.12: Mass of acid in mg per gram extract for experimental run 1 Southern Star	53
Figure 4.13: Mass of acid in mg per gram extract for experimental run 2 Southern Passion	54
Figure 4.14: Mass of acid in mg per gram extract for experimental run 3 African Queen	54

Figure 4.15: Mass of acid in mg per gram extract for experimental run 4 Southern Promise	55
Figure 4.16: Area of aroma compound for experimental run 2	55
Figure 4.17: Area of aroma compound for experimental run 3	56
Figure 4.18: Area of aroma compound for experimental run 4	56
Figure 5.1: a. Linalool b. Myrcene	60
Figure 5.2: P-y diagram for linalool (Morotomi et al., 1999) and myrcene (Bogel-Łukasik et al., 2009).	60
Figure 5.3: P-x diagram for linalool (Morotomi et al., 1999) and myrcene (Bogel-Łukasik et al., 2009).	61
Figure 5.4: a. Alpha-humulene, b. Trans-caryophyllene and c. Limonene	61
Figure 5.5: P-xy diagram for α -humulene and trans-caryophyllene (Michielin et al., 2009), limonene and linalool (Fonseca et al., 2003).	62
Figure 5.6: Southern Star extract during experimental run 1	62
Figure 5.7: Density of CO ₂ (Nist Chemistry Webbook, 2021)	64
Figure 5.8: Yield vs time for experimental run 1	65
Figure 5.9: Yield vs mass of CO ₂ for experimental run 1	65
Figure 5.10: Yield vs time for experimental run 2	66
Figure 5.11: Yield vs mass of CO ₂ for experimental run 2	66
Figure 5.12: Yield vs time for experimental run 3	67
Figure 5.13: Yield vs mass of CO ₂ for experimental run 3	67
Figure 5.14: Yield vs time for experimental run 4	68
Figure 5.15: Yield vs mass of CO ₂ for experimental run 4	68
Figure 5.16: Yield vs time for experimental run 5	69
Figure 5.17: Yield vs mass of CO ₂ for experimental run 5	69
Figure 5.18: Yield vs time for run 1 and run 5	70
Figure 5.19: Yield vs time for experimental run 6	70
Figure 5.20: Yield vs mass of CO ₂ for experimental run 6	71
Figure 5.21: Hop acid profile of each hop variety	76

Figure 5.22: Mass of acids in mg per gram extract for experimental run 5 Southern Star at 200 bar and 250 bar	76
Figure 5.23: Mass of acid in mg per gram extract for experimental run 6 Southern Star at 2 hours and 4 hours	77
Figure 5.24: Mass of acid in mg per gram extract for experimental run 2 and run 5	78
Figure 5.25: Hop aroma profile of each hop variety	78
Figure 5.26: Aroma profile at 150, 200 and 250 bar for experimental run 1	79
Figure 5.27: Aroma profile at 150, 200 and 250 bar for experimental run 1	79
Figure 5.28: Aroma profile at 150, 200 and 250 bar for experimental run 5	80
Figure 5.29: Aroma profile at 150, 200 and 250 bar for experimental run 5	80
Figure 5.30: Aroma profile for experimental run 6 at 1, 3 and 5 hours	81
Figure 5.31: Aroma profile for experimental run 6 at 1, 3 and 5 hours	81
Figure 5.32: Fraction of aroma at 150, 200 and 250 bar for experimental run 1	83
Figure 5.33: Fraction of aroma at 150, 200 and 250 bar for experimental run 5	83
Figure 5.34: Fraction of aroma at 150, 200 and 250 bar for experimental run 1	84
Figure 5.35: Fraction of aroma at 150, 200 and 250 bar for experimental run 5	84
Figure 5.36: Mass of acid in mg per litre mixture ethanol and hops	86
Figure 5.37: Mass of acid in mg per gram extract of Southern Passion	87

LIST OF TABLES

Table 1.1: Thesis outline	8
Table 2.1: Composition of constituents in SA hops (Hops, 2019)	11
Table 2.2: GC-MS analysis of German hop extract (Zekovic et al., 2006).	14
Table 2.3: GC-MS of hop extracts from the T90 pellets: Hallertau Mittelfruh (Kupski et al., 2017).	15
Table 2.4: Steam distillation vs rapid solvent extraction for hop essential oils (Lam et al., 1986)	20
Table 2.5: Composition of hops to hop extracts (O'Rourke, 2003).	21
Table 2.6: Advantages and disadvantages of CO ₂ (Beckman, 2004)	22
Table 3.1: Materials used during experiments	29
Table 3.2: Literature data	33
Table 3.3: Hot water extraction	38
Table 3.4: Materials	38
Table 3.5: Extraction conditions estimation	40
Table 4.1: Vapour-liquid equilibrium data for linalool and myrcene	42
Table 4.2: Regressed data for linalool	42
Table 4.3: Regressed data for myrcene	43
Table 4.4: Peng-Robinson parameters for linalool + CO ₂	43
Table 4.5: Peng-Robinson parameters for myrcene + CO ₂	43
Table 4.6: Summary of conditions for extraction	44
Table 4.7: Experimental runs summary	45
Table 4.8: Material balance for experimental run 1	46
Table 4.9: Mass data summary for all pilot plant experimental runs	47
Table 4.10: Hop standard for HPLC analysis	53
Table 4.11: HPLC results for boiling hops	57
Table 5.1: Sovova model parameters for experimental run 1	72
Table 5.2: Sovova parameters for experimental run 5	72

Table 5.3: Martinez model parameters summary	74
Table 5.4: Profile of SA extract vs profile worldwide extract (Zekovic et al., 2006)	85

GLOSSARY

Cultivar	Plant produced in cultivation
Fractionate	Divide into fractions
Hop-extracts	Developed from CO ₂ extraction
Humulus Lupulus L	Species of flowering plant in the hemp family
Miscella	Mixture containing extracted oil
Phytopharmaceutical	Medicines with pure active substances that come from plants
Terroir	Environmental factors that affect crop's phenotype

ACRONYMS

AB Inbev	Anheuser-Busch InBev
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
NIST	National Institute of Standards and Technology
SAB	South African Breweries
ScCO ₂	Supercritical CO ₂

Chapter 1: Introduction

This chapter outlines the background, problem definition, aims and objectives of the research project. It gives an overview of the entire document's structure, including the scope of the subsequent pages, chapter by chapter. Each chapter starts with a preamble and an introduction, followed by the relevant material for that chapter. Each chapter ends with chapter outcomes.

1.1 Introduction

Hop cones are the flowers of the *Humulus Lupulus* plant. The hops species form part of the Cannabaceae family of flowering plants. Hops are said to have an Asian origin. This is due to China being the only country worldwide where all three-hop species grow naturally. These hop species include *H. Lupulus*, *H. Japonicus*, *H. Yunnanensis* (Olsovska et al., 2016). The first hop species are grown in temperate areas. The second hop species is a Japanese hop. The last hop species is native to Yunnan, a Chinese province.

Due to hops having a unique flavour, it thus forms part of the primary ingredients when brewing (Haunold, 2010). Although hops are used in only minute quantities in brewing, their impact on the taste of beer is significant. Hops are valued for their content of two major compounds: those with a strong characteristic aroma (volatile compounds such as monoterpenes: myrcene, limonene) and those with a characteristic bitterness such as alpha and beta-acids. This is due to each hop cultivar having a unique composition of hop acids and aromas. Therefore brewers must consider hop variety, geographical origin and year of production before selecting the hop for brewing (Ocvirk et al., 2016).

According to Haunold 2010, about 30 countries grow hops commercially; however, only 15 countries are responsible for the international hop trade. In 2006, about 60% of the world's hop production and trade came from Germany and the United States. South Africa is a minor player on the world stage in supplying hops to the world market. Figure 1.1 illustrates the ranking of each hop producing country. Although China is the origin of hops, it ranked third in hop production worldwide.

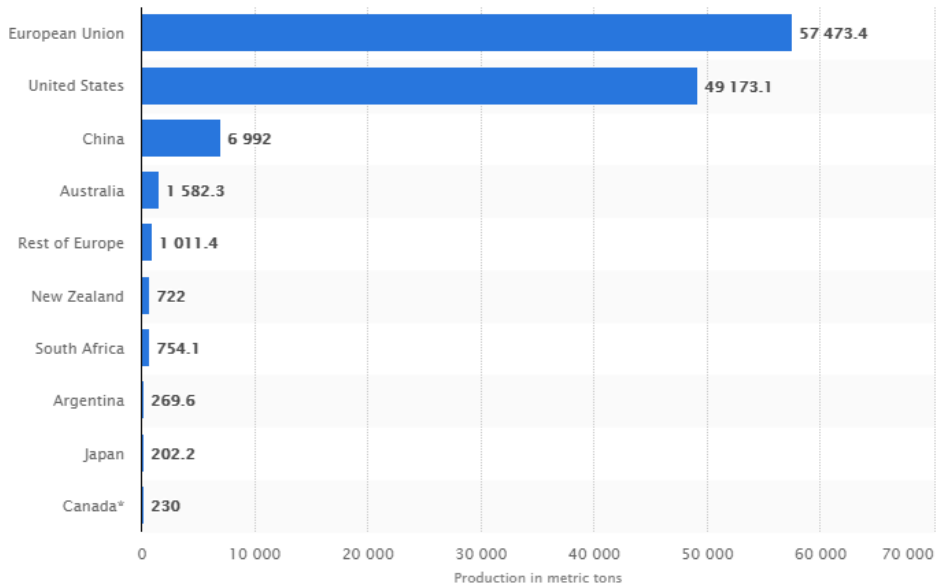


Figure 1.1: Production volume of hop worldwide in 2018, by country in metric tons (Shahbandeh, 2021).

1.2 South African hops

It was in the 1650s when hops were introduced in South Africa. Around 1652, Jan van Riebeeck planted the first hop garden, which brewed its first beer two years later. In the 1970s, the local strain, Southern Brewer, was cultivated, and the South African hop industry began to develop. This strain was adapted to the growing conditions in George, which led to the commercial production of hops. Over the years, more varieties were cultivated, and today the industry thrives (South African Breweries, 2015).

1.3 Hop types and processing

To preserve the hop cone, it is processed into different forms. This includes hop powder, hop pellets, enriched hop powder/pellets, hop extracts, isomerized hop extract, speciality hop powder/pellets, speciality hop extracts and hop oil (Kupski et al., 2017). Hop extracts contain two fractions: resin, which promotes the bitter taste in beer, and the volatile fraction, which promotes the aromatic side to the beer.

The two standard methods of producing hop extracts are using an organic solvent or supercritical CO₂ (Hoshino et al., 2018). Of these, the clear advantage of using supercritical fluid extraction is that one has more control of the operating parameters, which results in cleaner cuts of the fractions. The yields obtained with the two methods are best established experimentally. However, it can be shown that scCO₂ achieved much higher selectivities, while organic solvents tend to be much less selective. This is not to say that scCO₂ has a higher solvent power; its solvent power is comparatively much lower. However, it is recycled

efficiently, and thus, operationally, a much higher extract can be obtained using a much smaller amount of scCO₂.

1.4 Problem statement

In George, South Africa, which lies 34° south of the equator, hops thrive. Although George's summer days are three hours shorter than the northern hemisphere, South Africa has successfully grown hops (Carr, 2017).

South Africa is a minor contributor to the international hops market; however, the market for hops has grown beyond the standard requirements of the brewing industry. Therefore, it is interesting to explore advantages that local hop varieties may have over the traditional known varieties in non-traditional applications. As the hop industry expands there is a need for hop processing. Some of these applications include the non-alcoholic beverage and the phytopharmaceutical industries. Even in the brewing industry, the requirements of craft brewers for unique and consistent characteristics, and convenience of use, necessitates exploration of methods of enrichment and standardisation of specific compounds. The unique terroir that is characteristic of the region may impart unique characteristics to the local varieties. For this reason, it is necessary to explore the distinct advantages that the South African hops may have over other well-known varieties worldwide.

Four varieties are grown locally, each with a distinct set of characteristics in terms of the factors that contribute to their aroma and bitterness. Currently, in South Africa, there are no commercial supercritical CO₂ plants for the processing of hops. This work aims to explore the feasibility of extracting and fractionating the aroma and bitter compounds from the local hop varieties and fractionating the extracts into aroma rich and acid-rich fractions. Carbon dioxide is the solvent of choice, as it has many significant advantages over traditional organic solvents. Therefore, this project uses supercritical CO₂ via a high-pressure pilot plant to perform total and fractional extraction. This experimental work will include the use of a co-solvent and a control experiment as a comparison basis. The extraction kinetics will be modelled. Lastly, these extracts will be compared to those extracts found worldwide.

1.5 Purpose of the study

Currently, South African Brewery (SAB) hop farms and private growers harvest below 1% of worldwide production. This is roughly 855 tonnes, of which 735 are used at SAB and local craft breweries (Booyesen, 2017). The remaining 120 tonnes is exported around Africa for SAB beers (Bizcommunity, 2017). In 2016, Anheuser-Busch InBev (AB InBev) – SAB invested R610 million into South Africa's agricultural landscape. This was to finance 800 new

farmers and 20 commercial farmers to produce hops, barley and maize (Booyesen, 2017). As the South African hop farms expand, brewers will become more and more interested in the South African hop profile and its uniqueness.

In South Africa, to date, there are no commercial supercritical CO₂ plants. By conducting this project, the results will indicate whether having a supercritical CO₂ plant for the processing of hop cones will be technically feasible.

1.6 Aim and objectives

This study aims to determine the characteristics of South African hop extracts obtained using supercritical carbon dioxide and the feasibility of fractionation into aromatic and bitter fractions of hops.

Objectives

- Establish the likely conditions for extraction of the different fractions of aroma and α -acid compounds using theoretical considerations and literature data.
- Fractionate the extract into heavy and light fractions, and determine the yields and grades of the different fractions.
- Determine the composition of CO₂ extracts of the four varieties of South African hops and how they compare with similar extracts obtained from traditional varieties known internationally.
- A comparison of the hops components present in an aqueous solution made up from pellets to that of hop extract.

1.7 Hypothesis

It is hypothesised that it is possible, by using CO₂ as a selective solvent, and by carefully controlling extraction conditions (pressure and temperature) in the extraction vessel, to fractionate extract hops to obtain an aroma rich volatile extract, and the resinous heavier fraction, which is rich in the bitter alpha- and beta- acids. Further, it is expected that (i) the fractions will be of a quality suitable to replace wet and dry hopping, respectively, during the brewing process, and also that the composition of South African hop varieties may show a unique and distinguishing composition unlike those obtained from other geographical regions.

1.8 Research question

- 1) At what extraction conditions can the two fractions be obtained?

- 2) What is the spectrum of components contained in SA scCO₂ hops extracts, and how does it compare to those of hop extract found in literature?
- 3) How do the hop components in an aqueous solution made up of pellets compare to hop extract?

1.9 Approach

To answer objective two, an experimental approach is necessary to establish how to run the experiments to maximise the data produced and efficiently answer the research question. The extraction conditions are of utmost importance to promote sufficient extraction. The extraction conditions are obtained from literature. Literature suggests a temperature above 40°C results in thermal degradation; thus it was decided to extract at T = 40°C, P1= 150 bar, P2 = 200 bar and P3 = 250 bar

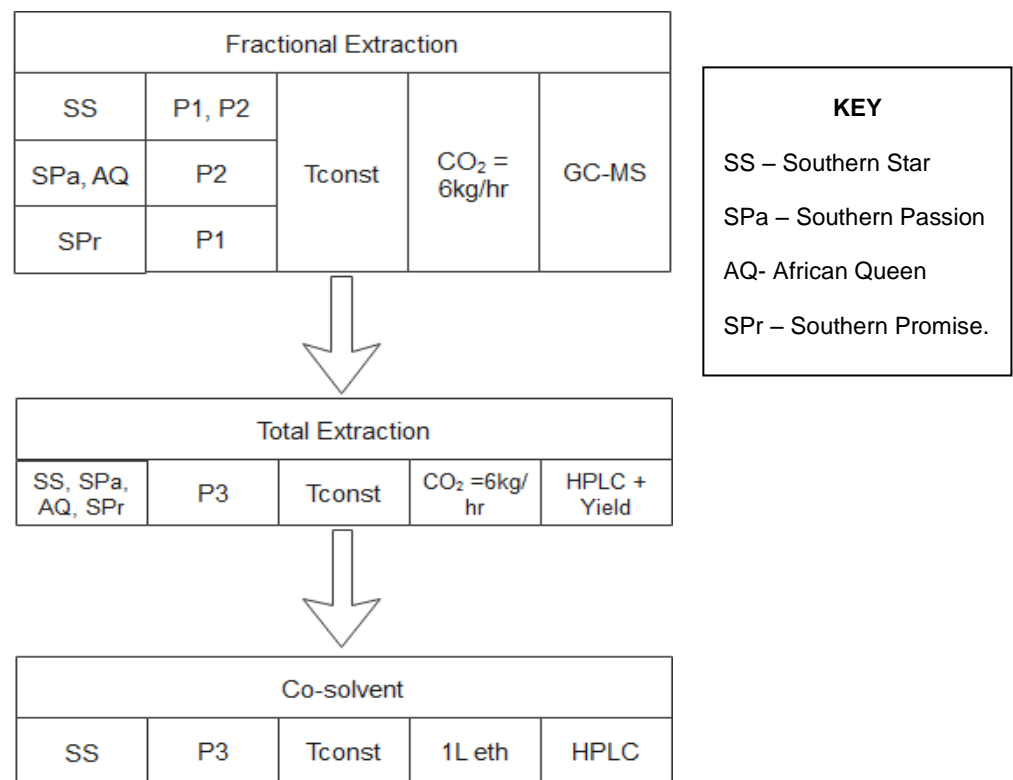


Figure 1.2: Approach to experimental work

1.9.1 Fractional extraction

The fractional extraction started with the Southern Star being split into its light and heavy components at P1 =150 bar and P3 = 250 bar. This step started by loading the ground hops into the extraction basket. The plant was pressurized to P1= 150 bar and set to T= 40°C. A 30 minute static extraction period was allowed. It is after that that dynamic extraction began; after 60 minutes, the first sample was collected. This

is continued until no significant increase in the extract was evident. The pressure was then raised to P2= 200 bar, and after every 60 minutes, a sample was collected. These samples were sent for GC-MS analysis to see the effect of pressure at a constant temperature. The next part in fractioning was on the remaining hop types. For Southern Passion and African Queen, the pressure was set at P2 = 200 bar. The sampling procedure continued until there was no change in extract produce. The final hop type was Southern Promise, and the pressure was set to P1= 150 bar. Each hop type was done on a separate day.

1.9.2 Maximum extraction

After fractionating, the pressure was increased to P3 = 250 bar, and total extraction began. After every 60 minutes, a sample was collected and weighed. The hop feed was not changed, but this step was necessary to calculate the global yield obtained with scCO₂. This stage is where the hop acids were extracted. This yield was compared to the yield obtained during other hop extractions found in literature. At the end of sampling, a sample was collected and labelled. These samples were stored in the fridge and later sent for HPLC analysis.

1.9.3 Co-solvent

At P3= 250 bar and T= 40°C after maximum extraction and the extract produced showed no increase, ethanol was weighed, and about 1 L was poured into the extraction basket and allowed to saturate the hop bed. A sample was collected. These samples are stored and then sent for HPLC analysis.

1.10 Motivation for study

The motivation behind this work lies in the development of the supercritical CO₂ extraction process in South Africa. This development would promote green technology. Extracting with supercritical has many advantages, such as the use of a tuneable solvent. This solvent is tuneable as temperature and pressure can be manipulated to achieve optimal extraction (Hoshino et al., 2018). The CO₂ in this process is recycled, thus saving cost and not adding any degrading effects on the environment.

An advantage of this work for craft brewers is that the hop extract will produce a homogenous bitter beer. The brewer would promote originality by creating new flavours when mixing various hop strains and presenting unique tasting beer. This would result in an increase in the production of craft beers, thus giving the market a boost.

1.11 Significance of the study

By completing this research, the profile of hop extracts obtained from South African hops using scCO₂ and scCO₂ with a co-solvent will be published for the first time. A profile of the separated hop volatile and bitter components obtained using scCO₂ solid phase fractionation will be published for the first time. This work will create a platform for a commercialised scCO₂ plant. This is beneficial as it affects the brewing industry and the exporting industry as South Africa would have an enriched unique hop extract that could enhance its value on the international market. Craft brewers will be able to promote originality, and it will be proudly South African

1.12 Delineation of the study

- This study will be done on hops grown in George, South Africa only.
- The only solvents used include CO₂ and ethanol only as they are generally accepted solvents.

1.13 Current state of knowledge and avenues for further research

By surveying the literature, it concludes that:

- Extraction of hop-extract using supercritical fluid extraction is a technically feasible process. The final product achieved is of a higher quality and flexible application compared to other extraction methods.
- South African has, until recently, been a minor producer of hops, only for a small domestic market. The recent decision by a significant world beer producer to use locally produced hops to produce local beer has stimulated the interest of SA hops internationally. The unique territory (climate and soil characteristics), in addition to a unique strain of hops (Southern Brewer), bred locally over nearly a century, combine to make the local hops a unique produce worth studying. The character of its extracts has not been reported in open literature.
- To the author's knowledge, there is no established plant concentrating on hops extracts in South Africa.

1.14 Thesis outline

Table 1.1: Thesis outline

Chapter	Outline
1: Introduction	In this section, the project is introduced by stating the problem statement. This chapter states the aim and objectives, forming the guideline and providing the structure for completing this work.
2: Literature review	This section focuses on the South African hop types, different soil types, climate and various factors that influence the successful growth of the hops. When estimating the conditions for extraction, literature data was provided on previous hop experiments and the equation of state for the theoretical calculation was selected. For objective 2, data on hop extraction and fractionation were provided, indicating that the scCO ₂ of hops is feasible. Hop analysis of hop extract was discussed and part of objective 3.
3: Materials and methods	This chapter discussed how the aim and objectives were carried out. A description of the pilot plant was provided. This chapter also discussed the method used for thermodynamic modelling (objective 1), pilot plant extraction (objective 2), the chemical analysis method (objective 3) and the method used for the control experiment (objective 4)
4: Results	The results chapter included the data obtained for the thermodynamic model (objective 1). The data from the pilot plant included mass data, extraction condition and time (objective 2). This data was used to model the extraction kinetics; these models include the Broken-Intact-Cells model and Martinez model. The results from the analysis are provided and that aided in characterising the hop extract (objective 3). Lastly, the HPLC analysis for the control experiment was provided (objective 4).
5: Discussion	In this chapter, all the results are discussed. The mass and yield data is compared to that found in literature (objective 2). The GC-MS and HPLC results are discussed in terms of trends and fractions produced (objective 3). The kinetic models are discussed in terms of absolute average relative deviation. The HPLC analysis for the control experiment was discussed (objective 4).
6: Conclusion	This chapter concluded the work and provided a summary of the overall project. It highlighted the project outcomes.

Chapter 2: Literature review

This chapter provides a background on how hops are grown and the forms of hop products. It discusses estimating conditions for extraction using theoretical calculations and literature data (objective one). For objective two, it discusses extraction methods and describes the principle of supercritical CO₂ extraction. For objective three to be fulfilled, the hop composition is provided and will be further discussed in chapter 5. A comparison of brewing with hop cones, hop pellets and hop extracts was completed to fulfil objective four.

2.1 Introduction to hops

Many factors influence where and how hops grow. In this section, three factors are discussed and how these factors impact hops. This theory is to highlight the uniqueness of having hops growing in South Africa.

2.1.1 Growing hops

In the mid-700s AD, the cultivation of hops for the brewing of beer occurred in the Hallertau region of Germany (Northern Hemisphere). Due to the plant having open-pollinated seeds, they vary in character and growing locations. The plant's flowering is dependent on its latitude, soil and climate (Dodds, 2017).

Latitude – hops generally grow 40°-55° north or south of the equator. The day length is a function of latitude. South Africa lies at 33.9°S. However, George South Africa lies outside the accepted latitude range (Dodds, 2017).

Soil – the soil in which hops grow ranges from light sandy soil to clay. The optimum soil required is light textured and well moist but free from water logging. It is necessary to consider the soil pH as it can evoke deficiencies or toxicity. It is thus, recommended to adjust the soil before planting the hops. The roots of hops need to be moist during the growing stage. These are deep-rooted plants, but the feeder root is in the upper soil (Dodds, 2017)

Climate – the dormancy in hops requires cold winters. When summer days become shorter, the hops enter the resting phase. The first stage is called the onset of dormancy. The second stage is called the break of dormancy and requires adequate chilling. The required threshold temperature ranges from 4.4 – 6°C for 30 – 60 days. The hop plant is sensitive to the wind; it is thus essential to consider sheltering from the wind. The strong winds might damage the leaf and result in the loss of cone-bearing laterals. The plant can be protected from strong winds by mountains or hills. Alternatively, windbreakers could be created by using a mesh system (Dodds, 2017).

2.1.2 Hops uses

Medicine- hops are used in Chinese traditional medicine to treat insomnia, restlessness and appetite loss. They use hop extracts to treat various illnesses such as leprosy, tuberculosis and asbestosis. The polyphenol, Xanthohumol has anticancer, antioxidation and anti-inflammatory properties (Lee et al., 2012).

Pharmaceutical- the hop cone contains active agents, namely hop acids (alpha acids, beta acids and iso-alpha acids). The alpha acid, humulone suppresses cyclooxygenase activity and prevents the decrease in bone loss. Hops have been processed into oral dosages to concentrate the acid dosage while preventing nausea and undesired side effects (Corporation, 2006).

2.2 South African hop varieties

African Queen- This African bred has a high alpha content. The aroma profile includes dank, blueberries, stone fruit, black current, gooseberries, bubble-gum, lemongrass and chillies. These hops are appropriate for all hop-forward beers, which include American and Belgian styles. It compliments subtly blended beers; these include coffee, fruit and spices (Hops, 2019).

Southern Passion- This pedigree is a diploid seedling originally from Saaz and Hallertauer crossing. The aroma profile includes passion fruit (granadilla), guava, red berries, melon, calendula and grapefruit. This hop compliments various beer styles, including lightly hopped session beers and all hop-forward beers of American and Belgian origins such as pale ales, IPAs and Saisons (Hops, 2019).

Southern Star- This pedigree is a diploid seedling selected from Outeniqua and a South African male hop. The aroma profile consists of tangerine, blueberries, pine, tropical fruit, dank and white pepper. This hop works well in all hop-forward beers as a bittering hop with clean bitterness (Hops, 2019).

Table 2.1 shows the percentage composition of each hop. This data shows that the African Queen is the hop variety that has the highest alpha acid content. All the hops have less than 1% linalool. The African Queen and Southern Star have similar percentage compositions, and the Southern Passion and Southern Aroma have similar percentage compositions. The fourth hop type is yet to be explored.

Table 2.1: Composition of constituents in SA hops (Hops, 2019)

Constituent	African Queen	Southern Passion	Southern Star
Alpha acids	10-17%	5-11.2%	12-18%
Beta acids	4-6%	7-8%	4-6%
Cohumulone	22-27%	16.6-20.2%	25-30%
Total oil (ml/100g)	0.7-1.5%	0.7-1.3%	1.4-1.7%
Humulene	21-30%	17-35%	21.9-32.8%
Caryophyllene	10-13%	10-13%	11.2-14.6%
Farnesene	5-7%	0.4-2.6%	4.5-12%
Linalool	<1%	<1%	<1%
Myrcene	25-29%	20-35%	27.5-38.9%

2.3 Thermodynamic modelling

When estimating the conditions for extraction two methods was used. The first method included the development of a thermodynamic model and the second method included using data from previous hop extraction experiments to estimate a range of suitable conditions. A thermodynamic model is a tool used to predict extraction conditions based on vapour-liquid equilibrium data. It consists of various components that are described below. The development of the model is further explained in Chapter 3.

2.3.1 Equations of state (EOS)

The standard fugacity model calculation for supercritical fluid extraction is a cubic equation of state (EoS). These include Peng-Robinson (PR, Peng and Robinson, 1976) and Soave-Redlich-Kwong (SRK, Soave, 1972). Mukhopadhyay (2002) states that PR EOS is as efficient as the van der Waals equation; PR EOS provides good qualitative phase behaviour and quantitative illustration of the variation in the system. The PR equation:

$$P = \frac{RT}{v-b} - \frac{a(T)}{V(v+b)+b(v-b)} \quad \text{Equation 2-1}$$

Where a and b for pure components are calculated using T_c , P_c and ω the acentric factor

$$b = 0.07780 \frac{RT_c}{P_c} \quad \text{Equation 2-2}$$

$$a(T) = a(T_c)\alpha(T_r, \omega) \quad \text{Equation 2-3}$$

$$a(T_c) = 0.45725R^2 \frac{T_c^2}{P_c} \quad \text{Equation 2-4}$$

$$\alpha = [1 + k(1 - Tr^{\frac{1}{2}})]^2 \quad \text{Equation 2-5}$$

$$k = 0.3746 + 1.5426\omega - 0.26992\omega^2 \quad \text{Equation 2-6}$$

$$\omega = -1.000 - \log_{10}\left(\frac{P_s}{P_c}\right)_{T_c} - 0.7 \quad \text{Equation 2-7}$$

The Soave Redlich-Kwong equation has been modified numerous times. This equation is modified for real gases and liquids. The following equation can predict molar volume below the critical temperature and also at high pressure above the critical temperature (Mcneill et al., 2019):

$$P = \frac{RT}{\{v-b_o-b_1(T)*\exp\left[\frac{-k(T)}{v}\right]\}} - \frac{a}{[v(v+b_o)]} \quad \text{Equation 2-8}$$

Where a is assumed to be constant and:

$$b_o = 0.2632 * v_c \quad \text{Equation 2-9}$$

$$b_1(T) \cong v_c[-2030.1Z_c^4 + 1983.8Z_c^3 - 738.112Z_c^2 + 121.162Z_c - 6.2489] \quad \text{Equation 2-10}$$

$$k(T_c) \cong v_c[-22.92Z_c^4 + 19.098Z_c^3 - 6.1684Z_c^2 + 0.7599Z_c + 1.0437] \quad \text{Equation 2-11}$$

2.3.1.1 Mixing rules

Several modifications of the mixing rules were suggested, thus increasing the equation's application (Mukhopadhyay, 2002). Mixing rules calculate the energy and co-volume parameters (Zamudio, 2014). The following equations are van der Waals mixing rules:

$$a = \sum_i \sum_j y_i y_j a_{ij} \quad \text{Equation 2-12}$$

$$b = \sum_i \sum_j y_i y_j b_{ij} \quad \text{Equation 2-13}$$

Where a_{ij} and b_{ij} are calculated by:

$$a_{ij} = (a_{ii}a_{jj})^{0.5}(1.0 - k_{ij}) \quad \text{Equation 2-14}$$

$$b_{ij} = 0.5(b_i + b_j)(1.0 - n_{ij}) \quad \text{Equation 2-15}$$

Where k_{ij} and n_{ij} are binary interaction parameters.

2.3.1.2 Binary interaction parameters

The binary interaction parameters (k_{ij} and n_{ij}) are determined when the thermodynamic model is fitted to experimental data. Zamudio (2014) states that the k_{ij} is the most crucial parameter. When working with non-polar mixtures, it will be high and low for polar mixtures. There are estimation techniques available for interaction parameters; however, it is not often

used. Alternatively, mixing rules can be combined to improve vapour liquid equilibrium predictions. The Gibbs free energy model and non-quadratic mixing rules provide the best results (Valderrama, 2003). However, van der Waals is commonly used in industrial applications (Kontogeorgis and Folas, 2009).

2.3.2 Data regression

The larger the experimental data, the greater the chance of uncertainty that may be distributed to the other parameters. This results in unreliable data. With the van der Waals, only two parameters would need to be evaluated (k_{ij} and n_{ij}) (Mukhopadhyay, 2002). Data regression can be done using AspenPlus. This is represented by the equation (Lombard, 2015):

$$Q = \sum_{n=1}^{NDG} W_n \sum_{i=1}^{NP} \left[\left(\frac{T_{exp} - T_{calc}}{\sigma_{T_i}} \right)^2 + \left(\frac{P_{exp} - P_{calc}}{\sigma_{p_u}} \right)^2 + \sum_{j=1}^{NC-1} \left(\frac{X_{exp,ij} - X_{calc,ij}}{\sigma_{y,ij}} \right)^2 \right] \quad \text{Equation 2-16}$$

NDG is the number of data groups, NP is the number of data points, and NC is the number of components. The measured property is the standard deviation and weight of the data group (Lombard, 2015).

2.3.3 Phase equilibrium calculation

The Fugacity method used to calculate phase equilibrium is on the foundation of equality of each component in each phase while temperature and pressure are constant. For a binary mixture with CO₂, the solubility in scCO₂ is defined as (Gordillo et al., 2005):

$$y_2 = \frac{P_2^{sat}}{\widehat{\phi}_2^{FP}} \exp \frac{(P - P_2^{sat})v_2^s}{RT} \quad \text{Equation 2-17}$$

Where

P_2^{sat} – solid sublimation pressure

v_2^s –solid molar volume

$\widehat{\phi}_2$ –equation of state

2.4 Composition and yield of extracts worldwide

The following literature survey aids in achieving objective one. This data is further analysed in Chapter 3.

In Chile, del Valle (2003) extracted hop pellets using a Thar Designs SFE-1 L unit. The investigation deduced that a pressure >200 bar a minimal increase in extract occurred and

temperature >40°C caused thermal degradation. At P = 200 bar and T = 40°C, the oleoresin yield was $146 \frac{g}{kg}$, α -acid = $577 \frac{g}{kg}$ and other compounds = $169 \frac{g}{kg}$ (del Valle et al., 2003).

In Serbia, Zekovic (2007) used SFE for five varieties of hops. The five varieties included Magnum, Hallertau Tradition, Spalt Selekt, Aroma and K-62; the total extraction yield (%) for each was 34.7, 16.6, 11.2 and 17.2, respectively (Zekovic et al., 2006).

In Brazil, Kupski (2017) used supercritical CO₂ extraction at a pressure range of 100-200 bar with increments of 50 bar and a temperature range of 35-55°C with increments of 10°C. In this work, the researcher modelled the extraction kinetics. The models used included the Broken-Intact-Cell (BIC) model and the shrinking core model. The highest yield obtained was 7.1% at P= 200 bar and T= 55°C. The BIC model produced a better result than the shrinking core model (Kupski et al., 2017).

Hoshino (2018), in Japan, used hops-extract-ethanol solutions and fractionated it using a counter-current column. A 50-120 bar pressure range and a temperature range of 40-80°C was used. The maximum purity and highest selectivities were $P_f = 0.99$ and $R_f = 0.27$ at P= 80 bar and T= 60°C (Hoshino et al., 2018).

2.5 Hop analysis found worldwide

In this section data on hop extracts analysis is provided. The South African hop extract will be compared to the following analysis. To have a fair comparison the analysis provided below has to be of hop extract that was produced at the same extraction conditions as to the extraction conditions used in this work.

The following GC-MS analysis is on hop extract produced at 150 bar and 40°C. This data is published by Zekovic (2006):

Table 2.2: GC-MS analysis of German hop extract (Zekovic et al., 2006).

Retention time	Compound	Magnum	Hallertau Tradition	Spalt Selekt	Aroma	K-62
8.88	β -Caryophyllene	2.21	tr.	0.16	0.09	0.1
9.33	α - Humulene	10.35	0.47	0.88	0.5	0.79
9.57	β - Kubebene	-	tr	0.37	0.06	-
10.07	Calarene	-	0.05	-	0.08	0.05
10.16	δ -Cadiene	-	0.14	-	0.13	0.15

tr <0.05%

The following hop extract analysis was published in an article by Kupski (2017):

Table 2.3: GC-MS of hop extracts from the T90 pellets: Hallertau Mittelfruh (Kupski et al., 2017).

Compounds	45°C and 150 bar	55°C and 200bar
Lupulone	25.46	22.22
Humulene epoxide	4.84	5.44
Betamethasone valerate	5.58	5.14
Stigmasterol	2.67	2.61
Humulene	3.18	3.85

2.6 Hops around the world

The section below discusses the background of hops found worldwide. Many countries have been growing hops for years and these are the traditional hop varieties found on the hop market.

2.6.1 China

Based on historical findings, the origin of hops was in Asia; however, hops are said to have a Chinese origin as this is the only country worldwide with all species of hops (*H. Lupulus*, *H. Japonicus*, *H. Yumnanensis*) grows naturally. The growing and cultivation of hops date back to 1500 to 1000 year BC by the Slavic tribes (Olšovská et al., 2018).

2.6.2 USA

In early 1629, hops were grown in North America (New Netherlands) and Virginia in 1648. Between 1800 and 1849, New England and New York grew nearly 1 500 000 pounds of hops. The development of the industry occurred after the Civil war. In the Pacific Coast, hops were grown between 1859 and 1869. This region, in 1909, became the leading hop producer in the United States (Edwardson, 1952).

2.6.3 Europe

Saint Arnold brewed with the aqueous extract (*vide infra*), and this bitter taste led him to try hops. Saint Arnold was the first monk to brew beer with hops. During the 6th century and through the early Middle Ages, monks brewed strong beer in North-western Europe (De Keukeleire et al., 1992).

2.6.4 Australia

In the 18th century, hops were grown, and this was closely related to the colonisation of the continent. In 1794, John Boston started brewing beer without hops. The growing of hops was challenging at first, but James Squires succeeded in cultivating hops with the suitable soil and right site. Due to the difficulty in growing hops right up to the 19th century, hops were

imported from England. The first growing areas followed the routes of settlers, and these included New South Wales and Victoria. In Tasmania (42°S), the growing of hops was successful from the start as the location, climate and light are well suited (Barth-Haas Group, 1860).

2.7 Hop forms and products

Hops grown commercially are processed into different forms depending on their harvest and intended use. Factors such as user preferences also play a significant factor. The different forms are described below.

2.7.1 Hop cones

A hop cone is a mature female flower that grows laterally. The lupulin glands are found on the cone. These glands produce hop acids (alpha acids and beta acids) and essential oils. A crown of rhizomes is found on the hop plant; rhizomes are the storage organ of the plant. The rhizome crown is the underground stem that has buds and roots (Dodds, 2017).

2.7.2 Hop pellets

Due to storage and sensitivity to oxidation, hop cones are pelletized. When pelletizing, the lupulin glands are entirely broken (Maye et al., 2016). The commonly used hop pellet is a "Type 90" pellet (Hughes & Hart, 2018). The Type 90 pellet gets the name from reducing 100% raw hops into 90%, as there are some losses during purification and processing. The Type 45 pellets are doubled enrichment of Type 90 pellets. Hop pellets are stored between 0-5°C; this ensures aroma compounds' conservation and reduces the deterring of alpha acids (Bamforth, 2006).

2.7.3 Hop extracts

The advantage of hop extract is a reduction in mass as well as volume in natural hops. There is a reduction in cost for transportation and storage (Bamforth, 2006). In 1997, Russian researchers investigated the extraction of hops using liquid CO₂. It was found that CO₂ at 5-15°C and pressures of 60-65 bar the mild solvent power extracted hop oils and soft resins. Shortly after, in Germany, supercritical CO₂ extractions at 40-60°C and 200-250 bar showed that supercritical CO₂ is more potent than liquid CO₂ (Moir et al., 2018).

2.8 Hops composition and structures

Figure 2.1 illustrates the make-up of a hop cone; it consists of bract and bracteole, which are leaf-like structures. This structure is all around the hop and is attached to the central axis.

Beneath the bract and bracteole, the lupulin glands are found, and it contains the essential oils and resin (Olsovska et al., 2016).

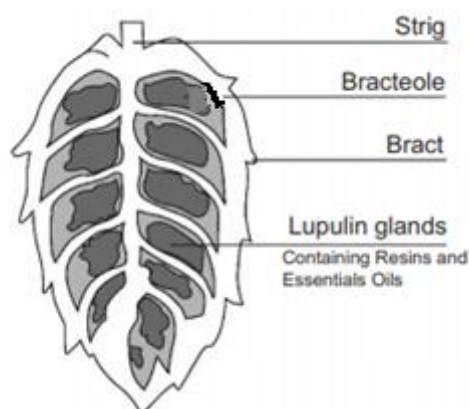


Figure 2.1: Hops anatomy (Olsovska et al., 2016)

2.8.1 Hop essential Oils

The hop essential oil is one of two significant compositions of the hop; mainly, hop essential oils and total resin. This hydrocarbon terpenoid fraction is categorised into monoterpenes (β -Myrcene) and sesquiterpenes (humulene, β -caryophyllene and farnesene). The terpenoids are sensitive to oxidation and thus produces oxygenated products. These products include alcohols, acids, and esters combined with aldehydes, ketones, and epoxides (Cattoor, 2012). Traditionally essential oils from hops were extracted using steam distillation. The steam would pass through the ground hops, and the oil would be removed from the condensate. To isolate the essential oil, liquid CO_2 can be used; however, the extract obtained has low stability, and physical changes occur during storage (Hrnčič et al., 2019). This fraction of the hop is commonly used as a sedative as they treat sleeping disorders. Essential oils antimicrobial activity has been studied, and tested and isolated hop oils have shown activity towards gram-positive bacteria (Olsovska et al., 2016).

2.8.2 Total Resin

Figure 2.2 illustrates the composition of the total resin found in the female hop cone's lupulin gland. Soft resin contributes the most significant component and is further split into alpha acids and the beta fraction. Hard resin contributes about 3-5% and consists of Xanthohumol and uncharacterised hard resin. The hop extract will be characterised in terms of hop aroma and hop acids.

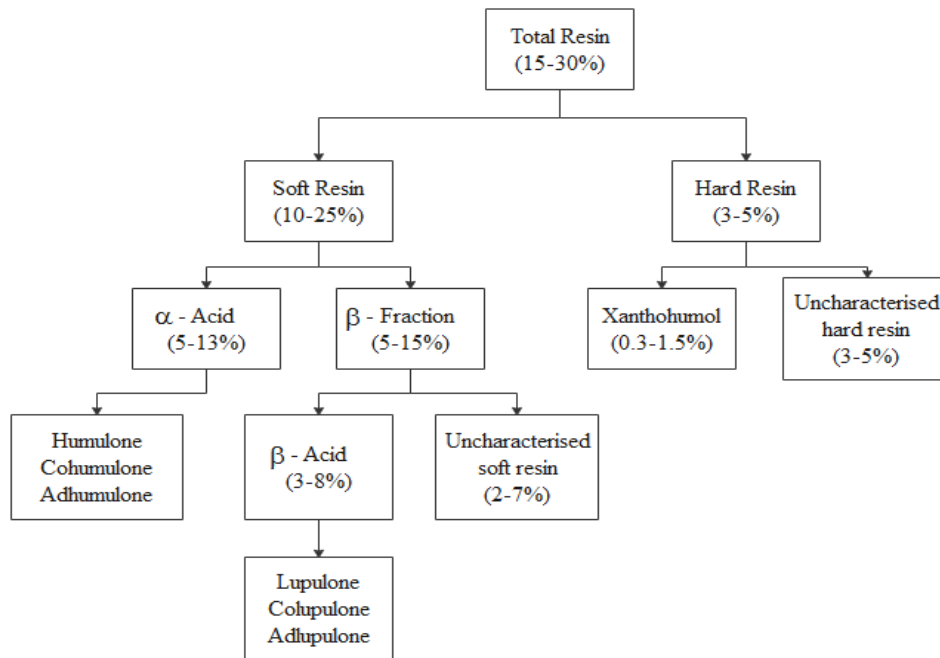


Figure 2.2: Hop Composition (Almaguer et al., 2014) and (Palamand & Aldenhoff, 1973)

2.8.2.1 Soft Resin

According to Almaguer (2014), soft resin produces a yellow thick viscous fluid. Soft resin is soluble and thus contributing to the bitter taste. This fraction is called soft resin, as the components are soluble in low boiling point alkanes. Besides the alpha and beta acids, the soft resin contains uncharacterised soft resin. This is the remaining portion after the alpha acids have been precipitated and the beta acids have crystallized (Stevens, 1966).

2.8.2.2 Alpha acids

The alpha acids in the soft resin can easily be separated, as their salts are insoluble in methanol (Stevens, 1966). In beer, the essential component is the α -acid, where humulone is the dominant component. Acids, in their essence, are tasteless; however, the isomerisation of acids produces a bitter taste. Isomerisation occurs when the acids are boiled. It can thus be deduced that the bitterness of any beer depends on the iso-alpha acid (De Keukeleire et al., 1992). Cohumulone ($C_{20}H_{28}O_6$) does not crystallize at room temperature; however, during absorption, it resembles humulone (Stevens, 1966). According to Cattoor (2012), high levels of cohumulone produces a low hop quality; thus, knowing the composition of acids is essential. Cattoor (2012), states that n-humulone is 35-70%, cohumulone is 20-65% and adhumulone is 10-15% of the total acid level. The amount of each is dependent on the variety (Cattoor, 2012).

2.8.2.3 Beta acids

The β -acid differs from the α -acid as an extra prenyl side chain, thus making it less acidic than alpha acids substitutes the tertiary alcohol located at C-6. Lupulone ($C_{26}H_{38}O_4$) is a colourless crystalline acid that is stable in the presence of air; however, colupulone does not crystallize (Stevens, 1966). The beta acids are sensitive to oxidation; however, a stable hulupone forms during the oxidation. The hulupones contains a harsh, bitter taste (Cattoor, 2012). The β -acids are hydrophilic and insoluble in beer; thus, they do not contribute to the flavour of beer (Ablamowicz, 2015).

2.8.2.4 Hard resin

This fraction is not soluble in hexane; however, it is soluble in cold methanol and ether (Taniguchi et al., 2015). When storing hops, the percentage of soft resin decreases as hard resin increases (Almaguer et al., 2014). According to Taniguuchi (2015), a beer brewed with hard resin extract will produce a superior bitterness and better foam stability than soft resin. Xanthohumol is a well-known prenyl flavonoid. To many brewers, polyphenols are considered as harmful elements as it promotes colloidal instability. This instability is due to complexes with proteins forming, thus creating turbidity or haziness in beer. Brewers might want to reduce the amounts of polyphenols by adsorption by polyvinylpyrrolidone during filtration. The properties of the polyphenol depend on its molecular weight. If the polyphenol has a low molecular weight, it is a potent antioxidant, which reduces the power of the wort and beer; however, it protects against oxidation and improves taste. The high molecular weight polyphenols play a role in the colour of beer and haze formation. Polyphenols play a role in beer quality, flavour stability and slow down oxidation in beer (Jaskula-goiris et al., 2014).

2.9 Extraction methods for hops

There are two methods of extraction discussed in this section, namely organic solvent extraction and supercritical CO_2 . Each method is explained, and advantages and disadvantages are provided. Supercritical CO_2 extraction is used in this work to achieve aroma rich fractions and acid fractions (objective 2)

2.9.1 Organic solvent extraction

During solvent extraction, a high yield of essential oil is produced at a low cost. Traditionally, solvents such as hexane, ethanol, methanol or methylene chloride were used. However, only ethanol and liquid CO_2 can be used nowadays, as they meet the requirements for generally acceptable solvents for food-grade products (GRAS) (Bamforth, 2006). The whole

hop is mixed with a 90% ethanol/water solution in an extractor to perform the ethanol extraction. The ethanol travels through the hop bed and becomes enriched with hop components. The hop leaves are then called spent material. The polar hop material and ethanol mixture is known as 'miscella'. This mixture is then pumped to the evaporation stage, where the ethanol is removed via vacuum evaporation. This type of extraction results in a minor conversion of alpha acids to iso-alpha acids (0.5-1.5%). The alpha acid content of ethanol extract is 20-50%, and the beta acid content varies from 15-40% and the hop oil content from 3-12% (Bamforth, 2006). A study was done on the rapid solvent extraction of hop essential oils. This data was compared to the recovery of essential oils by steam distillation. This data indicated that solvent extraction had a much higher recovery rate.

Table 2.4: Steam distillation vs rapid solvent extraction for hop essential oils (Lam et al., 1986)

Hop variety	Steam distillation Hop essential (mg/100g of hops)	Rapid solvent extraction Hop essential (mg/100g of hops)
Nugget	249.59	611.39
Cascade	123.38	267.65
Galena	139.61	306.72
Styrian	101.37	244.74

However, the drawback with extraction using ethanol is that it is a broad spectrum solvent. It is thus not possible to fractionate more precisely the composition of the extract. An additional separation step is required (evaporation) to remove the solvent.

2.9.2 Supercritical fluid extraction

During $scCO_2$, the solvent becomes enriched in solute and transports the solute by diffusion. This process is further explained in section 2.10.1. The first factor that affects recovery is solubility. To extract, the compounds should be sufficiently soluble in the supercritical fluid. The extraction should occur at a pressure that makes the desired compound of extraction most soluble. If solubility is good in the initial stages of extraction, the extraction time is reduced. The solubility of any compound is depended on the volatility of the compounds being extracted and the solvating power of the solvent. The solvating power is a function of density. When the temperature is constant, and pressure is low, the solubility is low, thus raising the pressure increases the solvating power. When constant pressure and temperature increase, the solutes are more volatile and exceed the falling salvation power (Westwood, 1993).

The second factor is diffusion. This is the process of the solute diffusing from the matrix. The process is unknown but can be modelled to produce effective diffusion coefficients (Westwood, 1993).

The third factor is the matrix. This refers to the extraction; although many of the compounds in the matrix are extractable, some are not. These compounds are locked in the internal structures of the extraction material (Westwood, 1993).

2.9.2.1 ScCO₂ as a solvent

A supercritical fluid cannot be classified as a gas or liquid, but it is a substance above its critical temperature (T_c) and critical pressure (P_c). For CO₂ $T_c = 31.1^\circ\text{C}$ and $P_c = 73.8$ bar. By adjusting the temperature and pressure of the extraction, the density of the solvent can be tuned. This is a non-polar solvent with a quadrupole moment. Compared to other solvents, scCO₂ has both liquid-like capabilities at high diffusivities and low viscosities such as a gas. Supercritical CO₂ is a greener technology due to its sustainable properties. This is an inert and low toxic solvent. It reaches its critical state easily (Budisa & Schulze-Makuch, 2014).

2.9.2.2 Modifiers

According to Brondz (2017), using CO₂ in it's own is seldom done due to CO₂ being lipophilic and some compounds in the feed being polar. For this reason, a modifier is used in the extraction process, thus increasing the bond separation and extraction of polar compounds. Due to ethanol being a polar solvent, it has the ability to dissolve a range of hop components such as polyphenols and carbohydrates. There are other solvents used such as methanol, acetone, and ethyl acetate (Hrnčič et al., 2019). Ethanol is the least toxic solvent (Brondz et al., 2017). This is one of two generally accepted solvents.

The following table compares the composition of the whole hop cone, solvent extraction and supercritical CO₂ extraction. This data indicates that supercritical CO₂ extracts are much more enriched in total resin and contains less water and tannins in comparison to whole hops.

Table 2.5: Composition of hops to hop extracts (O'Rourke, 2003).

Composition	Whole hops	Organic solvent extract	Supercritical CO ₂
Total resin (%)	12 – 20	15 – 60	75 – 90
Alpha acid (%)	2 – 12	8 – 45	27 – 55
Beta acid (%)	2 – 10	8 – 20	23 – 33
Essential oils (%)	0.5 – 2	0 – 5	1 – 5
Hard resins (%)	2 – 4	2 – 10	5 – 11
Tannins (%)	4 – 12	0.5 – 5	0.1 – 5
Waxes (%)	1 – 5	1 – 20	4 – 13
Water (%)	8 – 12	1 – 15	1 – 7

2.9.2.3 Advantages and disadvantages of supercritical fluids

Beckman (2003) describes the advantages and disadvantages of scCO₂ into two categories. Table 2 highlights the advantages and disadvantages.

Table 2.6: Advantages and disadvantages of CO₂ (Beckman, 2004)

Factor	Advantage	Disadvantage
<p>The use of CO₂ in chemical processes: (Environmental and safety).</p>	<p>Safety:</p> <ul style="list-style-type: none"> • CO₂ is non-flammable. • It has a threshold limit value that is safe for the worker to be exposed to daily. • It is safe for human exposure. <p>Environmental:</p> <ul style="list-style-type: none"> • CO₂ is naturally abundant. • CO₂ is less toxic in comparison to other solvents. • CO₂ is inert (no reactions will occur). • CO₂ is a greenhouse gas that can be withdrawn from the atmosphere. • CO₂ is non-explosive 	<p>Safety:</p> <ul style="list-style-type: none"> • Due to vapour pressure >60 bar, high-pressure equipment is required. • Asphyxiate of bystanders could occur (no cases has been reported thus far). • Due to large compressibility, excess heat can be absorbed. <p>Environmental:</p> <ul style="list-style-type: none"> • An uncontrolled release of a large amount of CO₂ into the atmosphere.
<p>The use of CO₂ as a solvent.</p>	<ul style="list-style-type: none"> • This solvent cannot oxidise, as it is already completely oxidized. • No contamination can occur as CO₂ is benign. • CO₂ is an aprotic solvent. • It is immune to free radical chemistry. 	<ul style="list-style-type: none"> • CO₂ has a relatively high critical and vapour pressure. • CO₂ has a low dielectric constant. • Supercritical CO₂ dielectric constant ranges between 1.2-1.5. • CO₂ has low polarizability.

2.10 Process modelling

Mass transfer governs the process of extraction. Multiple factors influence the rate at which extraction occurs. These factors are described below.

2.10.1 Mass Transfer

A good understanding of mass transfer qualitatively and quantitatively is needed to design and simulate the supercritical fluid extraction process. When extracting from a solid matrix, the vessel is loaded, and the supercritical solvent passes through the bed in an upward or downward direction. A constant solvent to solute ratio is maintained. The process parameters should be maintained to ensure that the solvent can efficiently hold the solutes. The solutes should be transported by diffusion. The loading stage is where the solute concentration is highest, and this occurs at the beginning of extraction. This remains steady for a while, and after that, the concentration starts to fall. The constant rate period is characterised by the rate of solvent and the characteristics of the solid substrate. Many regimes exist depending on the mass transfer mechanism. The extraction regimes include constant rate (solubility controlled), falling rate phase 1 (diffusion-controlled), falling rate phase 2 (desorption controlled) regime. For dynamic extraction, the solid matrix characteristics determine the mass transfer mechanism. Other parameters that determine solubility include temperature, pressure and co-solvent concentration. The process variables include particle size, void fraction, flow rate, flow mode, porosity and extractor configuration (Mukhopadhyay, 2002).

2.10.1.1 Process parameters

The extraction rate is influenced by the solvent's thermodynamics state, which in turn affects the solubility (Mukhopadhyay, 2002).

Pressure- as pressure increases and the temperature remains constant, the solvent capacity increases. The extracted content in the solid matrix will decrease over time (Brunner, 1994). Brunner (1994) illustrated that during the decaffeination of coffee beans the amount of extract produced increases with time.

Temperature- a high extraction rate is due to high temperature if the pressure is not too low. The solvent power is dependent on temperature. When the pressure and density are low, increasing the temperature increases the solvent power. Temperature drives mass transfer; thus, more extract per unit time is produced (Brunner, 1994).

Density- at a constant temperature, the extraction rate increases with an increase in density. Density is a direct function of solvent capacity. An increase in density increases the solubility. Brunner (2014) states that the extraction rate will vary at different temperatures but the same densities (Brunner, 1994).

Solvent ratio- it is one of the most significant parameters. When increasing the solvent ratio, extraction rates can be significantly enhanced with minimal changes in the process parameters. With a low solvent ratio, the remaining solid substrate is high. Extraction rates are greatly influenced when the solvent ratio is medium. To understand the solvent ratio, it is discussed in terms of economics. Factors that influence the production cost includes when working with a high solvent ratio, and the extraction time may be shorter; however, the operating costs are high as the capital costs for high solvent ratios are expensive due to equipment requirements. The increase in the amount of extract reduces the production cost per unit quantity (Brunner, 1994).

Size of solid particle- the mass transfer of any extraction process depends on the transport rate. With a decrease in particle, size the extraction rate increases. However, if the particles are too small, it may hinder the fluid flow in the extraction. With smaller particles, the rate of mass transfer decreases. The particle's geometry is of utmost importance; thus, the size reduction or pre-processing method is advantageous (Brunner, 1994).

2.10.1.2 Mass transfer coefficients

There is no physical uniformity to illustrate the behaviour of the extraction of natural materials. Therefore, a mathematical model for the occurrence of mass transfer is futile. However, there are simplified models (Mukhopadhyay, 2002). Brunner (1994) states that the diffusion of extracted components is essential in the extraction process.

2.10.1.3 Broken-Intact-Cells Model

Broken-intact-cells model- this model was developed by Sovova and had three fitting parameters. In this model, the solute is in a non-extractable solid cell wall. The three stages consist of a constant extraction rate (CER). This stage is driven by convection and extraction rate, which is proportional to solubility. The second stage is the falling extraction rate (FER). This stage is governed by diffusion and convection. The third stage is the slow extraction period. This stage is driven only by diffusion (Rai et al., 2016). The following three equations are used to model the kinetics:

$$m(t) = \dot{M}_F * Y_S * t * [1 - \exp(-Z)] \quad t < t_{CER} \quad \text{Equation 2-18}$$

$$m(t) = \dot{M}_F * Y_S * [t - t_{CER} * \exp\left(\frac{Z * Y_S}{X * X_O} * \ln\left\{\frac{1}{1-r} * \left(\exp\left(\frac{W * \dot{M}_F}{M_S}\right) * (t - t_{CER}) - r\right)\right\}\right)] \quad \text{Equation 2-19}$$

$$t_{CER} \leq t \leq t_{FER}$$

$$m(t) = M_S * [X_O - \frac{Y_S}{W} * \ln\left\{1 + \left(\exp\left(\frac{W * X_O}{Y_S}\right) - 1 * \exp\left(\frac{W * \dot{M}_F}{M_S}\right) * (t - t_{CER}) * r\right)\right\}] \quad \text{Equation 2-20}$$

$$t > t_{FER}$$

Where:

$$t_{CER} = \frac{(1-r) * M_S * X_O}{Y_S * Z * \dot{M}_F} \quad \text{Equation 2-21}$$

$$t_{FER} = t_{CER} + \frac{M_S}{W * \dot{M}_F} * \ln\left[r + (1-r) * \exp\left(\frac{W * X_O}{Y_S}\right)\right] \quad \text{Equation 2-22}$$

$$Z = \frac{k_{Fa} * M_S * \rho_f}{\dot{M}_F * (1-\varepsilon) * \rho_s} \quad \text{Equation 2-23}$$

$$W = \frac{M_S * k_{Sa}}{\dot{M}_F * (1-\varepsilon)} \quad \text{Equation 2-24}$$

t_{CER} – time when extraction occurs inside intact cells (min)

t_{FER} – time when extraction of easily accessible oils ends (min)

W, Z and r – model parameters

\dot{M}_F – CO₂ mass flow rate (kg/hr)

Y_S – Apparent solubility $\left(\frac{kg_{oil}}{kg_{fluid}}\right)$

M_S – mass of extract free feed (kg)

ε – bed porosity $\left(1 - \frac{\rho_{bed}}{\rho_s}\right)$

ρ_f – density of fluid $\left(\frac{kg}{m^3}\right)$

ρ_s – density of solid $\left(\frac{kg}{m^3}\right)$

k_{Sa} and k_{Fa} – volumetric mass transfer coefficients (min⁻¹)

To optimise the average absolute relative deviation can be calculated by:

$$AARD \% = \frac{100}{N} \sum_{i=1}^N \left| \frac{\text{yield predicted} - \text{yield experimental}}{\text{yield experimental}} \right| \quad \text{Equation 2-25}$$

2.10.1.4 Martinez model

The Martinez model is a logistic model. The following equation is used to model the extraction kinetics (Martínez et al., 2003):

$$m_i(t) = \frac{m_t}{\exp(b_i * tm)} \left\{ \frac{1 + \exp(b_i * tm)}{1 + \exp[b_i * tm - t]} - 1 \right. \quad \text{Equation 2-26}$$

Where b_i and tm are adjustable parameters and m_t is the total mass of the group.

2.11 Brewing with hop cones vs hop pellets vs hop extract

When brewing with whole cones the lupulin glands are found inside the cone and would need to be broken before use. This results in low yields. In addition, when brewing, the cones absorb water and expand thus needing to be removed once the beer is produced. When brewing with hop pellets the lupulin glands are broken by milling and this results in an improved dispersion in beer. The CO_2 extracts are the purest hop product and a standardised dose can be used during brewing. This method produces the highest yield in comparison to pellets and cones (Schönberger, 2020).

2.12 Chapter outcomes

In this chapter, a discussion on how hops are grown, how factors such as latitude, soil and climate affect the growth of the hop is presented. For objective one, when estimating the conditions for hop extraction literature data indicated that a pressure range of 150 – 280 bar was used and a temperature range of 35°-55°C was commonly used. When developing a thermodynamic model, an equation of state is chosen and for this work, the Peng-Robinson equation of state was selected. For objective two, literature indicated that it is possible to achieve tune the solvent and extract the desired compounds using supercritical CO_2 . The use of a co-solvent is done to modify the CO_2 so that any polar hop components can be extracted. The composition of hops was discussed and an array of hop analyses was provided as this data will be used as a basis for comparison (objective three).

Chapter 3: Method and Methodology

This chapter describes the methods used to perform experimental work, i.e. extraction, fractionation, use of a co-solvent, and a controlled experiment. Firstly, the conditions for the pilot plant extraction needed to be estimated. This was done using two different approaches: performing a flash calculation and estimation by surveying literature experiments on hops. The materials and equipment used and the operation of the pilot plant, and sample analysis are described. The experimental work of hops extraction using supercritical CO₂ entailed measuring yield and extract composition. The extraction kinetics was modelled using the Broken-Intact-Cells model and the Martinez model. The extract composition was determined via HPLC and GC-MS analysis.

3.1 Introduction

The organisation of the investigation is shown graphically in Figure 3.1.

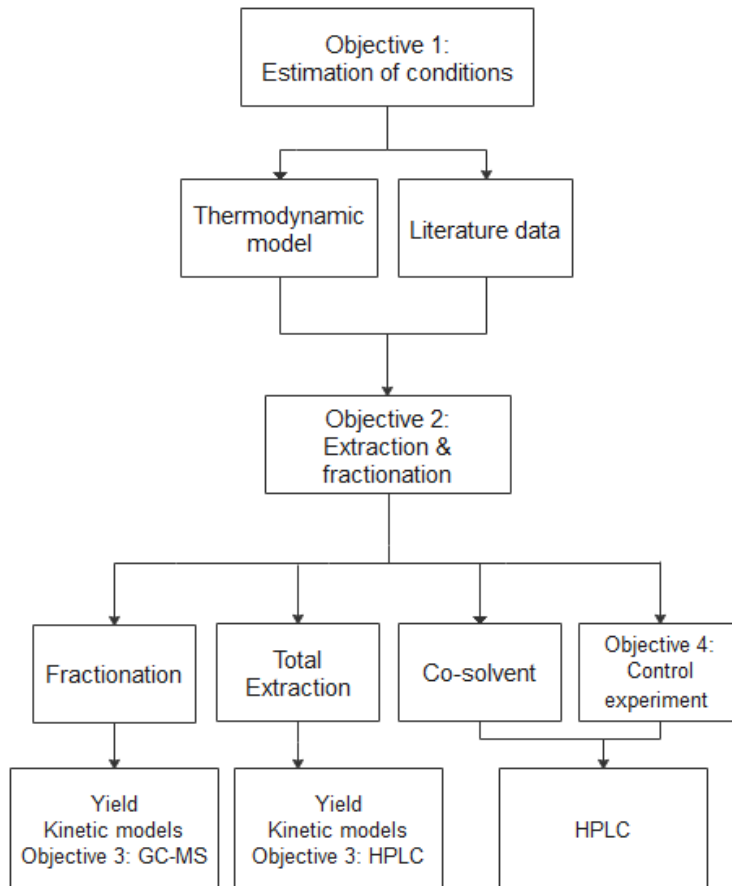


Figure 3.1: Overview of the method

Pilot plant experiments can be inordinately costly and time-consuming. This can be attributed to the long preparation periods, the time required to bring the plant to steady-state, and the long periods required in transition from one set of extraction conditions to another. The cost of the material required, as well as the solvent, is often significant. The utility and workforce availability are also significant factors. Therefore, the range of suitable conditions required to obtain results must be known as much as possible beforehand to avoid establishing these through trial and error. Therefore, before experimental work can be carried out, it is crucial to estimate feasible conditions for fractionation as accurately as possible. An alternative to carrying out trial and error experiments is to develop a thermodynamic model that is accurate enough to predict the solubilities of the significant components of the extracts over a range of parameters (T, P). The method by which this was accomplished is explained in detail in 3.4.1.1. While the solubilities are measured at equilibrium, the actual process is subjected to mass transfer, and as a result, estimations from equilibrium data are not always accurately predictive. It is expected that the thermodynamic model will predict much lower pressures but this data can be used to determine whether fractionation between hop components such as terpenes is feasible. Furthermore, literature data will also be used to confirm the range of conditions used in the experiments.

The experimental work was done in four parts. In Part I (fractionation), extraction was carried out at a constant temperature and three different pressure levels, starting at the lowest pressure and then increasing the pressure by 50 bar increments. In Part II, the final pressure was set so high as to be deemed to have extracted all useful components (maximum extraction). After that, in Part III, a co-solvent was introduced, and extraction was carried out one more time. The control experiment, in Part IV, was a lab-scale simulation of how hops are used in brewing. This was done to establish that the scCO₂ hops extract could impart the same profile as that obtained using the traditional hopping methods. This was done using a heating magnetic stirrer and a stainless steel vessel and boiling the hops similarly to how the hop pellets are boiled during brewing. The samples from each stage were collected. These samples were stored at -20°C before being sent for analysis. Each activity is described in detail below.

3.2 Materials

The hop pellets were purchased from the South African Breweries Limited (SAB). As shown in Table 3.1. The CO₂ was purchased from Air Liquide. The CO₂ cylinders had a dipstick and were connected at the start of the run. Ethanol was the co-solvent selected, as it was also present in the product in which hops extracts are used. The ethanol was purchased from Kimix.

Table 3.1: Materials used during experiments

Material	Supplier	Purity/ Type
Hops	SAB	<ul style="list-style-type: none"> • African Queen • Southern Star • Southern Passion • Southern Promise
CO ₂	Air Liquide	99.9%
Ethanol	Kimix	>99.9%

Every packet of hop pellets was vacuumed, sealed, and labelled with the hop type and the estimation of alpha and beta acids composition. The bag of hop pellets was opened in the morning of the run and ground or the day before and left under pressure with CO₂ in the extractor vessel. A ceramic mortar and pestle were used to grind the hops. This mortar had a capacity of 100 g. The pellets were ground to a particle size of 1 mm – 710 µm. This particle range was determined by sieving the ground hops. Grinding the pellets ensured that when extracting, the solvent could travel into the cell pockets. Each run had a feed of ± 1 kg ground hops.



Figure 3.2: Feed preparation

3.3 Equipment

Separex (France) manufactured the SFE-5 pilot plant. The first part of the plant consists of extractor vessels for extraction from solid material, and the second part consists of a counter-current column for fractionation of liquid feed. This work is focused on the extraction vessels.

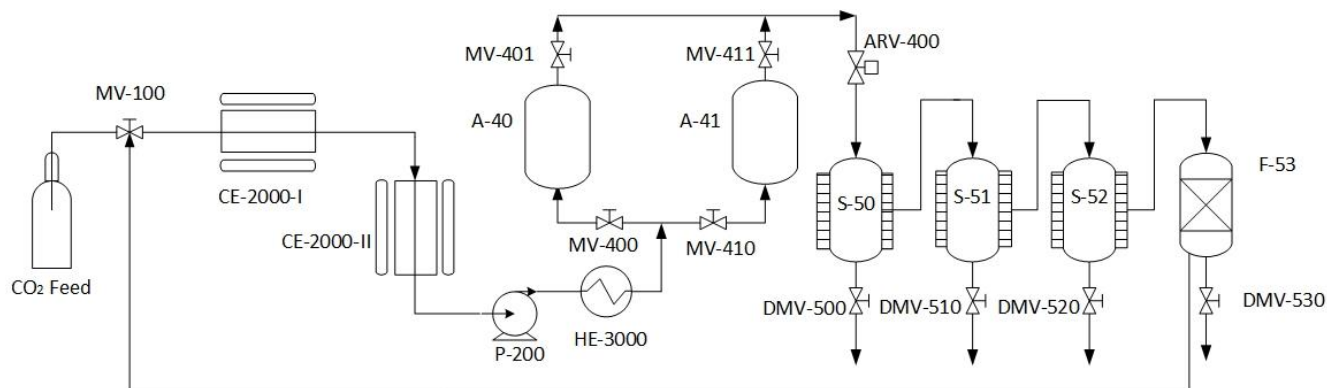


Figure 3.3: Schematic diagram of pilot plant

The pilot plant is located at the Cape Peninsula University of Technology (CPUT) in Cape Town, South Africa. The CO₂ feed was supplied in 30 kg bottles compressed to 50 bar pressure. This cylinder is connected from the outside of the building to the inside via a pipe. MV-100 is a three-way valve, which allows the CO₂ to flow to the condenser or the vent. CE-2000-I is the chiller with a max power of 4 kW at a temperature of 20°C. The chiller provides chilled water and glycol mix to the cooler CE-2000-II. P-200 is a piston pump. This is a volumetric pump for liquefied CO₂. It has a dual-head design and a control panel. HE-3000 is a heat exchanger with direct electrical heating. A-40 and A-41 are extractor vessels with extractor baskets. Its volume is 5 L, maximum pressure 700 bar and temperature 150°C. There is an automatic valve labelled ARV-400, which is used to build pressure in the extractor vessels. The equipment labelled S-50, S-51 and S-52 are jacketed separators with a maximum volume of 0.6 L, pressure 150 bar and temperature 100°C. F-53 is a filter with a maximum volume of 0.6 L, pressure of 100 bar and temperature of 100°C. The P&ID of the pilot plant is provided in Appendix A: P&ID of pilot plant

3.4 Methods

In this section, a breakdown of each method used to accomplish each of the objectives is discussed. The first section deals with the steps taken to estimate the conditions of extraction. This is followed by the method used for extraction, fractionation and use of a co-solvent. The final method focuses on the method used to conduct the control experiment.

3.4.1 Estimation of conditions

The estimation of conditions for extraction was objective one. Two different approaches were used to estimate the range of extraction conditions used in the experiment – a theoretical approach and an empirical approach. The theoretical approach was based on examining binary

phase diagrams of solute/CO₂ systems. To represent the terpenes, the solutes made up the bulk of the extract envisaged, such as myrcene and linalool. The empirical approach used published experimental data from experiments involving similar systems to estimate the range of possible conditions that would suit the experiment. The two results were not necessarily expected to coincide since the theoretical approach is based on equilibrium data and neglects the effect of extraction kinetics, which are likely to be a significant factor in this experiment.

3.4.1.1 Thermodynamic model

The thermodynamic models used in this work were based on the equations of state. This approach made it easier to use literature VLE data to fit the EoS models and also, in a few cases, to use published fitting parameters. The Peng-Robinson equation of state is the most frequently encountered model with successful estimations in similar work and was therefore adopted in this study.

Modelling Tools

A commercial process simulator, Aspen Plus, a product of AspenTech, is part of a much bigger package called Aspen One. Aspen Plus is a modelling environment for steady-state processes. The latest version, v10, also includes a facility for modelling specific units in batch mode. Aspen one also includes a recipe based modelling environment for a batch process, called Aspen Batch Process Developer. This was not used in this process, as the process was regarded as a semi-batch process, where only the extraction vessels were operated batch-wise, while the solvent circulation was modelled as a continuous recycle process.

Equations of state

In this work, a thermodynamic model of the hops/CO₂ system is an equation of state (EoS) that is used to predict the distribution of the various components of hops between the phases. The choice of the equation of state used was based on information found in the literature (Kwartiningsih et al., 2018). The Peng-Robinson was the most used for similar systems. This EoS was used to predict the equilibrium compositions of the liquid and vapour phases.

Collection of VLE

Vapour-liquid equilibrium data were obtained from literature sources including Morotomi et al., (1999), Bogel-Łukasik et al., (2009), Michielin et al., (2009) and Fonseca et al., (2003). The primary source of experimental VLE data was the National Institute of Science and Technology

(NIST) database. This is one of the most extensive databases of scientific information and is owned by the United States Government. It comes preinstalled with the Aspen Properties package and is available free from within the Aspen software. A portal to a second database, DECHEMA, is also available from within the Aspen properties environment but is only accessible at a premium. Physical property data for pure components and the binary experimental data were thus accessed through the NIST database.

Fitting model to experimental data

The Aspen Plus Properties package has a facility whereby binary VLE data can be entered, visualised, and fitted to thermodynamic models. The package comes with a large number of models already installed, and in addition, has a facility whereby custom models can be defined and stored. Determination of the goodness of fit is done automatically for every fitting calculation. Provision is made for the user to select a regression and convergence method, the number of iterations allowed, and the tolerance.

The VLE data will be searched for and saved for the simulation. The next step is using Aspen regression to complete the regression of each data set. Regression is used to fit the VLE data to the model selected. The P-xy graph will be plotted and analysed. The regressed data will then be used for a flash calculation, which is done in the simulation section of Aspen. The model will be tested using various conditions. These conditions include temperature and pressure. The outputs of these conditions are recorded. These parameters will then be used during a run on the pilot plant. The pilot plant results were analysed and compared to that of the simulation.

3.4.1.2 Literature data

It was required to estimate the pressure, temperature and CO₂ flow rates that would lead to a successful fractionation of the hops extracts into the aroma rich fraction and the alpha- and beta-acid-rich fractions. The pressure and temperature directly affect the density of the solvent, and thus its solvent power. The solvent flow rate affects the extraction kinetics – a higher solvent flow rate leads to faster extraction rates. Although scarce, some literature was found that gave reasonably reliable temperature and pressure starting points for the experiments. For the CO₂ flow-rate estimation, it was impossible to find systems of the same equipment scale in the literature. Therefore, the approach taken to equate the conditions in the extraction vessel to those found in the literature was to equate the fluid dynamics of the system found in the

literature to that of the pilot plant and the mass flow rate of CO₂ was scaled up. This is explained in more detail in the section on CO₂ estimation

Estimation of pressure and temperature

Three articles were considered for the estimation of the pressure and temperature; the table below summarises the pressure and temperature each author used. The third column indicated what the highest yield was for each paper. To see which combination of temperature and pressure gave the highest yield, the yield data of each article was plotted. This can be seen in Figure 3.4 - Figure 3.6

Table 3.2: Literature data

Authors	Conditions	Highest Yield
del Valle (2003)	P = 120, 160, 200, 240, 280 bar T = 40-60°C	16.1%
Kupski (2017)	P = 100, 150, 200 bar T = 35, 45, 55°C	10.1%
Zekovic (2006)	P = 150, 300 bar T = 40°C	13.35%

In Figure 3.4, the yield shows a downward trend at pressure 120 bar and 160 bar as temperature increases. According to del Valle (2003), this is a retrograde condensation phenomenon. This means that as the temperature is increased, the apparent solubility is reduced. This is a temperature increase induced retrograde, and thus the density of CO₂ decreases and thus the yield is much less. Between 200 bar to 280 bar, an increase in yield is observed; however, del Valle (2003) stated that pressures above 200 bar had a limited increase in yield.

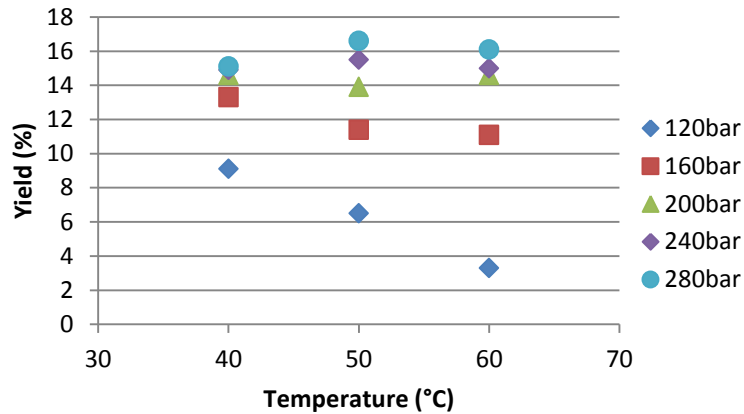


Figure 3.4: Effect of pressure on yield with an increase in temperature (del Valle 2003).

In Figure 3.5, at 35°C, the effect on the yield when increasing pressure is shown. The pressure is increased from 100 bar to 200 bar; however, the yield does not significantly increase. At 55°C and a 100 bar, the yield obtained was relatively insignificant, although it is expected to obtain terpenes at low pressure. As the pressure increases to 200 bar, the yield increases significantly; thus, a pressure of 150 bar was considered for this work.

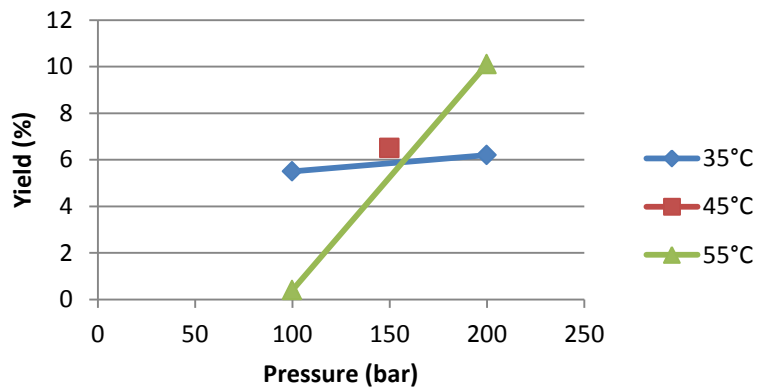


Figure 3.5: Effect of pressure at a constant temperature on yield (Kupski 2017).

Figure 3.6 considers yield at 150 bar and 300 bar at 40°C. This data shows that at 300 bar, a low yield is obtained, and at 150 bar, a high yield is obtained.

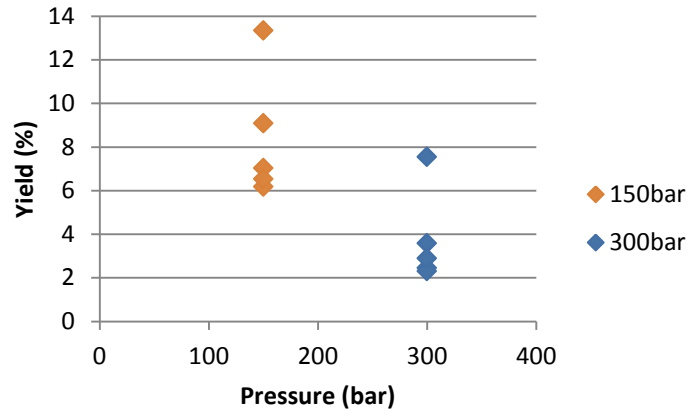


Figure 3.6: Effect of low and high pressure on yield (Zekovic 2006).

The preliminary runs assisted with determining the time required for the extraction to reach completion. The time was determined by extracting until no significant increase in extract produced was obtained. Based on these two runs, an extraction time of 2 hours was decided. For pressure, a range of 150 bar, 200 bar and 250 bar was decided. Due to Zekovic (2006) obtaining the highest yield at 150 bar and this data showing an insignificant yield at 100 bar. According to Kupski (2017), the highest yield was achieved at 200 bar. According to del Valle (2003), it was concluded that pressures above 200 bar had no significant increase in yield; however, based on preliminary runs, 250 bar showed an increase, and thus this pressure was included. A temperature of 40°C was decided on as del Valle (2003) stated that any temperature above 40°C caused thermal degradation.

Estimation of CO₂ flow-rate

To properly compare the data obtained in this work with that found in the literature, it was essential to establish the same fluid dynamics in this experiment as was observed in the reference literature. Therefore, to estimate a CO₂ flow rate, a published article was used to calculate the superficial velocity. In Kupski's (2017) article, a CO₂ flow rate of $3.25 \times 10^{-5} \frac{kg}{s}$ was used. Using the pressure and temperature during that extraction, the density of CO₂ was found on the NIST database. The density and mass flow rate were used to calculate the volumetric flow rate. Using the dimensions of the extractor vessel, the extractor area was calculated and used with the volumetric flow rate to calculate the superficial velocity. Using the dimensions of the pilot plant extractor, the area was calculated and used with the superficial velocity to calculate the volumetric flow rate. Using the pressure and temperature for the fractional extraction, the density of CO₂ could be extracted from the NIST database. Using the

density and volumetric flow rate, it was then possible to estimate the mass flow rate of CO₂ for a pilot plant scale extraction. Figure 3.7 summarises the calculations done to estimate the CO₂ flow rate used during all six runs.

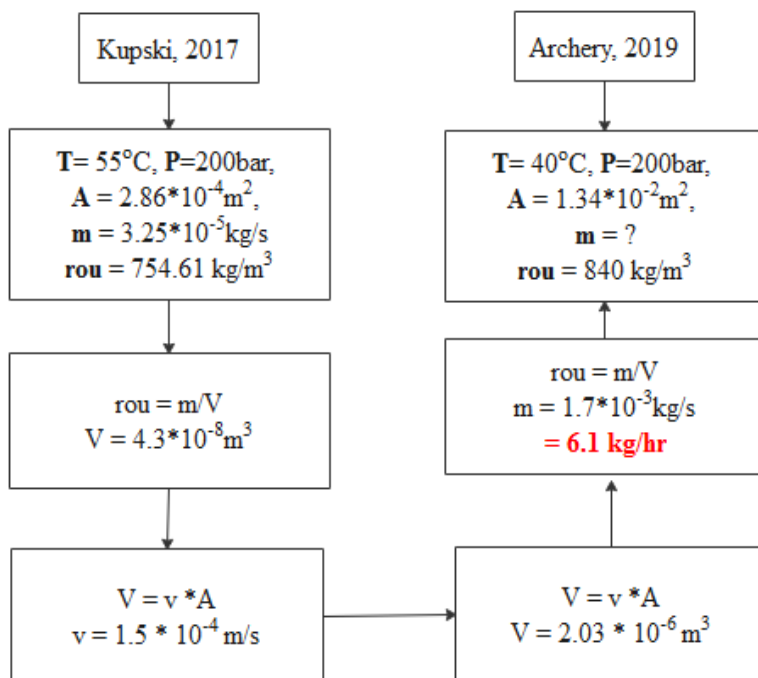


Figure 3.7: CO₂ flow-rate estimation

For the estimation of the CO₂ flow rate, an upscale of Kupski’s (2017) data was done, resulting in a mass flow rate of 6 kg/hr. The calculation for estimating the CO₂ flow rate is provided in Appendix C: Estimation of CO₂ flow rate.

3.4.2 Pilot plant extraction

In this section, a breakdown of objectives 2 and 3 are provided. The fractional separation is performed first. At each pressure, a sample is collected, and mass is recorded, and after the 2 hours, a sample of fractional separation extract is collected for GC-MS analysis. Once the experiment has reached the highest pressure (250 bar), samples are collected and weighed for the maximum extraction yield, and this sample is sent for HPLC analysis. Finally, the co-solvent is introduced, and a sample is collected for HPLC analysis.

Objective two: Perform fractionation from hops (a solid matrix)

- a. *Stage 1:* fractional separation using pure scCO₂. This stage entails splitting hop components into its fractions, in other words, volatile from acids. This is done by

sampling every 30 minutes at a set pressure, i.e. 150 bar and 200 bar. At the 2 hour mark, the sample collected is sent for GC-MS analysis

- b. *Stage 2*: determining the separability of volatiles from non-volatiles. This establishes the extent to which the volatiles can be separated from the alpha and beta acids.

Objective three: Perform maximum extraction under the given experimental conditions to provide data for the hop composition

- a. *Stage 1*: the maximum extraction possible at the given conditions using pure scCO₂. During this stage, the pressure is set to the highest (250 bar), and after every 30 minutes, a sample is collected. After 2 hours, the total mass is weighed and recorded. The sample at the end of the run is sent for HPLC analysis.
- b. *Stage 2*: where total extraction-using scCO₂ and a co-solvent (ethanol) is done. The ethanol is added into the vessel, and after some time, a sample is collected and stored for analysis.
- c. The last stage is where the extract is compared in yield and composition to that of the USA, European and South American origin.

The hop pellets were ground and sieved. The mass of the ground hops was weighed and recorded. The thermal equipment was set. The thermal equipment includes the chiller, heater, extractor vessel and separators. This equipment can be seen in Figure 3.3. Once thermal equilibrium is reached, the experiment can begin. The ground hops were loaded into the extraction basket labelled A41. The CO₂ from the cylinder was cooled so it could liquefy and be pumped via P200. Once the liquid CO₂ is pumped, HE3000 then heats it to operating conditions. The supercritical CO₂ entered the extractor basket at the bottom. A contact time of 30 minutes was allowed (static extraction). The CO₂ became enriched with hop extracts. This was followed by dynamic extraction, and samples were collected from S50, S51 and S52 every 30 minutes. These samples were weighed and labelled according to temperature, pressure and CO₂ flow rate. The samples were kept in a fridge until they could be analysed.

3.4.3 Control experiment

This section of the work aimed to determine the aroma and bitterness profile of a hop variety when the brewing process is simulated. This simulation did not include barley as the focus was on how the hop profile of the pellets may differ from that of the hop profile of the hop extract

produced via supercritical CO₂. A detailed method for this experiment is provided in Appendix B: Control Experiment

Objectives:

- To simulate the hopping in the boiling process to obtain the aroma and bitterness profile of a beer made with the same hops.
- To analyse the profile of aroma and bitterness compounds.

The following recipe was extracted from BeerSmith recipes. The hops used are Southern Passion. This recipe was selected as it uses South African hops to brew. This recipe is attached below.

Table 3.3: Hot water extraction

Brewing recipe (BeerSmith Cloud, 2021)		Lab-scale water extraction (simulated brewing)	
Water	29.19 L	Water	1 L
Hops	127.57 g	Hops	4.37 g

The recipe quantities were converted and then used to scale down to a lab experiment. The table below summarises the apparatus and materials used during this experiment.

Table 3.4: Materials

Apparatus	Material
Thermometer	Water
Scale	Hops
Stainless steel vessel	
Hot plate	

Figure 3.8 shows the set-up for the boiling of the hops. The temperature of the water was measured regularly.



Figure 3.8: Set-up for the control experiment

For this experiment, 1 L water was boiled to 100°C. A mass of 1.5 g hop pellets was weighed and loaded into the boiling water. A period of 30 minutes was allowed for boiling. After the 30 minute boiling, 0.97 g of hops were added to the mixture. This mixture was boiled for an additional 15 minutes. Once completed, a water bath was used to cool the mixture. A sample was collected (wet hopping sample). Lastly, 1.9 g of hops were added, and this mixture sat for 2 hours. Another sample was collected (dry hopping sample). These samples were sent for HPLC analysis.

3.4.4 Analysis

The analysis for both the aroma (GC-MS) and hop acids (HPLC) was outsourced. The GC-MS was done at Stellenbosch University and the HPLC analysis was done at the Oxidation centre at the Cape Peninsula University of Technology. The methods discussed below were extracted from articles provided by the analyst.

3.4.4.1 HPLC

All HPLC analysis was carried out on a Column: YMC-Pack Pro C18 with the dimensions 150 mm x 4.6 mm, 5 µm. The system was equipped with a quaternary pump, a temperature-controlled column chamber, an autosampler and a degasser membrane. The method carried out follows the method in the article titled “*An improved HPLC method for single-run analysis of the spectrum of hop bittering compounds usually encountered in beers*” (Oladokun et al., 2016).

3.4.4.2 GC-MS

The GC-MS analysis was done on a Capillary Column Model Number: Zebron 7HG-G007-11ZB-WAX. This column has a maximum temperature of 250°C with a nominal length of 30 m, a nominal diameter of 250 µm and a nominal film thickness of 0.25 µm.

GC method

For the GC method the oven had an initial temperature of 40°C and an initial time of 8 minutes. The run time was 38.57 minutes with a maximum temperature of 250°C and an equilibration time of 0.25 minutes.

3.5 Chapter outcomes

The outcomes of Chapter 3 are as follows. With regards to Objective 1, it was established that the conditions of extraction will be estimated using a combination of literature data, theoretical modelling and validation of the selected conditions. The theoretical model will indicate the range of conditions in which the targeted compounds are soluble in scCO₂, while the literature data will provide the actual conditions used in previous works. The experimental validation of selected conditions using a pilot plant facility will fulfil the first objective.

Table 3.5: Extraction conditions estimation

Condition	Estimation
Pressure (bar)	150, 200 and 250
Temperature (°C)	40
CO ₂ flow rate (kg/hr)	6

To fulfil the second objective, the following set of experiments were conducted:

These will ensure that an adequate set of data is available to enable a comprehensive analysis of the data concerning the influence of the relevant parameters on the composition of the various fractions.

To fulfil the requirements of the third objective, the volatile fraction will be analysed using gas chromatography, while the alpha and beta acids will be analysed using HPLC.

Finally, to fulfil objective four, two samples of the boiling of hop pellets will be sent for HPLC analysis.

Chapter 4: Results

This section presents the results from the experimental work performed to satisfy all the objectives described in Chapter 1 Sections 1.6. Objective one was aimed at estimating the conditions suitable to conduct fractionation of hops extracts. A pressure range of 150, 200 and 250 bar and 40°C was used. The second objective was to experimentally determine the degree of enrichment of the aroma rich and alpha and beta acid-rich fractions of the four hops strains. This resulted in mass data and hop extracts produced at the estimated conditions for analysis. The modelling of the extraction kinetics is presented using the Broken Intact Cells model and the Martinez model. Objective three was to determine the composition of the hop extract via analysis and compare it to the extracts found worldwide. The analysis included GC-MS for the aroma components and HPLC for the acid profile. The final objective was to conduct a control experiment. These results indicated that the hop extract produced was much richer in hop acids.

4.1 Introduction

The results found in this section follow the order of the objectives. It summarises the data obtained when estimating conditions. The mass data and physical properties obtained during experiments are used to calculate yield and fit kinetic curves. The analysis is the final aspect of the results. This chapter only presents the results.

4.2 Estimation of conditions

In this section, the results for the estimation of conditions for experimental work are presented. It includes the thermodynamic model data and the data from validation runs. The validation runs highlights the yield obtained and at which conditions. The thermodynamic model has the regressed data and P-xy graphs. The P-xy diagrams are discussed in chapter 5. The simulation data obtained during the thermodynamic model is summarized in a table.

4.2.1 Theoretical estimation: Use of VLE thermodynamic modelling

For the thermodynamic model, the EoS used was Peng-Robinson. Myrcene and linalool were the two compounds that the NIST database had complete VLE data sets. Myrcene is a monoterpene, and linalool is an oxygenated terpene.

4.2.1.1 Regression data

Regression is completed by using the regression function on Aspen and then selecting the data set and running the software. The vapour-liquid equilibrium data is presented in Table 4.1. The regression results for linalool are presented in Table 4.2 and for myrcene in Table 4.3.

Table 4.1: Vapour-liquid equilibrium data for linalool and myrcene

Compound	Pressure (bar)	Temperature (°C)	Vapour fraction (Y)	Liquid fraction (X)
Linalool	8.1	40	0.0003	0.91
	15.2		0.0005	0.83
	22		0.001	0.76
	28.1		0.0015	0.69
	42.4		0.0024	0.55
	61.7		0.003	0.37
	70.8		0.005	0.29
	76.4		0.0057	0.21
Linalool	40	50	0.0003	0.66
	60		0.0004	0.48
	79.9		0.0009	0.31
	90		0.002	0.19
	97.8		0.0079	0.06
Myrcene	70.4	40	0.0028	0.49
	79.9		0.0056	0.41
	85.7		0.0078	0.35
	90.2		0.0109	0.30
	93.5		0.0219	0.20
	94.6		0.032	0.14

Table 4.2: Regressed data for linalool

Experimental	Regressed	Difference	%Difference
39.85	45.56	5.71	14.32
39.85	47.27	7.42	18.63
39.85	46.89	7.04	17.68
39.85	48.76	8.91	22.37
39.85	48.01	8.16	20.48
39.85	47.85	8.00	20.07
39.85	45.87	6.02	15.11
39.85	44.92	5.07	12.73

Table 4.3: Regressed data for myrcene

Experimental	Regressed	Difference	%Difference
49.85	50.54	0.69	1.39
49.85	50.47	0.62	1.24
49.85	50.46	0.61	1.23
49.85	50.17	0.32	0.65
49.85	49.75	-0.10	-0.20
49.85	48.50	-1.35	-2.71

Table 4.4 and Table 4.5 show the Peng-Robinson parameters and standard deviation obtained after regression of the data.

Table 4.4: Peng-Robinson parameters for linalool + CO₂

Parameter	Component i	Component j	Value
PRKBV/1	Linalool	CO ₂	0.068
PRLIJ/2	Linalool	CO ₂	1.37E-04

Table 4.5: Peng-Robinson parameters for myrcene + CO₂

Parameter	Component i	Component j	Value
PRLIJ/1	Myrcene	CO ₂	0.13
PRKBV/1	Myrcene	CO ₂	0.19

4.3 Experimental validation

Two validation experiments were conducted to establish how pressure influences the yield. These experiments were done at $T = 40^{\circ}\text{C}$ and $\text{CO}_2 = 10 \text{ kg/hr}$. The hop type used was Southern Passion. The first experimental run was done at a pressure of 150 bar and had a total mass of extract of 53.12 g; this extraction occurred for 2.15 hours. The second experimental run was done at a pressure of 250 bar and achieved a total mass of extract of 109.3 g; the extraction time for this experimental run was 2.15 hours. The feed for these experimental runs was 1 kg. Experimental run 1 obtained a yield of 4.33%, and experimental run 2 obtained a yield of 9.09%. The total yield was 13.42%. This confirms that when increasing the solvent density the extraction rate increase and this increases yield (Kupski et al., 2017). Kupski (2017) obtained a yield of 7.1% at 150 bar however, in this validation run it was much lower (4.33%) this is due to the CO_2 flow rate being much higher. Therefore, in the final experimental runs, the CO_2 flow rate was set to 6 kg/hr.

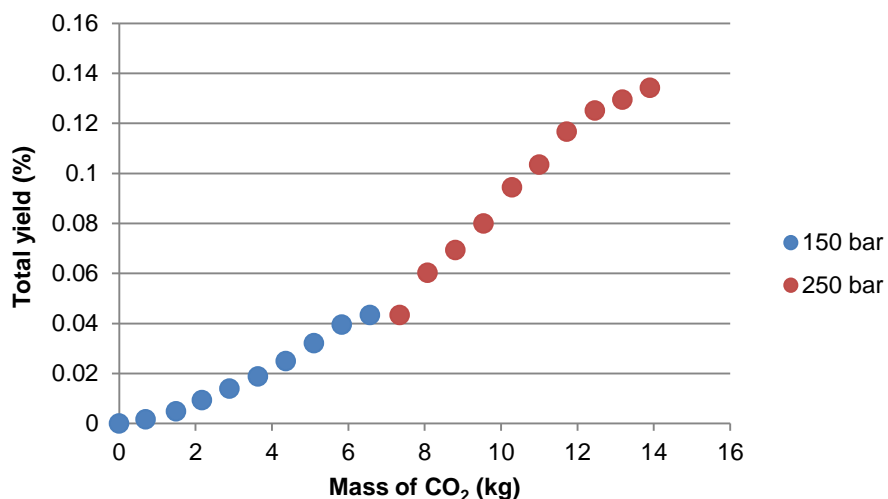


Figure 4.1: Yield of the validation experiment

4.3.1 Summary of conditions used

Table 4.6 shows the conditions used during each experimental run. This conditions were stated in Chapter 3 and was used as stated in Table 3.5.

Table 4.6: Summary of conditions for extraction

Condition	Estimation
Pressure (bar)	150, 200 and 250
Temperature (°C)	40
CO ₂ flow rate (kg/hr)	6

4.4 Experimental work

This section deals with the data collected during experiments. The material balance data are tabulated and used to calculate yield. Other data were collected during experiments, such as extraction time and mass of CO₂ used during each experiment. Kinetic models were also fitted using the same data. The results of pilot plant experiments were conducted at the conditions described in section 4.4.1.

4.4.1 Summary of experimental runs

Table 4.7 shows each feed for each experiment and the range of pressures used to extract from that hop type. The table also gives the total hours used to perform the extraction.

Table 4.7: Experimental runs summary

Experiment	Hop type	Feed (g)	Pressure (bar)	Total extraction time (hr)
Run 1	Southern Star	1006	150, 200, 250	5.30
Run 2	Southern Passion	1014.4	200, 250	4
Run 3	African Queen	911.27	200, 250	5
Run 4	Southern Promise	1002.2	150, 250	4
Run 5	Southern Star	1002.5	150, 200, 250	6
Run 6	Southern Star	975.8	250	5

All experiments were done at constant temperature ($T = 40^{\circ}\text{C}$) and constant CO_2 flow rate ($\text{CO}_2 = 6 \text{ kg/hr}$). A minimum extraction time of an hour per pressure was allowed in each pressure range, except in experimental run 6, the extraction occurred for 5 hours. Experimental run 6 was done to determine the maximum yield possible.

4.4.1.1 Experimental run 1

A feed of 1006 g of Southern Star was loaded. During this experiment, the extraction period of 2 hours at 250 bar was cut short when the plant had an emergency shutdown, and thus the extract collected was after 90 minutes.

4.4.1.2 Experimental run 2

In experimental run 2, the feed was Southern Passion, and for both 200 and 250 bar, the extraction period lasted for 2 hours for each pressure.

4.4.1.3 Experimental run 3

The feed for this run was African Queen. During experimental run 3, at 200 bar, an extraction of 2 hours was tested and then an additional 3 hours for 250 bar. This is due to the plant showing steady operation.

4.4.1.4 Experimental run 4

The feed for the experimental run 4 was Southern Promise. It had 2 hours per pressure. These pressures included 150 bar and 250 bar.

4.4.1.5 Experimental run 5

Experimental run 5 was done as a rerun of experimental run 1. This was to check the effect of the final 30 minutes, which was not completed initially in experimental run 1.

4.4.1.6 Experimental run 6

The feed was Southern Star. This experiment was done to check the extraction time required to extract all hop compounds. The experiment lasted 5 hours at 250 bar.

4.4.2 Fractional extraction: Material balance and yield

During pilot plant experimental runs, the mass of the extract was weighed and recorded. Table 4.8 shows how this mass data was logged.

Table 4.8: Material balance for experimental run 1

Press (bar)	Time (min)	Yield (%)	Mass of extract (g)	Mass of CO ₂ (kg)
150	30	0.039	39.320	6.75
	60	0.086	46.88	6.93
	90	0.104	18.42	10.32
	120	0.124	19.93	13.75
200	150	0.136	12.74	17.27
	180	0.162	25.27	20.55
	210	0.173	11.84	23.84
	240	0.181	7.52	27.43
250	270	0.188	7.47	29.95
	300	0.194	6.1	32.6
	330	0.204	10.07	35.25
Total		20.43	205.56	

Table 4.9 shows the mass data obtained during all experimental runs completed on the pilot plant. Each experimental run has a different feed. Experimental run 5 is the duplicate run of experimental run 1.

Table 4.9: Mass data summary for all pilot plant experimental runs

Experimental run	Mass of extract (g)			Yield (%)			Total mass of extract (g)	Total yield of a run (%)
	150bar	200bar	250bar	150bar	200bar	250bar		
Run 1: Southern Star	124.55	57.37	23.64	12.38	5.7	2.35	205.56	20.43
Run 2: Southern Passion	-	122.44	44.56	-	12.07	4.39	167	16.46
Run 3: African Queen	-	120.48	39.02	-	13.22	4.28	159.5	17.50
Run 4: Southern Promise	132.36	-	25.85	13.21	-	2.58	158.21	15.79
Run 5: Southern Star *	126.25	64.44	40.91	12.59	6.43	4.08	231.6	23.10
Run 6: Southern Star	-	-	198.85	-	-	20.03	198.85	20.03

(*) – Duplicate experimental run

4.5 Kinetic models

The kinetic models include the BIC model. This model was fitted to experimental runs 1 and 5. These experimental runs include extraction at 150, 200 and 250 bar. Each pressure was maintained for two hours each before increasing the pressure. The Martinez model was fitted to all the experimental runs, and it has 2 adjustable parameters. A correctional factor was introduced to improve the fit.

4.5.1 Broken-Intact-Cells model

The Broken-Intact-Cells model has three zones: the constant extraction rate, falling extraction rate and the diffusion-controlled. During this work, the data indicated that constant extraction was maintained. This is due to increasing pressure before the falling extraction rate begins. Therefore, the focus was on the first equation for the model, as this equation is used to fit data obtained during constant extraction. By visual inspection of Figure 4.2 and Figure 4.3, the constant extraction rate is seen. This model was fitted to experimental runs 1 and 5.

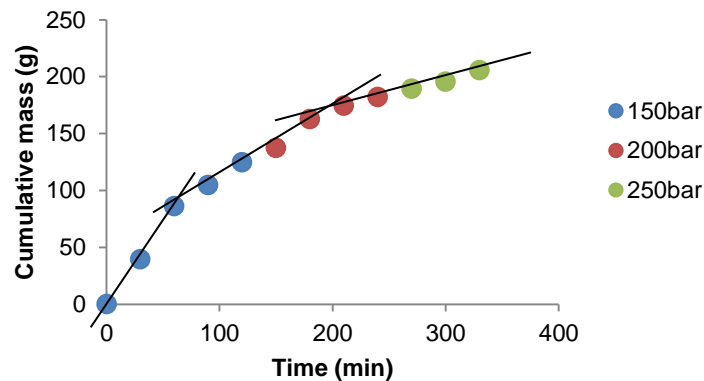


Figure 4.2: Constant extraction for experimental run 1

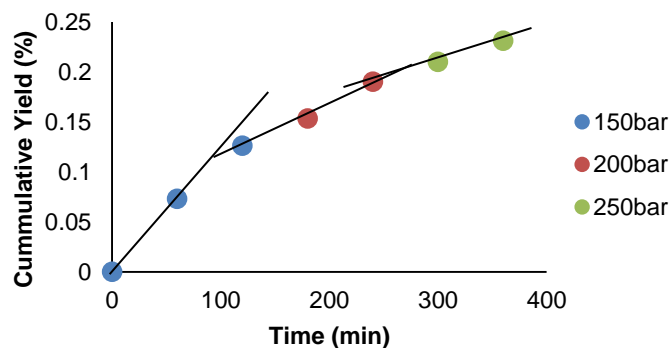


Figure 4.3: Constant extraction for experimental run 5

The following figures show how accurately the model predicts the mass data obtained during the experimental work.

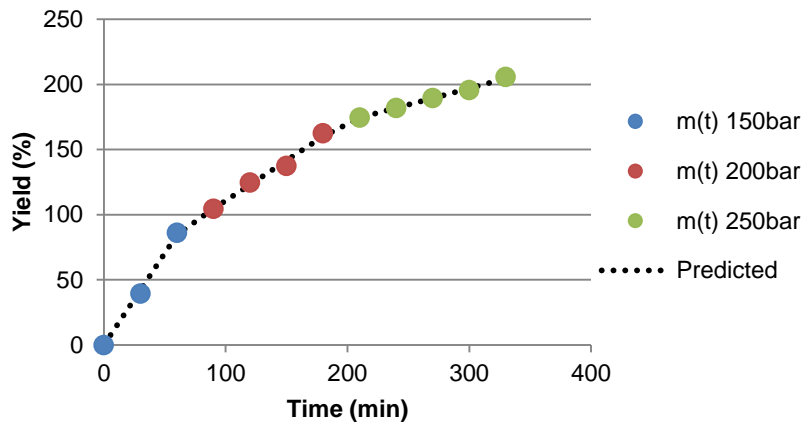


Figure 4.4: BIC model for experimental run 1

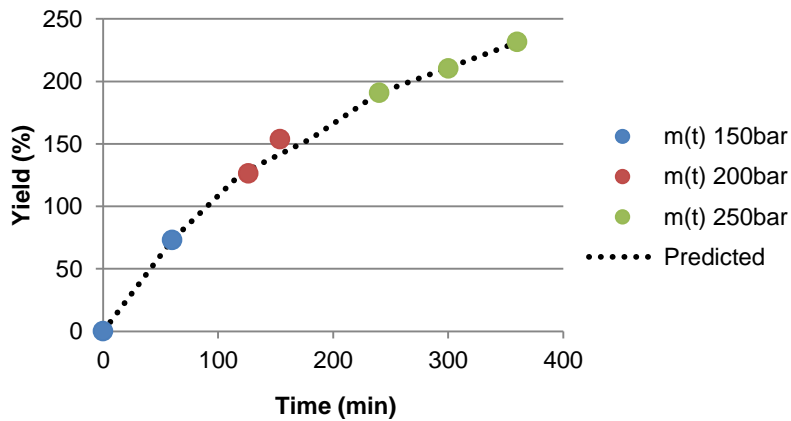


Figure 4.5: BIC model for experimental run 5

4.5.2 Martinez model

The following figures show the comparison of the Martinez model for each experimental run. The plot shows mass data from the lab vs mass data calculated. The regression line shows the fit of the predicted to the actual mass data.

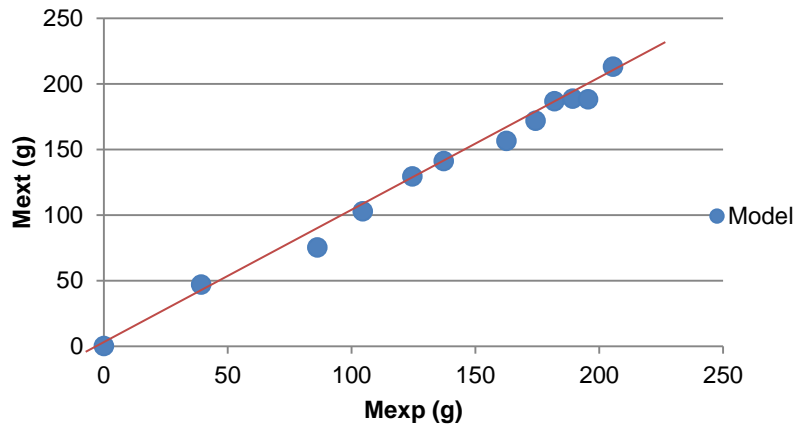


Figure 4.6: Martinez model for experimental run 1

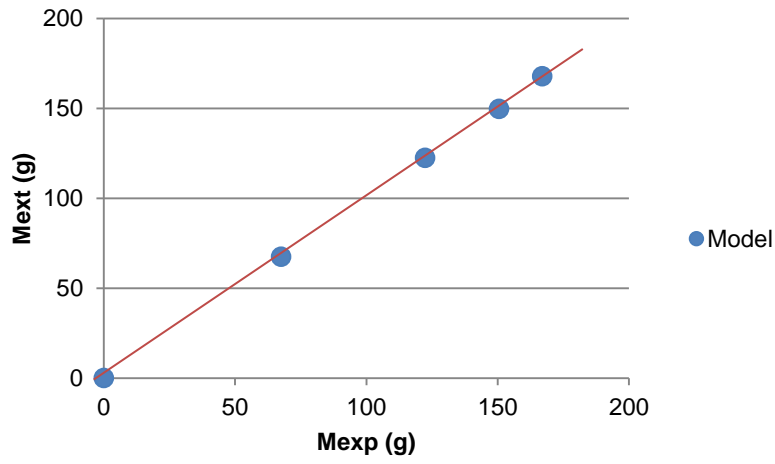


Figure 4.7: Martinez model for experimental run 2

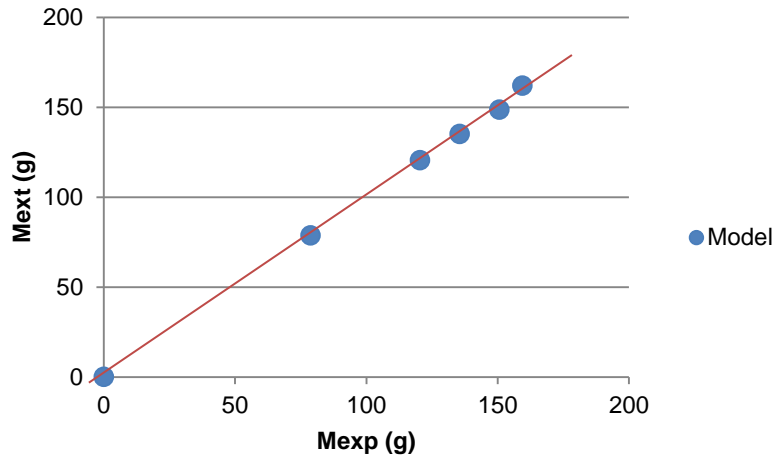


Figure 4.8: Martinez model for experimental run 3

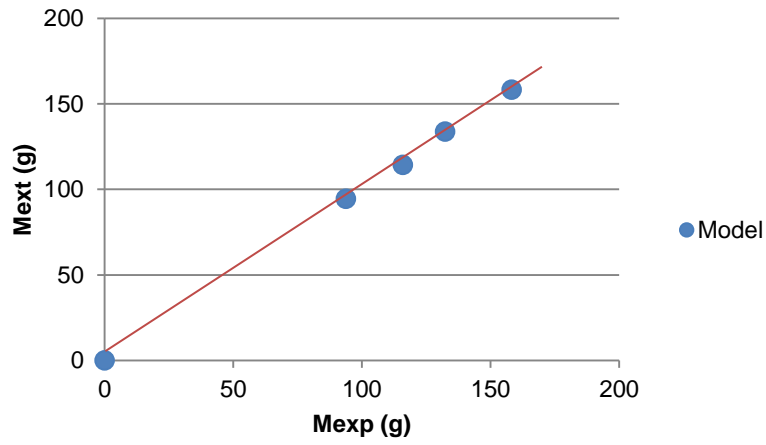


Figure 4.9: Martinez model for experimental run 4

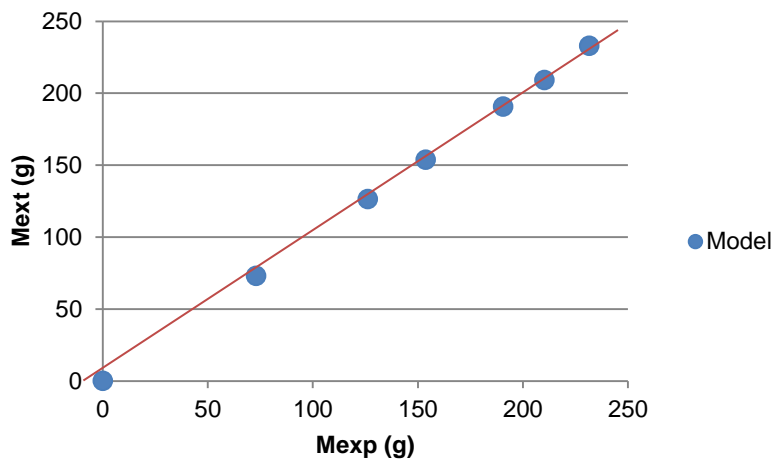


Figure 4.10: Martinez model for experimental run 5

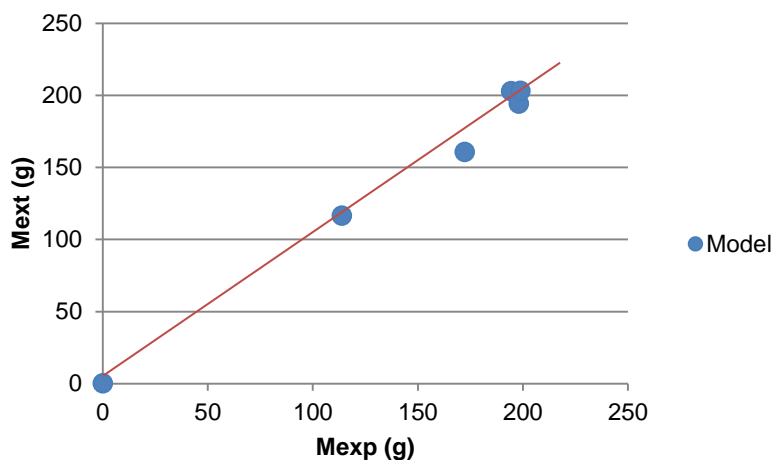


Figure 4.11: Martinez model for experimental run 6

4.6 Analysis

The HPLC analysis was done at the Oxidation centre at the Cape Peninsula University of Technology, and the GC-MS analysis was done at Central Analysis Facility (CAF) at Stellenbosch University. The results of each analysis are presented below.

4.6.1 HPLC

This analysis was done to quantify the alpha acids and beta acids within the hop extract produced via supercritical CO₂ extraction. During this experimental work, 6 experiments were completed, and the HPLC analysis was done on the heavier fraction, i.e. the fractions obtained at the higher pressure during that specific experiment. The standard used during the analysis

was the International Calibration Extract 4 (ICE-4). This standard is the international calibration extract hop standard of the European Brewery Convention (EBC) and the American Society of Brewing Chemists (ASBC).

Table 4.10: Hop standard for HPLC analysis

International Calibration Extract 4 (ICE-4)	
Cohumulone 10.98 %	Colupulone 13.02 %
N+adhumulone 31.60 %	N+adlupulone 13.52 %
Total α-acids 42.58 %	Total β-acids 26.54 %

Table 4.10 shows the composition of the total alpha and beta acids. It identifies the specific acid compound.

This data was used to profile the hop types. It was used to graphically illustrate the effect of time, pressure and hop type. This data can be compared to the data found in literature; however, it compares total alpha and beta acids. Figure 4.12 shows the acid profile of Southern Star extracts at 250 bar with an extraction time of 90 minutes. The total alpha acids content was 63 mg/g sample and beta acid content of 10.95 mg/g sample. This hop type is classified as a high alpha acid hop.

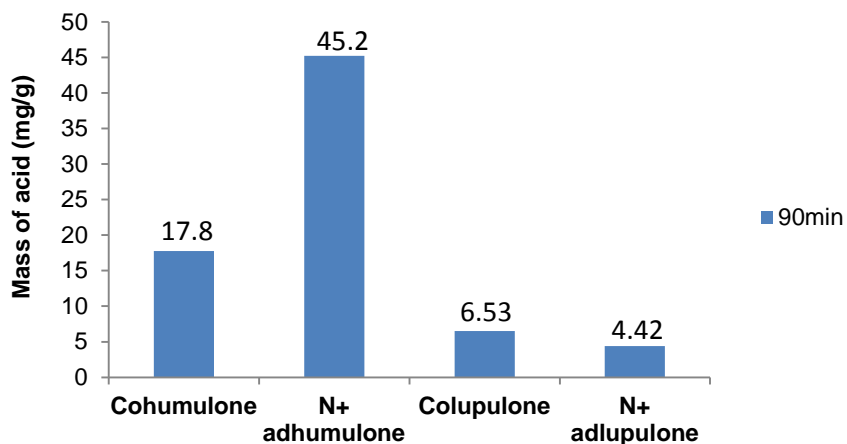


Figure 4.12: Mass of acid in mg per gram extract for experimental run 1 Southern Star

Figure 4.13 shows the acid profile of Southern Passion extract at 250 bar with an extraction time of 2 hours. The total alpha acids content was 61.08 mg/g sample, and the beta acids content was 17.79 mg/g.

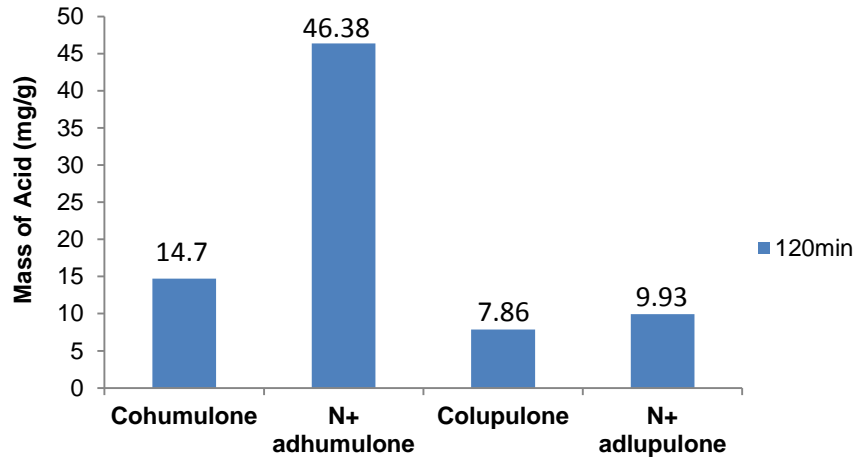


Figure 4.13: Mass of acid in mg per gram extract for experimental run 2 Southern Passion

Figure 4.14 shows the acid profile of African Queen extract at 250 bar for 3 hours. The total alpha acids content was 58.3 mg/g sample, and beta acids were 17.56 mg/g.

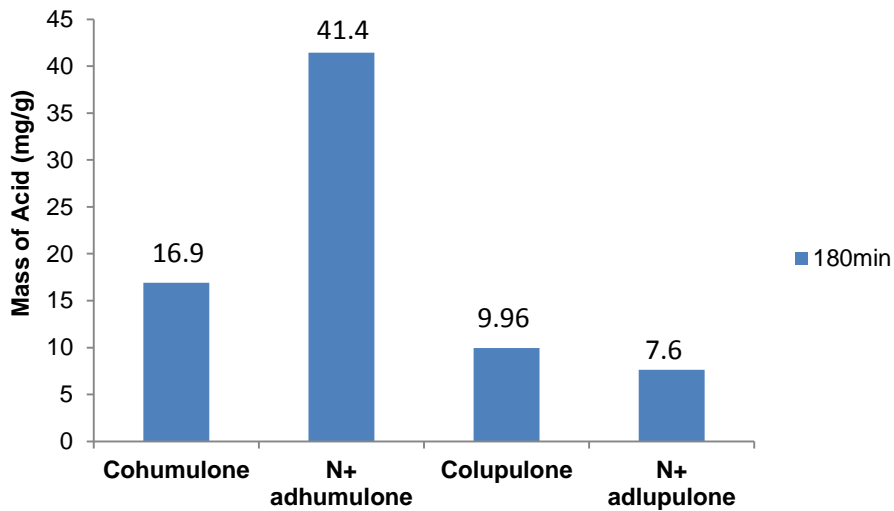


Figure 4.14: Mass of acid in mg per gram extract for experimental run 3 African Queen

Figure 4.15 shows the acid profile of Southern Promise extract at 250 bar for an extraction time of 1 hour. This total alpha acid content was 46.9 mg/g sample and beta acid 16.3 mg/g. This is an aromatic hop type.

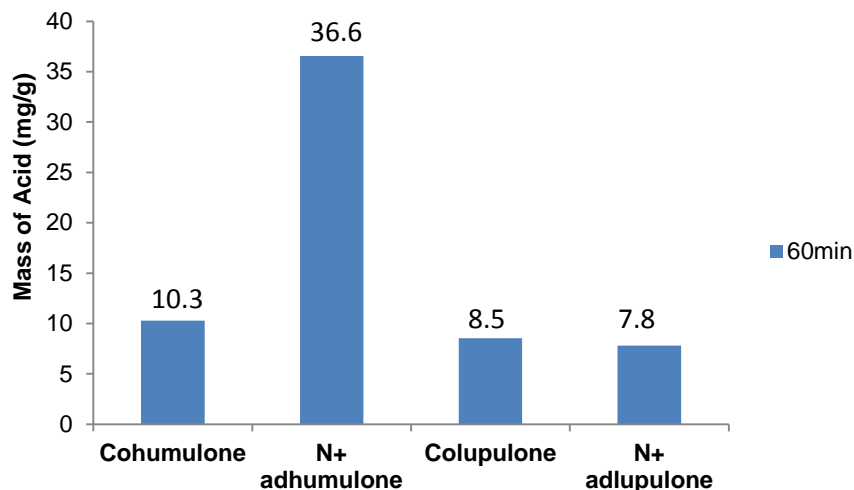


Figure 4.15: Mass of acid in mg per gram extract for experimental run 4 Southern Promise

4.6.2 GC-MS

This analysis was done to quantify the volatile compound within the hop extract produced via supercritical CO₂ extraction. During this experimental work, 6 experiments were completed, and the GC-MS analysis was done on the lighter fraction, i.e. the fractions obtained at a lower pressure during that specific experiment; however, with experimental runs 1, 5 and 6, the extract samples include extracted obtained at 150, 200 and 250 bar.

Figure 4.16 shows the volatile profile of experimental run 2, for Southern Passion at 200 bar for 2 hours.

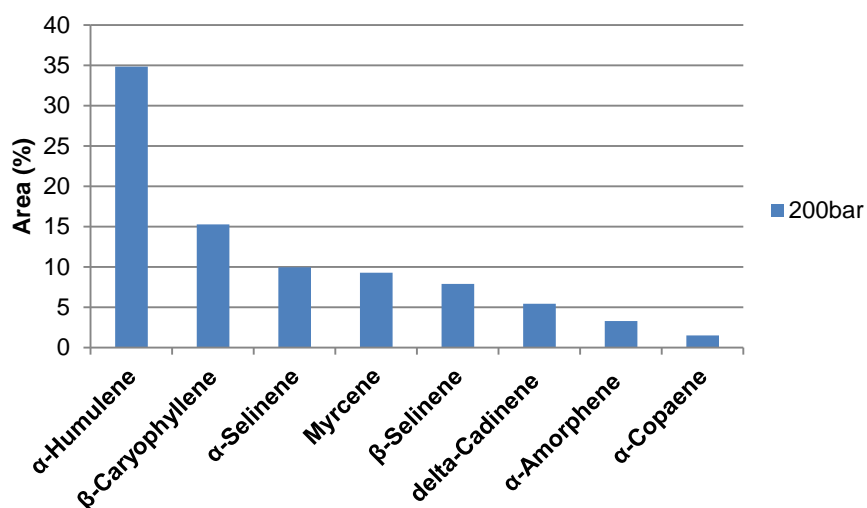


Figure 4.16: Area of aroma compound for experimental run 2

Figure 4.17 shows the volatile profile of experimental run 3. This is for African Queen at 200 bar for 2 hours

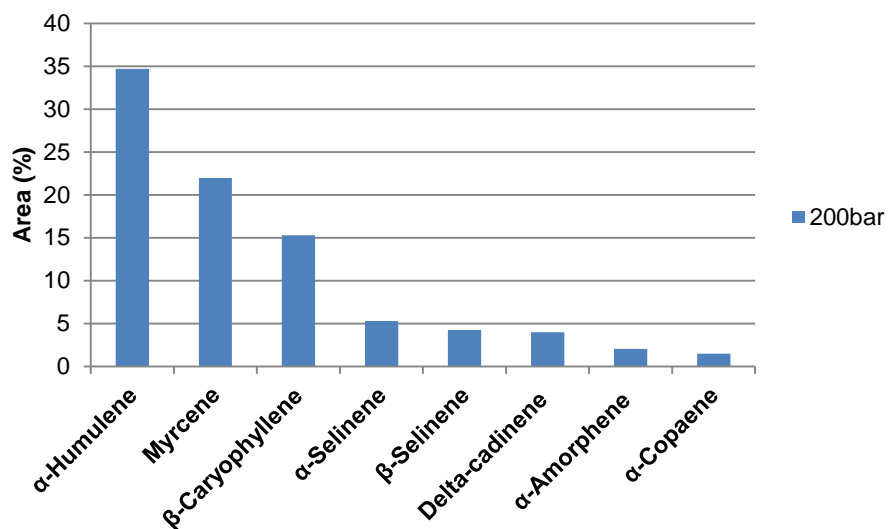


Figure 4.17: Area of aroma compound for experimental run 3

Figure 4.18 shows the volatile profile of experimental run 4. This run was for Southern Promise at 150 bar for three hours.

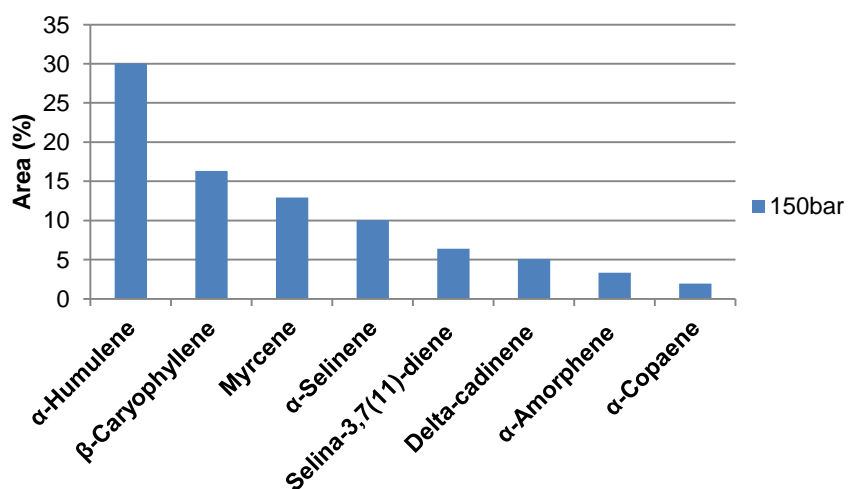


Figure 4.18: Area of aroma compound for experimental run 4

4.7 Control experimental

For the control experiment, the samples were sent for HPLC analysis. This result is further discussed in chapter 5.

Table 4.11: HPLC results for boiling hops

Hop acid (mg/L)	Wet hopping	Dry hopping
Cohumulone	1.16	0.44
N+ Adhumulone	2.48	1.86
Colupulone	-	-
N+ Adlupulone	-	-

4.8 Chapter outcomes

In this chapter, the results obtained during experimental work and analysis was presented. For objective one, the theoretical estimation of conditions resulted in data for pressures 150, 200 and 250 bar at constant temperature (40°C). The data obtained during this simulation focused on the behaviour of the volatile compounds namely myrcene and linalool. With objective two, the experimental fractionation resulted in material balance data for 150, 200 and 250 bar samples. These samples were sent for HPLC and GC-MS analysis to fulfil objective three. The analysis data included the aroma profile and hop acid profile. This data was used to characterise the profile of the South African hop extracts. The experimental data were used to model the extraction kinetics and these models included the BIC and Martinez model. With objective four, the control experiment HPLC data indicated a decrease in hop acids after dry hopping. This data is further discussed in Chapter 5.

Chapter 5: Discussion

This chapter presents an in-depth discussion of the results presented in Chapter 4. The data discussed include that relating to material balances, chemical analysis and extraction kinetics. From this discussion, conclusions were drawn relating to the conditions required to achieve the fractionation of hop extracts (objective one), the extent of fractionation that can be achieved (objective two), the composition of the fractions (objective three), and the hop components present in an aqueous solution (objective four). The overall conclusion from this work will be presented in the final chapter.

5.1 Introduction

Beer hop extraction is not a completely new proposition. Many authors have investigated the extraction of hops in the open literature such as Zekovic (2006), del Valle (2003) and Kupski (2017). However, the investigation of the separation of aroma from bitter components remains unanswered. Baskette, in a non-peer-reviewed publication, shows how 4 different hop types were extracted at 150 and 300 atmospheres, to yield two fractions. Although the compositions of the fractions are not given, one can speculate on the possibility of their different compositions. Whether the compositions differed sufficiently to be applied differently, as bittering and aroma agents separately, were not commented upon. The aim of this work was to shed light on this question.

Bizaj et al (2021) experimented with different solvents – CO₂, SF₆, propane and dimethyl ether. The separation of aroma and bitter components was not the aim of this study. However, the results indicate that the separation of alpha and beta acids were not an easy separation to achieve with these solvents (Bizaj et al., 2021).

5.2 Estimation of conditions

Two methods were used to estimate the extraction conditions. The first method was using theoretical estimation: VLE calculations by developing a thermodynamic model and doing a flash calculation to check at which temperature and pressure fractionation occurred. The second method was surveying literature and comparing the effect of the used conditions and the yield achieved by each author.

The first step in developing this model entailed listing the major components found in hops, and finding as many of their relevant physical properties as possible. As can be expected,

experimental values of many of the required properties, such as solubility in CO₂, and the VLE of the binary systems of the relevant compounds in CO₂ could not be found in the literature. These properties could thus be estimated using group contribution methods, within the Aspen property package. The procedure entailed constructing a molecular structure on a dedicated section of the screen and invoking a programme to calculate the bond energies, thus defining a specific molecule. Some of the relevant structures were already supplied inside the NIST Database, with some of the relevant physical properties. The NIST database included some hop aroma compounds, such as myrcene and linalool, which had complete VLE data.

The next step was to decide on the package to use to estimate, interpolate, and model the physical properties. Since the compounds involved were largely non-polar, an equation of state simple enough to require one or two fitting parameters, but robust enough to accurately model the VLE behaviour of all the compounds was required. The Peng-Robinson EoS fitted this requirement. It is also one of the most commonly used for modelling the VLE behaviour of hydrocarbons in general.

When performing the flash calculation the CO₂ pressure was set to 150 bar and 40°C as an estimate. The mass flow rate was set to 6 kg/hr. For the hop feed, an estimate for the mass fraction was set to 0.6 for myrcene and 0.4 for linalool. For the vessel, the pressure was set to 150 bar and 40°C. The outlet streams were named extract and bottoms. These streams were set to the operating conditions of the separators during pilot plant operation. The pressure was set to 50 bar and 100°C. The simulation results were assessed; it was noted that any pressure between 100-250 bar results in all the compounds either completely recovered in the extract stream or bottoms stream. Any pressure between 80-90 bar resulted in an error in simulation. The only pressure range that resulted in successful fractionation was between 71-78 bar. The optimal fractionation condition was found to be 77 bar. This pressure was much lower than that of the pressures used in literature. This difference is due to extraction being governed by mass transfer. When simulating equilibrium data factors such as plant matrix (internal cells) is not considered. However, this simulation was used to determine whether fractionation between volatile compounds is possible. When looking at terpenes, it would be an advantage to segregate oxygenated terpenes from monoterpenes. This simulation provided data that showed that the separation of linalool from myrcene is possible at 77 bar.

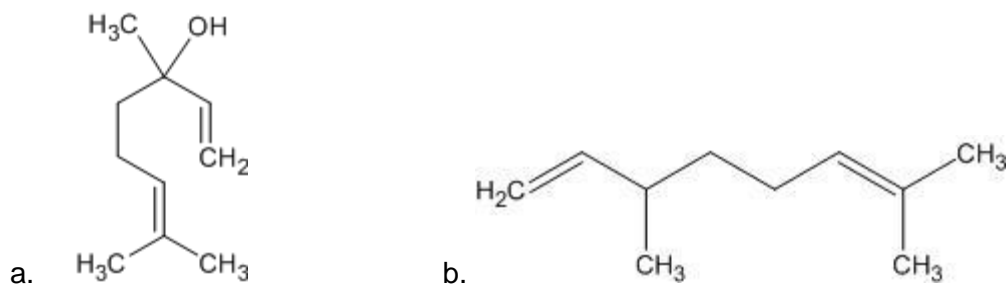


Figure 5.1: a. Linalool b. Myrcene

Figure 5.2 shows the vapour fraction for linalool and myrcene. From this data it can be seen that linalool and myrcene at 40°C behave similarly.

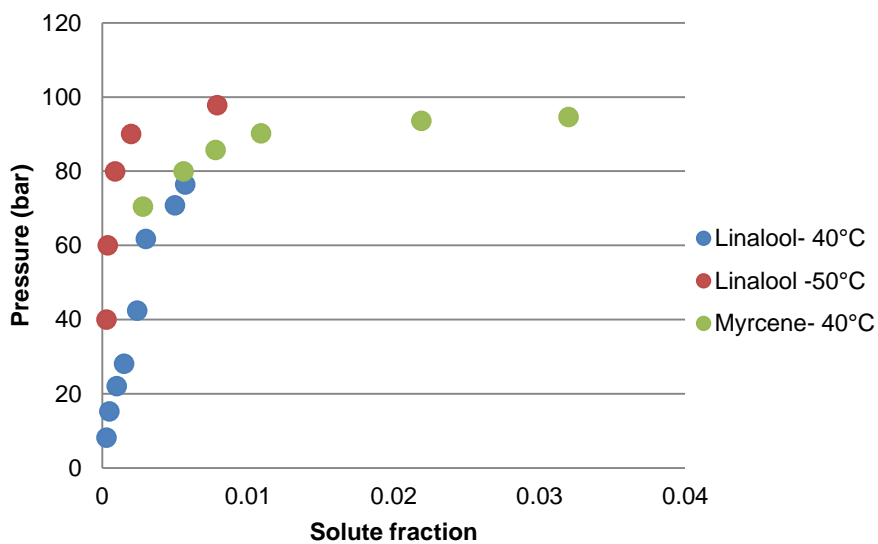


Figure 5.2: P-y diagram for linalool (Morotomi et al., 1999) and myrcene (Bogel-Łukasik et al., 2009).

Figure 5.3 shows the liquid fraction of linalool and myrcene.

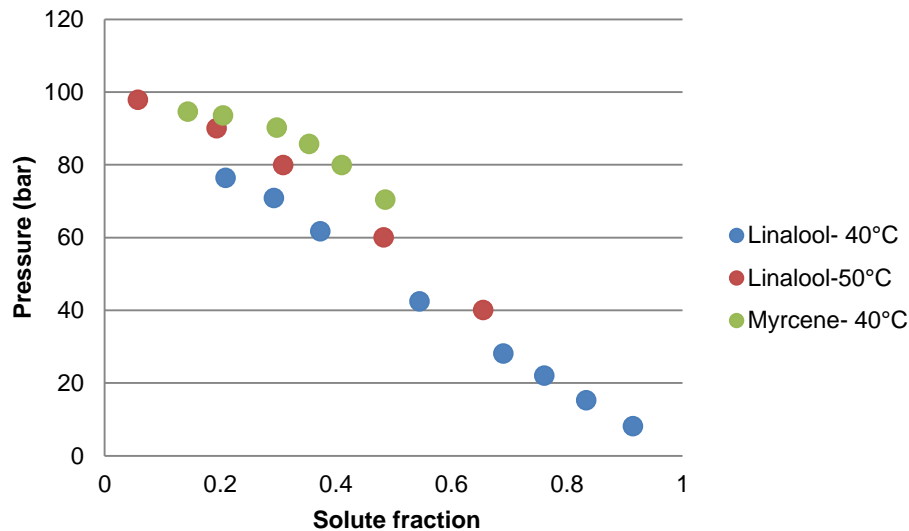


Figure 5.3: P-x diagram for linalool (Morotomi et al., 1999) and myrcene (Bogel-Łukasik et al., 2009).

Figure 5.4 shows the structures for alpha-humulene, trans-caryophyllene and limonene.

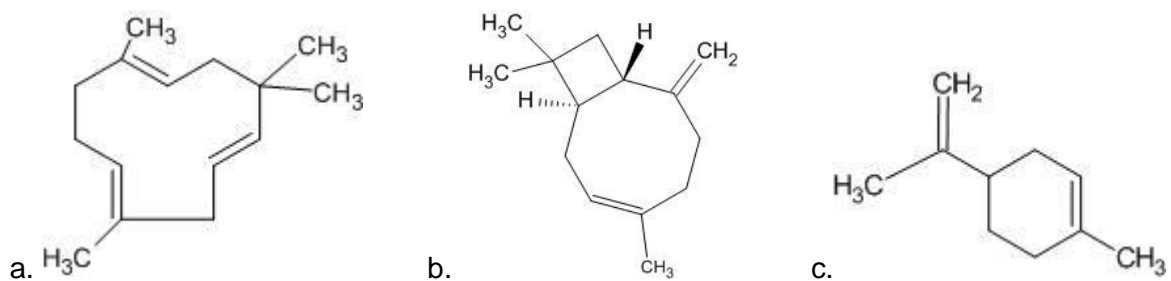


Figure 5.4: a. Alpha-humulene, b. Trans-caryophyllene and c. Limonene

Figure 5.5 shows the vapour-liquid equilibrium data from α -humulene, trans-caryophyllene, linalool and limonene. From this figure, it is noted that α -humulene and trans-caryophyllene has a similar solubility and linalool and limonene has a similar solubility. This experimental VLE data suggests that it should be possible to separate linalool and limonene from α -humulene and β -caryophyllene. It is of industrial interest to separate the oxygenated terpenes from the volatile fraction. Although this data suggests that group separation might be possible it is important to note that equilibrium data does not reflect experimental data

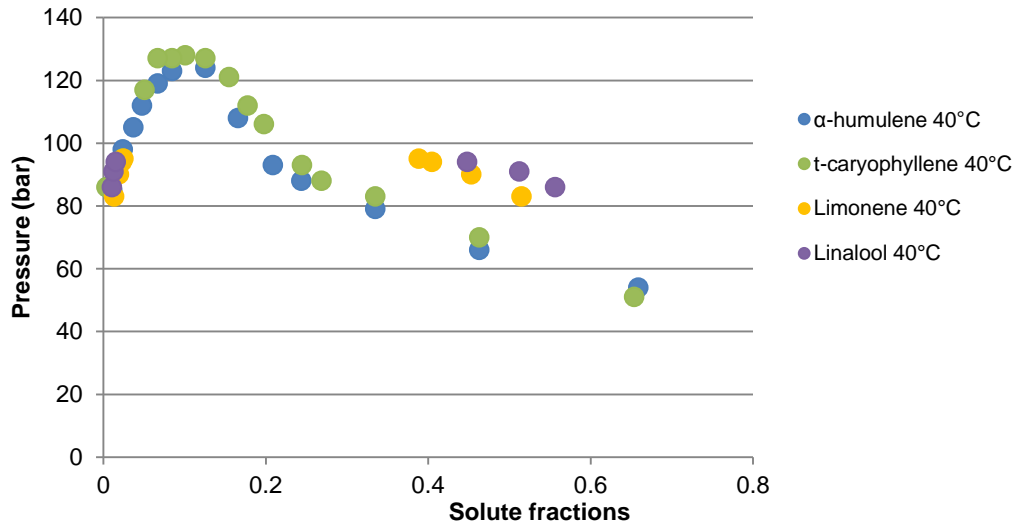


Figure 5.5: P-xy diagram for α -humulene and trans-caryophyllene (Michielin et al., 2009), limonene and linalool (Fonseca et al., 2003).

5.3 Experimental work

The experimental work produced the material balance data. This data was used to calculate yield and plot the trends. The experimental data can be found in Appendix E: Lab journal

5.3.1 Fractional extraction: Material balance

The order of the hop type for extraction was according to the acid profile. Southern Star had the highest acid content, and Southern Promise had the lowest of the four.



Figure 5.6: Southern Star extract during experimental run 1

For each experiment at the first pressure, the extracted mass was the highest compared to the other sampling times for the remaining pressures, i.e. for experimental run 1 at 150 bar – 132.36

g and experimental run 5 at 150 bar – 126.25 g. By the second pressure, the masses decrease significantly. This ranged between 25.86 – 64.44 g. This is due to the exposed free oil at the start of extraction. By mechanically breaking the cell walls of the hop, the solvent can easily travel within the cell walls and carry the solutes. Some solutes may be located in the internal cell walls of the hop materials. For this reason, grinding is an essential step as it reduces the particle size and thus increases the surface area for the solvent to penetrate the hop and easily enrich hop components. Kupski (2017) used a range of 0.71 mm – 0.43 mm. In this work, the particle size was reduced to 1 mm – 710 μm . Another reason for the larger mass is the 30 minutes static extraction, and it is during this period, all the volatile components are easily extracted and thus when sampling larger mass is obtained. Kupski (2017) observed 90 minutes to ensure the solvent was enriched in hop components. In this work, 30 minutes was sufficient, due to the pilot plant's ability to maintain the pressure in the vessel. As the pressure increases, the mass of extract in each time interval decreases due to all the extractable components being extracted at previous pressures. It was noted that after 6 hours, there was no significant change in the mass of extract being produced; thus, it can be concluded that maximum extraction lasts for 6 hours. The co-solvent (ethanol) was introduced once maximum extraction was completed. These samples were sent for HPLC analysis. This data indicated the no hop acids were detected in this sample. The co-solvent aided in making the CO_2 more polar to extract any remaining compounds that the CO_2 might not have extracted. This result indicates that using CO_2 as a solvent for the extract of hops will result in the complete extract of all hop compounds present in the hop pellet. However, the spent hop are not completely invaluable as it may be used for potting soil. This is due to the spent hops being rich in nitrogen. The spent hops are suggested to be mixed with brown materials such as sawdust (Community Composting, 2014). Guo-qing (2005) extracted flavonoids (Xanthohumol) from waste hops with a modifier (ethanol). The flavonoid recovery increased as the ethanol percentage added increased (Guo Qing et al., 2005). This would suggest that using pure ethanol would be efficient as 80% ethanol was used in that study. Although this study indicated that, the use of a modifier does extract hop polyphenols. It is important to note that in this work the ethanol use was 99% pure. The modifier was used to extract any usable hop compounds however the analysis indicated that no hop compounds were present and this may be due to all compounds being extracted at 250 bar.

5.3.2 Fractional yield

The yield was calculated for each pressure. The yield was calculated by

$$Yield = \frac{\text{Mass of extract}}{\text{Feed of hops}} \quad \text{Equation 5-1}$$

Each experiment has two figures—one illustrating the yield vs time and the other yield vs mass of CO₂. From the figures, it was noted that as the solvent's density increases, the total yield increases. This trend was observed by Kupski (2017) and del Valle (2003). In Figure 5.7, at 150 bar the density of CO₂ is 780 kg/m³ but as the pressure is increased to 200 bar the density increases to 840 kg/m³ and at 250 bar, CO₂ has a density of 880 kg/m³. At a constant temperature and increasing pressure the solvating power of CO₂ is increasing, however, for constant pressure and increasing temperature the solvating power of CO₂ is decreased (Pereira & Meireles, 2010). The solubility of the solutes strongly depends on the solvating power of the solvent.

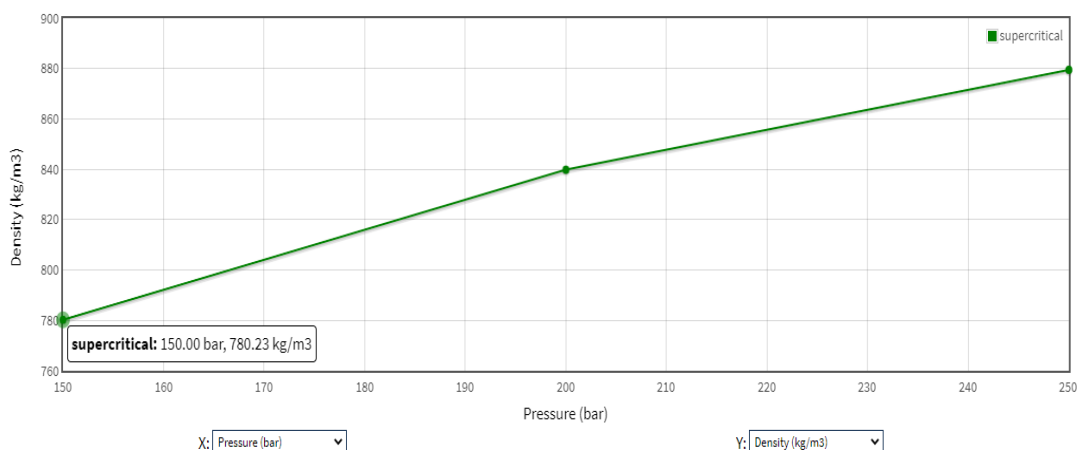


Figure 5.7: Density of CO₂ (Nist Chemistry Webbook, 2021)

The temperature and CO₂ flow rate were kept constant, and only the pressure was increased by 50 bar increments. For experimental run 1, a total yield of 20.43% was achieved. At 150 bar, a yield of 12.38%, 200 bar 5.7% and 250 bar 2.43%.

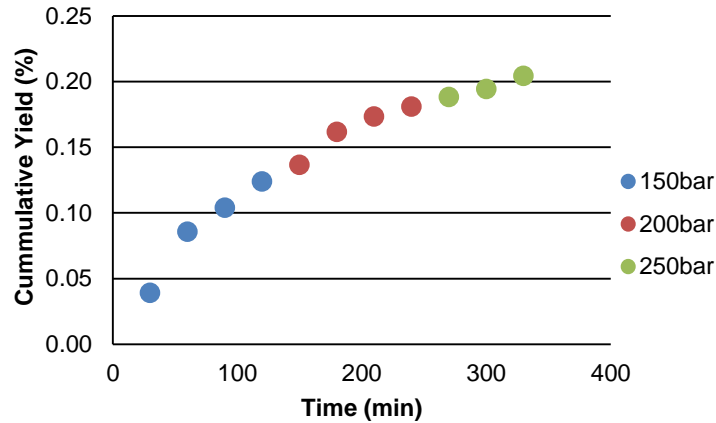


Figure 5.8: Yield vs time for experimental run 1

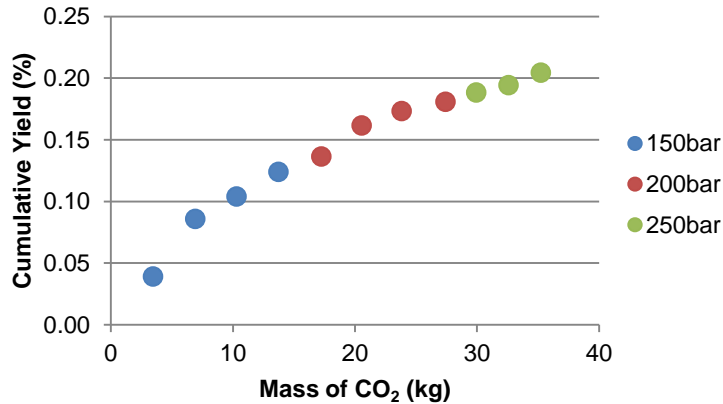


Figure 5.9: Yield vs mass of CO₂ for experimental run 1

The yields obtained at 250 bar ranged from 2.35 - 4.08%. Although the density of CO₂ is highest at 250 bar (880 kg/m³), this data following the statement by del Valle (2003) that any P>200 had no significant increase in the yield obtained.

For experimental run 2, a total yield of 16.46% was achieved. The yield per pressure was 200 bar 12.1% and 250 bar 4.39%. In all of the experimental runs, the data points show a constant extraction rate.

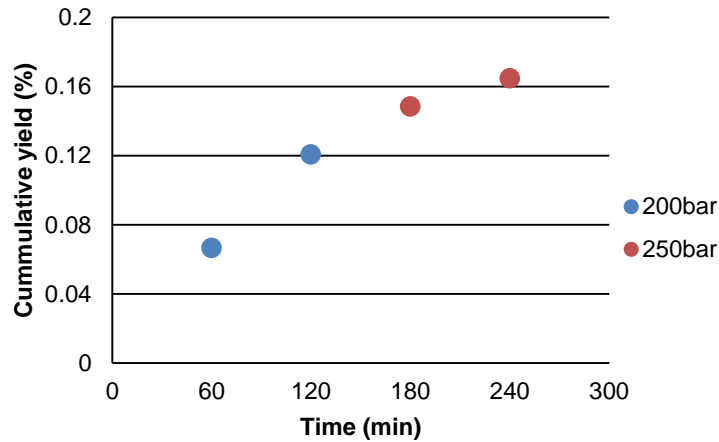


Figure 5.10: Yield vs time for experimental run 2

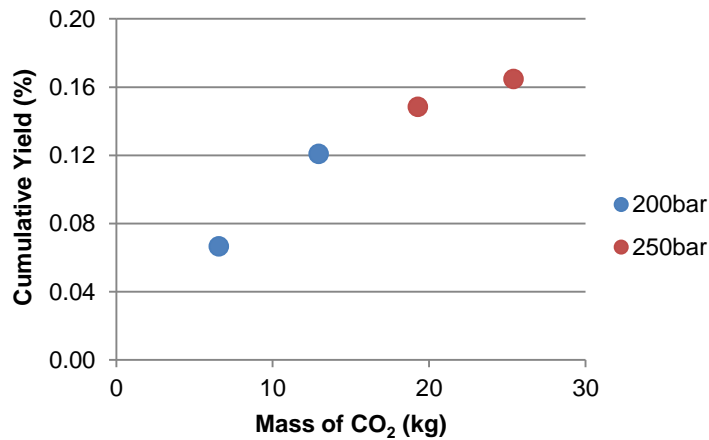


Figure 5.11: Yield vs mass of CO₂ for experimental run 2

In experimental run 3, a yield of 17.5% was achieved. At 200 bar, a yield of 13.22% and 250 bar 4.28% was obtained. At 250 bar, the extraction lasted for 3 hours instead of two hours. This is due to the plant pilot operating steadily compared to the previous experimental runs where an emergency shutdown occurred. The reason for the shutdown was unclear, as the previous two experimental runs data had no visible errors. During fractional extraction, experimental run 3 had the highest yield (13.22%). This yield was obtained at 200 bar after 2 hours. Kupski's (2017) highest yield was 7.1% at 200 bar and 55°C after 3 hours. This was achieved in an extraction column with a diameter of 1.91 cm and a height of 16.8 cm. This difference can be explained in terms of CO₂ density. At 200 bar and 40°C, the density of CO₂ is 840 kg/m³ and at 200 bar and 55°C, the density is 754.61 kg/m³. With an increase in temperature the density of CO₂ decreases therefore, the yield is lower with an extended extraction time. The data

published by del Valle (2003) a yield of 14.6% at 200 bar and 40°C after 4 hours was achieved. This was achieved in a vessel of 0.2 L. This is slightly higher than the yield achieved in this work and this may be due to the extended extraction time (additional 2 hours) or the hop variety used.

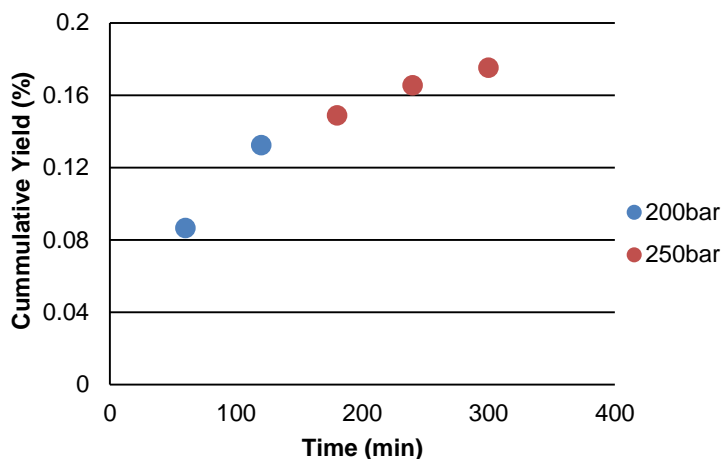


Figure 5.12: Yield vs time for experimental run 3

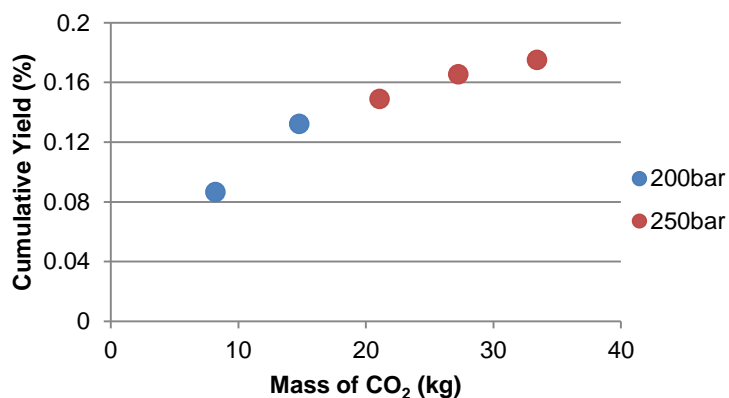


Figure 5.13: Yield vs mass of CO₂ for experimental run 3

In experimental run 4, a yield of 15.79% was achieved. During this experimental run, an unexpected shutdown occurred at 250 bar; thus, there is only data for one hour for that pressure range. At 150 bar, the extraction time was 3 hours and produced a yield of 13.21%. Zekovic (2006) achieved a yield of 13.35% at 150 bar and 40°C after 2.5 hours in vessel size of 0.2 L. It was expected that the yield of this run would be slightly higher than that of Zekovic as an additional 30 minutes for extraction was allowed. However, it is slightly lower and this may be due to the hop variety used.

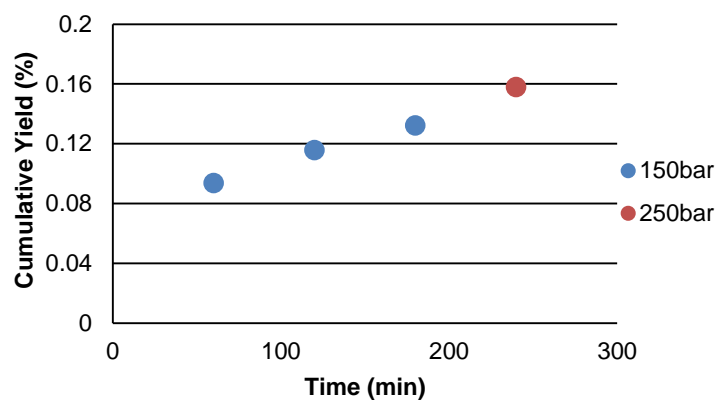


Figure 5.14: Yield vs time for experimental run 4

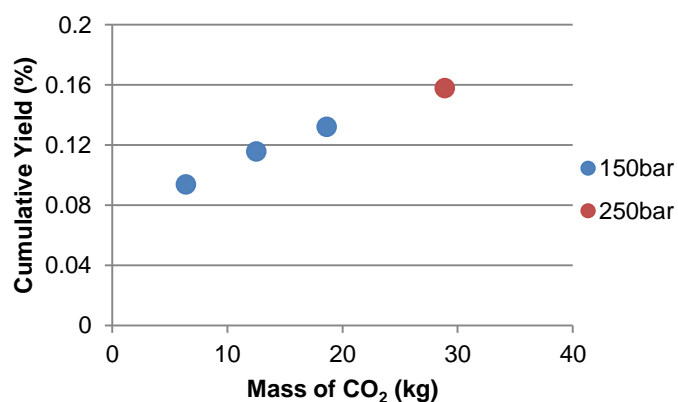


Figure 5.15: Yield vs mass of CO₂ for experimental run 4

Experimental run 5 achieved a yield of 23.1%. This experiment was done as a duplicate for experimental run 1. The yield obtained at 150 bar after 2 hours was 12.59%. For the second pressure, 200 bar, a yield of 6.2% was obtained. For the final pressure, a yield of 4.08% was obtained.

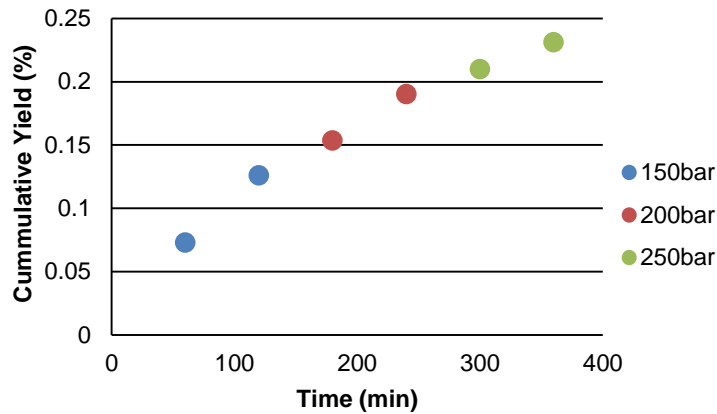


Figure 5.16: Yield vs time for experimental run 5

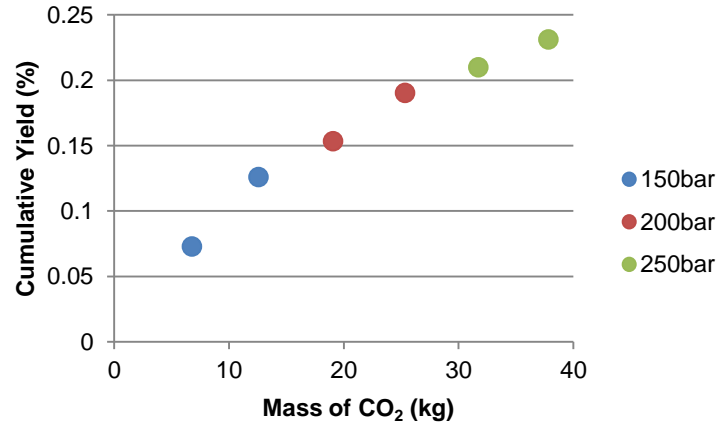


Figure 5.17: Yield vs mass of CO₂ for experimental run 5

Figure 5.18 shows experimental run 1 vs experimental run 5 in terms of yield. The square symbol represents run 1 and the circle symbol represents run 5. When comparing the mass of extract for experimental runs 1 and 5 at 150 bar experimental run 5 produced 0.21 g more than experimental run 1, at 200 bar run 5 produced 0.5 g more than run and at 250 bar run 5 produced 1.65 g more than experimental run 1. It is important to note that during experimental run 5 at 250 bar an additional 30 minutes of extraction occurred. The small difference in mass indicates the repeatability of this work.

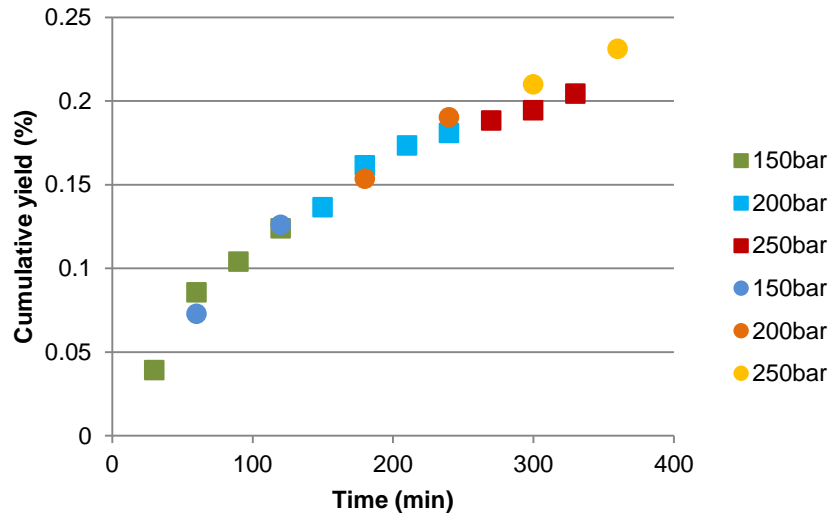


Figure 5.18: Yield vs time for run 1 and run 5

Experimental run 6 was completed to determine the total extract produced at a pressure of 250 bar. A yield of 20.03% after 5 hours was achieved.

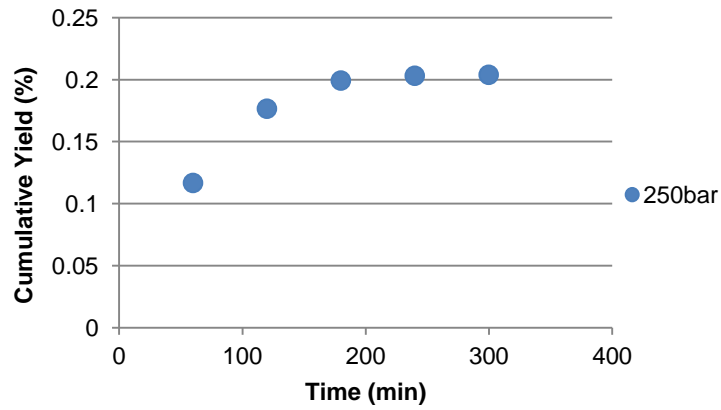


Figure 5.19: Yield vs time for experimental run 6

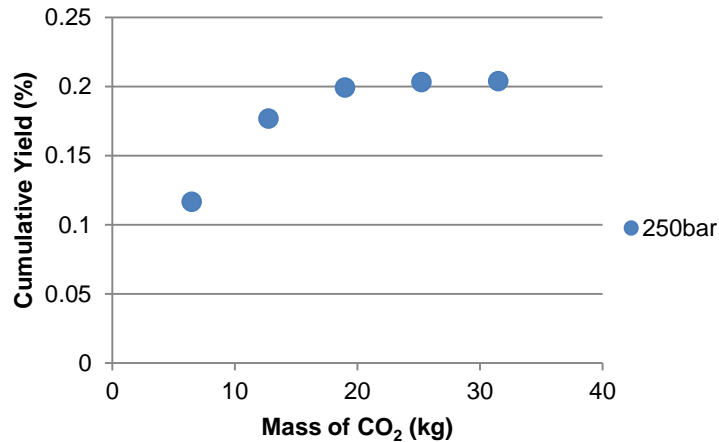


Figure 5.20: Yield vs mass of CO₂ for experimental run 6

5.4 Kinetic models discussion

The extraction kinetics followed constant extraction; thus, the Broken-Intact-Cells model was fitted for experimental runs 1 and 5. For all 6 experiments, the Martinez model was fitted

5.4.1 Broken-Intact-Cells model

The Broken-Intact-Cells model was applied to experimental runs 1 and 5. This is due to each pressure range staying in the constant extraction zone. The constant extraction zone is maintained by increasing the pressure before the falling rate begins. The Sovova model $m(t) = \dot{M}_F * Y_S * t * [1 - \exp(-Z)]$ $t < t_{CER}$ Equation 2-18, was used due to the equation meeting the requirements of modelling extraction kinetics within the constant extraction zone. This equation has one adjustable parameter, Z. Once Z was obtained, it was used to estimate $k_F a$ (Volumetric mass transfer coefficient). To fit the equation, the slope of each constant extraction section was determined using a trendline on Excel. Y_s is the apparent solubility; it was estimated from the slope of a plot of mass of extract vs mass of CO₂. Using all the parameters and an estimated Z value, the mass was predicted using the model equation. The error between the mass obtained during lab and mass calculated was minimized by changing the Z adjustable parameter. Microsoft Excel was used to calculate the parameter, and MS Excel solver was used to estimate the adjustable parameter Z. This was done by calculating the error and using Solver to minimize the error by adjusting parameter Z. Table 5.1 and Table 5.2 summarizes all the parameters calculated to fit the model equation to the experimental data. All the calculations for this model is provided in Appendix D: Kinetic modelling.

Table 5.1: Sovova model parameters for experimental run 1

	Experimental						Model	
Time	Mass (g)	Slope	Y_s	Z	k_{Fa}^a	k_{Fa}^b	Mass (g)	AARD%
0	0	1.4115	12.22	1.5E-03	6.3E-05	4.4E-05	0	3.5
30	39.32	1.4115	12.22	1.5E-03	6.3E-05	4.4E-05	42.34	
60	86.2	1.4115	12.22	1.5E-03	6.3E-05	4.4E-05	84.69	
90	104.62	0.6169	5.44	1.1E-03	4.3E-05	3E-05	104.62	1.4
120	124.55	0.6169	5.44	1.1E-03	4.3E-05	3E-05	123.13	
150	137.29	0.6169	5.44	1.1E-03	4.3E-05	3E-05	141.63	
180	162.56	0.6169	5.44	1.1E-03	4.3E-05	3E-05	160.14	
210	174.4	0.2505	2.70	9.3E-04	3.4E-05	2.4E-05	174.4	0.3
240	181.92	0.2505	2.70	9.3E-04	3.4E-05	2.4E-05	181.91	
270	189.39	0.2505	2.70	9.3E-04	3.4E-05	2.4E-05	189.43	
300	195.49	0.2505	2.70	9.3E-04	3.4E-05	2.4E-05	196.94	
330	205.56	0.2505	2.70	9.3E-04	3.4E-05	2.4E-05	204.46	

a- Constant hop bed

b- Hop bed filled entire vessel

Table 5.2: Sovova parameters for experimental run 5

	Experimental						Model	
Time	Mass (g)	Slope	Y_s	Z	k_{Fa}^a	k_{Fa}^b	Mass (g)	AARD%
0	0	1.218	10.76	1.1E-03	4.6E-05	3.2E-05	0	0.00
60	73.08	1.218	10.76	1.1E-03	4.6E-05	3.2E-05	73.08	
120	126.25	0.4592	4.26	1.1E-03	4.1E-05	2.9E-05	126.25	0.00
180	153.8	0.4592	4.26	1.1E-03	4.1E-05	2.9E-05	153.80	
240	190.69	0.3381	3.27	1.0E-03	3.8E-05	2.6E-05	190.69	0.15
300	210.31	0.3381	3.27	1.0E-03	3.8E-05	2.6E-05	210.98	
360	231.6	0.3381	3.27	1.0E-03	3.8E-05	2.6E-05	231.26	

a- Constant hop bed

b- Hop bed filled entire vessel

The bed porosity ($\varepsilon = 0.55$), the density of the bed ($\rho_{bed} = 0.2$), the density of solid and density of CO₂ at each pressure ($\rho_{solid} = 0.65$, $\rho_{spent} = 0.28$) was calculated and used to calculate $k_F a$. the particle size was between 710 μm – 1mm. The values of $k_F a$ ranged from $2.6 \cdot 10^{-5}$ to $6.2 \cdot 10^{-5}$ min^{-1} . To compare the $k_F a$ values the k_f (mass transfer coefficient per specific surface) is calculated by:

$$k_f = \frac{k_F a}{a_o} \quad \text{Equation 5-2}$$

Where:

$$\begin{aligned} a_o &= \frac{2}{d_p} * (1 - \varepsilon) && \text{Equation 5-3} \\ &= \frac{2}{0.001} * (1 - 0.55) \\ &= 900 \text{ m} \end{aligned}$$

The k_f values ranged from $7 \cdot 10^{-8}$ to $2.67 \cdot 10^{-8}$. Kupski (2017) fitted extraction data to three kinetic models. These models included the BIC, shrinking core and a model based on equilibrium. For the BIC model, the k_f values ranged from $4.31 \cdot 10^{-8}$ to $1.56 \cdot 10^{-7}$ (Kupski et al., 2017). The k_f values are lower than that of Kupski's. The values of $k_F a$ are influenced by the external mass transfer process by shifting the internal mass transfer and the variations found in the hop bed or the solvent (Del Valle & De La Fuente, 2006). The absolute average relative deviation was calculated. For run 1 at 150 bar a value of 3.5%, 200 bar a value of 1.4% and 250 bar a value of 0.3%. Although no deviation was expected, this may be due to pilot plant scale being close to that of the industrial scale and assumptions made based on literature may not be true for this process. In Kupski (2017) the error for the BIC model ranged between 2.3 to 3.3%. For run 5, the model fitted adequately, and no deviation was found for 150 and 200 bar; however, for 250 bar, a value of 0.15% for AARD was calculated.

5.4.2 Martinez Model

This logistic model has two adjustable parameters, t_m and b . To improve the fit of the model, a correctional factor, A , was introduced. This model was fitted to each run. All the calculations for this model is provided in Appendix D: Kinetic modelling.

Table 5.3: Martinez model parameters summary

Exp	Model parameters				
	Press (bar)	tm (min)	b (min ⁻¹)	A	AARD%
Run 1	150	-3928.22	0.001004	1.00035	9.45
	200	-6687.5	0.000578	1.0012	2.65
	250	-6794.62	0.000601	1.00069	2.61
Run 2	200	-3070.59	0.001038	1.00038	0.016
	250	-11589.6	0.000328	1.0021	0.511
Run 3	200	-5482.02	0.00076	1.00056	0.086
	250	-17186.2	0.00024	1.0015	1.08
Run 4	150	-10559.8	0.00035	1.0018	0.99
	250	-9308.68	0.00038	1.0022	3.94E-08
Run 5	150	-3984.33	0.00099	1.00032	0.13
	200	-5674.01	0.00072	1.00055	0.01
	250	-10206.6	0.00047	1.00067	0.59
Run 6	250	-7185.61	0.00079	1.00024	4.46
	250	-30975.5	0.00016	1.0012	2.07

All the values of tm were negative, and the values for b was in the range of 10⁻³ to 10⁻⁴ and A = 1. The negative values for tm indicated a decrease in extraction (Jokić et al., 2011). The b value for each pressure range was in the ranges of 0.0001 to 0.0009 except for the b value in experimental run 1 at 150 bar (0.001) and experimental run 2 at 200 bar (0.001). The b values of these experiments were slightly high. Jokić (2011) fitted soybean data to the Martinez model and the b value for two pressures (200 bar and 300 bar) were slightly higher than the other experimental runs. This was explained by the crossover phenomena observed for seed oils at those pressures while the temperature remained constant (Jokić et al., 2011). The crossover phenomena is explained as the region where the solute is highly soluble in the solvent (June-wells, 2018). The same conclusion cannot be made for this work as all the data of the other experimental runs at 150 bar and 200 bar do not fall within the same range i.e. experimental run 4 at 150 bar (b = 0.00035) and experimental runs 3, 5 and 6 when b = 0.00076, 0.00072 and

0.00079 respectively. The AARD% was calculated; for experimental run 1 at 150 bar, a value of 9.45% was achieved. Although this seems high in many literature articles such as Kupski (2017), a mean error = 9.5% was acceptable. For the other pressure ranges, the deviation was 2 or smaller than 1 was calculated. The comparative plots are shown in Figure 4.6 to Figure 4.11. These are plots of experimental data vs predicted data. The middle line indicates how accurately the model equation fitted the data. In an article by Bizaj (2021) the supercritical and subcritical extraction of Slovenian hops was performed. In this article, the extraction kinetics was modelled and fitted to the Crank model. Although this model fitted adequately the author stated that the model relies on time and does not account for any factors that describe the extraction flow rate (Bizaj et al., 2021).

5.5 Analysis data

Two types of analysis were done on the hop extract. HPLC for the hop acids and GC-MS for the volatile aroma compounds

5.5.1 HPLC

Figure 5.21 shows the hop acid profile of each hop variety at 250 bar. The extraction times were for Southern Promise (60 minutes), Southern Star (90 minutes), Southern Passion (120 minutes) and African Queen (180 minutes). When observing this data it was noted that Southern Passion contained the highest amount of adhumulone. Other than this difference it was noted that these four South African varieties has a similar hop extract profile. The hop acid profiles are similar to one another.

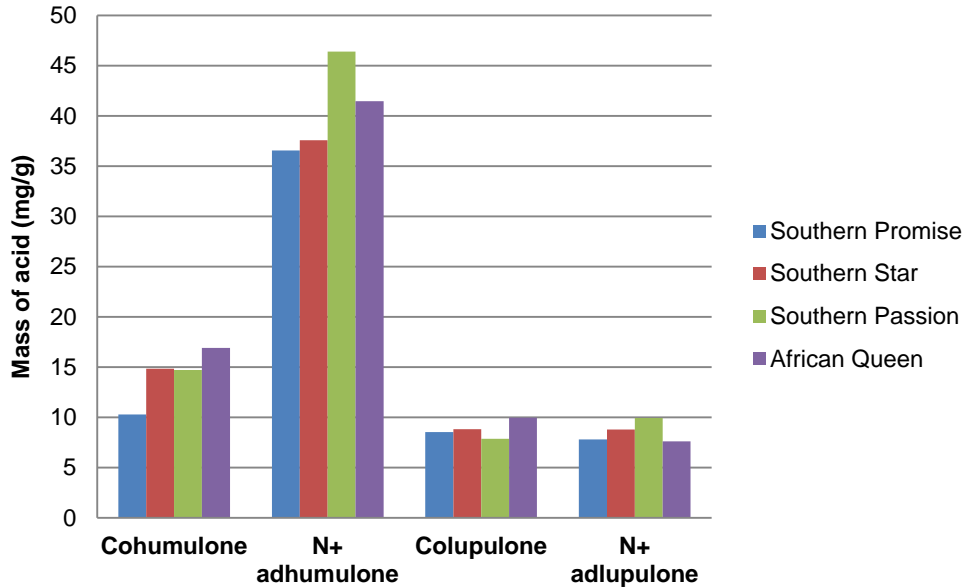


Figure 5.21: Hop acid profile of each hop variety

Figure 5.22 shows how the extract differs with an increase in pressure. Experimental run 5 was done on Southern Star. The green bars represent extract at 200 bar for 2 hours, and the purple bar represents extract at 250 bar for 2 hours, both on the same hop type. From this figure, it can be deduced that at a lower pressure, more beta acids are extracted, and once the pressure is increased, the number of alpha acids are increased. Zekovic (2006) analysed acids in a hop extract sample obtained at 300 bar and the highest alpha acid content (%) was the Magnum hop at 41%.

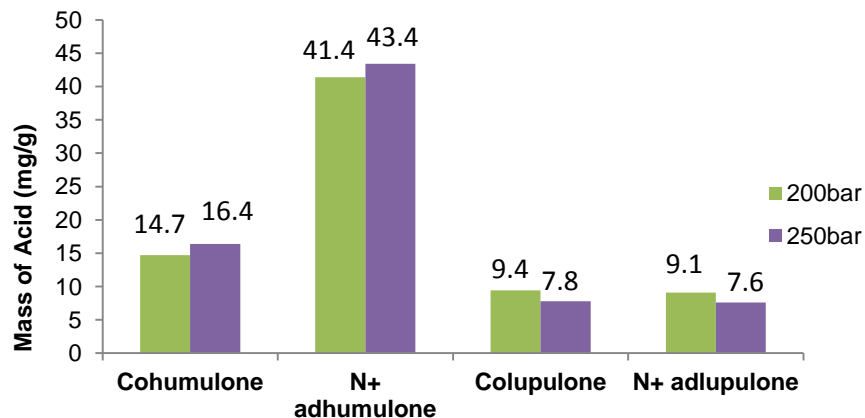


Figure 5.22: Mass of acids in mg per gram extract for experimental run 5 Southern Star at 200 bar and 250 bar

Figure 5.23 shows the effect of extraction time on the composition of acids. Experimental run 6 was done on Southern star at 250 bar. The blue bars represent extracts after 4 hours, and the red bars represent extract after 2 hours by doubling the extraction time, the alpha acids composition increases. This increase in alpha acids and decrease in beta acids is considered as an advantage for the brewer as an extract with high alpha acids and low beta acids are desired. This is due to beta acids being hydrophobic and therefore less soluble in water (Čulík et al., 2009). However, this extract would be slightly more expensive to produce. In an article by Langezaal (1990), the alpha acid content of a hop grown in a pharmacognostic garden in the Netherlands was analysed. This data look at the alpha acid content of the extract that was extracted at one, two and three hours. The extract was produced at 40°C and 200 bar. For the first hour, the alpha acid content was 25% and the beta acid content was 10%. For the second hour, the alpha acid content was 27% and the beta acid content was 8%. For the third hour, the alpha acid content was 23% and the beta acid content was 8% (Langezaal et al., 1990).

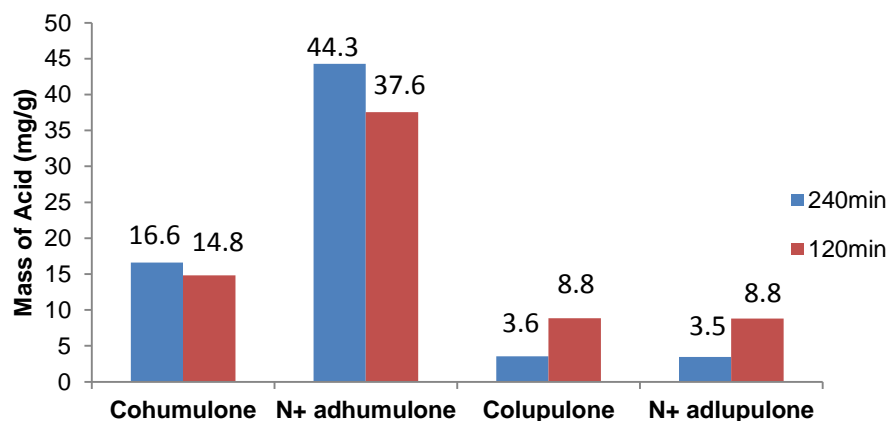


Figure 5.23: Mass of acid in mg per gram extract for experimental run 6 Southern Star at 2 hours and 4 hours

Figure 5.24 shows how the hop type differs when extracted at the same pressure for the same extraction time. Experimental run 2 was done on Southern Passion at 250 bar for 2 hours, and experimental run 5 was done on Southern Star at 250 bar for 2 hours. The red bars represent Southern Passion, and the green bars represent Southern Star. The profiles are similar; however, when calculating the total acids, Southern Passion (61.09 mg/g alpha acids) is higher than Southern Star (59.77 mg/g alpha acids). When comparing the total beta acids, Southern Passion (17.79 mg/g beta acids) had a higher composition than Southern Star (15.36 mg/g beta acids).

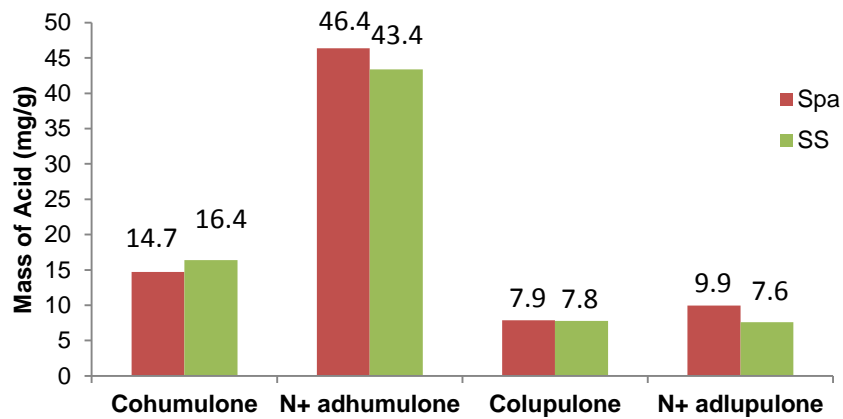


Figure 5.24: Mass of acid in mg per gram extract for experimental run 2 and run 5

5.5.2 GC-MS

The GC-MS results were arranged according to the most abundant compound. This was determined by calculating the area of each compound. The most abundant volatile compounds included alpha-humulene, beta-caryophyllene, myrcene, delta-cadinene, alpha-selinene, and alpha-amorphene. Figure 5.25 shows the aroma profile of each hop type. From this figure, it is noted that Southern Star is rich in alpha-humulene and African Queen contains the highest amount of myrcene. These differences may seem minor, however, its influence on the flavour of the beer is major.

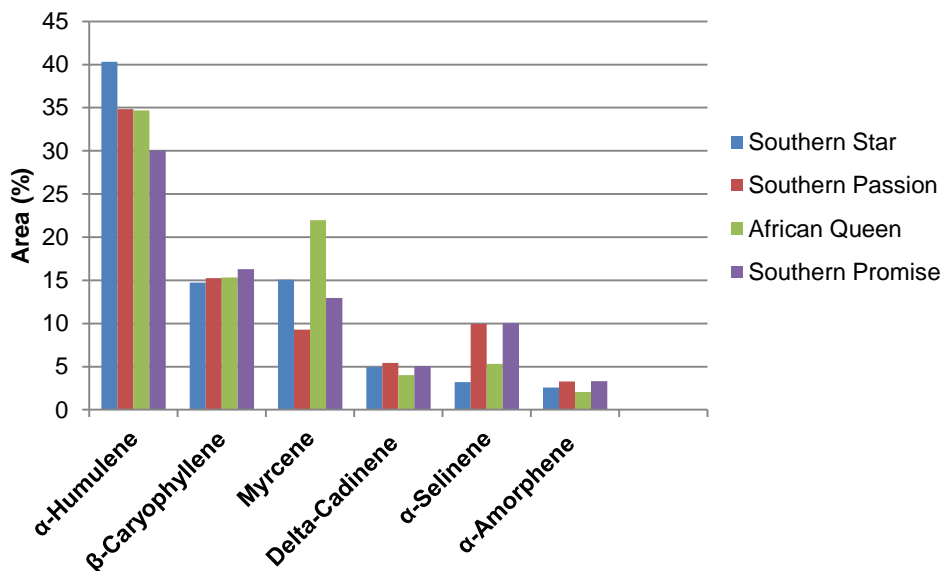


Figure 5.25: Hop aroma profile of each hop variety

In Figure 5.26 during experimental run 1 at 150, 200 and 250 bar, all volatile compounds decreased as pressure increased except myrcene. Myrcene is found in large quantities in hops this is due to it forming in the hop cone until the hop reaches the maturation phase (Shellhammer, 2021). Although the amount of myrcene continues to increase the amount of beta-caryophyllene and humulene does not increase (Craft beer and brewing, 2021). The following two figures show the area of the volatile compound at 150, 200 and 250 bar.

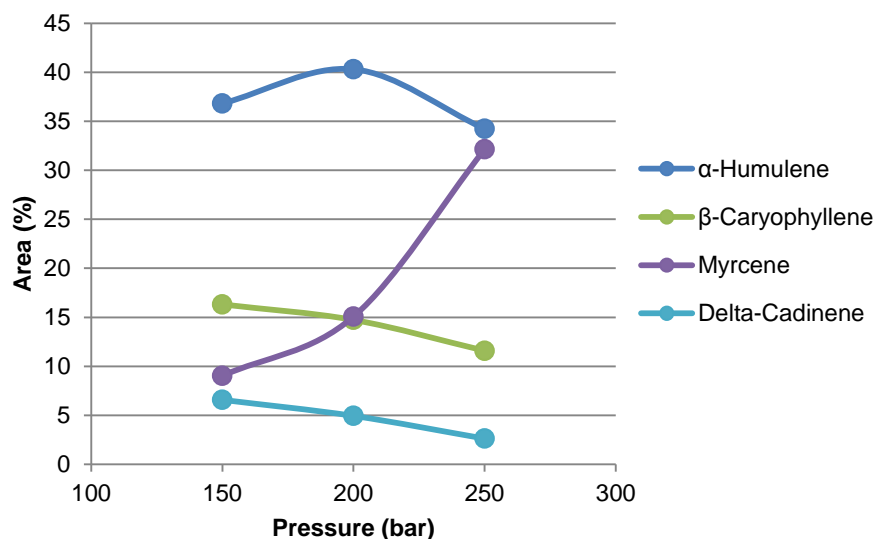


Figure 5.26: Aroma profile at 150, 200 and 250 bar for experimental run 1

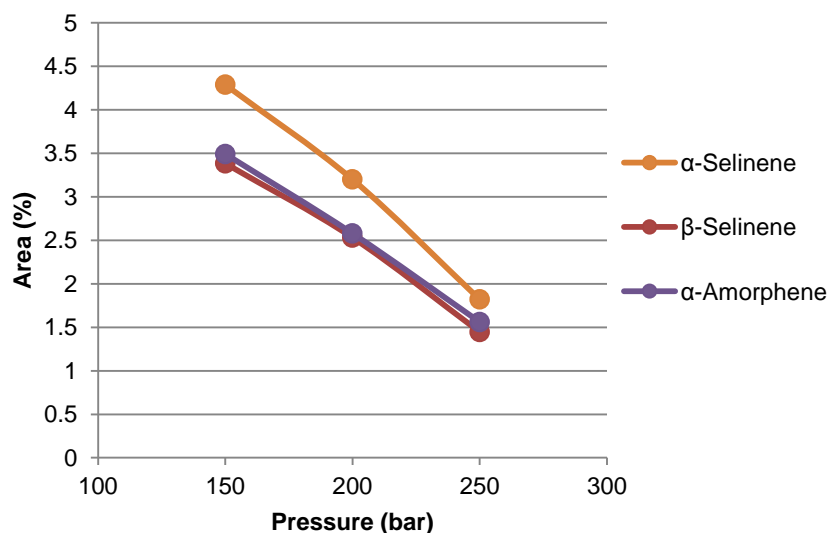


Figure 5.27: Aroma profile at 150, 200 and 250 bar for experimental run 1

Figure 5.28 and Figure 5.29 is the duplicate experiment of the experiment run 1. The same trend is observed.

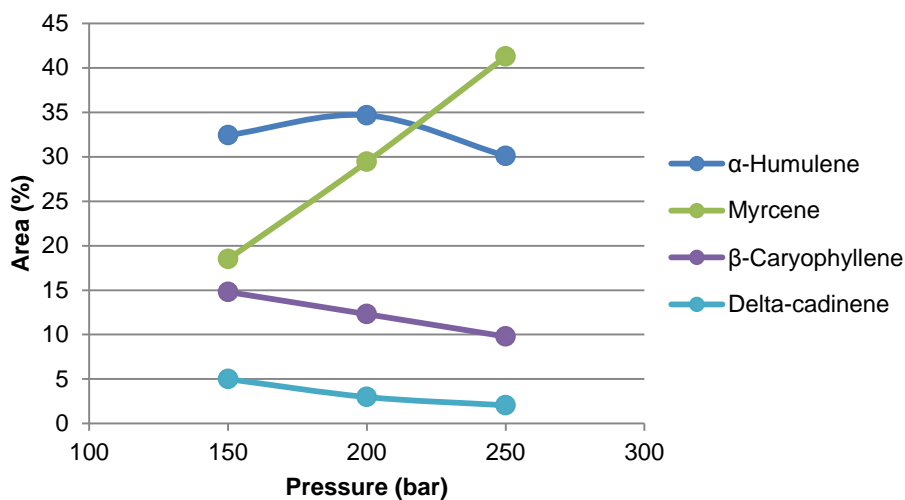


Figure 5.28: Aroma profile at 150, 200 and 250 bar for experimental run 5

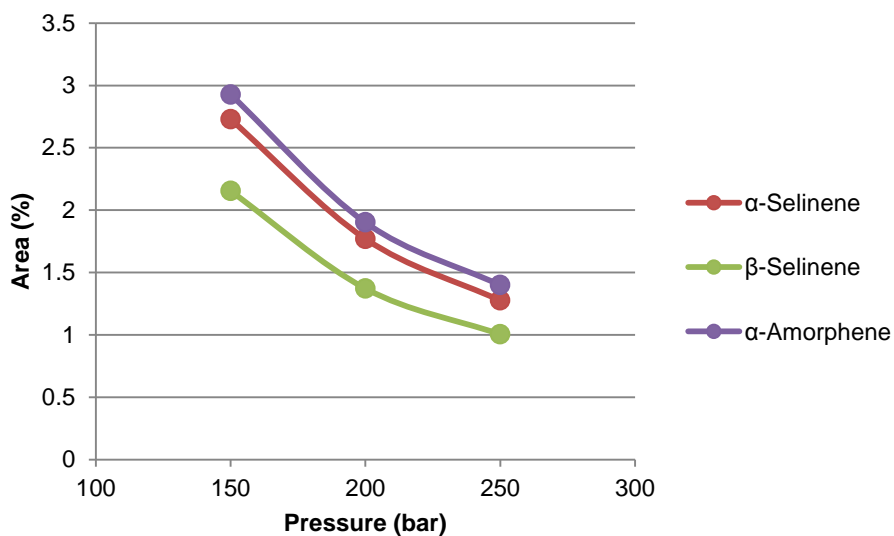


Figure 5.29: Aroma profile at 150, 200 and 250 bar for experimental run 5

Figure 5.30 and Figure 5.31 shows how the extract differs as extraction time is increased. Most volatile compounds decrease as the extraction time increases. These light compounds are easier extracted, and after one hour, most of them are extracted except myrcene. In Figure 5.31, alpha-seliene shows the same trend as myrcene.

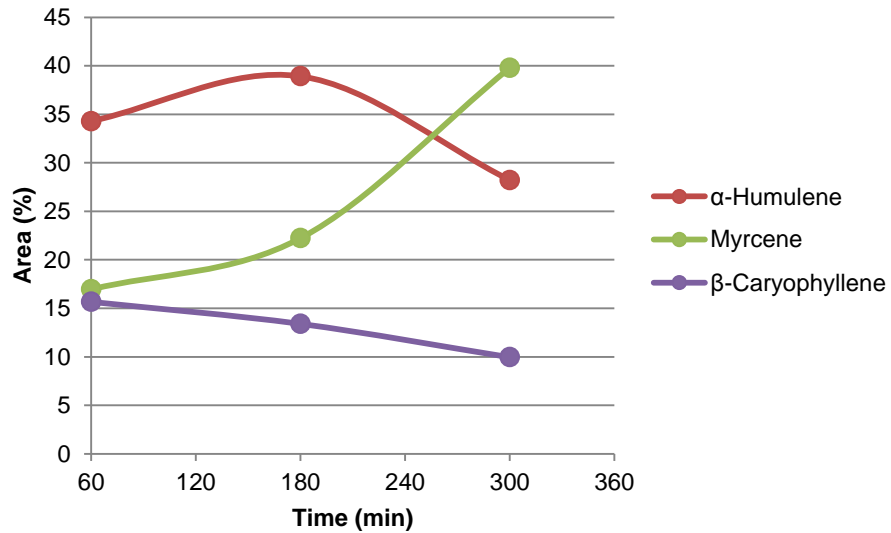


Figure 5.30: Aroma profile for experimental run 6 at 1, 3 and 5 hours

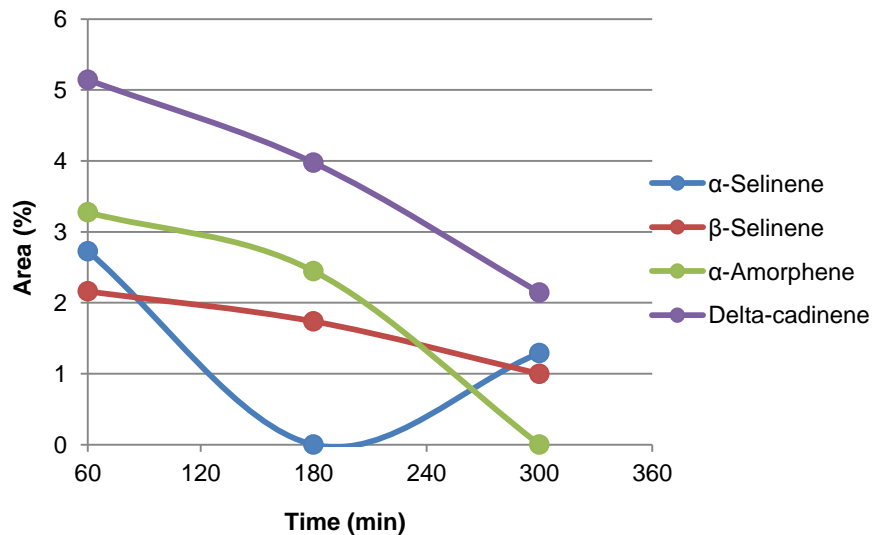


Figure 5.31: Aroma profile for experimental run 6 at 1, 3 and 5 hours

Figure 5.32 - Figure 5.35 are for experimental runs 1 and 5. It shows the different fractions achieved at 150, 200 and 250 bar. The fraction at 150 bar differs from the 250 bar fraction as the composition of each compound is different, i.e. at 150 bar, myrcene is found in low amounts in the fraction, but at 250 bar, high amounts in the fraction. Similarly, delta-cadinene is found in high amounts at 150 bar but low amounts at 250 bar. This indicated that fractional extraction was successful as each fraction has different profiles. When looking at Figure 5.32 and Figure 5.33 it is noted that the order of the compounds at 150bar is reversed at 250 bar. This is an

advantage as the brewer can brew beers with different hop aroma profiles from the same hop variety. If the brewer prefers an extract, where the hop aroma compounds are similar in quantities the 200 bar hop extract would be appropriate for this. These fractions can also be used as their dosage is standardised and will be achieved if the extraction is repeated. When looking at Figure 5.34 and Figure 5.35 these compounds are found in much less quantities. The 150 bar fraction contains the highest amount of these compounds. Although the compounds are found in similar amounts in each fraction, the brewer could use the preferred dose and achieve a beer with a unique aroma. The data for experimental runs 1 and 5 are similar thus providing confidence in the accuracy of the repeatability of this work. The sum of squares error was calculated between runs 1 and 5 for 150 bar it is 0.14, for 200 bar it is 0.05 and for 250 bar it is 0.04. The error is calculated to determine whether the fractions during run 1 can be duplicated (run 5). These low values indicate that these fractions are similar and can be duplicated

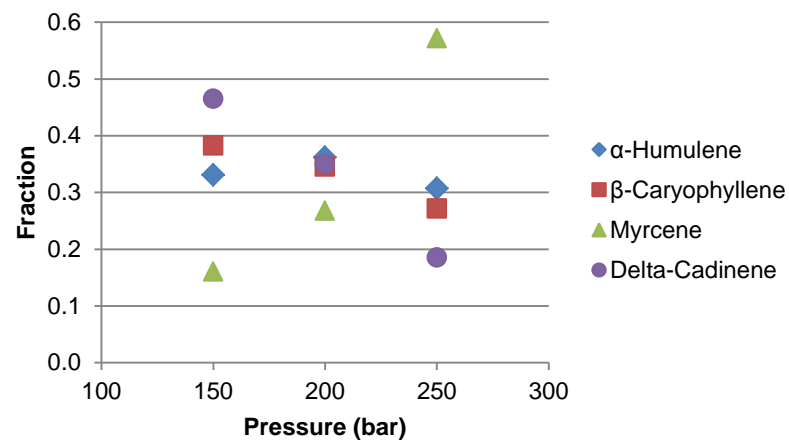


Figure 5.32: Fraction of aroma at 150, 200 and 250 bar for experimental run 1

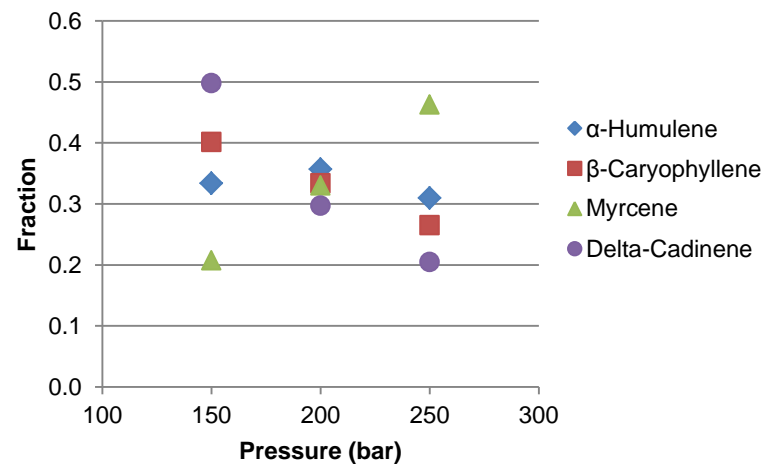


Figure 5.33: Fraction of aroma at 150, 200 and 250 bar for experimental run 5

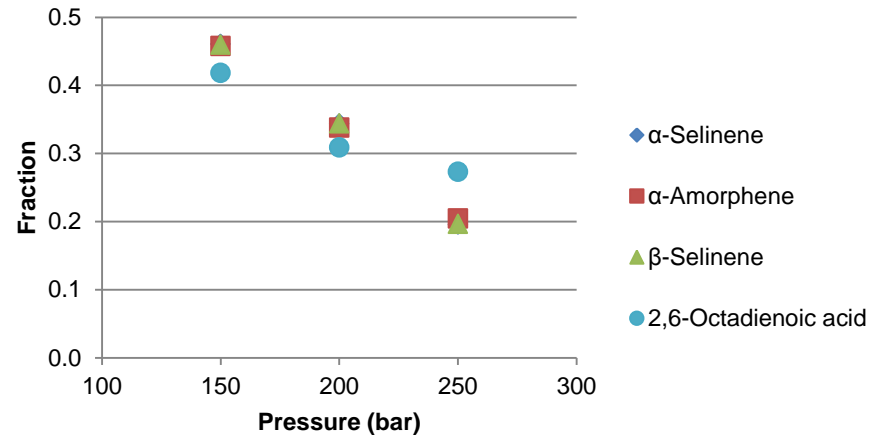


Figure 5.34: Fraction of aroma at 150, 200 and 250 bar for experimental run 1

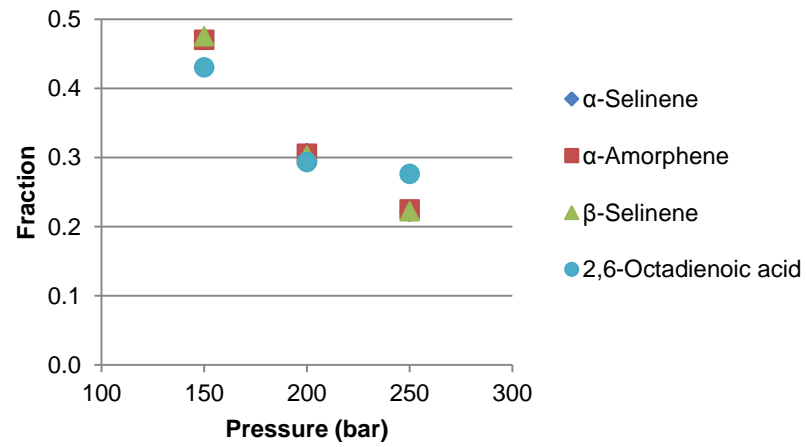


Figure 5.35: Fraction of aroma at 150, 200 and 250 bar for experimental run 5

5.6 Comparison of SA extract to worldwide extract

To compare the profile of the South African hop extract to that of the extract found worldwide, the extract should be produced at the same conditions as the South African hop extract. Zekovic (2006) performed extraction at 150 bar and 40°C. In the article, the GC-MS data was published and thus, it could be compared to the extract produced in this work. The hop types used in that article had a German origin. The most abundant compound in the aroma of the German Magnum hop was alpha-humulene with a compound percentage area of 10.35%, the South African hops: Southern Star and Southern Promise had a compound percentage area of 36.81% and 30.05%, respectively. The amount of beta-caryophyllene, alpha-humulene and delta-cadinene found in the South African hop extract are much higher than the German hop extract. This difference indicates that the South African hop extract has a highly aromatic profile.

Table 5.4: Profile of SA extract vs profile worldwide extract (Zekovic et al., 2006)

150bar	Compound area (%)						
	Southern Star	Southern Promise	Magnum	Hallertau Tradition	Spalt Selekt	Aroma	K-62
Beta-Caryophyllene	16.31	16.30	2.21	-	0.16	0.09	0.1
Alpha-Humulene	36.81	30.05	10.35	0.47	0.88	0.5	0.79
Calarene	0.045	-	-	0.05	-	0.08	0.05
Delta-Cadinene	6.57	5.07	-	0.14	-	0.13	0.15

5.7 Control experiment

Figure 5.36 shows the acid profile when boiling hops. The blue bars represent the acid composition when the mixture was boiled for 30 minutes. The red represents the profile of the mixture after dry hopping. This entailed adding hops and allowing the mixture to stand for 2 hours, then filtering and sampling it. In both samples, there were no beta acids detected. This may be due to beta acids being insoluble in the water and thus being removed after filtration. In the boiling stage, the alpha acids were higher than the dry hopping stage. Although adding more hops would suggest an increase in hop acids, this analysis indicated a decrease in hop acids.

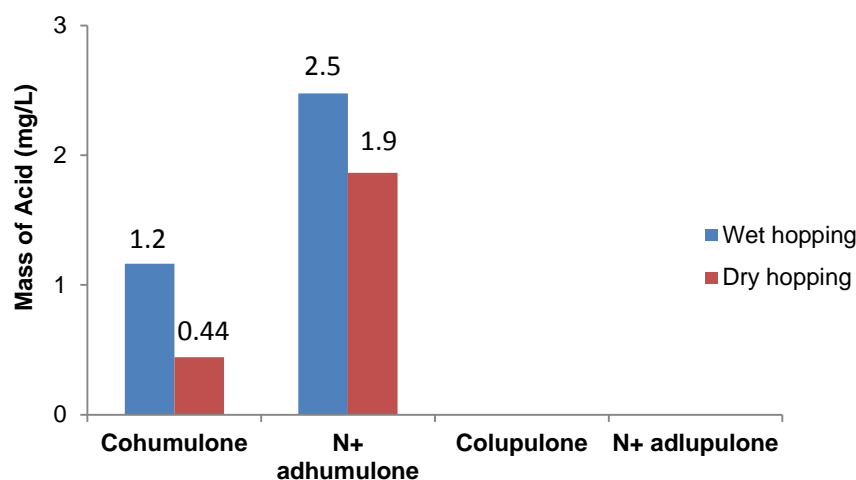


Figure 5.36: Mass of acid in mg per litre mixture ethanol and hops

According to Hopsteiner (2017), dry hopping changes the hop acid composition. If a beer contains a high International Bitterness Unit (IBU) when dry hopping the hop pellets absorb iso-acids and add alpha acids. The hop acids are soluble and change the hop composition (Hopsteiner, 2017). In the Brauwelt International Journal the effects of dry hopping on beer bitterness, IBU and pH were studied. The data for dry hopping indicated that when dry hopping a beer that has a low IBU more hop acids are present when adding fewer hop pellets (Maye & Smith, 2018). This would suggest that knowing the IBU of the beer before dry hopping is important as it can be used to calculate the amount of hop pellets required to either increase hop acid or decrease the hop acid. The hop composition depends on the type of beer that is being brewed.

When comparing Figure 5.36 and Figure 5.37 it is noted that the hop acids profile differ significantly. In the hop extract, the ratio of alpha acids to beta acids is 3.4 whereas in the aqueous solution only alpha acids were detected. The advantage of this is that the brewer has a concentrated amount of hop acids and much less, hop extract in comparison to pellets

that will be used when brewing. Although the profiles differ, the hop extract can be used as a standardised dose and the beer will thus have a homogeneous bittering profile for each batch that is brewed. In the aqueous solution, no beta acids were detected and this is seen as an advantage for the brewer however, this method requires the filtration process and also results in spent waste which adds to the waste that the brewer will need to discard.

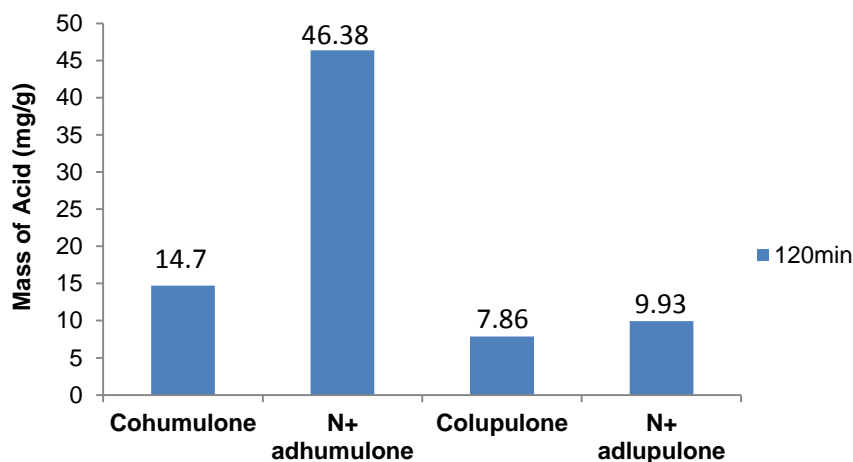


Figure 5.37: Mass of acid in mg per gram extract of Southern Passion

When brewing a beer, typically for 19 L, about 14 – 42 g of hop pellets are used, with an additional 14 – 28 g added during the last stage of boiling for the beer's aroma (Colby & Spencer, 2013). However, when using hop extract, for every 20 L of beer, only 1 ml of hop extract is used (Smith, 2016). Other than the minimized quantity, hop extract eliminates the stage of late hopping and reduces the amount of waste hops the brewer is left with after filtering the beer

5.8 Chapter outcomes

The chapter focused on interpreting and discussing the results obtained during experimental work and analysis. The experimental work was completed at the conditions established through theoretical estimations and literature data. The experimental work produced yield that was compared to that of the yield produced by other authors at similar conditions. The highest yield (13.22%) was achieved at 200 bar which was similar to the yield of del Valle (2003) where a yield of 13.9% at 200 bar was achieved. The experimental data were modelled and was discussed in terms of AARD% and the fit of values of the adjustable parameters. The models showed an adequate fit. The HPLC analysis shows how the acid profile differed with pressure, time and hop type. This data indicated that the South African hop extracts are rich in adhumulone (alpha acid). The GC-MS analysis showed that various fractions was obtained at 150, 200 and 250 bar during experimental runs 1 and 5. The GC-

MS data for experimental runs 1 and 5 were compared and indicated successful fractionation. Lastly, the analysis of the hop extract at 150 bar was compared to German hop types. This data indicated the South African hop extract was much more aromatic than the German hop extract.

Chapter 6: Summary and conclusion

This project focused on the supercritical CO₂ extraction of South African hops. Currently, in South Africa, there are no known commercial scCO₂ for the processing of hops. This work aimed to determine the characteristics of South African hop extracts obtained by supercritical CO₂ and the feasibility of obtaining aromatic and bitter fractions of hop extract. The objectives included estimating the conditions for fractionation, fractionating the hop into aromatic and bitter fractions, determining the composition of SA CO₂ extracts and compare it to the extracts found worldwide, use a co-solvent to establish if the hop residue contained any valuable compounds. Lastly, a control experiment was conducted to compare hop components present in an aqueous solution made up from pellets to that of extract.

6.1 Summary

For this project to be completed, it was necessary to first check literature and find the gap in scCO₂ hop extraction. This data indicated that the production of hop extraction is feasible and has been done around the world by many authors such as del Valle (2003), Kupski (2017), Zekovic (2006) and a few others. Although literature indicated that hop extraction is feasible not much data was found on fractionating hops into its components (aroma and acids). This work looked at producing the hop extracts of South African hops and also fractionating the hop. To achieve this, extraction conditions were of utmost importance and two methods were used (theoretical estimation and surveying literature). This entailed collecting VLE data and performing a flash calculation (theoretical estimation) and collecting data in published articles to compare extraction conditions with yield (literature estimation). Once the conditions were successfully estimated the method for extraction, fractionation and the use of a co-solvent was devised. These methods considered raw materials, process operation and the planning which data collection was needed to achieve the aim. The experimental work consisted of 6 experimental runs of which the first four experiments were done on a different South African hop variety. Experimental run 5 was done as a duplicate of experimental 1 and experimental run 6 was done to determine the total extract that can be produced. During experimental run 1 after maximum extraction, a co-solvent (ethanol) was used to determine if any hop compounds remained after extraction with scCO₂. For the control experiment, the method followed a recipe from Beer Smith and it was conducted at a lab scale. This experiment did not include all the steps in brewing as the focus was on determining the hop acid profile produced when using hop pellets. These experiments resulted in mass data, extraction kinetics data and samples that could be sent for analysis. The mass data were used to calculate yield and compare to hop experiments found in literature. The process conditions were fitted to two kinetic models, the Broken-Intact-Cells

model and the Martinez model. These models used fitted using Microsoft Excel Solver. The hop extract samples were sent for GC-MS and HPLC analysis. The GC-MS analysis checked the aroma profile of the hop extracts produced at the lower pressure (150 bar) and the HPLC analysis checked the hop acids profile of the hop extracts produced at the higher pressure (250 bar). All the data produced during these experiments were discussed and compared to literature. The hop extracts were characterized and compared to hop extracts of German origin.

6.2 Conclusion

For objective one, the estimation of conditions was completed. Although the theoretical estimation data only included two hop components (myrcene and linalool). This method produced data that was used to determine whether the separation between volatile compounds was possible. Objective two was completed as data was produced for the scCO₂ of South African hops. The fractionation of hops in aroma rich fractions and acid-rich fractions was difficult to achieve. However, the data indicated that hop extracts that were produced at three different pressures had a different aroma profile from each other. The data produced for objective three indicated that the South African hop is different from the hop extract analysis found in literature. This is due to the South African hop extract containing higher amounts of the commonly found hop aroma compounds such as alpha-humulene and beta-caryophyllene. The hop acid profile of the hop pellets boiled indicated a big difference from the hop extract. However, this is advantageous as hop extract contains a concentrated standardized profile. The work was successful in producing South African hop extracts of four local varieties and fractionating to produce hop extract of different profiles.

6.3 Project outcomes

This project resulted in producing yield data for the supercritical CO₂ extraction of South African hops at a pilot plant scale. This data indicated the technical feasibility of producing South African hop extracts and fractionating the hops from a solid matrix. The analysis of these hop extracts resulted in the characterization of the aroma and hop acids profile of four South African hop varieties. Two kinetic models were developed and the volumetric mass transfer coefficient was calculated. This work produced hop extract fractions that contained different aroma profiles. The South African hop extract also showed that its profile differs from traditional hop varieties. The production of these hop extracts could be a platform to standardized the South African hop extract and benefit brewers.

6.4 Recommendations

This project was produced hop extract and for future work this extract can be used to brew beer of various aromas and compare the flavor and taste to that of the exist beer types. Another recommendation for this work is to use various co-solvents and compare the hop compounds extracted. These hop compounds can be used in comestic industries and not just in food and beverage.

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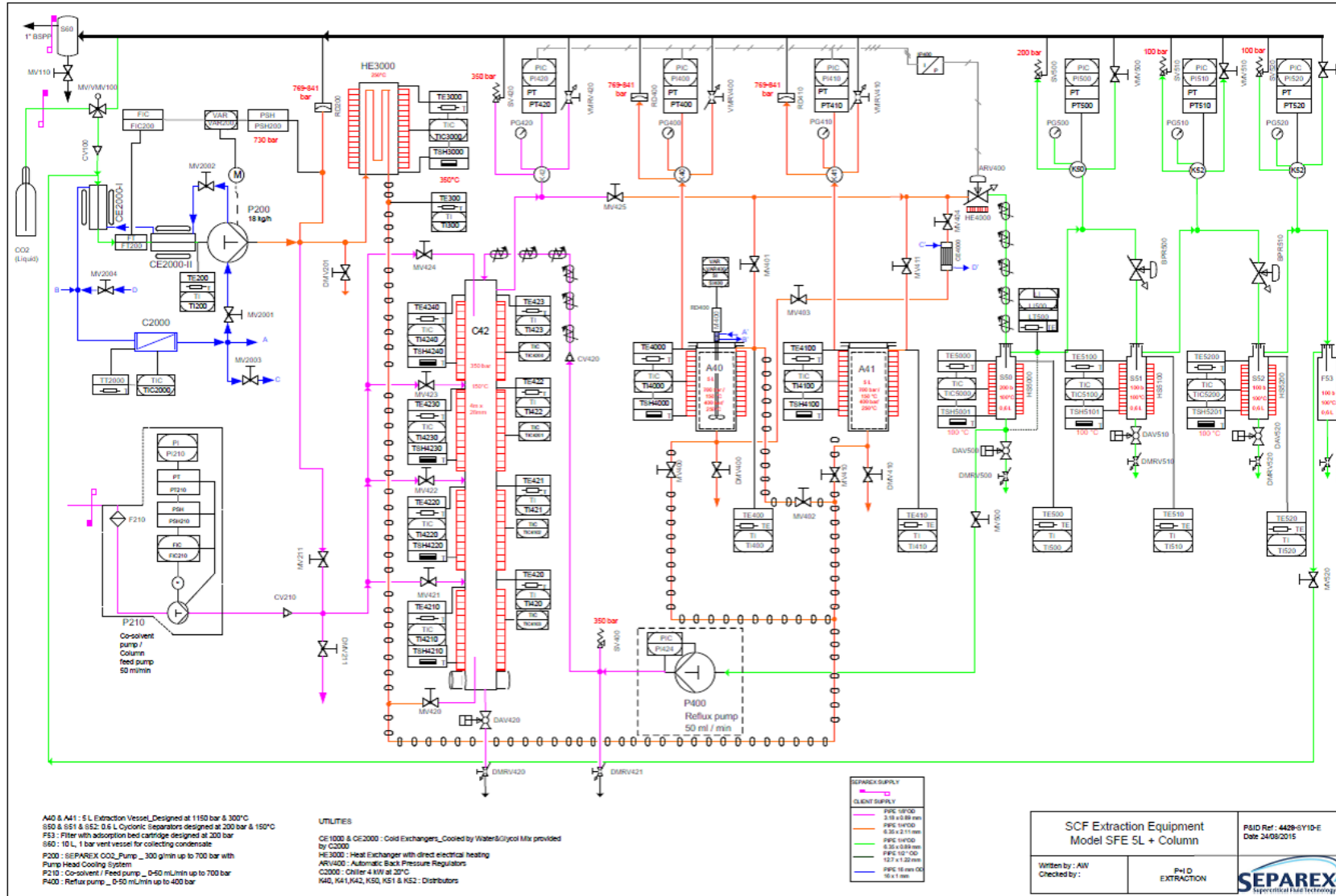
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Appendix A: P&ID of pilot plant



Appendix B: Control Experiment

Method:

- 1 L water was boiled to 100°C.
- A mass of 1.5 g hop pellets was weighed and loaded into the boiling water.
- A period of 30 minutes was allowed for boiling.
- An additional 0.97 g of hops were added to the mixture.
- Boiled for an additional 15 minutes.
- A water bath was used to cool the mixture.
- A sample was collected.
- 1.9 g of hops were added, and this mixture sat for 2 hours.
- Another sample was collected.
- HPLC analysis.

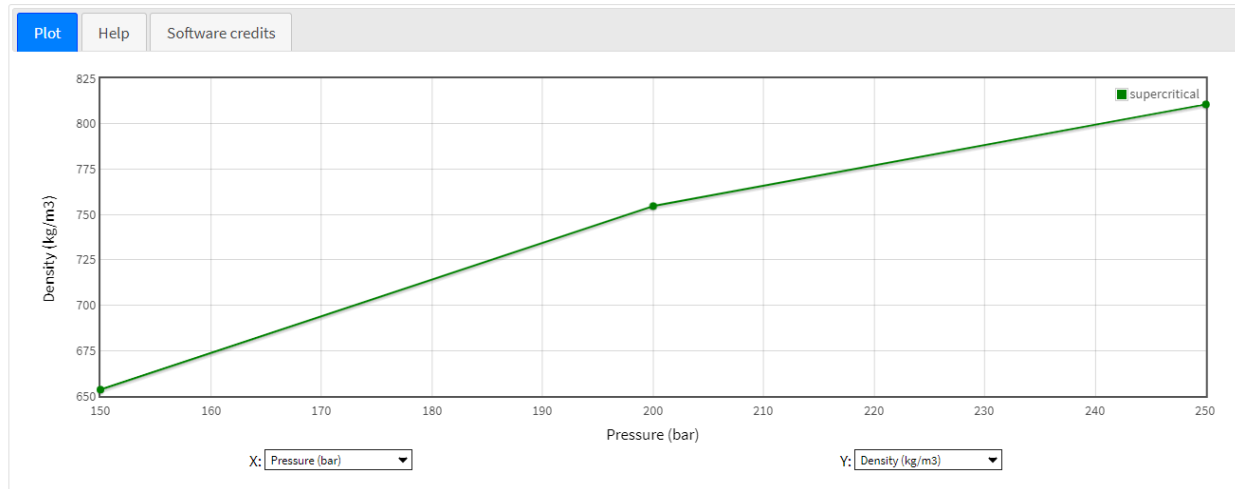
Appendix C: Estimation of CO₂ flow rate

The following calculations were done to calculate the CO₂ flow rate for the experimental runs. The data was extracted from Kupski's article.

Kupski	
Temperature (°C)	55
Pressure (bar)	200
Internal diameter (m)	0.0191
CO ₂ flow-rate (kg/s)	$3.25 * 10^{-5}$

The density of CO₂ was extracted from the NIST database. This can be seen in the figure below.

Isothermal Data for T = 55.000 C



To calculate the volumetric flow rate, the density with the mass flow rate was used.

$$\rho = \frac{\dot{m}}{\dot{V}}$$

$$754.61 = \frac{3.25 * 10^{-5}}{\dot{V}}$$

$$\dot{V} = 4.3 * 10^{-8} \frac{m^3}{s}$$

Using the internal diameter, the area cross-sectional area was calculated as follows

$$A = \frac{\pi * d^2}{4}$$

$$A = \frac{\pi * (0.0191)^2}{4}$$

$$A = 2.86 * 10^{-4} m^2$$

Using the continuity of flow equation, the velocity can be calculated.

$$\dot{V} = A * v$$

$$(4.3 * 10^{-8}) = (2.86 * 10^{-4}) * v$$

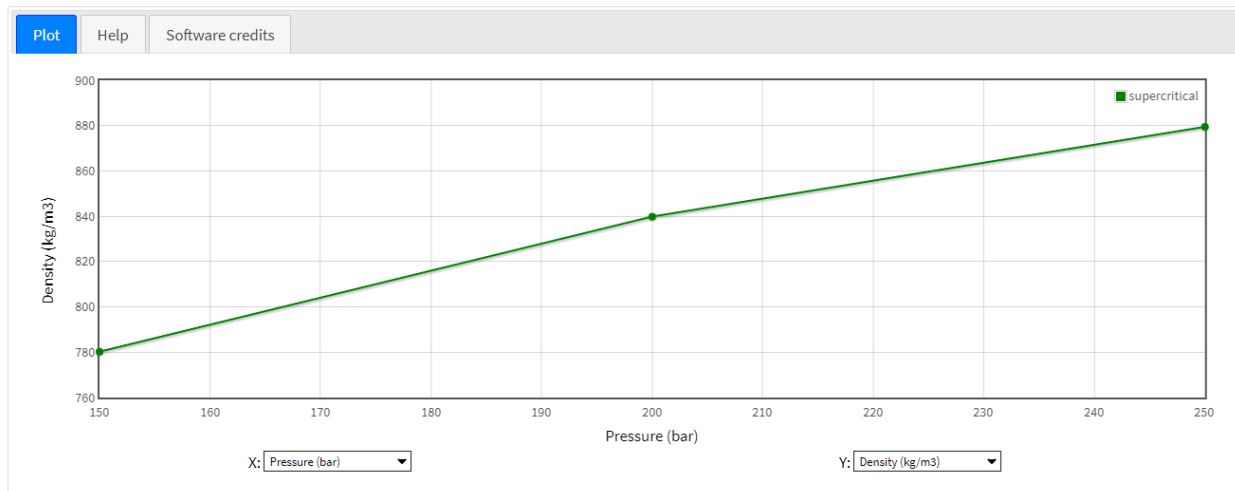
$$v = 1.5 * 10^{-4} \frac{m}{s}$$

This for work, the following data was used.

Temperature (°C)	40
Pressure (bar)	200
Internal diameter (m)	0.131
Velocity (m/s)	$1.5 * 10^{-4}$

The density was extracted from the NIST database

Isothermal Data for T = 40.000 C



Using the internal diameter, the area cross-sectional area was calculated as follows

$$A = \frac{\pi * d^2}{4}$$

$$A = \frac{\pi * (0.131)^2}{4}$$

$$A = 0.0134 m^2$$

Using the continuity of flow equation, the velocity can be calculated.

$$\dot{V} = A * v$$

$$\dot{V} = (0.0134) * (1.5 * 10^{-4})$$

$$\dot{V} = 2.03 * 10^{-6} \frac{m^3}{s}$$

To calculate the mass flow rate, the density with the volumetric flow rate was used.

$$\rho = \frac{\dot{m}}{\dot{V}}$$

$$840 = \frac{\dot{m}}{(2.03 * 10^{-6})}$$

$$\dot{m} = 0.0017 \frac{kg}{s}$$

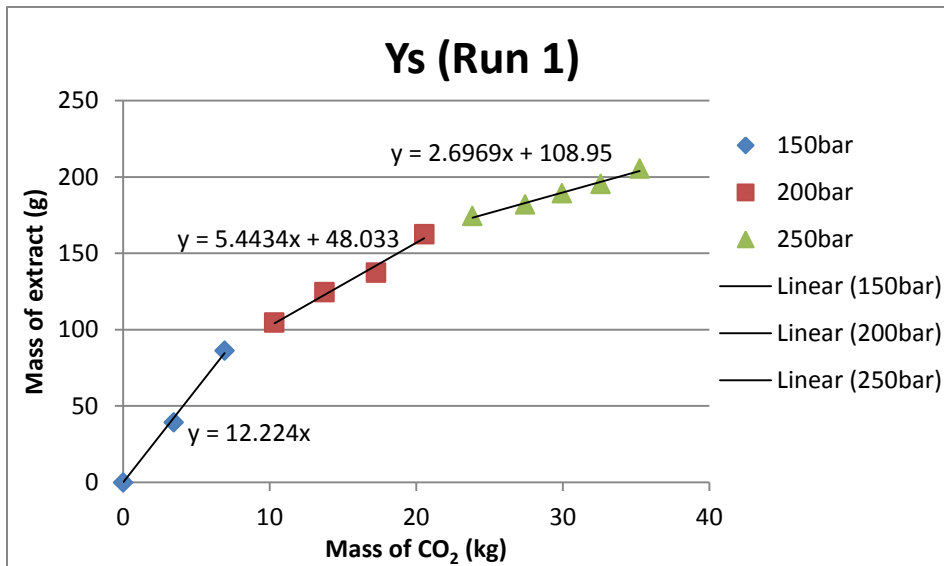
$$\dot{m} = 6.1 \frac{kg}{hr}$$

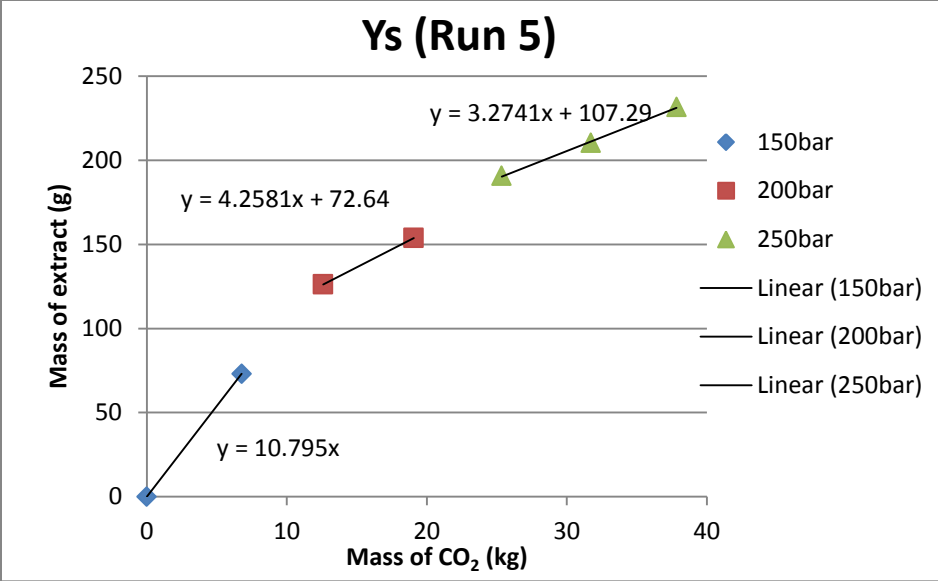
Appendix D: Kinetic modelling

Sovova Model sample equation

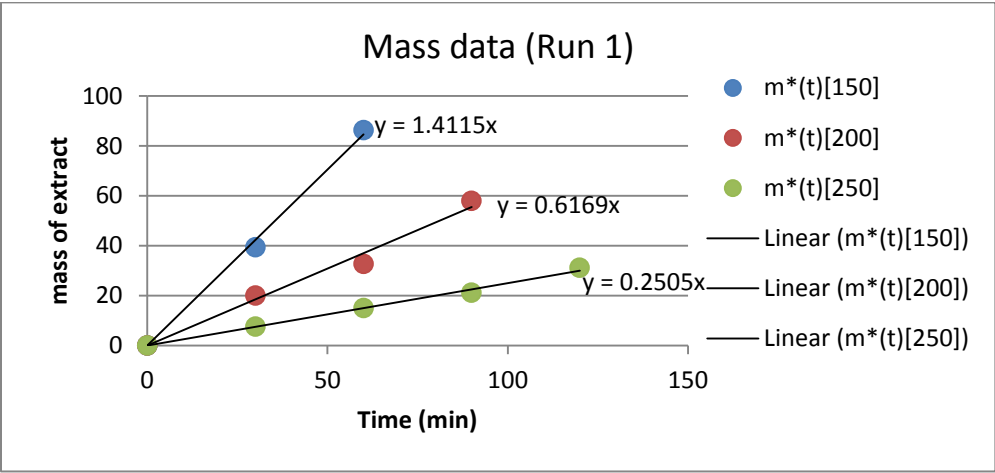
From the Sovova model equation, it was noted that by estimating the slope of the mass of extract data, it would be possible to estimate Z by using Excel Solver. This was done by guessing an initial Z value and using MS Excel solver to get the guesstimated value closer to the actual slope value. Once Z was estimated, the volumetric mass transfer coefficients were calculated.

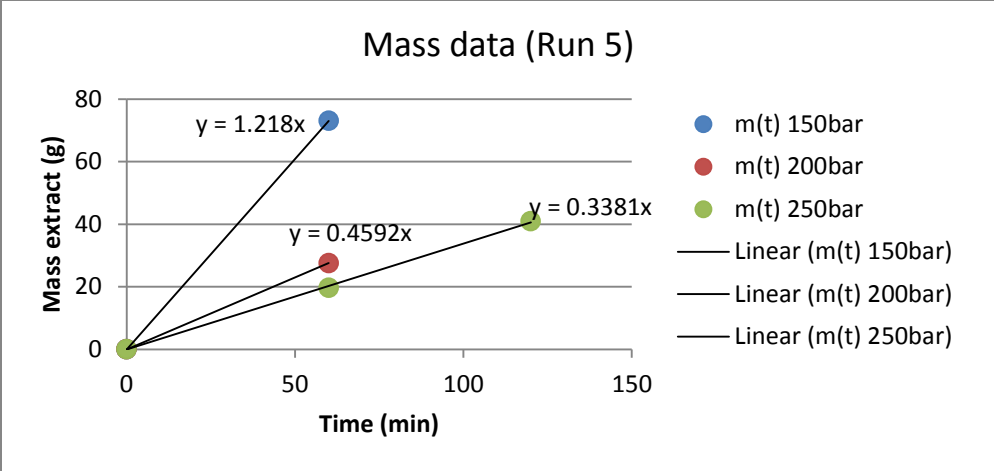
The first step was estimating Ys. This is calculated from the slope of mass of extract vs mass of CO₂





Then the slope of each mass data set was calculated by the plotting mass of extract vs time. This can be seen below





Using the slope and Ys, it was possible to predict the Z by using MS Excel solver and the mass data obtained during experiments; the following table summarizes the steps

Run 1

	Time (min)	m(t)	t*	m*(t)[150]	m*(t)[200]	m*(t)[250]	mF	Y(s)	Slope	Z	Predicted	Predicted yield	AARD%	
150bar	0	0	0	0			100	12.22	1.4115	0.00115	0.0	0		
	30	39.32	30	39.32			100	12.22	1.4115	0.00116	42.3	42.35	0.0714	3.57
	60	86.2	60	86.2			100	12.22	1.4115	0.00115	84.4	84.44	0.0209	
200bar	90	104.62	0		0		100	5.44	0.6169	0.00113	0.0	104.62	0.0000	1.43
	120	124.55	30		19.93		100	5.44	0.6169	0.00113	18.5	123.13	0.0116	
	150	137.29	60		32.67		100	5.44	0.6169	0.00113	37.0	141.63	0.0307	
	180	162.56	90		57.94		100	5.44	0.6169	0.00113	55.5	160.14	0.0151	
250bar	210	174.4	0			0	100	2.70	0.2505	0.00093	0.0	174.4	0.0000	0.26
	240	181.92	30			7.52	100	2.70	0.2505	0.00093	7.5	181.9	0.0000	
	270	189.39	60			14.99	100	2.70	0.2505	0.00093	15.0	189.4	0.0002	
	300	195.49	90			21.09	100	2.70	0.2505	0.00093	22.5	196.9	0.0074	
	330	205.56	120			31.16	100	2.70	0.2505	0.00093	30.1	204.5	0.0054	

Run 5

	Time (min)	m(t)	t*	m*(t)[150]	m*(t)[200]	m*(t)[250]	mF	Y(s)	Slope	Z	Predicted	Predicted yield	AARD%	
150bar	0	0	0	0			100	10.76	1.218	0.00113	0	0		
	60	73.08	60	73.08			100	10.76	1.218	0.00113	73.08	73.08	1.06E-11	0.00
200bar	120	126.25	0		0		100	4.26	0.4592	0.00108	0	126.25	2.25E-16	6.50E-04
	180	153.8	60		27.55		100	4.26	0.4592	0.00108	27.552	153.80	1.30E-05	
250bar	240	190.69	0			0	100	3.27	0.3381	0.00103	0	190.69	1.49E-16	0.15
	300	210.31	60			19.62	100	3.27	0.3381	0.00103	20.286	210.98	3.17E-03	
	360	231.6	120			40.91	100	3.27	0.3381	0.00103	40.572	231.26	1.46E-03	

The following parameters were estimated and collected from NIST

PHYSICAL PROPERTIES				BED CHARACTERISTICS		Extractor dimensions	
Density Spent (g/cm3)	0.29	Density CO2 150bar (g/cm3)	0.78	ε	0.56	Dia (m)	0.131
Density of Hops (g/cm3)	0.65	Density CO2 200bar (g/cm3)	0.84	Particle dia	0.00071	Len (m)	0.38
Hop feed (g)	1002	Density CO2 250bar (g/cm3)	0.88				
Spent hops (g)	904						
humidity (%)	0.17	Density of hop bed	0.2012				

Using this data, it was possible to calculate kfa.

$$Z = \frac{k_F a * m_s * \rho_F}{m_F * (1 - \epsilon) * \rho_S}$$

The following table is for run 1

Time	kFa	kFa
0	4.72E-05	3.29E-05
30	4.74E-05	3.30E-05
60	4.72E-05	3.29E-05
90	4.31E-05	3.00E-05
120	4.31E-05	3.00E-05
150	4.31E-05	3.00E-05
180	4.31E-05	3.00E-05
210	3.38E-05	2.35E-05
240	3.38E-05	2.35E-05
270	3.38E-05	2.35E-05
300	3.38E-05	2.35E-05
330	3.38E-05	2.35E-05

The following table is for run 5

Time	kFa	kFa
0	4.64E-05	3.23E-05
60	4.64E-05	3.23E-05
120	4.11E-05	2.86E-05
180	4.11E-05	2.86E-05
240	3.75E-05	2.61E-05
300	3.75E-05	2.61E-05

360	3.75E-05	2.61E-05
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Martinez model

For the model, each term was calculated separately. An initial value for b, t_m and A was guesstimated. Using Excel solver and the AARD, the gussed values were adjusted to reduce the error. The following table shows the summarized calculation

150 bar		200 bar		250 bar	
m_t (g)	1000	m_t (g)	1000	m_t (g)	1000
b (min^{-1})	1.00E-03	b (min^{-1})	5.78E-04	b (min^{-1})	6.01E-04
t_m (min)	-3.93E+03	t_m (min)	-6.69E+03	t_m (min)	-6.79E+03
A	1.00E+00	A	1.00E+00	A	1.00E+00
term 1	5.16E+04	term 1	4.77E+04	term 1	5.93E+04
Num	1.02E+00	Num	1.02E+00	Num	1.02E+00

	T	m_{exp}	Deno	-1	m_{ext}	AARD(%)	
	0	0			0		
150bar	30	39.32	1.019	9.09E-04	4.69E+01	1.93E-01	9.4
	60	86.2	1.018	1.46E-03	7.52E+01	1.28E-01	
	90	104.62	1.018	1.99E-03	1.03E+02	1.85E-02	
	120	124.55	1.017	2.51E-03	1.29E+02	3.88E-02	
200bar	150	137.29	1.019	2.95E-03	1.41E+02	2.72E-02	2.6
	180	162.56	1.019	3.28E-03	1.57E+02	3.70E-02	
	210	174.4	1.019	3.60E-03	1.72E+02	1.49E-02	
	240	181.92	1.018	3.91E-03	1.87E+02	2.69E-02	
250bar	270	189.39	1.014	3.18E-03	1.89E+02	4.09E-03	2.6
	300	195.49	1.014	3.17E-03	1.88E+02	3.74E-02	
	330	205.56	1.014	3.59E-03	2.13E+02	3.69E-02	

This method was repeated for all 6 runs

Appendix E: Lab journal



Faculty of Engineering

Department of Chemical Engineering

Extraction Vessel A41

Experimental run summary

**Aroma and Bitter Extracts from South African Hops: Characterisation
and Extraction Process Development using Supercritical CO₂**

Name: Vianka Archery

Student Number: 215040333

Year: 2021

Lab summary report

Feed preparation

Every packet of hop pellets was vacuumed, sealed and labelled with the hop type and the estimation of alpha and beta acids composition. The bag of hop pellets was opened in the morning of the run and ground on the day before and left under pressure with CO₂. A ceramic mortar and pestle were used to grind the hops. This mortar had a capacity of 100 g. The pellets were ground to a particle size of 1 mm – 710 µm. This particle range was determined by sieving the ground hops. Grinding the pellets ensured that when extracting, the solvent could travel into the cell pockets. Each run consisted of ± 1 kg hops.



Trial run: Southern Star

This run was on the 19th of April 2021 at 07:14 am. A purge of the system was done, and the pressure was set to 150 bar. A feed of 1118.53 g was loaded. At 11:05 am, static extraction occurred for 30 minutes. At 11:40, the pressure was increased to 150 bar as some pressure was lost during static extraction, and then dynamic extraction began. At 12:10, the first sample was collected with a mass of 39.91 g. Sampling occurred at a 30-minute interval. At the 1 hour mark, an extract with a mass of 50.12 g was collected. After running for 90 min, the plant tripped, and this feed was compromised. At this point, a mass of 11.73 g was collected.

The yield was calculated by

$$Yield = \frac{\text{Mass of extract}}{\text{Feed of hops}}$$

This run resulted in a 9% yield for a running time of 2 hours. These 2 hours consisted of 30-minute static extraction and 1.30-minute dynamic extraction. According to an observation, the pressure in separators 1, 2 and 3 was above 70 bar, which could have been the reason for the

emergency shutdown. The plant was depressurized, and a 1 L ethanol was loaded; this was left overnight. The following day, the pressure was built to 250 bar, and after 60 minutes, a sample was collected for HPLC analysis.

Boiling water experiment

It was decided to halt pilot plant experiments as the reasons for the emergency shutdown was unclear. This experiment was done on the 22nd of April 2021. This experiment aimed to determine the aroma and bitterness profile of a beer brewed from a hop variety. A recipe for this experiment was extracted from BeerSmith.

For this experiment, 1 L water was boiled to 100°C. A mass of 1.5 g hop pellets was weighed and loaded into the boiling water. A period of 30 minutes was allowed for boiling. After the 30 minute boiling, 0.97 g of hops were added to the mixture. This mixture was boiled for an additional 15 minutes. Once completed, a water bath was used to cool the mixture. A sample was collected. Lastly, 1.9 g of hops were added, and this mixture sat for 2 hours. Another sample was collected. These samples were sent for HPLC analysis.



Run 1: Southern star

This run was done on 20th April 2021. The hop type was Southern star with a mass of 1006 g of ground hops. The hops were loaded, and pressure was increased to 150 bar. At 09:51 am, static extraction began for 30 minutes. At 10:24 am, dynamic extraction began, and the first sample was collected at 10:54 am with an extract mass of 39.32 g. Sampling occurred every 30 minutes for 2 hours. The masses recorded were 60 min = 46.88 g, 90 min = 18.42 g and 120 min = 19.93 g. The total yield for this pressure was 12.38%

The pressure was increased to 200 bar. A 30-minute sampling procedure was followed. At 30 min = 12.74 g, 60 min = 25.27 g, 90 min = 11.84 g and 120 min = 7.52 g. This pressure resulted in a yield of 5.7%

The pressure was increased to 250 bar. A 30-minute sampling procedure was followed. At 30 min = 7.47 g, 60 min = 6.1 g and 90 min = 10.07 g. At 15 min before reaching 120 min, the plant tripped. This pressure resulted in a yield of 2.35%. The speculation was that the plant had an emergency shutdown due to long hours of operation as no error was visible from pilot plant data.



Run 2: Southern Passion

Due to the plant emergency shutdown, it was decided to complete the remaining hop types at 2 pressures for 2 hours. Run 2 was completed on the 28th of April 2021. The hop type was Southern passion with a mass of 1014.44 g. The run began at 6:30 am. After grinding the hops, purging the system and loading the hop feed at 09:59 am. The pressure was increased to 200 bar for static extraction. During static extraction, a pressure drop of at least 15 bar was noted during previous runs. Thus it was decided to set the pressure to 230 bar. Dynamic extraction began at 10:56, and at 11:56, the first sample was collected. To keep the plant at a steady-state, samples were now collected at a one-hour interval. At 60 min = 67.55 g and 120 min = 54.89 g., the yield of this pressure was 12.08%.

The pressure was increased to 250 bar for two hours. At 60 min = 28.11 g and 120 min = 16.44 g. This pressure resulted in a yield of 4.39%



Run 3: African Queen

This run was done on the 30th of April 2021. The feed was African queen hops with a mass of 911.27 g. At 09:40 am, the feed was loaded, and static extraction began at 10:21. At 10:56 am, dynamic extraction began, and sampling followed after an hour. At 60 min = 78.8 g and 120 min = 41.65 g. This pressure resulted in a yield of 13.21%.

The pressure was increased to 250 bar. For this pressure, an extraction time of three hours was tried. At 60 min = 15.11 g, 120 min = 15.08 g and 180 min = 8.82 g. This pressure had a yield of 4.28%



Run 4: Southern promise

This run was done on the 4th of May, 2021. This feed was Southern promise, with a mass of 1002.19 g. This hop type was ground the Friday and loaded into the vessel. It was left isolated at a pressure of 8bar over the weekend. At 07:27 am, static extraction began, and by 08:01,

dynamic extraction began. At 60 min = 93.87 g, 120 min = 22.06 g and 180 min = 16.43 g. This pressure had a yield of 13.2%

The pressure was increased to 250 bar. After 60 min an extract of 25.85 g was collected. The plant then tripped. It was speculated that the chiller might have leaked. This had a yield of 2.5%.



Run 5: Southern star rerun

This was a rerun of run 1. This feed was Southern star with a mass of 1002.45 g. At 07:55 am, static extraction began. By 08:30, dynamic extraction started. At 60 min = 73.08 g and 120 min = 53.17 g. This pressure resulted in a yield of 12.59%

This pressure was increased to 200 bar. At 60 min = 27.55 g and 120 min = 36.89 g. This run had a yield of 6.42%.

This pressure was increased to 250 bar. At 60 min = 19.02 g and 120 min = 21.29 g. This run had a yield of 4.08%.



Data summary

Summary of mass and yield data

Hop Run	Mass of extract (g)			Yield (%)			Total mass of extract (g)	Total yield of run (%)
	150bar	200bar	250bar	150bar	200bar	250bar		
Trial run	101.76	-	-	9.1	-	-	101.76	9.1
Run 1	124.55	57.37	23.64	12.38	5.7	2.35	205.56	20.43
Run 2	-	122.44	44.56	-	12.07	4.39	167	16.46
Run 3	-	120.48	39.02	-	13.22	4.28	159.5	17.50
Run 4	132.36	-	25.85	13.21	-	2.58	158.21	15.79
Run 5 (rerun)	126.25	64.44	40.91	12.59	6.43	4.08	231.6	23.10
Run 6	-	-	198.85	-	-	19.92	198.85	19.92

Data summary discussion

The trial run had the lowest yield, and this is expected as the run lasted for 90 minutes at Pset = 150 bar. The highest yield was achieved at run 5, which was the rerun of run 1. This rerun has a 3% higher yield than run 1 due to run 1 stopping before collecting the two-hour sample at 250 bar. This was due to the emergency shutdown of the plant. At 250 bar, run 5 had a mass of extract of 40.19 g, whereas run 1 had 23.64 g. This indicates that in the last hour, the mass of extract doubled. Run 2 and run 3 have similar yields. Both of these runs were 2 hours at pressures with different hop types. Run 4 was used to evaluate the effect of running time on the yield. When comparing the masses at 150 bar, run 4 had about 6-7 g more than the other runs for the extra hour of extraction. Run 3 at 250 bar lasted 3 hours; however, that mass is just under the mass in run 4 at 250 bar, which had an extraction time of 2 hours. Thus, running three pressures for 2 hours each will result in the best yield.

Material balance

Trial run 150bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
30	283.58	39.91	39.91	0.0357
60	333.7	90.03	50.12	0.0448
90	345.43	101.76	11.73	0.0105

Run 1 data at 150bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
30	285.62	39.32	39.32	0.0391
60	332.5	86.2	46.88	0.0466
90	350.92	104.62	18.42	0.0183
120	370.85	124.55	19.93	0.0198

200bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
30	259.14	12.74	12.74	0.0127
60	284.41	38.01	25.27	0.0251
90	296.25	49.85	11.84	0.0118
120	303.77	57.37	7.52	0.0075

250bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
30	254.3	7.47	7.47	0.0074
60	260.4	13.57	6.1	0.0061
90	270.47	23.64	10.07	0.01

Run 2 data at 200bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
60	311.18	67.55	67.55	0.066
120	366.07	122.44	54.89	0.054

250bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
60	272.29	28.12	28.12	0.027
120	288.73	44.56	16.44	0.016

Run 3 data at 200bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
60	322.73	78.83	78.83	0.087
120	364.38	120.48	41.65	0.046

250bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
60	258.16	15.11	15.11	0.017
120	273.24	30.19	15.08	0.017
180	282.07	39.02	8.83	0.0097

Run 4 data at 150bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
60	341.53	93.87	93.87	0.0937
120	363.59	115.93	22.06	0.0220
180	380.02	132.36	16.43	0.0164

250bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
60	273.14	25.85	25.85	0.0258

Run 5 data at 150bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
60	316.79	73.08	73.08	0.0729
120	369.96	126.25	53.17	0.0530

200bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
60	272.05	27.55	27.55	0.0275
120	308.94	64.44	36.89	0.0368

250bar

Time (min)	Mass extract (g)	Mass without bottle (g)	Mass	Yield
60	260.09	19.62	19.62	0.0196
120	281.38	40.91	21.29	0.0212