Optimisation of Qcide® oil extraction and separation

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Declaration

I declare that this is my own work and has not been submitted elsewhere in whole or in part to obtain other degree award. The content of this thesis is the result of author work and the contribution of others has been acknowledged in the Statement of Contribution of Others.

Tristan Gibbs

September 2023

Statement of Contribution of Others

This research was conducted under the supervion of Prof Yinghe He and he has made academic guidance and editorial contributions to this thesis. The co-supervisor was Dr Yang Liu. Research and Analysis support was provided by Nathan Simms with his contributions responsible for substantial portions of Chapter 5.

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1 Background

Essential oils (EO's) have been used since antiquity for a variety of applications including as medicines, for rituals, and as aromatics. Historical evidence points to the use distillation apparatus by several ancient cultures from 3000BC, with some research indicating the use of aromatic plants as far back as 4500BC [1]. This chapter will explore the uses of essential oils in modern times with a focus on historical observation of the advantages and disadvantages that EO application represents. The explorations of essential oil in this chapter are to provide a general background of EO's that is necessary to understand the research and application of Qcide[1] oil, which is an EO of particular interest.

1.1 Essential Oils

EO's are natural oils obtained from raw plant material and animal excretions [2]. EO composition is highly variable depending on the source and extraction method. A single EO can be comprised of as many as 300 different compounds including; hydrocarbons, terpenes, acids, esters, ketones, and lactones [3]. The variability of EO's leads to use in a broad range of fields including medicinal use, public health, animal health, crop protection, and consumer products. One of the driving factors for EO development in these sectors is the overuse, environmental toxicity of, and resistances to, currently used synthetic compounds, such as Organophosphates, Spinosyns, Neonicotinoids, Carbamates, and Pyrethroids [4, 5].

1.1.1 Medicinal Use

While medicinal EO's have been used globally since ancient times, they saw declining use in western countries after the discovery of antibiotics in the early 20th century [6]. There has been an emergence of medicinal EO research in recent decades as the medicinal benefits of EO's have been explored, and antibiotic resistance has been observed [2, 7, 8]. Recent studies have shown certain EO's to have strong antibiotic, antifungal, and antioxidant properties, this has led to development of EO based preventatives, medicines, and therapies [9-14]. As of 2011 the World Health Organisation data values the global market of herbal medicines at USD$60 billion, with steady growth [15].

1.1.2 Public Health

Public health is the science and process of preventing disease, prolonging life, and improving the quality of life through organised efforts and informed choices of society,

[1] Qcide tradename is owned by Bio-Gene Technology Limited

organisations, communities, and individuals. One important initiative in the scope of public health is the control of disease carrying insects (Diptera: Culicidae) which can spread Malaria and other neglected tropical diseases (NTD) such as Dengue Fever, Urban Yellow Fever, Chikungunya, and Zika [16]. Due to the global harm represented by these significant public health issues, there has been extensive study into the efficacy of EO's to combat these disease vectors. Souza *et al* [16] reviewed the adulticide and repellent activity of EO's reported in 43 articles published between 2001 and 2018. The review found that many plant species produce EO's with strong adulticide, insecticide and repellent activity. However, EO's can have a reduced period of active effectiveness due to the volatility of the active components. This highlights the need for investigation into specific product formulations capable of maximising efficacy or longevity. Soares de Oliveira *et al* [4] also conducted an extensive review articles published between 2000 and 2019 that reported the repellent characteristics of EO's. The review includes EO's from 31 families of plants, and found a variety of reported larvicidal, pupicidal and ovicidal effects including; neurotoxicity [17], neurotransmission inhibition [18], morphogenesis disruption, feeding inhibition, and mortality by paralysis.

1.1.3 Animal Health

Animal health has many similar considerations to public health, where it is important to stop the spread of parasites and infections from insects such as ticks, blowflies, and fleas [19, 20]. Protection from, or eradication of, these vectors is an important consideration for the health of the animals and the integrity of the animal products. The economic cost of animal health losses and reduced yield in production animal systems can be considerable, with studies in Indian livestock in 2007 putting the cost at US$498.7 million per annum [21]. One of the considerations in the field of animal health, is the use of EO's in feedstock nutrition, disease control, and genetic improvement [22, 23].

1.1.4 Crop Protection

Crop protection is the science, practice and management of forestry and crops in order to protect them from destructive elements such as disease, weeds and pests. In 2009 the global market of pesticides used for crop protection was USD$43 billion [24], and in 2010 EO-based pesticides made up 4.5% of the global pesticide market [25]. The use of EO's as biopesticides is a growing market due to the global trend towards greener and more sustainable pest control technologies, and the unfavourable effects of currently used synthetic pesticides that include the development of pest resistance, impact on non-target species, negative environmental and

public health effects, and unacceptable pesticide residues in food and other consumer products [5, 25]. In contrast EO-based formulations have shown low toxicity to non-target species [14], low mammalian toxicity [20, 26], and reduced chemical persistence [20, 27, 28]. EO based crop protection has been extensively researched and has been found effective as herbicides [2, 28, 29], antifungals/antibacterials [28, 30], and pesticides [2, 14, 20, 26, 31]. Limitations of EO based pesticides include; slow action or activation, short residual effect requiring higher rates or repeat applications, limited shelf life, challenges in growing biomass required for EO production, and high probability of batch variation based on climate, season and extraction method [20, 25, 28, 31].

1.1.5 Consumer Products

EO's have been used traditionally in consumer products for millennia. More recent novel applications for their use have been investigated as part of various scientific studies. Consumer products that utilise EO's include cosmetics, perfumes, household pharmaceuticals, insecticides, fungicides, food preservatives, food flavourings, toiletries, cleaning products, and household chemicals [2, 3, 20, 32]. A modern focus of ongoing EO research is their application in food preservation, as a replacement for synthetic based preservatives which have been found to have pollutive effects and undesirably long persistence that facilitates microbial resistance [2, 3]. The primary research undertaken on EO's in this field seeks to take advantage of the antioxidant and antimicrobial effects exhibited by EO's obtained from sources such as Betel leaf [3], Lemongrass [33, 34], Sweet basil [32], and other plants that produce oil with high phenolic content [34-37]. The addition of EO's as a food preservative can be performed in many ways; via direct addition to foodstuffs [33, 35, 37], coating or washing [34, 37], proximity aerosols [37], and encapsulation/coating of food packaging [36, 38]. The use of EO's in consumer products has many advantages similar to those discussed previously, however some disadvantages also apply. The volatility of EO's can be beneficial in avoiding undesirable persistent residues in food, but this can also limit the effective duration of EO based preservation. The selective antimicrobial effect that particular EO's exhibit is important in ensuring that unintended toxicity is not a problem, however this may require the use of specific EO's and places a burden of extensive research on the use and safety of any new EO.

1.1.6 Essential Oil Application Summary

EO's are highly variable in both their composition and uses. EO's have been in use since ancient times and are under intense scrutiny for emerging and novel use. The specific

composition of an EO can differ depending on the source of the EO, the extraction method used, and even the time of year that the EO is harvested [39]. Novel EO applications provide many advantages over synthetic chemicals and traditional EO use; however, there are challenges associated with their development. The specificity of EO's and their applications requires extensive research into the source, extraction, composition, application, toxicity, and commercial viability of each potential EO and their proposed uses.

1.2 Qcide Oil

"Qcide" is the trade name given to an oil collected from a cultivar of *Eucalyptus cloeziana* found in Australia. The primary chemical component of Qcide is tasmanone, which is a naturally occurring β-triketone. Qcide oil is heavier than water with a bright yellow to orange colour (Figure 1-1). The oil has a mildly acrid odour that is evocative of fusty gum tree mulch. Qcide oil composition by GC-FID area%, can be grouped into broad chemical categories, with an example shown in Table 1-1.

Figure 1-1: Qcide Oil showing typical colour after extraction.

Table 1-1: Analysis results of several Qcide oils by Gas Chromatography with Flame Ionisation Detection, mean and standard deviation of Area%. Analysis performed at Southern Cross University using analysis method developed at Southern Cross University [40].

Compound	Area %
Monoterpenes and Monoterpenoids	15.70 ± 2.41
Sesquiterpenes and Sesquiterpenoids	3.01 ± 0.62
Tasmanone	73.57 ± 3.12
Other Triketones	2.06 ± 1.09

1.2.1 Qcide Applications and Research

Qcide oil is currently under commercial development by Bio-Gene Technology Ltd as a natural insecticidal compound [41, 42]. The development of Qcide aims to utilise the effectiveness of its insecticidal properties in commercial end-use products, and to use the novel insecticidal mode of action to assist in the control of chemically resistant insect strains. The insecticidal activity of Qcide has been the subject of patents for the control of a range of pest species. It has also been reported in other publications [43].

EO's containing β-triketones studied in recent years have been found to display promising activity as herbicides, insecticides, antimicrobials, and antibacterials. The study of β-triketone oils primarily originated from the study of EO's derived from the Myrtaceae family of plants, which have traditionally been harvested for eucalyptus oil high in monoterpenoids like pinenes and eucalyptol [44, 45]. A plant species that has been previously noted for its significant β-triketone content is *Leptospermum scoparium* (Manuka myrtle) a native plant species of Australia and New Zealand which contains β-triketones in concentrations up to 35%. The specific chemical composition of *L. scoparium* EO differs depending on the growing region [39]; but the β-triketones leptospermone, isoleptospermone and flavesone are present as primary chemical components of these EO's [5, 39, 46]. The application of *L. scoparium* EO has shown efficacy as an antibacterial/antimicrobial agent [39, 46], mosquito larvicide [47], and herbicide [5, 48]. Jeong *et al* [46] tested the acaricidal activity of *L. scoparium* EO against a control of diethyltoluamide (DEET) and found that, among the three species of mites tested, the EO was up to 70 times more effective than DEET. Investigation of EO herbicidal activity has found that the β-triketones inhibit the activity of p-Hydroxyphenylpyruvate dioxygenase (HPPD), an enzyme which disrupts the biosynthesis of carotenoids in plants resulting in "bleaching" and potentially death of the plant [5, 48].

1.2.2 Qcide Extraction

At time of writing there is no evidence of previous research into the development, extraction, and separation of Qcide oil at a commercial scale. As Qcide is still in the developmental stages, the current extraction methodology is broadly based upon that used for tea tree oil production. This extraction method uses batches of up to ˜2.5 tonnes of harvested *E. cloeziana* biomass as the solid matrix which is subjected to steam distillation that is driven by steam generated from an external boiler. The heating process generates an internal condensate that contains Qcide oil, which is collected into large separation vessels. As the Qcide oil is immiscible in and heavier than water, the oil is gradually separated from condensate through gravity settling. This method produces high quality Qcide oil, however this extraction process has several disadvantages. Using current parameters of low relative steam flowrate at 100°C the distillation duration required is currently too long, with evidence that significant amounts of oil is not being extracted from the biomass. This method also creates a viscous hydrosol residue within the distillation vessel that can cause fouling in the distillation equipment. In order to improve the viability of Qcide oil for commercial production at scale, it is imperative that significant improvements are made to the extraction methodology of Qcide from *E. cloeziana*.

1.3 Project Objectives

With limited research currently available into the extraction of high tasmanone EO's, the current studies aim to build a knowledge base around the key processing conditions that will be important to facilitate the most efficient extraction of Qcide oil from *E. cloeziana* biomass for commercial production. This project seeks to examine the extraction process of Qcide oil in order to optimise the processing conditions, composition and overall yield of Qcide oil derived from *E. cloeziana*. The optimisation of the extraction process aims to address one of the primary barriers for EO products, the requirement for access to high volumes of oil with consistent composition and in a cost effective manner [2]. With these outcomes as the primary project objectives, this project will;

1. Examine the current technologies available for whole tree EO extraction and the viability for implementation in Qcide oil extraction,
2. Examine the key processing parameters of the selected extraction methodology with regards to how they directly affect the quality, rate, and yield of Qcide extraction,

3. Examine the effect, viability, and implementation of *E. cloeziana* biomass pre-treatment prior to the extraction process,
4. Explore and report the effects of the researched and implemented processing parameters to support recommendations to improve the efficiency of Qcide extraction for commercial production.

1.4 Thesis Outline

Following this Chapter 1, the thesis is divided into the following chapters.

Chapter 2 reviews relevant literature in the field of EO extraction. It details the methods of extraction with examples of existing research and seeks to present the advantages and disadvantages associated with each method. This chapter will present a critical analysis of the extraction processes in regard to viability for Qcide extraction, and will propose the methodology for the specific experimentation undertaken as part of this project.

Chapter 3 describes the specific experimental aims, project limitations, experimental equipment, and experimental processes that are used throughout this project. This chapter will describe in detail the methods of extraction and analysis that are used during this project.

Chapter 4 presents the effects of distillation temperature and steam input flowrate on the rate and yield of Qcide oil during steam distillation extraction.

Chapter 5 presents the effects of comminution and steam input flowrate on the rate and yield of Qcide oil during steam distillation extraction.

Chapter 6 combines the conclusions of both experiments in order to present the amalgamated results and formulate recommendations for the improvement of Qcide oil extraction for commercial purposes.

2 Literature Review

Essential oils (EO's) are obtained from a variety of methods depending on the particular oil and the technological limitations of the extraction process. This chapter will review essential oil extraction technologies and prior work over a range of oil types. This chapter will also explore the commonly used methods of biomass preparation that can be used in conjunction with extraction technologies. The advantages and disadvantages of each extraction method will be assessed in relation to some commonly extracted products. This chapter will critically assess the methods discussed in relation to the viability for Qcide extraction and will propose methodology for the extraction experimentation during this project.

2.1 Methods for Extraction of Essential Oils

Essential oil extraction is a process by which chemical components are separated from the source material, typically a solid matrix of plant biomass. EO extraction can be conducted using a range of methods which are generally categorised under two broad terms; Distillation and Solvent Extraction.

2.1.1 Distillation

This category of extraction involves separation of the EO from the biomass by taking advantage of the volatility or steam volatile properties of the EO components. The transmission of EO is facilitated by movement of a gaseous solvent (typically steam) through the solid matrix and collecting the condensate which will contain the desired components. Distillation methods include Hydro Distillation, Steam Distillation, Hydro Diffusion, and Vacuum Distillation. The efficacy of each distillation extraction method will depend on a variety of factors, but most notably on the specific biomass and EO composition.

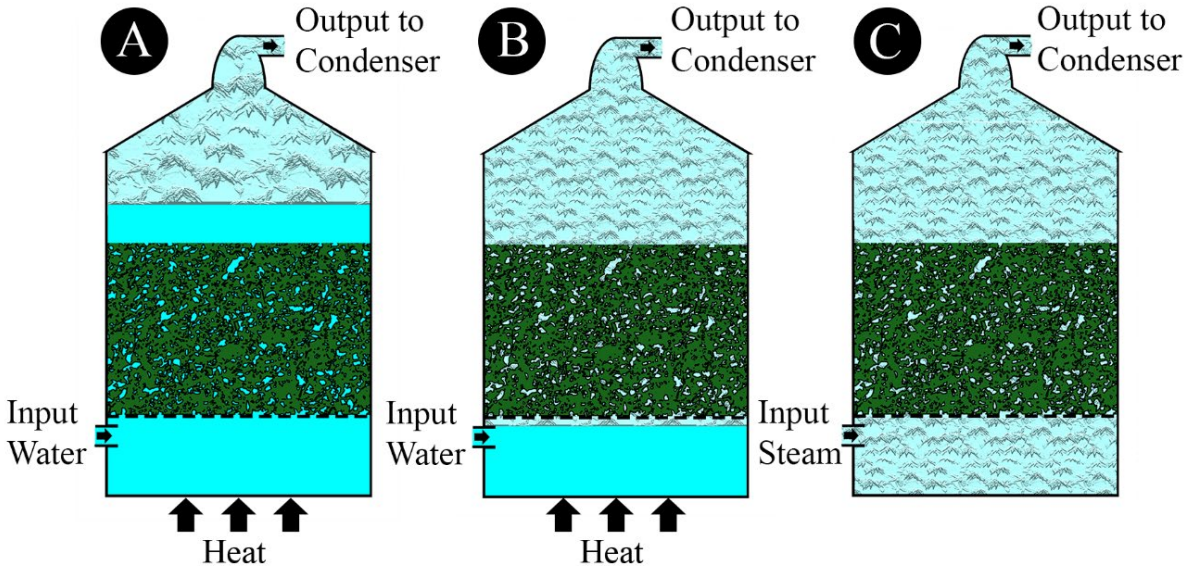

Figure 2-1: Diagrammatic representation of different distillation methods; A) Hydro Distillation, B) Steam Distillation, C) Steam Distillation with a satellite steam source.

2.1.1.1 Hydro Distillation

Hydro distillation (or water distillation) is the process of boiling a suspension of biomass in water and condensing the resulting vapour. A diagrammatic representation of hydro distillation is shown Figure 2-1A. The condensate contains an immiscible mixture of EO and water, which can then be separated. Hydro distillation is the simplest method of EO extraction and is suited to applications where other distillation methods are not viable, either due to their complexity or high capital cost. It is also preferable to use hydro distillation for application where full water immersion is required, such as when steam contact would cause biomass to agglomerate into impenetrable clusters [2, 49].

Hydro distillation can have a range of drawbacks depending on the equipment and biomass combinations used for processing [2, 49]. Extended application of heat and water can lead to hydrolysis and thermal degradation of EO components, resulting in reduced yields and undesirable colour/fragrance of the EO's. Biomass located in close proximity to the heat source can char, which will lead to degradation of the plant material and collected EO's. The charring effect can also be facilitated by biomass that will form dense agglomerations that would fall to the base of the distillation vessel, or by processing biomass that is rich in gum or mucilage, which will cause a thickening of the distillation water. These effects can also apply when the distillation equipment is not charged with enough water to complete the distillation, allowing the vessel to run dry and char the biomass.

Hydro distillation can be used to extract a wide variety of EO's such as tea tree oil, eucalyptus oil, and herb oils [2]. Because of the simplicity and versatility of hydro distillation it is often used as a starting point for laboratory studies, or as a comparison for other more complicated extraction technologies.

2.1.1.2 Steam Distillation

Steam distillation differs from hydro distillation in that the biomass is not immersed or suspended in water. Steam for the distillation can be generated within the still (Figure 2-1B), separated from the solid matrix by a barrier such as a perforated plate or mesh screen, or can be supplied by a satellite boiler (Figure 2-1C). Generating the steam within the distillation vessel does not increase the capital cost of the process as much as the addition of a satellite boiler and can be achieved by modification of hydro distillation equipment. This type of modification does come at the cost of reduced biomass capacity within the distillation vessel. Since the steam is generated within the same vessel as the biomass there is considerably less water charged at the beginning of the process, which requires water to be continuously added to the process or a cohobation line be added to the process.

Many studies into the extraction of EO's have been conducted using steam distillation, with both steam distillation and hydro distillation often being used as a comparison point for more advanced extraction techniques. The efficacy of steam distillation in comparison to that of hydro distillation is reported to have high variance depending on the particular biomass, and the specific physical composition of the biomass, that are used in the extraction process. Mohammed *et al* [50] compared steam and hydro distillation for *Carum carvi* (Caraway) and *Anethum graveolens* (Dill) seeds, finding that the yield of EO was lower using steam distillation as was the presence of oxygenated components. The authors claim that these results concur with several previous studies, however recent studies by Garcez *et al* [51] found no significant difference in yield between steam and hydro distillation of *Anethum graveolens*.

When studies have specified leaf material for distillation extraction, it is evident that the difference between steam and hydro distillation is determined by the plants used and the oils that these plants contain. Studies that targeted *Laurus nobilis* (Bay laurel) and *Platycladus orientalis* (Chinese arborvitae) found that hydro distillation yield was greater, however, a better quality of oil could be obtained using steam distillation [52, 53]. A change in the chemical composition between the two distillation methods was also observed and attributed to thermal modification, or degradation of EO's in prolonged contact with boiling water. Hydro distillation involves constant contact with water that facilitates absorption of specific EO

components that can either retard or expedite the transport of specific components through the distillation equipment. Shiwakoti *et al* [54] found that there was no significant difference in basil leaf EO composition between steam and hydro distillation, but oil yield was significantly higher from steam distillation. The authors postulate that the constant water contact facilitated by hydro distillation provides a heat buffer around the leaf, whereas steam distillation allows for greater heat contact which causes the oil glands within the leaf to rupture.

For many applications of EO extraction it is not practical to extract oil from only a particular part of the plant, so industrial extraction is often conducted on whole plant biomass. Steam distillation of *Origanum majorana* (Marjoram) was compared to hydro distillation and super critical fluid extraction processes [55]. It was found that steam distillation had a lower oil yield than the other methods, but extracted oil displayed antimicrobial activity against a wider variety of tested pathogens. The increased antimicrobial activity was attributed to the higher concentrations of α-terpinene, γ-terpinene and terpinene-4-ol that were present in EO extracted by steam distillation. Extraction of EO from *Rosmarinus officinalis* (Rosemary) plants showed opposite trends, in terms of yield, to that seen in Marjoram plants. These studies observed that steam distillation could extract Rosemary oil at a higher rate than hydro distillation, but at a lower rate than more advanced techniques like supercritical extraction [56, 57]. It is worth noting that the extraction time for these studies differ significantly, even between studies using the same plant, with effective extraction times ranging from 10 minutes [57] to 180 minutes [55, 56].

Steam distillation differs in many ways from hydro distillation but essentially involves progression/adaption of similar processes and equipment. There are inherent advantages and disadvantages of steam distillation when compared to other technologies, with the efficacy of steam distillation differing significantly according to the source biomass. EO yield and quality by steam distillation has been studied extensively over a range of plant types and the results diverge depending on biomass composition (seeds, stems, leaves, etc.), extraction parameters, and even growing locations and harvest times of biomass. In order to assess the performance of steam distillation in extracting a novel EO like Qcide it is necessary to conduct distillation across a range of parameters.

2.1.1.3 Hydro Diffusion

Hydro diffusion (as an extraction process) is a method that relies primarily on hydrodiffusion (the physiochemical process). This process involves fluid-soaked biomass releasing oil by osmosis through the plant cells and is therefore more suited to collecting oils

that have a greater water solubility rather than volatility [2, 49]. A traditional hydro diffusion still engages injection of low pressure steam above the biomass, with a condenser located below the biomass housing. Although traditional hydro diffusion is mentioned anecdotally in literature as a clean and efficient method of obtaining certain oils on a small scale, there are no reports of a significantly large or industrial extraction operation that currently uses this method. However, in recent years there has been increased study into hydro diffusion as a microwave assisted process [58-65].

Hydro diffusion with microwave assistance has been examined for a variety of plants and plant components. A critical review of 12 research papers by Kavi *et al* [61] found that the optimal extraction conditions would differ depending on the plant components used for extraction (leaves, peels, flowers, fruit, seeds, wood, rhizomes, etc), and that the extraction of EO for a plant could not be inferred from a previously studied different plant. The typical comparison between microwave assisted hydro diffusion and hydro distillation finds that the processes produce similar EO yields and composition. This trend is seen in a range of biomass types; orange peel [66], Bakhtiari savory [60], rosemary [63], and spearmint and pennyroyal [58]. Studies comparing microwave assisted hydro diffusion with steam distillation found that steam distillation produced a greater yield and higher quality oil in terms of phenolic content, however this came at the cost of much higher energy consumption [59, 62]. Microwave assisted hydro diffusion represents a more energy efficient extraction method, but does not show significant improvement from traditional methods, in terms of oil yield or quality. Hydro diffusion does show promise in extraction of specific EO components, and given its energy efficiency, has great potential for further study or integration with other processes.

2.1.1.4 Vacuum Distillation

Vacuum distillation is a method of extraction at reduced pressure, which allows the extraction of EO components at lower-than-normal temperatures. A significant point of vacuum distillation is that it allows the extraction of high boiling point organic components that exhibit significant decay at high temperatures [2, 67]. While vacuum distillation can be used as a method of oil extraction from biomass, there are very few instances where it is used as a standalone industrial process. Vacuum distillation is desirable as an extraction process due to the low input energy required during the process, and the selectivity of extraction, but requires significant equipment investment or modification [67]. Vacuum distillation is most commonly used in conjunction with other extraction processes in order to; purify the EO by

targeted extraction of desirable or objectionable components [49, 67, 68], or to fractionate EO's into component groups in order to separate chemical classes or suit product uses [67, 69-73].

Despite the advantages of low input energy and minimal component degradation, there has been relatively little study into the efficacy of vacuum distillation in comparison to other methods. Comparison between cold press extraction and vacuum distillation extraction of citrus EO found that the oil yield from vacuum distillation was much higher, but the extracted oil exhibited lower antioxidant capacity and antimicrobial activity against most tested bacteria [74]. A more recent study compared traditional hydro distillation with vacuum hydro distillation of oregano oil, and found that vacuum distillation achieved a higher oil yield and stronger antibacterial effect against *E. coli* and *S. aureus* [75]. In both these studies it was found that the vacuum distilled oil had a higher concentration of alcohols, phenolic derivatives, and terpenes; while the other methods produced a higher concentration of monoterpenes.

The low operating temperature used in vacuum distillation provides high thermal efficiency, with low degradation of EO components. These properties make vacuum distillation ideal for purification or isolation of EO components in sequence with other extraction methods. However, there are many disadvantages to vacuum distillation, particularly relating to cost, that make it difficult to use as a primary extraction process. The initial setup of vacuum distillation requires significant investment in process equipment including pumps to draw and maintain the vacuum environment, process vessels that can maintain the high volumetric flow required in vacuum distillation, and vessels and safety mechanisms that can prevent air ingress into the system [67].

2.1.2 Solvent Extraction

This category of extraction involves direct contact of a solvent with a solid matrix of biomass. Solvent extraction can be conducted using a range of methods including Maceration, Percolation, Decoction, Reflux Extraction, Soxhlet Extraction, Pressurised Liquid Extraction, Subcritical Fluid Extraction, Supercritical Fluid Extraction, and Hydro Distillation. The rate and efficacy of solvent extraction techniques are affected by several factors including solvent properties, solvent contact duration, solid matrix size, temperature, and agitation. While many solvent extraction processes produce a significantly higher yield of oil, the product may contain compounds in excess of the desired composition [3], and the product can require expensive or complicated means to separate from the process solvent. The physical properties of the product can also show significant variance from the desired product; such as colour, odour, viscosity, and semi-solid properties [76]. Depending on the solvent extraction method utilised, the

extraction could be a batch or continuous process, and may require solvents of varying volumes and price.

2.1.2.1 Maceration

Maceration is the process of extracting oil from a solid matrix by immersion in a solvent. This process can be conducted at room temperature to temperatures just below the boiling point of the solvent used. Maceration can be used as a precursor to other extraction techniques, and in some cases is required to facilitate the release of EO [2, 49]. Maceration can also be used when the purpose is to create a dilute product, like a tincture. While this eliminates the need to extract the EO from the process solvent, it reduces the solvent choice to accommodate high miscibility and low toxicity. The major disadvantages of maceration extraction are low oil yield compared to other techniques, long extraction time, a high solvent to biomass requirement, and the cost of recycling or disposing of extraction solvent. The oil extracted is highly dependent on the solvent used, which allows for selective compound extraction [77, 78], but also limits the choice of solvents depending on the range of compounds requiring extraction. In order to offset the disadvantages of this process, maceration is often paired with assistance techniques such as; comminution, microwave assisted extraction, ultrasonic assisted extraction, and enzyme assisted extraction [77-79].

2.1.2.2 Percolation

Percolation is a continuous, or semi-continuous process, by which an extraction solvent is passed through a solid matrix. This process relies on unsaturated solvent capturing EO from the solid matrix in a desorption process as it flows through the matrix. Similar to the maceration process, it can be used as a precursor process to other extraction techniques, or to create a dilute tincture. While percolation has been extolled as more efficient than maceration [79], an initial period of maceration is often required to raise the product yield. Percolation can also add complexity to the extraction process, with a powdered solid matrix requiring imbibition, or percolation cells required to prevent solvent loss and ensure contact efficiency [49]. Disadvantages of percolation extraction include long extraction times and a high ratio of solvent to solid matrix; however, efficiency can be increased when paired with comminution, microwave assisted extraction, and ultrasonic assisted extraction.

2.1.2.3 Decoction

Decoction is a batch solvent extraction process where the solid matrix is immersed in solvent and boiled for a fixed period, with the goal to promote elution of components into the

solvent and concentrating the solution by removing the vapour from the system. The difference between decoction and hydro distillation is that during decoction the vapour is discarded, whereas during hydro distillation the vapour contains the EO product. This extraction method operates at atmospheric pressure and primarily uses water as a solvent, resulting in an aqueous product that is infused with water soluble EO components [49]. In comparison to other solvent extraction methods, decoction has shown good relative affinity for extraction of alkaloid compounds [79], and mixed results for the extraction of phenolic and flavonoid components [80-82]. Decoction can suffer from the same disadvantages of other solvent extraction methods, potentially requiring long extraction times and a large ratio of solvent to solid matrix, but this is highly dependent on the biomass used and the desired product. It has also been seen that decoction results in a loss of the most volatile components, and decomposition of heat sensitive components [79, 80, 82]. Decoction can also be integrated with other technologies to increase efficiency; such as mixed solvent, microwave assisted extraction, ultrasound assisted extraction, and pulsed ohmic assisted extraction [83]. While these assisted extraction methods have often shown an increase in the energy efficiency, the end product can be altered dramatically, so detailed process analysis is required when adding assisted methods to extraction technologies like decoction.

2.1.2.4 Reflux Extraction

Reflux extraction is a batch solvent extraction process where the solid matrix is immersed in solvent and boiled for a fixed period under full reflux and atmospheric pressure conditions. The purpose of reflux extraction is to promote elution of components into the solvent through raised temperature conditions and specific solvent properties. While reflux extraction has traditionally been conducted with water in the production of tinctures, the process can be conducted at reduced temperatures, with increased yield, through the use of low boiling point solvents. Some solvents that have displayed high yield at reduced temperature are; petroleum ether [84], ethyl acetate [85], and ethanol-water combinations [76]. Integration with efficient heating methods such as pulsed ohmic extraction and microwave assisted extraction has shown that reflux extraction can be conducted with high energy efficiency [86], however performing extractions with these combinations on a large scale is a complicated and expensive proposition. Disadvantages of reflux extraction include; potentially long extraction times, high ratio of solvent to solid matrix, and thermal degradation of desirable compounds [85, 87, 88]. Another disadvantage that has been noted in studies is poor solvent to solid matrix contact,

making operations like comminution and mechanical agitation necessary for efficient reflux extraction [86].

2.1.2.5 Soxhlet Extraction

Soxhlet extraction is conducted by placing the solid matrix inside the extraction chamber of a Soxhlet Extraction Apparatus (Figure 2-2). The solid matrix is commonly reduced to a powder form and contained inside a thimble made of porous material. The extraction solvent is heated to boiling point in a separate chamber and is condensed above the extraction chamber, allowing the hot condensed solvent to fall upon the solid matrix. The solvent fills up the extraction chamber until a fixed volume is reached, at which time the solvent is removed from the extraction chamber via a syphon tube which drains into the solvent boiling chamber.

This extraction method relies on the permeation of the oil through the solid matrix and solubilisation into the extraction solvent. The solvent in the extraction chamber is refreshed continuously through a cycle of re-boiling, and the oil is concentrated in the boiling chamber of the Soxhlet Extraction Apparatus. Soxhlet extraction relies heavily on the solvent properties to extract oil from the solid matrix and requires careful investigation before a biomass-solvent combination is used. High oil yields gained from Soxhlet extraction is often offset by the specificity of the compounds extracted; such as methanol mixtures extracting high concentrations of terpenoids and phenolic compounds [80, 81, 84], and dichloromethane extracting high amounts of hydrocarbons but low amounts of aldehydes and ketones [89]. Because the extracted solution is constantly re-boiled in order to recycle the solvent, low boiling point solvents are used in order to keep the process temperature low and avoid the problem of thermal degradation of extracted compounds. Soxhlet extraction may require long extraction times, high ratio of solvent to solid matrix, and complicated separation of EO product from extraction solvents.

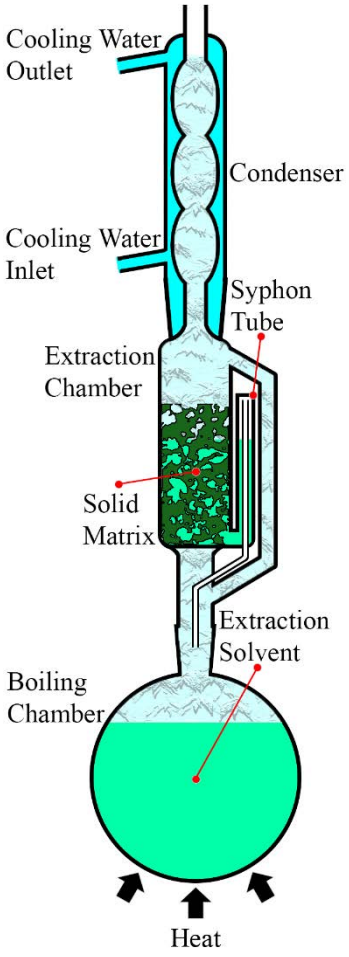

Figure 2-2: Soxhlet Extraction Apparatus

2.1.2.6 Subcritical Fluid Extraction

Subcritical Fluid Extraction is also known as Superheated Fluid Extraction, Pressurised Liquid Extraction, or Accelerated Fluid Extraction. Subcritical Fluid Extraction involves immersing the solid matrix in a solvent and applying high pressure and temperature to the system. The temperature is higher than the normal boiling point of the solvent, but the high pressure of the system keeps the solvent in its liquid state. The use of liquids above the usual boiling point increases the extraction rate by altering the fluid properties of the solvent; such as density, viscosity, surface tension and permittivity [49]. Of particular interest is the changing properties of water, for which the dielectric constant decreases dramatically with increasing temperature. This means that the polarity of water decreases to a point where it can behave similarly to methanol, allowing water to be used as an efficient 'green' extraction solvent. Cheng *et al* [90] extensively reviewed the study of subcritical fluid extraction. They found that EO was generally extracted more efficiently using other extraction methods, but subcritical fluid extraction resulted in a product that was more concentrated in oxygenated compounds. The review individually analysed many groups of chemical compounds (flavonoids, polyphenols, organic acids, glycosides, carbohydrates, EO's, alkaloids, quinones, terpenes, lignans, and steroids), finding the extraction efficacy of each to be dependent of the solvent and extraction parameters. Whilst subcritical extraction is almost unanimously seen as a strong methodology for extracting oxygenated compounds, it is often considered to be poor at extracting particular groups of compounds such as monoterpenes and sesquiterpenes [91-93]. In comparison to other solvent extraction methods, subcritical fluid extraction uses a lower amount of extraction solvent and is conducted over a shorter period to achieve optimal yields. The product of subcritical fluid extraction does require further treatment, which can be complicated or hazardous depending on the solvent used. Even water as solvent can require extensive treatment, with the extract often being presented as an oil-water emulsion.

2.1.2.7 Supercritical Fluid Extraction

Supercritical fluid extraction is conducted by immersing the solid matrix in a solvent under conditions where the temperature and pressure is maintained above the critical temperature and critical pressure of the solvent. A fluid in this critical region displays fluid properties between that of a liquid and gas, with higher diffusivity, lower viscosity, and lower surface tension than the solvents in a non-critical state. These properties facilitate efficient diffusion throughout the solid matrix, and high dissolution of oil components into the solvent. The nature of the critical fluid also allows for alteration of extraction condition, with small increases in pressure leading

to large changes in solvent density, which could raise the solvating ability of the supercritical fluid. For a solvent to be viable for supercritical extraction, the supercritical state should be achievable at relatively low temperatures. Supercritical fluid extraction is highly dependent on the solubility and thermodynamic properties of the solvent in the supercritical stage, which is well established for many possible solvents [94]. Several solvents have been proposed for supercritical extraction; such as Argon, Hexane, Pentane, Butane, Nitrous Oxide, Sulphur Hexafluoride and Fluorinated Hydrocarbons [95], however the majority of research of industrial supercritical fluid extraction is conducted using Carbon Dioxide (CO_2), which has many advantageous extraction properties in the supercritical range. The critical point of CO_2 is 31.2°C and 73.8Bar which allows extraction to be performed at relatively low temperatures, which protects thermally sensitive components from degradation. CO_2 is also inexpensive, non-combustible, readily available, and easily separated and recycled. The extraction using supercritical CO_2 is often seen to be a relatively selective process, with high yields and shorter extraction times when extracting specific components of EO's [56, 79, 94-97]. The selectivity of supercritical CO_2 extraction is of great benefit for extracting valuable components when only a narrow range desired, but if the product contains a large variety of components the extraction process can become more complicated. In order to increase the productive range of supercritical CO_2 extraction it is necessary to use a multi-pressure staged extraction process, or integrate other methods such as cosolvent addition [15, 49]. Installation of extra processing to supercritical CO_2 extraction can be complicated and costly, and is undesirable in a process that is already recognised as being prohibitively expensive to initiate [2]. Supercritical CO_2 extraction has also been found to be best at extracting low molecular weight and non-polar compounds, with CO_2 displaying poor solvating properties for components with molecular weights above $250_{g/mol}$, and very poor solvating properties for polar components [2, 49, 97].

2.2 Biomass Pre-treatment

In the extraction of essential oil from plant material the composition, yield, and rate of the oil can be dependent on how the plant material is handled prior to extraction. Pre-treatments are often conducted in order to reduce the extraction time or increase the yield, but there are some oils that require specific pre-treatment in order to perform extraction [2, 49, 98]. Pre-treatment of biomass can include seasonal harvesting and crop treatment, comminution, drying, and solvent treatment.

2.2.1 Seasonal Harvesting

One consideration in the production of essential oils is the timing of harvesting. This is especially pertinent when whole trees are harvested; as is the case of production of Tea Tree oil and eucalyptus oils such as Qcide oil. The oil content and biomass regrowth could be affected by many factors, such as seasonal rainfall, temperature, and incidence of pest and disease. A study of essential oil steam distilled from Damask Rose found that season variation and geographic distribution highly affected the composition of the essential oil [99], with higher concentration of oil found in biomass harvested during Spring compared to other seasons. Seasonal harvesting does not always have a dramatic effect on the composition of essential oil. Douglas *et al* [39] found that there was little variation in New Zealand Manuka oil composition due to the season, however there was effect on oil yields due to the seasonal effects on leaf to stick ratio in trees. This study, as well as others, have also suggested that fire ecology and regeneration cycles can contribute to the chemical composition of specific tree essential oil [100]. For species such as eucalyptus the frequency and timing of tree harvesting has an effect on the maturity of the collected biomass. Johnsons study of the *Eucalyptus gobulus* described the juvenile leaves as having a completely different shape, a less waxy texture to the leaf surface, and smaller pores [101]. Study of the impact of Patchouli leaf maturity on essential oil content found that semi-matured leaves yielded greater oil content compared to juvenile and fully mature leaves. Although there have been few studies into oil extraction in different stages of leaf maturity, there is some evidence to show an impact on oil yield and composition.

2.2.2 Comminution

Comminution, sometimes termed as Mechanical Size Reduction, is the act of reducing the average particle size of solid materials by crushing, grinding, cutting, or other processes. Comminution is used extensively in many industries as a critical step in industrial processes, and in the production of many food products such as sugar and flour. In steam distillation processes the goal of comminution is to increase the interfacial area between the solid matrix and the steam, as well as to reduce the diffusion resistance of the solid matrix. When comminution is conducted prior to industrial distillation it is important that the benefit exceeds the cost, whether associated with energy, labour or time requirements, or undesirable by-product creation.

As comminution is an important consideration for mass transfer in distillation operations there is extensive study into the particle size parameters during distillation extraction. The

reduction of the particle size of the solid matrix has shown positive effects on yield for many different types of biomass; such as, Sweet Flag rhizomes [102], African Ginger [103], Eucalyptus leaves [104], and Cumin seeds [105]. These studies show a consistent increase in oil yield with decreasing particle size, but do not address the input considerations of comminution or the effect of large scale distillation of small particle solid matrix. Another consideration is the effect of comminution on the chemical composition of the extracted oil. Kokotkiewicz *et al* [106] studied the extraction of oil from celery seeds and found that comminution had a negative effect on the phthalide content of the product, but did not take into consideration the effect on total yield or efficiency. Another study into distillation extraction by Chasteberry [107] found that the oil composition differed when comminution was applied, with whole fruits producing oil that was rich in monoterpene hydrocarbons, and comminuted fruits producing oil that was much higher in less volatile components.

2.2.3 Drying

Drying of biomass is conducted for many reasons depending on the plant, the extraction method, and the harvest timing. Drying of biomass can enable storage in a stable condition until oil extraction can be conducted and to prevent contamination and loss [49]. Drying as a pre-treatment for extraction could be conducted as a method of (i) removing water to allow permeation of process fluids into the biomass; (ii) disrupting the surface and cell structure of the biomass to facilitate oil and process fluid permeation; or (iii) inducing specific reactions within the biomass. In the extraction of hemp oil, drying of the biomass is conducted prior to extraction to induce oxidative reaction and facilitate the conversion of cannabinoid acids in the alcohol forms [98]. Drying is often found to have a negative impact on the yield of more volatile components, such as monoterpenes, but has also been found to have a positive effect on the total oil yield or at least a positive effect on the yield of higher molecular weight components of essential oil [108, 109].

Drying of biomass can be conducted through many methods, from small scale low complexity methods such as sun, shade, oven, and microwave drying, to complex industrial scale methods such as rotary, flash, disk, cascade, and superheated steam dryers. While there are many dryer designs using variations of inputs and configuration, laboratory studies will often use the small scale and low complexity options due to small sample size and controllability considerations. Sun and shade drying are often used as a simple method to test the effect of moisture content on oil extraction, but these methods are time intensive and prone to uncontrollable temperature and airflow fluctuations. Although inefficient, these methods of

drying have shown the benefit of biomass drying prior to distillation [110, 111]. Assisted methods such as microwave and oven drying are effective at accelerating the drying of biomass in preparation for oil extraction, however these methods can cause loss or degradation of desirable volatile components [111, 112].

2.3 Critical Assessment of Extraction Methods

For the extraction of EO's, there is a wide variety of possible extraction methods. These extraction methods range from traditional and widely used methods, to more innovative and experimental methods. Due to the extremely diverse and complex nature of EO's, there is no one extraction method that is superior to others in all cases, and even methods that do produce greater EO yields can be unsuitable for a number of reasons relating to cost, processing time or effect on the composition of the EO.

Hydro and steam distillation are well established and widely used industrial methods due to the relatively low complexity, low capital investment, and large processing volume. Hydro and steam distillation can extract a wide variety of EO components but relies on these components being steam volatile and immiscible in water. Components that do not meet these criteria can become trapped in the extraction equipment internal condensate, which can retard the extraction rate, or could complicate the separation of oil from the distillation condensate. These processes are usually run at high temperature, which increase the rate and yield of oil extraction, but can also cause hydrolysation and heat degradation of desirable components and result in adverse effects on product colour and odour.

Hydro diffusion is similar to hydro/steam distillation but is more suited to collecting components that have a high water solubility rather than volatility. This method relies on hydrodiffusion of the EO into the process fluid, which will collect a wide range of oil components, but the separation of the oil from the process fluid can become complicated or expensive. Hydro diffusion benefits greatly from the addition of energy efficient heating methods, such as microwave and electric pulse assistance, but increasing the temperature has the same advantages and disadvantages as traditional hydro and steam distillation.

Vacuum distillation is the process of hydro distillation, steam distillation, or hydro diffusion, operated at a pressure below atmospheric. The goal of vacuum distillation is to perform the particular distillation extraction at a lower temperature in order to reduce heat degradation of precious components in the EO. Due to the complexity and high cost of large scale vacuum distillation, it is not seen as a viable option for industrial scale extraction, and is

instead used primarily for fractionalisation and purification of products obtained by other extraction methods.

Solvent extraction uses solvents suited to the specific solvent extraction process and the chemical composition of the desired product. While there are many possible solvent choices, some of the more common solvents used are methanol, ethanol, dichloromethane, CO_2, and water. Maceration and percolation are solvent extraction processes that operate at lower temperatures and target components that are more soluble than volatile. These processes are ideal for acquisition of aqueous products, such as tinctures and tonics, but require more extensive separation processes to obtain pure EO products.

Soxhlet extraction uses similar principles of extraction as maceration and percolation, but benefits from the semi-continual circulation of extraction solvent through the Soxhlet apparatus. While Soxhlet extraction is often seen to be a more efficient process, the constant boiling of the extraction solvent can cause heat degradation of the product. To avoid heat degradation, Soxhlet extraction is often operated with low boiling point solvents, which may then require further processing to reduce toxicity in the product and effluent.

Decoction and reflux extraction are both processes that involve the complete immersion of the solid matrix in a solvent that is boiled for a set period. Both methods suffer from the thermal degradation of EO components and are usually unable to produce a pure product without further separation processing. While both of these extraction methods are used extensively in the small scale production of herbal medicines, there are extensive issues with operating these methods on an industrial scale, such as thermal degradation of components and poor heat efficiency during operation.

Subcritical fluid extraction takes advantage of favourable solvent properties by pressurising the solvent so it can be held at a temperature above its normal boiling point. Extensive review of this method shows strong yields of certain EO components are offset by weak extraction of other components. This method requires high-pressure, raised temperature, or large quantities of low boiling point solvent, making the equipment required complicated and expensive. Because the product of subcritical fluid extraction is not a pure oil product, the extensive post-extraction processing reduces the viability as an industrial process.

Supercritical fluid extraction takes advantage of a particular solvent's thermodynamic properties in the supercritical state, which includes high diffusivity, low viscosity, and low surface tension. While many solvents can be held in a supercritical state at acceptable process conditions, the primary solvent used for supercritical extraction is CO_2. CO_2 supercritical extraction has seen extensive study and frequently exhibits significantly high yield and purity

of EO components, as well as improved antioxidant activity with the extracted oil. Supercritical CO_2 extraction is also lauded as being energy efficient, environmentally friendly, relatively safe, and free of thermal degradation by-products. Despite these apparent advantages, supercritical CO_2 extraction is unsuitable for high volume EO extraction due to the initial equipment setup being prohibitively expensive. Further, while studies show supercritical CO_2 extraction to have a high yield of selected components, the process is poor at extracting polar and high molecular weight components, which would make this method completely unsuitable for the extraction of certain EO's.

2.4 Proposed Method for Qcide Extraction and Optimisation

The primary goal of this study is to investigate optimal conditions for the extraction of Qcide oil, for which the primary chemical component is Tasmanone. Early trials on Qcide oil extraction were conducted in far North Queensland using equipment and practices typically used in Tea Tree oil extraction, i.e., hydro/steam distillation. The results have shown Qcide oil to be both steam volatile and immiscible in water. These physicochemical properties of the oil, combined with the simplicity and practicality for its application in rural areas, makes hydro and steam distillation extraction currently the most suitable method for Qcide oil extraction. As steam distillation can in general extract oil at a higher rate than hydro distillation, this study will investigate the application of steam distillation to improve the feasibility in industrial scale operations.

The extraction method employed at the initiation of this study extracted less than 65% of available oil after 400 minutes of extraction. In order to improve the efficiency and viability of Qcide oil extraction, it is necessary to refine the process conditions to maximise the recoverable yield of Qcide oil while maintaining quality and reducing the required extraction time. Key controllable process conditions for hydro/steam distillation include solid matrix size, extraction temperature/pressure, and flowrate. The effect of these parameters on the extraction rate and tasmanone content of Qcide oil was evaluated using laboratory scale equipment at James Cook University laboratories.

3 Laboratory Equipment and Chemical and Data Analysis

3.1 Introduction

While steam distillation has been proven to be a suitable extraction method for Qcide oil, initial on-site trials have extracted less than 65% of available oil after 400 minutes of extraction. To improve the viability of industrial scale Qcide extraction, it is necessary to examine the effect of various processing conditions on the efficiency of oil extraction. This will allow the various process conditions to be optimised to improve the yield of Qcide oil while maintaining quality and reducing the required extraction time. With steam distillation, the controllable process conditions include solid matrix size, extraction temperature/pressure, and flowrate. This chapter will present and discuss biomass sample procurement and preparation, laboratory scale distillation equipment at James Cook University, chemical analyses and data analysis procedures that were used in the experimental investigation.

3.2 *Eucalyptus cloeziana* Biomass

Biomass used for the production of Qcide oil is obtained from a rare cultivar/chemotype of *Eucalyptus cloeziana*, grown on a tea tree farm site in the Mareeba shire. Biomass for these experiments was collected from coppiced *E. cloeziana* trees using custom made industrial tea tree harvesting equipment. The biomass was collected by JCU researchers immediately after harvesting, with individual samples of ˜1kg collected and compacted into 3 litre snap-lock plastic containers. The samples were transported in ice chilled containers to JCU, where they were stored at 4°C until distillation experiments were performed.

3.3 Distillation System

Steam distillation experiments were performed at JCU laboratories using a combination of custom made and procured equipment. A diagrammatic representation of the distillation setup is shown in Figure 3-1. Steam was generated using a 4Bar satellite boiler (Figure 3-3), and steam input flowrate was controlled using orifice plates and valves in the steam input line. Distillation was conducted inside a custom made stainless steel chamber (Figure 3-2) with the steam input separated from the biomass by a stainless steel perforated plate. The pressure, and therefore the temperature, inside the distillation chamber was controlled using a back pressure valve located on the output line (Figure 3-2G). The output line fed the output distillate into a custom made stainless steel Liebig condenser and the condensate was collected into 1L glass bottles. In order to increase the heat efficiency of the distillation apparatus, all heated

equipment was insulated where appropriate and practicable (Figure 3-2). The pipe insulation was 25mm thick Rockwool SPI with foil facing, and extra insulation was made with 50mm rockwool blanket. Other insulation was made in order to surround instruments in the distillation vessel lid to improve safety and heat efficiency. This extra lid insulation was made up of pouches, fabricated from kangaroo leather, which were filled with offcut rockwool insulation material. The pressure inside the distillation chamber was monitored using a 0 to 6 Bar pressure transducer (Figure 3-2E) and the temperature was monitored using a custom made T-type thermocouple (Figure 3-2D).

During distillation condensate would build up within the distillation chamber. The distillation chamber therefore required regular purging in order to prevent water-biomass contact, and flooding of the distillation chamber. The purge line was located in the base of the distillation chamber, consisting of a ball valve connected to a stainless steel Liebig condenser that drained the cooled purge material into a collection vessel. The purging was conducted manually as the purge fluid would cause fouling of any steam trap assembly that was used.

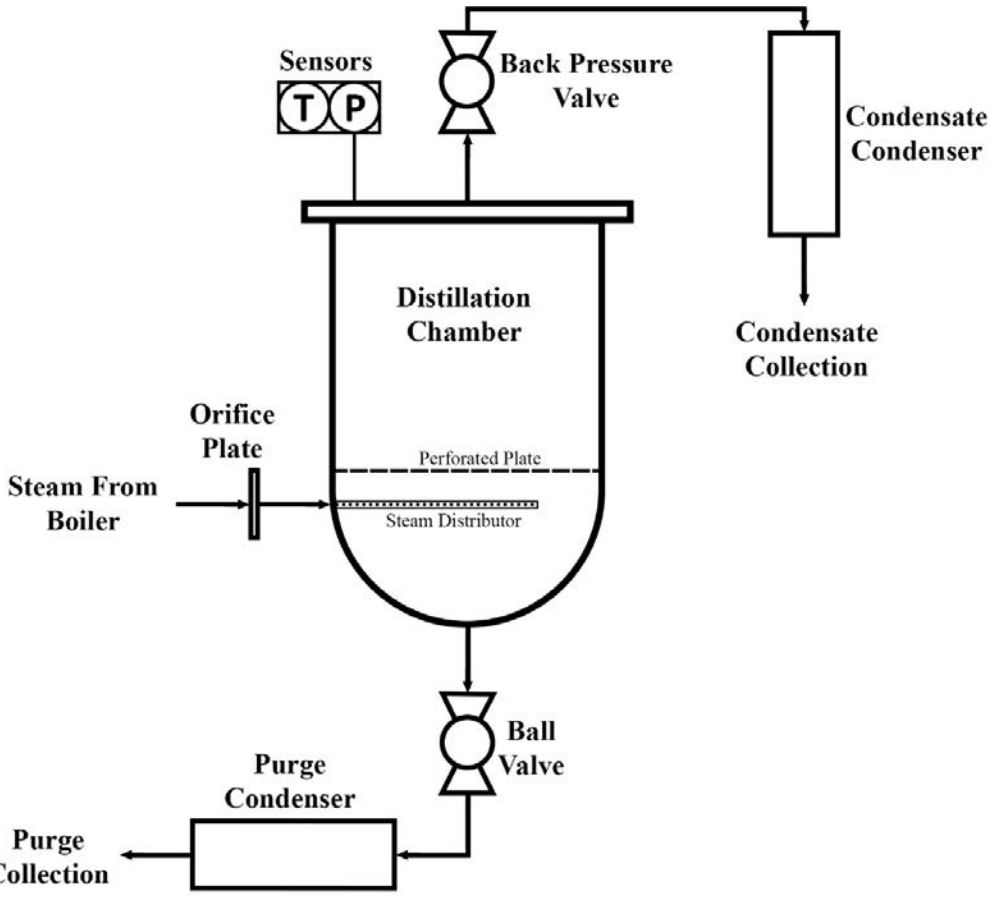

Figure 3-1: Diagrammatic representation of JCU laboratory distillation equipment.

Figure 3-3: Maxi24 Ghindi Benvenuto steam generator.

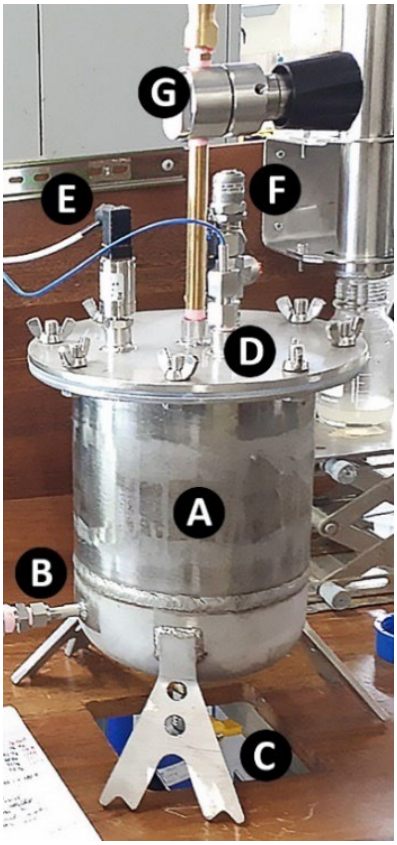

Figure 3-2: A) Custom made distillation vessel, B) Steam input pipe, C) Purge valve, D) TC custom thermocouple assembly, E) RS-PRO pressure transducer, F) Trident 5Bar relief valve, G) Tescom 44-2300 back pressure regulator.

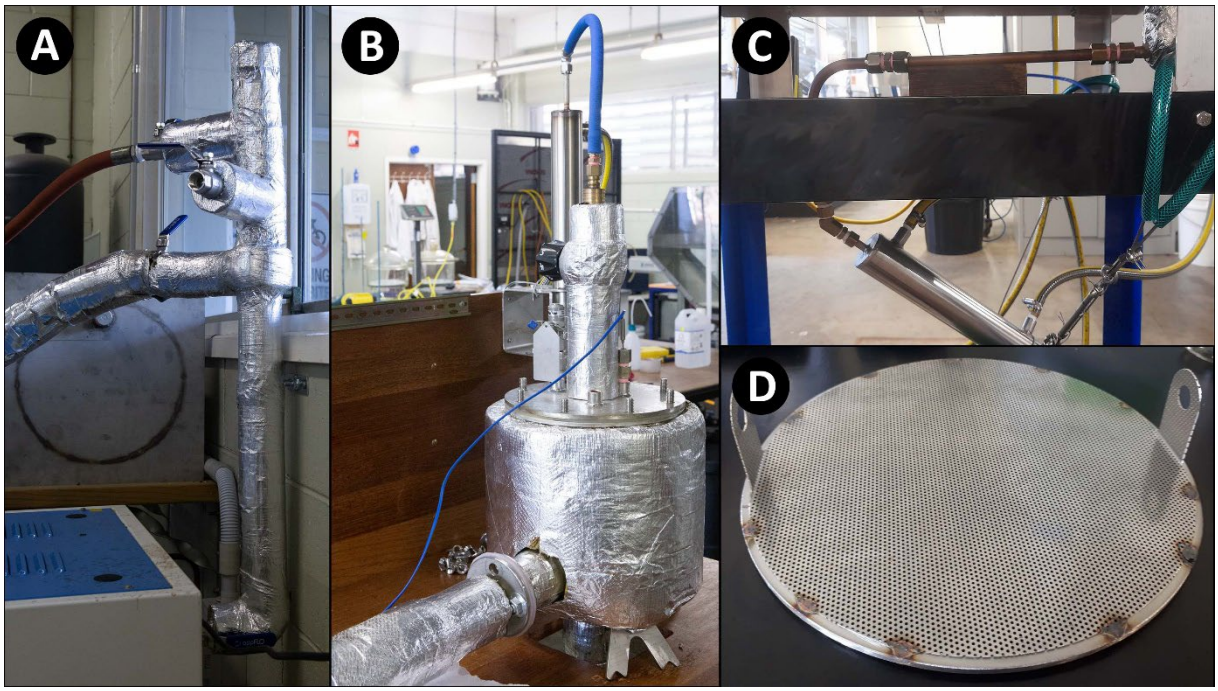

Figure 3-4: Laboratory distillation equipment including isulation using Rockwool insulation material; A) Maxi24 Ghindi Benvenuto steam generator and steam manifold, B) Custom made distillation vessel, steam input line includes orifice plate flange assembly, C) Purge line including condenser, D) Perforated plate used to separate biomass from steam distributor.

3.4 Chemical Analysis

Chemical analysis was conducted by Gas chromatography with Flame Ionisation Detection (GC-FID) Detection. Analysis was undertaken at the James Cook University Advanced Analytical Centre and at the Southern Cross Plant Science Analytical Research Laboratory. These analyses were conducted in order to establish a theoretical maximum yield of tasmanone from *E. cloeziana* biomass, to analyse the effect of condensate internal to the distillation vessel, and to assess the effects of distillation parameters on the composition of Qcide oil extracted by steam distillation.

3.4.1 James Cook University Advanced Analytical Centre (JCU-AAC)

GC-FID was performed at JCU to analyse samples for tasmanone content. GC was conducted using a Varian 1200L with flame ionisation detection. The GC column was a SGE GC column, SGE BPX35, 60m x 250µm ID, 25µm film. The GC conditions were as follows: split ratio 50:1, run time 30min, constant flow rate mode at ~1.0mL/min, column flow at 31cm/sec, column temperature 50°C - 240°C, ramp rate 9°C/min, injector temperature 250°C, detector temperature 300°C. Samples were prepared with HPLC grade ethanol at a concentration of 10mg/ml. Analysis was conducted using an external tasmanone standard provided by BioGene.

3.4.2 Southern Cross Plant Science Analytical Research Laboratory (SCPS-ARL)

GC-FID was performed at SCU to provide a detailed analysis of oil composition. The GCFID analysis performed at JCU was derived directly from the analysis method used at SCU. Oil samples were sent from JCU by overnight freight in ice cooled packaging and prepared for analysis by SCPS-ARL.

3.5 Data Analysis

3.5.1 Yield Calculation

In order to analyse the effect of varying conditions on the rate and quality of Qcide oil extraction, the data was interpreted into a format such that one point in an experiment can be easily compared to corresponding points within all experiments. The yield of each discrete sample was calculated as a percentage of the biomass weight per hour (Equation 3.1). The concentration of each discrete sample was calculated as a weight percentage of the discrete collected condensate sample (Equation 3.2). The accumulated oil yield was calculated by the total mass of oil collected as a percentage of the biomass weight (Equation 3.3). A normalised

accumulated yield was calculated as the total mass of oil collected expressed as a percentage of the biomass weight per kilogram of the total steam input (Equation 3.4).

Equation 3.1 shows the calculation of discrete sample yield (Y_n) where; $m_{oil\ n}$ is the mass of oil collected for the n^{th} sample, m_b is the mass of biomass used for the experiment, and t_n is the collection period for the n^{th} sample.

$$Y_n = 100 \times \frac{m_{oil\ n}}{m_b \times t_n} \quad 3.1$$

Equation 3.2 shows the calculation of the concentration of oil in each discrete condensate sample (C_n) where; $m_{oil\ n}$ is the mass of oil collected for the n^{th} sample, and $m_{condensate\ n}$ is the mass of the condensate collected for the n^{th} sample.

$$C_n = 100 \times \frac{m_{oil\ n}}{m_{condensate\ n}} \quad 3.2$$

Equation 3.3 shows the calculation of the accumulated oil yield ($Y_{accumulated\ n}$), up to and including the n^{th} sample, where; $m_{oil\ n}$ is the mass of oil collected for the n^{th} sample, and m_b is the mass of biomass used for the experiment.

$$Y_{accumulated\ n} = 100 \times \frac{\sum_1^n m_{oil\ n}}{m_b} \quad 3.3$$

Equation 3.4 shows the calculation of the normalised accumulated oil yield ($Y'_{accumulated\ n}$), up to and including the n^{th} sample, where; $m_{oil\ n}$ is the mass of oil collected for the n^{th} sample, m_b is the mass of biomass used for the experiment, and $m_{steam\ n}$ is the mass of steam input for the n^{th} sample. As the steam input was not directly metered, the total steam input was calculated by the sum of the mass of the collected condensate and the mass of the collected internal purge.

$$Y'_{accumulated\ n} = 100 \times \frac{\sum_1^n m_{oil\ n}}{m_b \sum_1^n m_{steam\ n}} \quad 3.4$$

Solvent extraction and chemical analysis were used to determine the concentration of tasmanone within *E. cloeziana* biomass. The concentration of tasmanone was used to estimate a theoretical maximum yield, and to determine tasmanone yield at discrete points in the experiments. The per mass theoretical maximum yield of tasmanone ($Yt_{b\ max}$) from *E. cloeziana* biomass was calculated using Equation 3.5,

Equation 3.5 shows the calculation of the theoretical maximum yield of tasmanone from *E. cloeziana* biomass, where; $C_{t\ GC}$ is the concentration of tasmanone in a sample analysed by GC-FID, $m_{extract}$ is the mass of oil extract obtained from solvent extraction, $C_{extract\ GC}$ is the concentration of oil extract in a sample prepared for GC-FID analysis, and $m_{b\ solvent\ extraction}$ is the weight of biomass used in the solvent extraction.

$$Yt_{b\ max} = \frac{C_{t\ GC} \times m_{extract}}{C_{extract\ GC} \times m_{b\ solvent\ extraction}} \quad 3.5$$

Equation 3.6 shows the calculation of the theoretical maximum yield of tasmanone from a single distillation experiment ($Yt_{D\ max}$), where; $Yt_{b\ max}$ is the theoretical maximum yield of tasmanone from *E. cloeziana* biomass calculated from Equation 3.5, and m_b is the mass of biomass used for the experiment.

$$Yt_{D\ max} = Yt_{b\ max} \times m_b \quad 3.6$$

3.5.2 Significance Testing

All hypothesis testing and statistical analysis of significance was conducted using t-analysis with a confidence interval of 95%.

4 Effect of Distillation Temperature and Steam Input Flowrate on the Extraction of Qcide Oil

4.1 Abstract

Qcide oil is an essential oil found in the leaves of a particular chemotype of *Eucalyptus cloeziana*, that has displayed promising attributes as a natural insecticide. This study seeks to identify favourable processing conditions required for the efficient distillation of Qcide oil from *E. cloeziana* biomass. The effects of distillation temperature and steam input flowrate was observed through 19 steam distillation experiments conducted at six different parameter points; low temperature with low steam flow (LT-LF), low temperature with high steam flow (LT-HF), medium temperature with low steam flow (MT-LF), medium temperature with high steam flow (MT-HF), high temperature with low steam flow (HT-HF), and high temperature with high steam flow (HT-HF). After 3 hours of distillation, the total accumulated oil at each parameter point was found to be; HT-HF (0.811±0.054 $wt\%_{biomass}$), MT-HF (0.668±0.062 $wt\%_{biomass}$), HT-LF (0.601±0.036 $wt\%_{biomass}$), LT-HF (0.524±0.038 $wt\%_{biomass}$), MT-LF (0.495±0.062 $wt\%_{biomass}$), LT-LF (0.340±0.018 $wt\%_{biomass}$). Analysis of unprocessed biomass approximated the oil content of *E. cloeziana* biomass to be 1.44 $wt\%_{biomass}$. Analysis of extracted oil found no clear detrimental effects of increased distillation temperature on the chemical composition of extracted oil.

4.2 Introduction

The primary aim of this experiment is to elucidate the most favourable processing conditions required for Qcide oil extraction from *Eucalyptus cloeziana* biomass using steam distillation. The experimental conditions investigated are (1) distillation temperature and (2) steam input flowrate. As there is little existing research available into the extraction of tasmanone-rich oil from *E. cloeziana* biomass it is important for this experiment to record extensive data that may not be immediately useful for the primary goal of the experiment. A secondary goal of this experiment is to start building a database that can be used for investigations into other parameters that may affect the extraction rate of Qcide oil.

4.3 Experimental

4.3.1 Distillation Biomass

Biomass used as the solid matrix for this experiment was collected and stored as per Section **3.2**, the biomass was collected on the 22nd of November 2020. Prior to distillation each biomass

sample was subjected to sieve analysis to record the particle size distribution of biomass collected directly from the industrial harvester. The biomass was classified using laboratory classification sieves (Figure 4-1) of aperture sizes 19.00mm, 9.50mm, 4.75mm, and 2.36mm. In order to ensure that the biomass would fit inside the laboratory distillation chamber, and to ensure that relatively large twigs would not facilitate steam channelling, any biomass that was larger than 38mm in length was reduced to 38mm using pruning shears. An amount, representative by ratio, of each size distribution was collected and recombined into an 800g biomass sample for use in distillation experiments.

Figure 4-1: Laboratory classification sieves.

During storage there was small amounts of liquid that accumulated in the 3L storage containers and there was some darkening of leaf material. The darkening effect was noted to be more apparent when storage length was increased, but this darkening did not appear to affect the leaf integrity. There did not appear to be noticeable changes to the accumulated fluid amount, the accumulated fluid viscosity, the accumulated fluid colour, the accumulated fluid odour, or the biomass odour.

4.3.2 Distillation

Distillation was conducted at JCU using laboratory equipment described in Section **3.3**. The distillation plan for this experiment was to collect condensate samples for six hours, with increased sampling during the first two hours of the process. Increased sampling was conducted in the early stages to investigate the early stage distillation dynamics. Condensate samples were collected for a series of periods following the sampling plan;

- Samples 1 – 4, 30 minutes of condensate for two hours following first condensate, and

- Samples 5 – 8, 60 minutes of condensate collected until six hours following first condensate, or until no oil was observed in the condensate.

This set of experiments examined the effect of temperature on the oil extraction of Qcide oil as well as the effect of different steam input flowrates. The distillation was conducted at low (˜12g/min) and high (˜18g/min) steam flow rates and at three temperatures; ˜105°C, ˜125°C and, ˜145°C. The oil was separated from all distillate samples by gravity and centrifugal separation and all oil and condensate samples were quantified by mass. All experiments were conducted in triplicate at all parameter points. A collection of the oil samples collected were sent to Southern Cross University for chemical analysis by GD-FID. To assess effect of internal condensate and the consequences of purging this fluid, a sample of the purge material was retained for chemical analysis at JCU-AAC.

4.3.3 Solvent Extraction from Biomass and Purged Internal Condensate

Solvent extraction was conducted on a portion of the biomass samples in order to calculate the tasmanone content of *E. cloeziana* biomass. Calculating the tasmanone content of the biomass allows the calculation of a maximum theoretical tasmanone yield for this series of experiments, and provides an important comparison point for future experimentation. The biomass for solvent extraction was selected in representative size fractions calculated from sieve analysis (Section 4.3.1) and proportions of leaf material and woody material were collected as to maintain a representative ratio of the two. The samples used for solvent extraction consisted of 25g of biomass, and the extraction was conducted on five separate biomass samples. The 25g samples comminuted prior to solvent extraction, which was conducted by maceration with 200ml dichloromethane, mixed for 48 hours. The dichloromethane was removed from the product using a rotary evaporator under reduced pressure. The oil was analysed for tasmanone content by GC-FID at the James Cook University Advanced Analytical Centre.

During distillation there was a requirement for the distillation chamber to be purged of internal condensate in order to prevent flooding. In these stages of experimentation, it is understood that Qcide oil has a low solubility in water, however there is anecdotal evidence to suggest that exposure to hydro-distillation water results in increased uptake of tasmanone and other high molecular weight components in the solution. A sample of purge water was therefore taken for chemical analysis to assess the tasmanone content and the consequences of heat inefficiency within the distillation chamber. 25g of purge water was used for solvent extraction

which was conducted by mixing with 200ml dichloromethane for 48 hours. The dichloromethane portion was separated and dewatered with sodium sulphate which was removed, with any small particulates, by vacuum filtration. The dichloromethane was removed from the product using a Heidolph rotary evaporator under reduced pressure. The purge oil product was analysed for tasmanone content by GC-FID at the James Cook University Advanced Analytical Centre.

4.3.4 Chemical Analysis

Chemical analysis was conducted by GC-FID at both JCU-AAC and SCPS-ARL as detailed in Section **3.4**. Analysis of solvent extraction oil was conducted at JCU-AAC. These analyses were conducted to establish a theoretical maximum yield of tasmanone from *E. cloeziana* biomass, and to analyse the effect of condensate internal to the distillation vessel. Analysis of oil extracted by distillation was conducted at SCPS-ARL. These analyses were conducted in order to assess the effects of temperature and flowrate on the composition of Qcide oil extracted by steam distillation.

4.4 Results and Discussion

4.4.1 Particle Size Distribution

Sieve analysis was conducted on every sample of biomass used in these experiments. As the particle size of the biomass was not a primary focus of these experiments, the data was not used for particle size calculation, but as a reference that can be used for any future experiments relating to this parameter. The mass percentage size distribution of each sample can be found in Appendix A.

4.4.2 Tasmanone Content of Biomass

Chemical analysis of the solvent extract was conducted at JCU-AAC to analyse tasmanone content in *E. cloeziana* biomass. The biomass used for the solvent extraction was collected as a subsample of biomass used for distillation, at the same size and leaf:wood ratios as was used in the distillation experiments. Five biomass solvent extractions were completed and analysed, the results of which are shown in Table 4-1. The solvent extracted ‘oil’ did not resemble steam distilled Qcide oil, as it was dark brown to black in colour and highly viscous. Typical Qcide oil contains more than 80% (by weight) tasmanone, and the extract collected by dichloromethane extraction/evaporation averaged 28.8 $wt\%_{tasmanone}$. This low concentration indicates that dichloromethane is extracting more chemical components than by steam

distillation extraction. While dichloromethane solvent extraction is an acceptable method for analysis purposes, it does not represent a 'true' extraction of Qcide. These extractions were used to find the total amount of tasmanone present in the whole biomass, and not just in the leaf material. Using Equation 3.5, the theoretical tasmanone yield by weight of *E. cloeziana* biomass was found to be 11.52 ± 0.43 $mg_{tasmanone}/g_{biomass}$, or 1.15 ± 0.04 $wt\%_{biomass}$. Distillations were conducted on 800g biomass samples, using Equation 3.6 estimates the maximum tasmanone yield ($Yt_{D\ max}$) of each distillation to be 9.22 ± 0.35g.

Table 4-1: Solvent extraction performed at JCU-AAC, *E. cloeziana* biomass extracted by dichloromethane maceration.

	Biomass Sample Weight	Extract Weight	Tasmanone Concentration in Extract	Tasmanone Concentration in GC Preparation	Theoretical Tasmanone Yield
Sample	$m_{b\ solvent\ extraction}$ [g]	$m_{extract}$ [g]	$C_{extract\ GC}$ [mg/ml]	$C_{t\ GC}$ [mg/ml]	$Yt_{b\ max}$ [mg/g]
1	24.9847	1.1804	10.14	2.50	11.65
2	24.9747	1.0396	10.34	2.95	11.88
3	25.0144	0.8069	9.99	3.43	11.07
4	25.1668	1.3217	9.98	2.29	12.05
5	25.1711	0.8234	10.23	3.43	10.97
				Mean and Standard Deviation	11.52 ± 0.43

This analysis was conducted on *E. cloeziana* biomass collected in November 2020 from a single growing region in North Queensland. Analysis of triketone-rich manuka oil has found that region and season can cause variation of the chemical composition within the essential oil [39]. In order to assess the accuracy of the tasmanone-biomass concentration potential, extended seasonal oil variation analysis would be required.

4.4.3 Distillation Oil Yield

Qcide oil collected from the distillation of *E. cloeziana* biomass was separated as pure oil from condensate through gravity and centrifugal separation. The distillation was conducted using 6 different temperature/steam flow rate settings; (1) low temperature with low steam input flowrate (LT-LF), (2) low temperature with high steam input flowrate (LT-HF), (3) medium temperature with low steam input flowrate (MT-LF), (4) medium temperature with high steam input flowrate (MT-HF), (5) high temperature with low steam input flowrate (HT-LF), and (6) high temperature with high steam input flowrate (HT-HF). The mean of all parameters is shown in Table 4-2. Initial experiments (HT-HF) were conducted using a low flow metering valve in the steam input line, and using an operating pressure approaching 400

kPa. During these experiments it was difficult to maintain a steady steam input flowrate, as the flowrate would fluctuate greatly as the pressure inside the boiler discharged and recharged. To maintain more consistent flowrate the target pressure for high temperature distillations was lowered to ˜300kPa from ˜400kPa. This made the experiments more reliable at the expense of a reduction of ˜6°C operating temperature.

Table 4-2: Parameter points for all experiments. Pressure, temperature and steam input flowrate is shown as the mean and standard deviation during the entire distillation experiment.

Test Parameters Code	Number of serials	Pressure [kPa]	Temperature [°C]	Steam Input Flowrate [g/min]
LT-LF	3	7.7 ± 1.8	102.0 ± 0.5	12.1 ± 0.2
LT-HF	3	25.1 ± 3.2	106.1 ± 1.6	19.2 ± 1.5
MT-LF	3	115.3 ± 23.6	121.9 ± 3.5	12.7 ± 0.5
MT-HF	4	125.5 ± 12.8	123.8 ± 2.6	19.6 ± 1.3
HT-LF	3	293.0 ± 32.3	142.3 ± 3.2	12.6 ± 1.1
HT-HF	3	387.0 ± 28.0	149.9 ± 2.7	19.1 ± 2.7

All the results of the distillation experiments are shown in Appendix B. The results are shown as a mean and the standard deviation of experiments conducted at each parameter point specified in Table 4-2.

Figure 4-2 shows the oil yield calculated by Equation 3.1 for samples taken at each time point, averaged across all repeat experiments. The HT-HF experiments show the highest initial yield of oil (˜0.89wt%$_{biomass}$/hour) which is over double the yield of MT-HF (˜0.44 wt%$_{biomass}$/hour), which is the next highest. The oil yield from the HT-HF experiments drops sharply over the first 120 minutes of sampling, where the oil yield drops below all other experiments. After 300 minutes of HT-HF distillation the condensate no longer has any retrievable oil, indicating an exhaustion of retrievable oil from the biomass at some point between the 240th minute and the 300th minute. The lowest early yields can be seen from the LT-LF sample, which shown no dramatic yield drop, but a gradual decline for the duration of the experiment. As the experiment duration extends, the oil yield of all other experiments declines faster than the LT-LF and by the 360th minute the LT-LF has the highest yield.

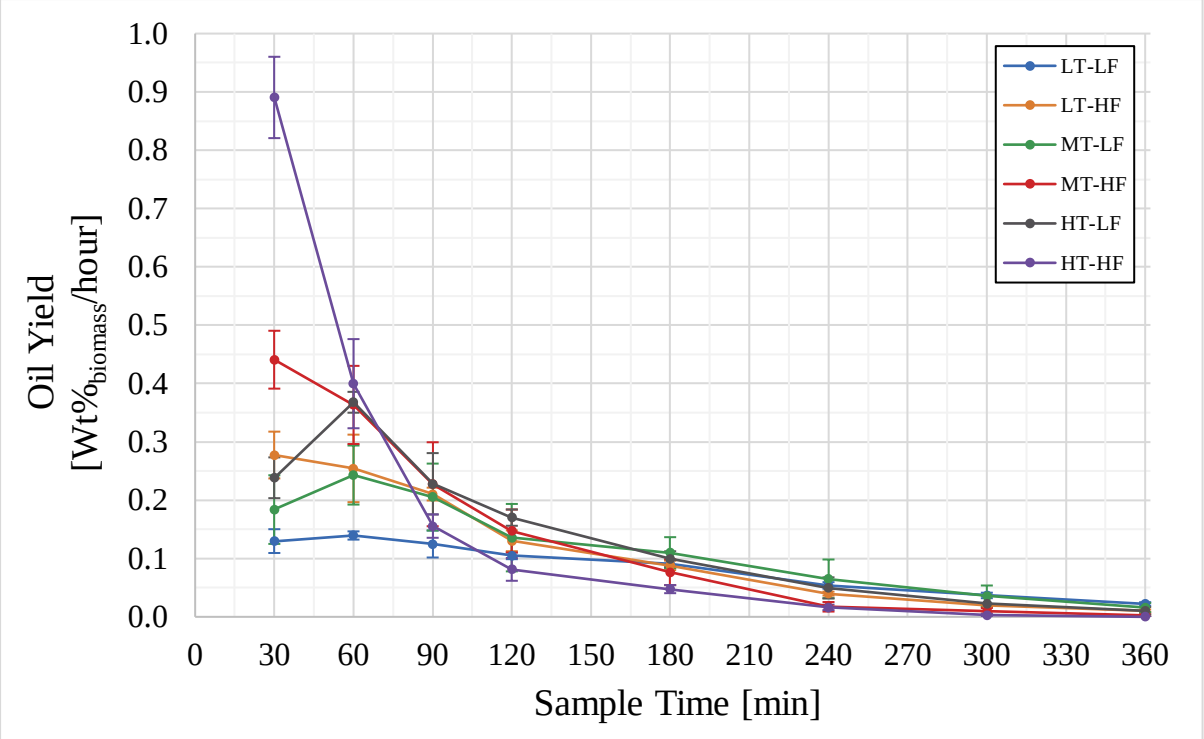

Figure 4-2: Sample oil yield displayed in wt% of the biomass per hour, showing mean and standard deviation of all experiments.

The effect of the high early yields is better illustrated in Figure 4-3, which shows the total accumulated oil over the experimental period, calculated with Equation 3.3. This shows the rapid increase of the collected oil in the HT-HF and the MT-HF distillations during the initial stages, followed by a plateau of the yield after 180 minutes. Although the individual yield of the HT-HF samples declines rapidly and substantially, the high initial yield means that the total yield is never eclipsed by any other experiment. At the other extreme, the LT-LF experiments do not see a dramatic decrease in the yield of individual samples over time, also does not approach the total yield of any other experiment due to the substantially lower initial yields. Economically it is important to achieve the highest possible oil yield with the lowest input, and time input is a very important factor in this equation. Evaluating the experiments by looking at only the amount of oil that was collected over the first 180 minutes of the experiments would indicate yields of: HT-HF (0.811±0.054 $wt\%_{biomass}$), MT-HF (0.668±0.062 $wt\%_{biomass}$), HT-LF (0.601±0.036 $wt\%_{biomass}$), LT-HF (0.524±0.038 $wt\%_{biomass}$), MT-LF (0.495±0.062 $wt\%_{biomass}$), LT-LF (0.340±0.018 $wt\%_{biomass}$). Figure 4-3 shows that there is an increase in accumulated yield, when increasing distillation temperature without changing the steam flowrate, and when increasing flowrate without changing the distillation temperature.

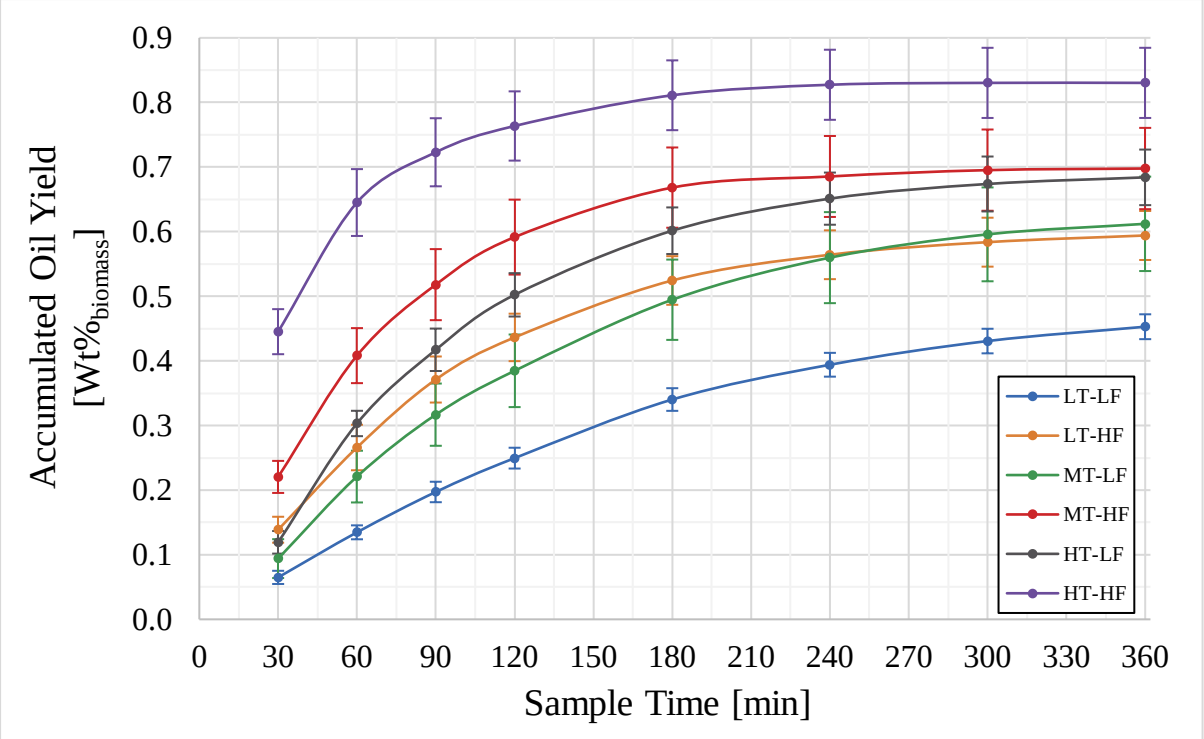

Figure 4-3: Total accumulated oil from samples in each experimental parameter set, displayed in wt% of the biomass, showing mean and standard deviation of all experiments.

Time is not the only factor influencing the economics of a distillation extraction. Steam input and condensate handling are both significant factors determining process optimisation. Figure 4-4 shows the oil concentration of each sample collected during experiments, calculated using Equation 3.2. Similar to the trends seen in the sample oil yields; the HT-HF, the MT-HF, and the LT-HF samples initially display high oil concentration which declines rapidly over the first 120 to 180 minutes, after which these three experiments produce similar low oil concentrations. The HT-LF, the MT-LF, and the LT-LF all increase in concentration until the 60th minute, after which the concentrations decline at a considerably slower rate than the HT-HF, MT-HF and LT-HF. After the 120th minute there is a clear distinction in the concentrations of the LF and the HF experiments, with the LF experiments producing condensate with a higher oil concentration; however after 360 minutes all experiment concentrations converge.

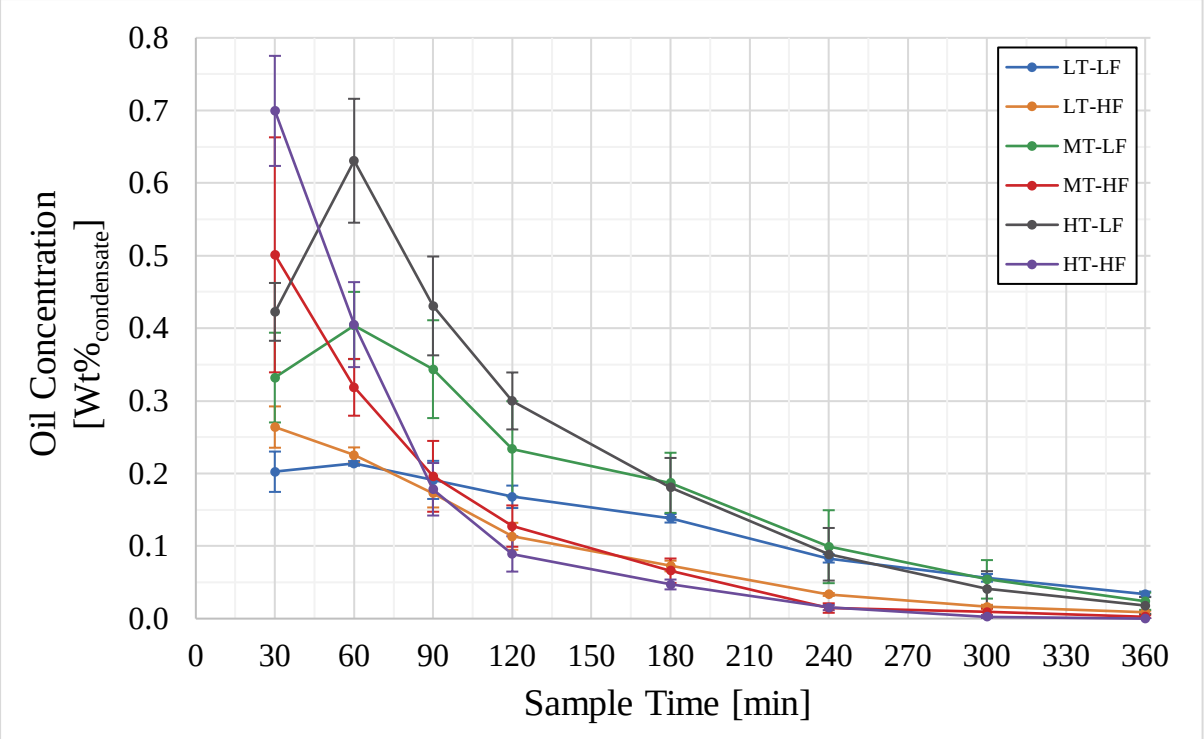

Figure 4-4: Sample oil concentration of distillation condensate samples, displayed in wt% of oil to sample condensate mass, showing mean and standard deviation of all experiments.

Figure 4-5 shows the accumulated oil yield normalised to the total steam input of the experiment. This gives an indication of the oil extraction efficiency with respect to the energy input. For each temperature setting, the high flow experiments start with a high yield of oil and a decline in yield from each subsequent time point. In comparison, the low flow experiments show an increase in oil yield from the first 30min samples to the 60min samples, followed by a more gradual decline in yield over the experimental period. As the temperature is increased there is an increase in the extraction efficiency, and this holds true for the experiment duration of both the LF and HF experiments. The effect of flowrate seems to have a much more dramatic effect on the extraction efficiency, with large gains in the early stages of the experiment and a rapid decline. There is a point for each temperature where the HF efficiency drops below that of the LF counterpart; this is 120min for the HT experiments, 120min for the MT, and 180min for the LT. In terms of total oil accumulation none of the low flow experiments eclipse their high flow counterparts, as the initial yield difference is far too great. The oil concentration in the condensate is greater only in the first 30 minute HF samples, with the oil concentration being consistently higher in low flow samples in the mid to latter stages of each experiment. This leads to some questions about the efficiency of experimental parameters, with Figure 4-5 implying that 120 min into distillation the HT-LF parameters represent a more efficient oil

output to steam input ratio, despite accumulating 51.7% less oil than the HT-HF experiment at the same time point.

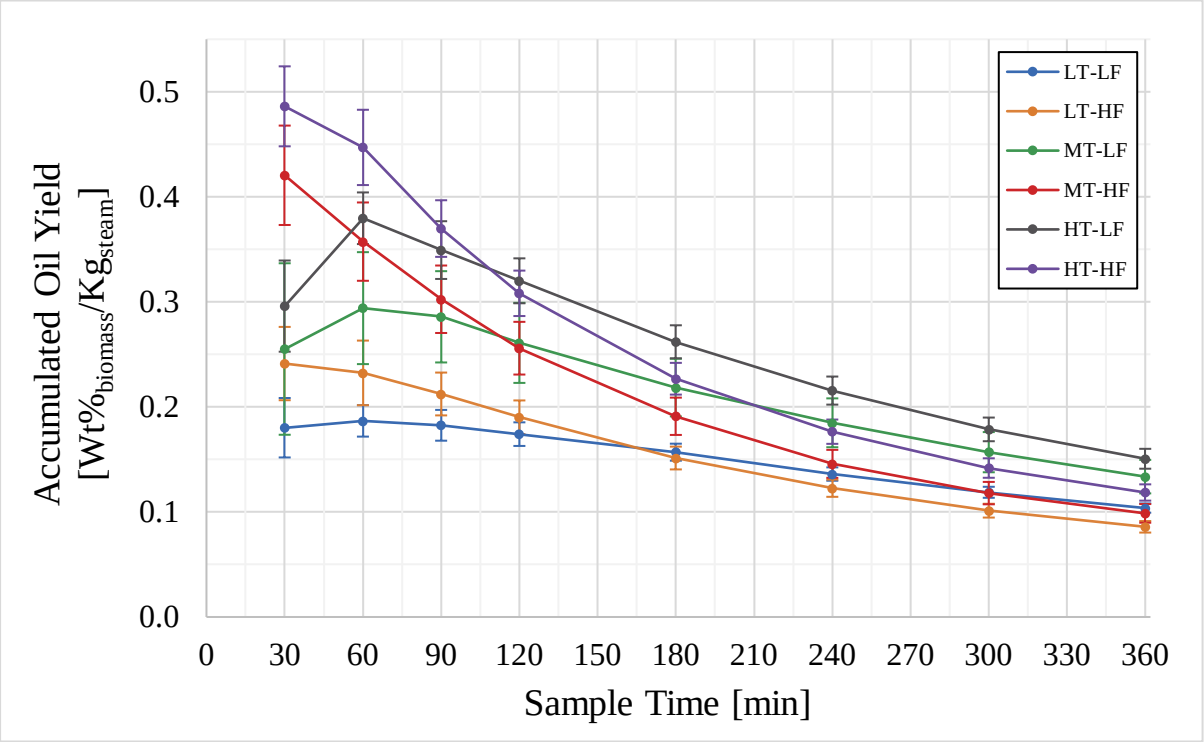

Figure 4-5: Total accumulated oil yield for each parameter set normalised to the total steam input during that experiment, showing mean and standard deviation of all experiments.

In order to assess the effect of increasing the temperature on the oil yield, it is important to make comparisons of the different temperatures while keeping the steam flowrate constant. Figure 4-2 shows that high flowrates there are large differences in the initial oil yield of individual samples due to temperature, with HT > MT > LT. The higher the experiment temperature, the more rapidly the yield of individual samples declines, until the comparative yield is LT > MT > HT after 180 minutes. Despite the reversal of sample yield, the relatively high early yield of the HT compared to MT (and the MT compared to LT) samples meant that the total accumulated oil yield (Figure 4-3) at high flowrate was always significantly greater in HT experiments. This is true for the entire experimental duration and for comparisons between HT and MT, HT and LT, and MT and LT. At low steam flowrates the apparent differences between temperature points were less pronounced, but followed a similar trend to the high flow counterparts. At low flowrates the individual sample yield was greatest in the second condensate sample (30th minute to the 60th minute), and declined over the experimental duration. The sample yields were initially HT > MT > LT and remained this way until the 120th minute. The higher the temperature of the experiment, the faster the decline in oil yield, but a full reversal (LT > MT > HT) was not seen until the 300th minute. While the HT-LF

accumulated yield was always significantly higher than the LT-LF, the HT-LF increase over the MT-LF is only significant from 60min to 120min, and the MT-LF is significantly higher than the LF-LF beyond the 30th minute. These results show a clear trend of increasing oil accumulation with increasing distillation temperature, especially at higher steam input flowrates.

The same compartmentalised analysis needs to be undertaken for the three distillation temperatures in order to clearly define the effect of steam flowrate on the oil yield. For both the LT and the HT experiments an increase in the steam flowrate always results in a significant increase in the accumulated oil yield. In the first hour of the MT experiments, there is substantially more oil collected in the MT-HF distillation when compared to the MT-LF experiment (Figure 4-2), which results in a significantly higher amount of accumulated oil until the 4th hour of distillation. When looking at Figure 4-3 there is a visual distinction between experiments of the same temperature with differing flowrate, with the higher flowrates showing a much sharper increase in accumulated oil during the initial stages. In the first three hours of distillations the HT-HF accumulated 97.7% of the oil that was collected over the entire 6 hour distillation, as compared to the HT-LF distillations which accumulated 87.9%. This is also seen in the MT and the LT experiments; with the MT-HF accumulating 95.8% compared to the MT-LF 80.8%, and the LT-HF accumulating 88.3% compared to the LT-LF 75.1%. These results show a clear trend of increasing oil yield and accumulation rate with increasing steam flowrate.

During distillation experiments the internal condensate was purged to prevent flooding, compounding heat inefficiency, and biomass-liquid contact. The amount of purge water collected was recorded and is shown in Figure 4-6. Generally, the amount of purge required was greater in higher temperature experiments, with most other experiments fairly consistent in purge amount for the experiment duration. The purge amount for each experiment, as a percentage of the total steam input was; HT-HF 40.3%, HT-LF 54.4%, MT-HF 30.6%, MT-LF 46.9%, LT-HF 24.2%, and LT-LF 38.0%. To determine potential loss of tasmanone from the system, a sample of purge water was subjected to solvent extraction and analysed by GC-FID.

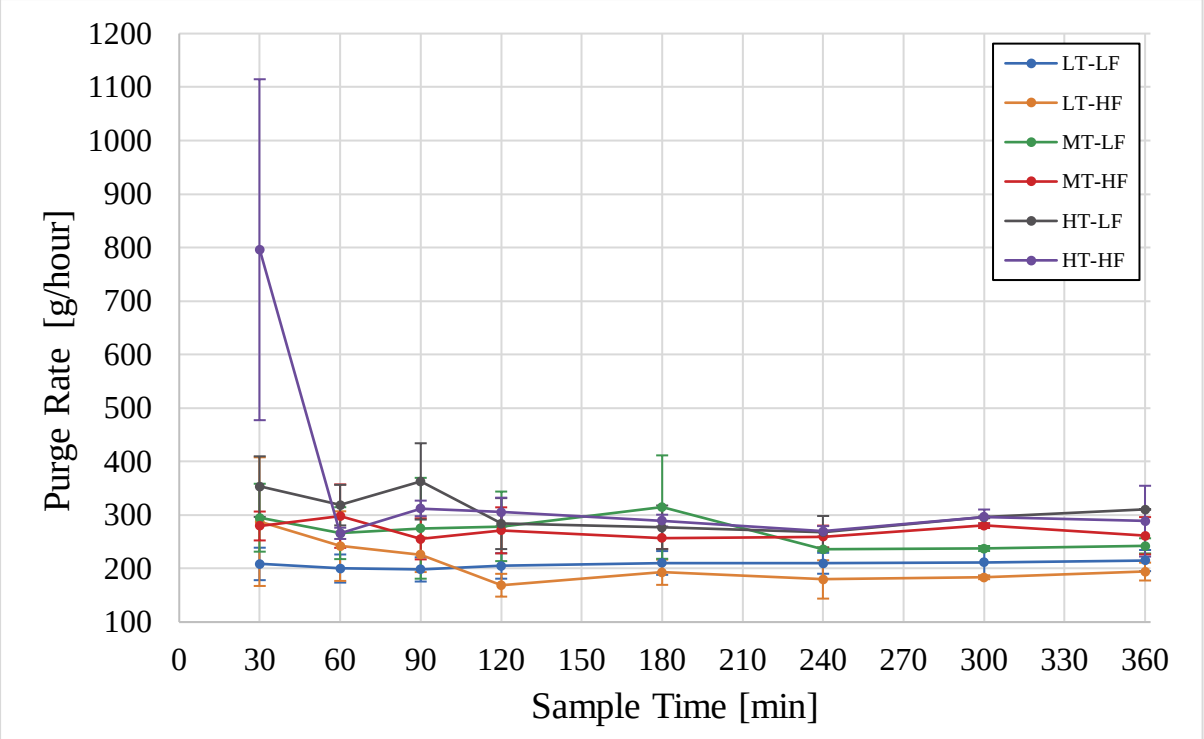

Figure 4-6: Purge water collected at each sample point during temperature/flowrate experiments, showing mean and standard deviation of all experiments.

4.4.4 Tasmanone Content of Purge Water

Chemical analysis of purge water extract was conducted at JCU-AAC in order to analyse tasmanone content of the distillation vessel purge water. A sample was taken from the purge water of a MT-HF experiment. After solvent extraction and evaporation, the remaining 'oil' was a very dark (approaching black) quasi-solid that had a pungent sweet smell. The GC-FID analysis was conducted at the same time as the five biomass extracted samples shown in Table 4-1. The concentration of tasmanone in the purge water was found to be 3.93 $mg_{tasmanone}/g_{purgewater}$, or 0.39 wt%. This concentration is high when compared to the concentration of oil in distillation condensate. This indicates that despite the low water solubility of Qcide under normal conditions, there is a considerable amount that can be retained in water after prolonged exposure to distillation conditions. This water retention of desirable components indicates that extraction methods that use large amounts of liquid water, such as traditional hydro-distillation and hydrodiffusion, would be ineffective at extracting Qcide into an easily separatable product.

While the sampling approach for the purge water may have been inadequate, the apparent richness of tasmanone in the purge water does help explain some of the trends seen in the oil yields. In the HT-HF and MT-HF experiments there was a tendency of the planned penultimate and ultimate samples to contain no oil, which would indicate a depletion of the biomass. Table

4-1 indicates that the tasmanone concentration in biomass should be approximately 1.15wt%$_{biomass}$, which assuming 80% tasmanone content of Qcide would indicate an oil content of approximately 1.44wt%$_{biomass}$. However, Figure 4-3 indicates that the experiments that imply biomass depletion extracted a total oil quantity of 0.70wt%$_{biomass}$ to 0.83wt%$_{biomass}$. The presence of tasmanone in the purge water would explain why all the oil was not extracted in the distillation condensate, despite the apparent biomass depletion. Despite the presence of tasmanone in the purge water, the retrieval of this is not easily achieved through physical processes. The purge water could not be separated by gravity settling or centrifugal separation but was retrieved by solvent extraction. The primary conclusion that can be reached by the presence of substantial tasmanone in the purge water is that heat efficiency within the distillation chamber is extremely important to the efficient steam distillation extraction of Qcide oil. It is clear that heat loss in the chamber results in an increased amount of required purge which accumulates undesirable amounts of important components. Reducing the heat loss, either by vessel or process engineering, should result in a greater yield of Qcide.

4.4.5 Chemical Composition of Distillation Oil

Chemical analysis of oil obtained from steam distillation experiments was conducted at SCU using GC-FID. Detailed chemical analysis identified 21 compounds that could be grouped into 4 broad categories: Monoterpenes and Monoterpenoids, Sesquiterpenes and Sesquiterpenoids, Tasmanone, and Other Triketones. The peak area % content by these 4 categories are shown in Table 4-3, Table 4-4, Table 4-5, Table 4-6, Table 4-7, and Table 4-8.

Table 4-3: Chemical composition of low temperature – low flow (LT-LF) oil samples collected during distillation experiment. Constituent chemicals are grouped into monoterpenes, sesquiterpenes, and triketones.

LT-LF

Experiment Time [minute]	30	60	90	120	180	360
	Peak Area %					
Monoterpenes and Monoterpenoids	14.156	3.687	1.398	0.723	0.332	0.132
Sesquiterpenes and Sesquiterpenoids	2.300	1.803	1.392	1.280	1.563	1.165
Tasmanone	71.716	80.992	83.153	82.422	81.058	76.744
Other Triketones	7.483	10.352	11.130	12.126	14.435	18.903

Table 4-4: Chemical composition of low temperature – high flow (LT-HF) oil samples collected during distillation experiment. Constituent chemicals are grouped into monoterpenes, sesquiterpenes, and triketones.

LT-HF

Experiment Time [minute]	30	60	90	120	180	360
	Peak Area %					
Monoterpenes and Monoterpenoids	8.860	2.327	1.001	0.551	0.286	0.180
Sesquiterpenes and Sesquiterpenoids	1.385	1.232	1.477	1.571	1.905	1.932
Tasmanone	78.818	88.958	89.182	88.269	87.014	84.213
Other Triketones	7.372	5.235	6.017	6.828	7.689	9.101

Table 4-5: Chemical composition of medium temperature – low flow (MT-LF) oil samples collected during distillation experiment. Constituent chemicals are grouped into monoterpenes, sesquiterpenes, and triketones.

MT-LF

Experiment Time [minute]	30	60	90	120	180	360
	Peak Area %					
Monoterpenes and Monoterpenoids	13.792	1.725	0.736	0.236	0.149	0.066
Sesquiterpenes and Sesquiterpenoids	2.928	2.088	1.259	0.959	0.671	0.558
Tasmanone	70.089	84.650	85.329	85.735	85.026	80.690
Other Triketones	7.461	8.371	9.361	10.050	11.550	15.856

Table 4-6: Chemical composition of medium temperature – high flow (MT-HF) oil samples collected during distillation experiment. Constituent chemicals are grouped into monoterpenes, sesquiterpenes, and triketones.

MT-HF

Experiment Time [minute]	30	60	90	120	180	360
	Peak Area %					
Monoterpenes and Monoterpenoids	10.716	1.386	0.435	0.213	0.150	0.110
Sesquiterpenes and Sesquiterpenoids	1.869	1.638	1.369	1.057	0.972	0.924
Tasmanone	79.458	88.578	89.336	88.416	87.070	86.350
Other Triketones	4.142	5.386	5.901	7.138	8.440	9.131

Table 4-7: Chemical composition of high temperature – low flow (HT-LF) oil samples collected during distillation experiment. Constituent chemicals are grouped into monoterpenes, sesquiterpenes, and triketones.

HT-LF

Experiment Time [minute]	30	60	90	120	180	360
	Peak Area %					
Monoterpenes and Monoterpenoids	16.963	0.882	0.356	0.222	0.123	0.108
Sesquiterpenes and Sesquiterpenoids	3.733	2.149	1.082	0.767	0.526	0.307
Tasmanone	67.598	83.592	84.383	83.880	83.315	81.583
Other Triketones	6.499	9.588	10.805	11.875	13.318	15.316

Table 4-8: Chemical composition of high temperature – high flow (HT-HF) oil samples collected during distillation experiment. Constituent chemicals are grouped into monoterpenes, sesquiterpenes, and triketones.

	HT-HF					
Experiment Time [minute]	30	60	90	120	180	360
	Peak Area %					
Monoterpenes and Monoterpenoids	8.521	0.370	0.186	1.311	0.312	0.296
Sesquiterpenes and Sesquiterpenoids	1.779	1.079	0.560	0.575	0.596	0.269
Tasmanone	76.871	81.456	79.513	78.077	73.187	71.962
Other Triketones	5.596	14.467	16.721	16.469	22.378	20.891

Figure 4-7 shows the Peak area % of tasmanone for each oil sample extracted and separated during the processing experiments. All experiments show an initial increase in the tasmanone peak area % followed by a decline over the experimental period. Curiously there is not a clear trend in the Tasmanone peak area in relation to the temperature or flowrate of the specific experiment. The HT-HF experiment shows the lowest tasmanone peak area% of all the experiments, is highest at 60minutes and dramatically decreases before stabilising in the later stages. The MT-HF and LT-HF experiments showed the greatest tasmanone peak area% of the experiments, plateauing at 30 – 60minutes before slowly declining up to the 360th minute. Figure 4-7 indicates that the relative composition of tasmanone in the oil is not strongly dependant on the steam flowrate or the distillation temperature, at least not up to 150°C. If the HT-HF data was to be removed from Figure 4-7: GC-FID analysis of oil collected during distillation experiments. Peak area % for Tasmanone (Tas).Figure 4-7 there would be a clear indication that higher flowrates result in an increase in the relative composition of tasmanone in oil, to explore this possibility increased oil analysis should be conducted on any future experimentation involving flowrate analysis.

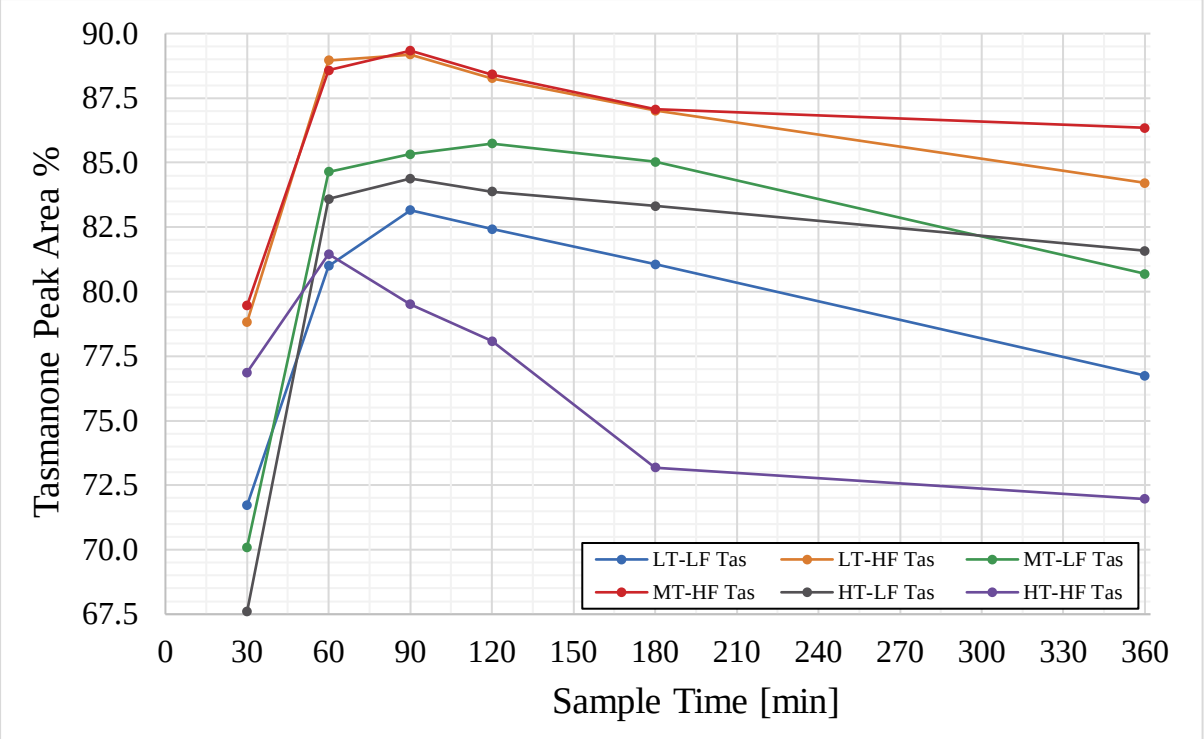

Figure 4-7: GC-FID analysis of oil collected during distillation experiments. Peak area % for Tasmanone (Tas).

Figure 4-8 and Figure 4-9 show the peak area % of Monoterpenes and Sesquiterpenes (respectively) for each sample during experiments. For all experiments the drop in monoterpenes is rapid during the initial stages, with peak area % approaching zero in the late stages. The large initial extraction of these components is likely due to the higher comparative volatility of terpenes, and the smaller molecular size facilitating faster permeation through leaf material. The drop in monoterpene concentration is stronger as the process temperature and flowrate is increased, with the relative monoterpene concentration of the 60 and 90 minute samples in the order: HT-HF < HT-LF < MT-HF < MT-LF < LT-HF < LT-LF. The peak area% of the sesquiterpenes also appears to show a dependence on the distillation temperature. The HT experiments both see a rapid drop in sesquiterpene content in the first 90 minutes followed by a slower decline for the experimental period. The MT experiments show the same sesquiterpene trend as the HT, but after 60 minutes the relative content in the MT remains higher than the HT for the sampling duration. The LT experiments not only see a slower decline in sesquiterpene content during the initial stages, but also see an increase in the relative content in the mid to late sampling period, with the LT sesquiterpene content higher than the MT and HT after the 60th minute of experiments. Looking at Figure 4-13 and the total monoterpene and sesquiterpene content of the oils, there does appear to be a relationship between the relative content and the physical appearance of the oil. While there does not seem to be a specific

threshold for a colour change in the oil, the higher terpene concentrations appear to manifest as green/brown colouration in the oil.

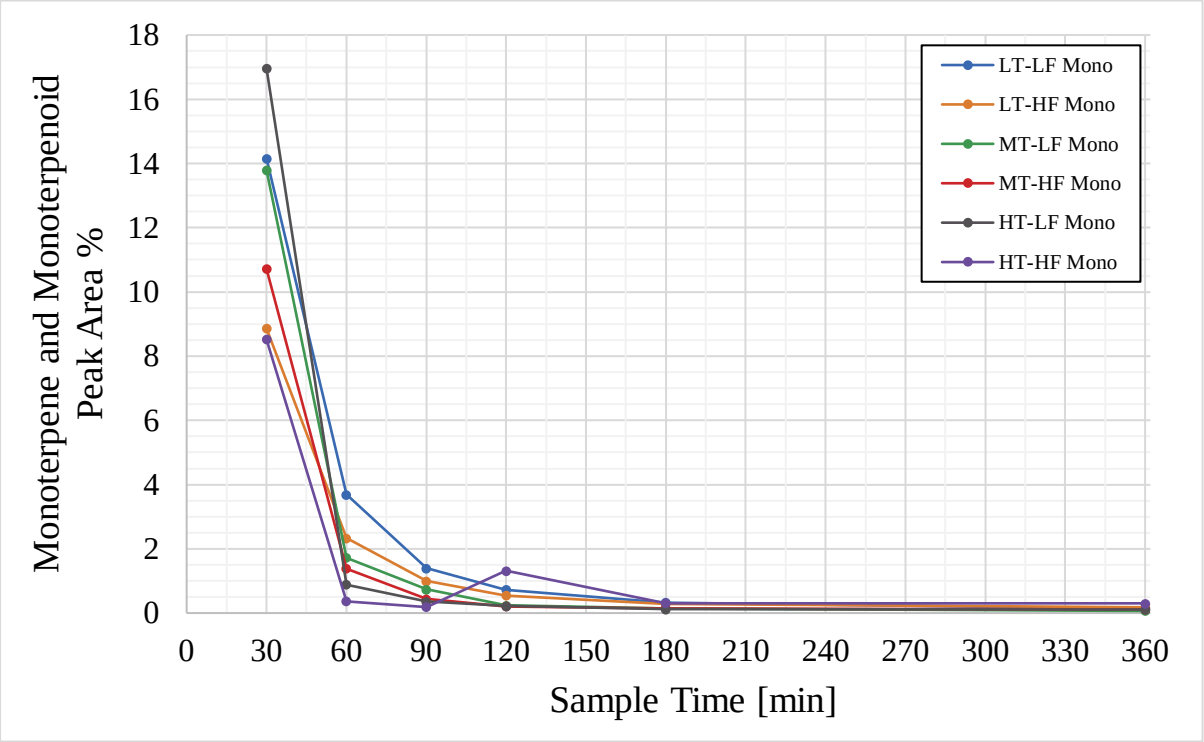

Figure 4-8: GC-FID analysis of oil collected during distillation experiments. Peak area % for Monoterpenes and Monoterpenoids (Mono).

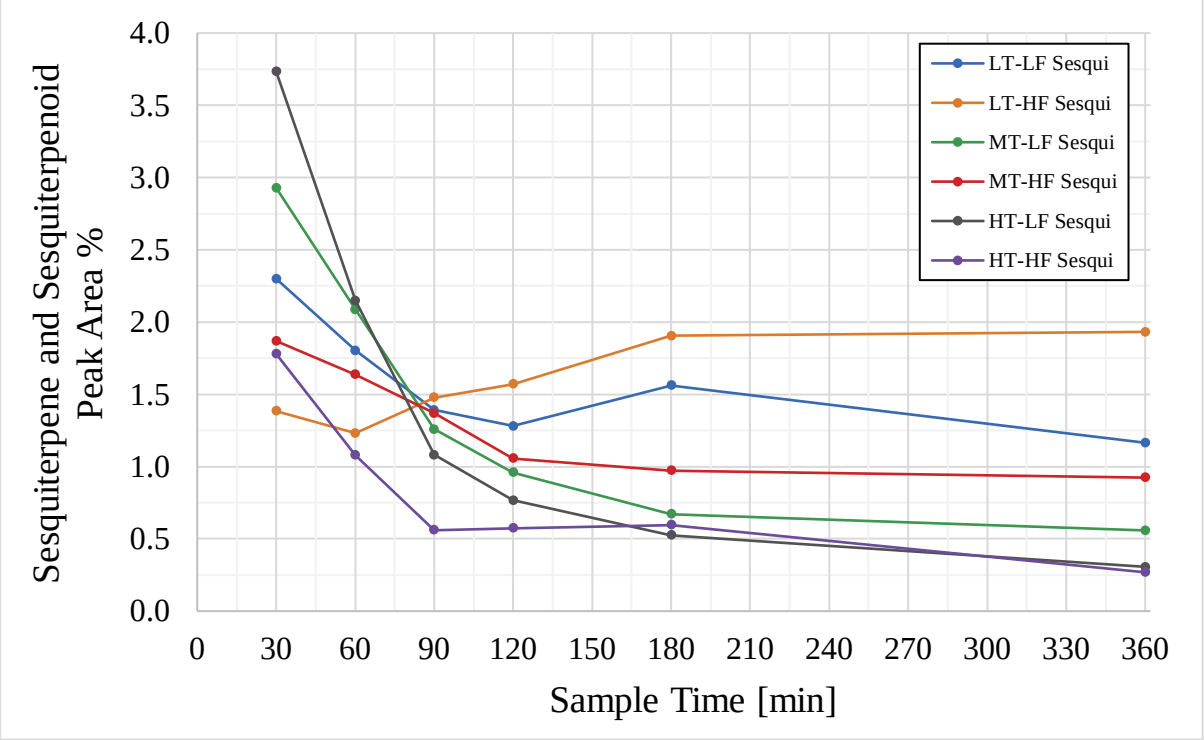

Figure 4-9: GC-FID analysis of oil collected during distillation experiments. Peak area % for Sesquiterpenes and Sesquiterpenoids (Sesqui).

Figure 4-10 shows the peak area% of triketones, other than tasmanone, for each sample during experiments. Curiously this figure appears to show trends that are counter to what can be seen in Figure 4-7, which does not show any clear relationship between content and flowrate or temperature. While all experiments see only small concentrations of other triketones during the early stages, the relative amount increases through the experiment duration, this is likely due to the lower volatility of triketones and the large molecular size of triketones resulting in slower permeation through leaf material and slower transport through distillation. When looking at the total triketone content of the collected oils, as is shown in Figure 4-11, there is only a small difference in the total triketone content between experiments. All experiments show a dramatic increase in triketone content at the 60th minute of distillation, and plateau at approximately 95 peak area % for the experimental duration. The HT-HF experiment does appear to show decreased amounts of tasmanone which is balanced out by an increased amount of other triketones, however this is not seen in the other HT experiment which contains lower amounts of other triketones than the LT-LF experiment.

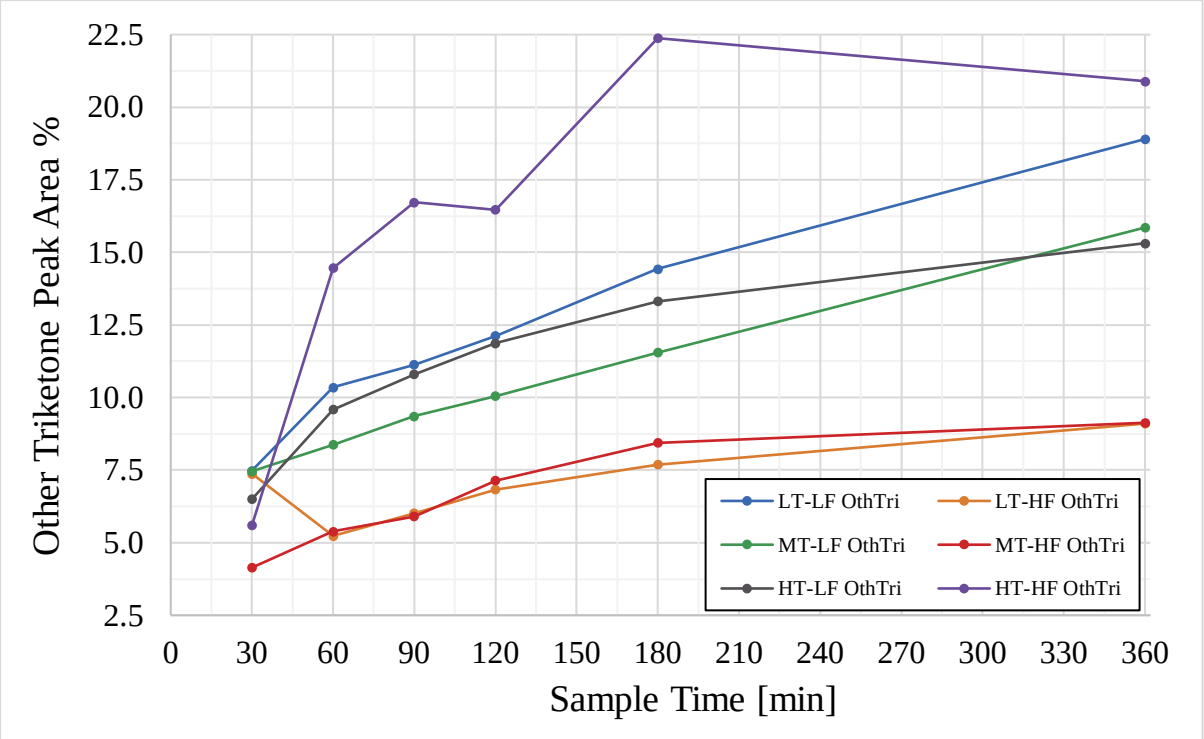

Figure 4-10: GC-FID analysis of oil collected during distillation experiments. Peak area % for Triketones other than Tasmanone (OthTri).

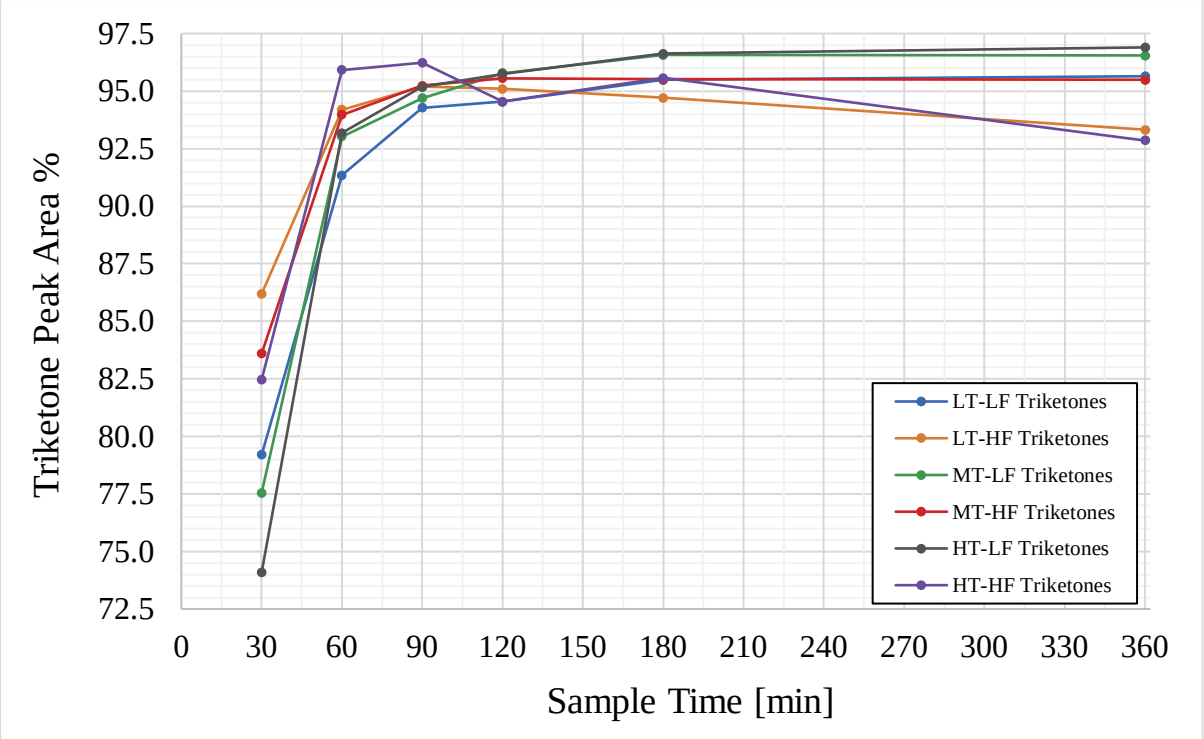

Figure 4-11: GC-FID analysis of oil collected during distillation experiments. Peak area % for all Triketones.

A common issue regarding steam distillation is the formation of undesirable by-products [113]. Looking at the decreasing tasmanone concentration from the 6th hour of distillation, it is expected that extended hydrolysis is causing degradation of some components and formation of new components. Comparing the three plots shown in Figure 4-7, Figure 4-10, and Figure 4-11 shows a reciprocal increase in other heavy components as the tasmanone concentration decreases; this could be indicative of tasmanone degradation but there is no clear trend to support degradation by the experimental parameters.

4.4.6 Tasmanone Yield of Distillation Oil

The maximum theoretical yield of tasmanone from the biomass samples was calculated (in Section 4.4.2) to be 11.52 ± 0.43 $mg_{tasmanone}/g_{biomass}$, or 9.22 ± 0.35g of tasmanone for a distillation of 800g biomass. Using the distillation oil yield shown in Section 4.4.3 and the tasmanone content calculated in Section 4.4.5, the accumulated yield of tasmanone during each experiment was calculated. Figure 4-12 shows the yield of tasmanone during each experiment set, as a percentage of the theoretical maximum ($Yt_{D\ max}$). This tasmanone accumulation tends to be rapid in the early stages of the experiments, with a plateau in the latter stages. The most prominent plateau is shown by the HT-HF experiment; which not only saw a rapid decline in oil extracted during the later distillation stages (Figure 4-2), but also extracted oil with a smaller tasmanone concentration (Figure 4-7). Despite this the HT-HF experiments showed the highest

yield of tasmanone due to the dramatically higher extraction of oil in the first 2 hours of distillation. Despite the difference in tasmanone concentrations discussed in Section 4.4.5, the difference in tasmanone concentrations were not dramatic enough to change the trends discussed in Section 4.4.3. As discussed in Section 4.4.4, there is likely a considerable amount of tasmanone that was removed in the purge water, which would explain why the tasmanone accumulation plateaus at an amount that is considerably lower than theoretical maximum yield.

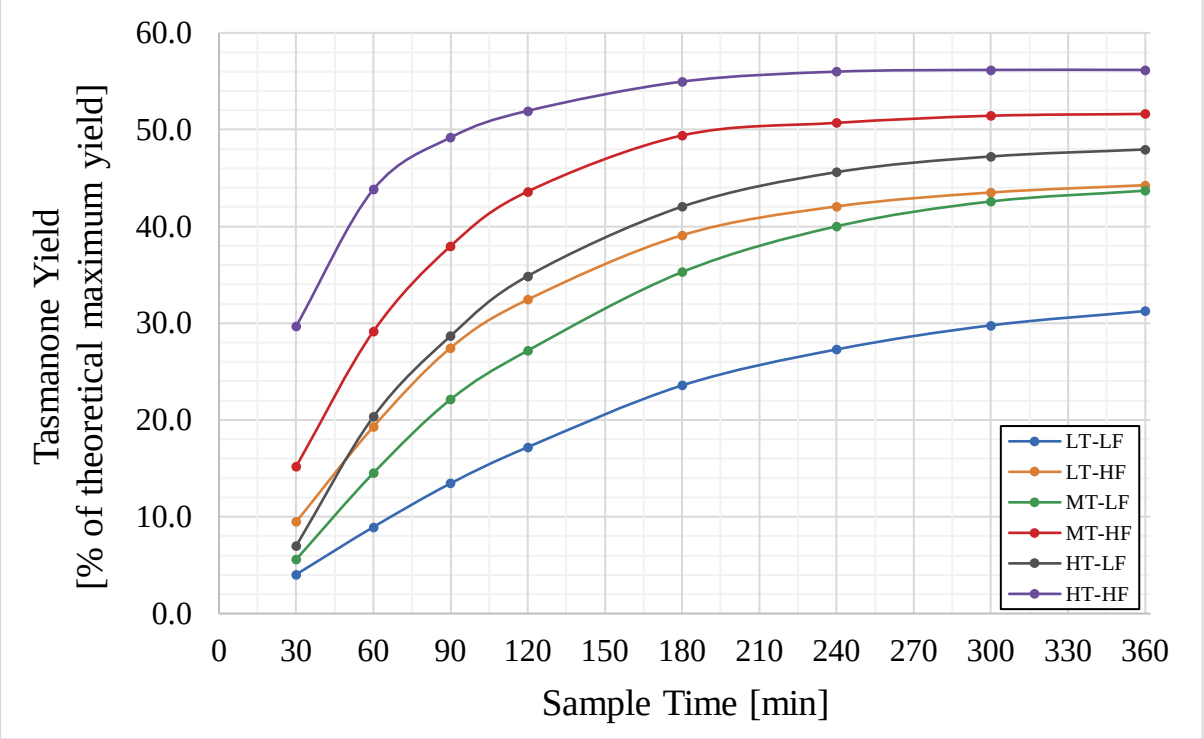

Figure 4-12: Accumulated tasmanone yield from each experimental parameter set, displayed in % of the maximum theoretical yield ($Yt_{D\,max}$).

Looking at each flowrate setting for the experiments shown in Figure 4-12 there is noticeable trend of increased overall yield, and increasing yield rate, as the distillation temperature is increased. When looking at each temperature setting, there is also a dramatic increase in the overall yield and yield rate when increasing the steam input flowrate. An important effect of increasing the steam input flowrate is that it increases the early yield to such a rate that the yield plateau occurs earlier, indicating a much more time efficient distillation. Figure 4-12 shows that there is a similar accumulated tasmanone yield profile in both the LT-HF experiment and the HT-LF for the first two hours of distillation, but as the distillation time increases the yield of the HT-LF experiment plateaus to a greater degree. This indicates that while both increasing temperature and flowrate have positive effects on the yield and rate of tasmanone extraction, temperature may have a more positive effect on the amount of extracted tasmanone and flowrate has a more positive effect on the yield and time efficiency of the overall

extraction process. After the full 360 minute distillation cycle the highest tasmanone yield was achieved by HT-HF (56.17%), then MT-HF (51.62%), HT-LF (47.96%), LT-HF (44.27%), MT-LF (43.70%), and LT-LF (31.26%). As the purpose of these experiments is to find favourable conditions for Qcide extraction, it is important to investigate the efficient tasmanone yield in the first 3 hours of extraction. After 180 minutes of distillation the highest tasmanone yield was achieved by HT-HF (54.97%), then MT-HF (49.40%), HT-LF (42.05%), LT-HF (39.09%), MT-LF (35.29%), and LT-LF (23.57%).

4.4.7 Visual and Olfactory Observations

A total of 19 distillation experiments were conducted at 6 different parameter points (Table 4-2). For each parameter set, a set of samples were chosen that visually represented the amount and physical properties of the whole parameter set, these oil samples are shown in Figure 4-13. The samples displayed in Figure 4-13 show a distinct colour evolution with some of the samples differing from the usual physical appearance range of Qcide oil described in **Section 1.2: Qcide Oil** and shown in Figure 1-1. The oils collected during these experiments display a variety of physical properties, even between short time points with a single experiment. Figure 4-13A (LT-LF) is the closest in colour and aroma to oil currently collected by industrial distillation. The floral biomass aroma is fairly consistent across samples shown in Figure 4-13A (LT-LF), Figure 4-13B (LT-HF), Figure 4-13C (MT-LF), and Figure 4-13D (MT-HF); with a more pleasant odour in the samples that display a brown colour or greenish tinge. The change in colour is an indication of the change in chemical composition, with transition from brown/green to yellow coinciding with a drop in monoterpene content. Samples shown Figure 4-13E (HT-LF), Figure 4-13F (HT-HF) and the latter samples in Figure 4-13D (MT-HF) display a darker colour with an orange tinge rather than yellow. These samples also have an acrid odour, tending toward ash-dirt rather than the aroma of cut biomass. The tendency toward a more orange colour and acrid odour seems to coincide with a higher content of ‘other triketones’ in the oil, as in present in the HT-LF, HT-HF, and latter MT-HF samples.

Another readily observed physical property of the extracted oil is the level of adhesion to the glass vials. While most of the collected ‘pure’ oil will readily form a thin coating on the inside of storage vials, there is a tendency of higher heat oil samples to adhere to glass more readily. This can be seen in Figure 4-13 where sample in E and F show larger masses of oils adhering to the upper reaches of the vials. When the images in Figure 4-13 were captured, all samples had been left undisturbed in a dark cabinet for 30 days. While wettability testing was not within the scope of these experiments, the adhesion tendency of Qcide oil is an important

factor in the post distillation collection of oil. Some anecdotal observations of the physical oil behaviour during these distillations include;

- a tendency of 30min and 60min sample to form irregular oval shapes yellow in colour with a dark coating,
- a tendency of high temperature condensate producing oil that more readily clings to the inside of separation vessels both glass and plastic, and
- a tendency of rapidly cooled condensate samples forming oil droplets that readily cling to any solid surface within the condensate.

These observations were not explored during these experiments; however, they may indicate important Qcide properties for future analysis.

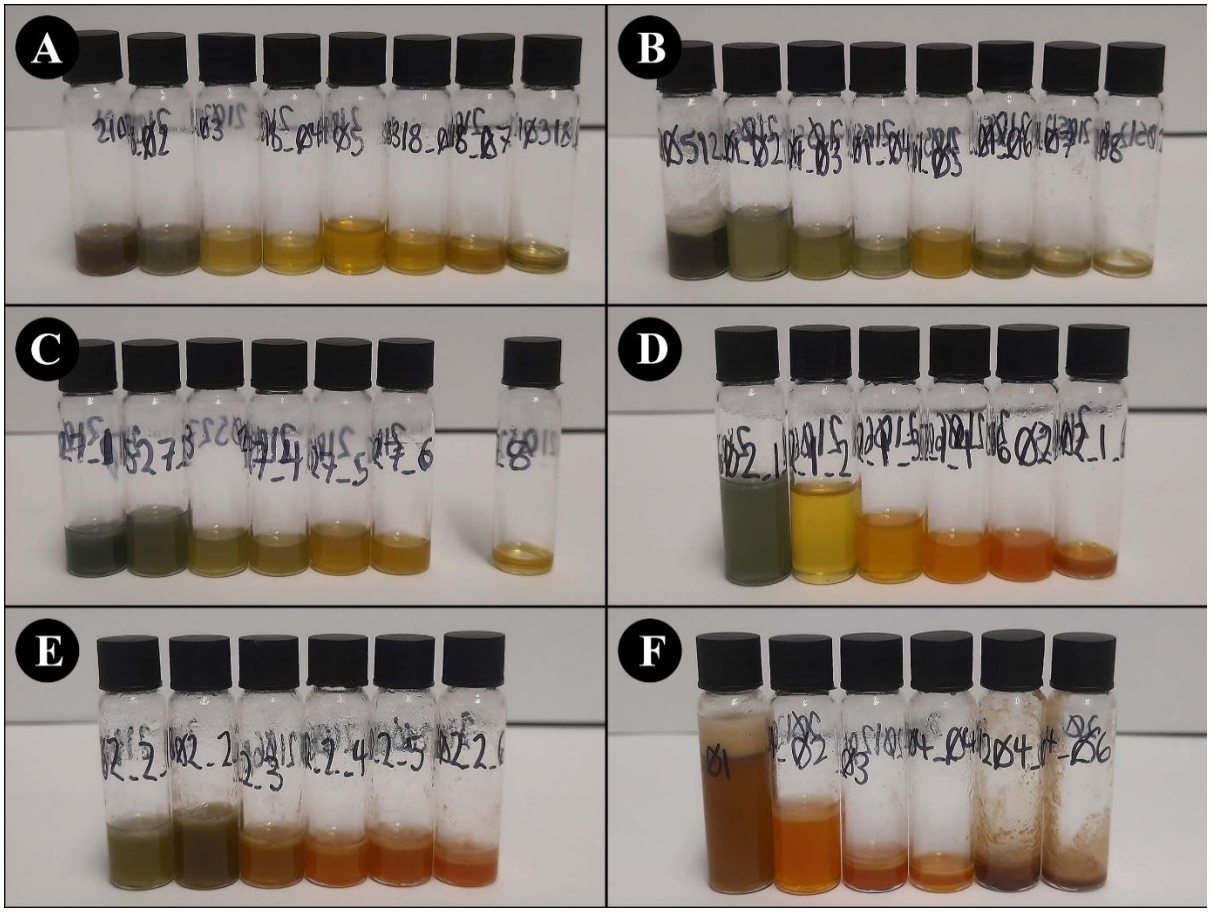

Figure 4-13: Extracted oil samples chosen as a physical reprentation of the oil collected by each parameter experiment. A) LT-LF, B) LT-HF, C) MT-LF, D) MT-HF, E) HT-LF, and F) HT-HF.

4.5 Conclusion

During this study, 19 steam distillation experiments were conducted at six different parameter points: low temperature with low steam flow (LT-LF), low temperature with high steam flow (LT-HF), medium temperature with low steam flow (MT-LF), medium temperature with high steam flow (MT-HF), high temperature with low steam flow (HT-HF), and high temperature with high steam flow (HT-HF). Solvent extraction was also conducted on *E. cloeziana* biomass in order to quantify the tasmanone content of the biomass and estimate the theoretical oil yield. The tasmanone concentration in the biomass was found to be 1.15 ± 0.04 $wt\%_{biomass}$ which leads to a Qcide oil approximation of 1.44 $wt\%_{biomass}$. It was found that after 180 minutes of distillation the accumulated oil yield could be significantly increased, both by increasing the distillation temperature and the steam input flowrate. It was also shown that there was a substantial increase in the extraction rate when increasing the steam flowrate. After 180 minutes of distillation, the total accumulated yield of the HT-HF experiments was 21.4% greater than the next highest yield and was 105.9% greater than the LT-LF control experiments. The accumulated yields in descending order was; HT-HF (0.811±0.054 $wt\%_{biomass}$), MT-HF (0.668±0.062 $wt\%_{biomass}$), HT-LF (0.601±0.036 $wt\%_{biomass}$), LT-HF (0.524±0.038 $wt\%_{biomass}$), MT-LF (0.495±0.062 $wt\%_{biomass}$), LT-LF (0.340±0.018 $wt\%_{biomass}$). Beyond 180 minutes of distillation, the extraction efficiency drops rapidly, especially in higher temperature/flowrate experiments. These experiments indicate that, in order to increase the efficiency of Qcide extraction, distillation should be limited to 3 to 4 hours due to the dramatically reduced extraction efficiency after this period.

The results indicate that Qcide extraction is greater in higher temperature (up to 150°C) and higher steam flowrate without detrimental effects on the oil composition. The tasmanone yield and extraction rate was found to be greater as the distillation temperature and steam input flowrate is increased. After three hours of distillation the tasmanone yield in descending order was; HT-HF (54.97%), MT-HF (49.40%), HT-LF (42.05%), LT-HF (39.09%), MT-LF (35.29%), and LT-LF (23.57%). The efficiency of tasmanone extraction was dramatically reduced after 3 hours of distillation, especially in the higher flow and higher temperature experiments, but the order of experimental tasmanone yield did not change after extending the distillation to 6 hours.

During distillation, internal condensate was purged in order to prevent flooding of the equipment, and contact between the fluid and biomass during distillation. Analysis of a purge water sample found a tasmanone content of 0.39% in that sample, which was comparable to

some of the condensate samples collected during distillation. The nature of the purge water made the separation of tasmanone-rich oil difficult, which indicates the presence and content of the purge water to be undesirable. This undesirable product would be exacerbated by such processes as traditional hydrodistillation and hydrodiffusion and can be eliminated by increased heat efficiency in the process.

5 Effect of Comminution and Steam Input Flowrate on the Extraction of Qcide Oil

Abstract

Current interest in Qcide oil is focussed around the fast and efficient extraction of the oil from *Eucalyptus cloeziana* biomass. This may be achievable both by alteration of process parameters and pre-treatment of biomass. This study seeks to investigate the effect of biomass comminution and variation of steam input flowrate on the extraction yield of Qcide oil during steam distillation. Steam distillation experiments were conducted at ˜125ºC for six hours at two different steam input flowrates and three different levels of comminution. The steam input flowrates were ˜12 g/min for low flow and ˜18g/min for high flow. Comminution was conducted to produce biomass with three different mean surface diameters; (1) 5.57mm for no reduction, (2) 2.91mm for moderate reduction, and (3) 1.55mm for high reduction. The 14 experiments were broken up into 6 unique parameter sets: no reduction with low flow (NR-LF), no reduction with high flow (NR-HF), moderate reduction with low flow (MR-LF), moderate reduction with high flow (MR-HF), high reduction with low flow (HR-LF), and high reduction with high flow (HR-HF). These experiments found a consistent increase in Qcide oil yield with an increase in steam input flowrate. After 3 hours of distillation the yield of the HF experiments was consistently higher than the LF experiments with the same mean surface diameter; NR-HF 1.05 ± 0.04 $Wt\%_{biomass}$, NR-LF 0.76 ± 0.07 $Wt\%_{biomass}$, MR-HF 1.07 ± 0.13 $Wt\%_{biomass}$, MR-LF 0.87 ± 0.08 $Wt\%_{biomass}$, HR-HF 1.19 $Wt\%_{biomass}$, and HR-LF 0.98 $Wt\%_{biomass}$. Increased flowrate resulted in the oil yield being increased by 36.72% for the NR experiments, 22.71% increase for the MR experiments, and a 21.09% increase in the HR experiments. These experiments determined that for each flowrate there was a general trend of increasing oil yield with decreasing mean surface diameter; however, this trend did not hold true for every size reduction, and when the oil yields were determined to be higher the results were only significant when comparing the no reduction experiments to the high reduction experiments.

5.1 Introduction

Chapter 4 investigated effects of temperature and steam input flowrate on the extraction of Qcide oil from *E. cloeziana* biomass and found that increased temperature and flowrate both have a positive effect on the extraction rate and quantity of Qcide oil. This chapter will

investigate the effect of comminution and steam input flowrate on Qcide extraction rate and quantity.

5.2 Experimental

5.2.1 Distillation Biomass

Biomass used as the solid matrix for this experiment was collected and stored as per Section 3.2, the biomass was collected on the 16th of July 2020. In this experiment, the primary goal was to investigate the effect of biomass comminution on the yield of Qcide oil, so detailed size analysis and manipulation was conducted on the biomass prior to distillation, this is detailed in Section 5.2.2.

5.2.2 Particle Size and Surface Area Calculation

The purpose of these experiments was to determine the effect of comminution on the distillation extraction of Qcide oil. Particle size distribution by sieve analysis was used to determine the initial particle size of the biomass, and to determine the extent of comminution. The initial biomass size was calculated from the biomass that was derived directly from the industrial harvester. All experiments conducted using this particle size are referred to as no reduction trials (NR). In order to test the effect of comminution, the particle size of the samples was reduced before distillation. The first size reduction was conducted by reducing the mean particle size to approximately one half that of the no reduction trials, and are referred to as moderately reduced trials (MR). The second size reduction was conducted by reducing the mean particle size to approximately one quarter that of the no reduction trials, and are referred to as highly reduced trials (HR).

The particle size distribution was calculated by sieve analysis using laboratory classification sieves of aperture sizes 19.00mm, 9.50mm, 4.75mm, and 2.36mm (Figure 5-1). Sieve analysis was conducted on biomass samples of approximately 1000g using mechanical shaking, and the distribution was considered complete when the change in mass increments were less than 1%. Once the mass fractions of a sample were determined, a representative sample was taken from each size group to prepare an 800g sample for steam distillation. For samples that required comminution of biomass, this was achieved by using a Anko FP9053KB-GS food processor to chop biomass for 90 second intervals. Portions of biomass were comminuted from the greatest particle size fraction until the required mean particle size was achieved.

Figure 5-1: Laboratory Classification Sieves with Biomass Separated into Representative Size After Sieve Analysis.

In this experiment series the particle size was determined by the surface mean diameter calculated through methods described by Allen [114]. This method allows for calculation of a surface mean diameter for particles that are not cuboid or spherical and takes into account the sphericity, surface area, and density on the particles under investigation. The surface area of the biomass used in distillation is an important factor as the available interfacial area for oil transport is critical for distillation. The equations used to calculate the surface mean diameter, the surface area, and the sphericity of particles are shown below:

$$D_s = \frac{1}{\sum_{i=1}^{n} \frac{x_i}{D_{pi}}} \tag{5.1}$$

$$A_w = \frac{6}{\phi \rho_p} \sum_{i=1}^{n} \frac{D_{pi}}{x_i} \tag{5.2}$$

$$\phi = \left(\frac{d_p}{d_s}\right)^2 \tag{5.3}$$

D_s is the surface mean diameter

x_i is the mass fraction of the biomass on the i^{th} sieve

n is the number of sieves used (including the base pan)

D_{pi} is the average aperture size between sieve i and sieve i+1

A_w is the specific surface area of the biomass particles

ϕ is the sphericity of the biomass particles

ρ_p is the density of biomass particles

d_p is the volume equivalent diameter of biomass particles

d_s is the surface equivalent diameter of biomass particles

The surface area and density calculations were conducted using image analysis with ImageJ, size measurements using digital callipers, and mass measurements using OHAUS pioneer series scales. The thickness of the leaves was measured across the base, centre, and top of each leaf so that an average leaf thickness could be calculated. After calculating the surface area and average thickness of the analysed leaves, the density was calculated using the measured mass. The biomass samples were visually assessed and were found to contain approximately 90% leaf material, as such the calculated leaf density was assumed to be the density of the entire biomass.

5.2.3 Distillation

Steam distillation experiments were performed at JCU laboratories using equipment described in Section 3.3. Distillation was conducted on 800g biomass samples that were prepared as described in Section 5.2.2**.** Distillation was conducted at ˜125°C for a six-hour period which was initiated after the first drop of condensed distillate was collected. Condensed distillate samples were collected every hour, after the first drop was detected, and at each sampling time the internal condensate was purged. The purge was conducted manually by opening the purge valve and allowing the liquid purge water to flow into the collection vessel. Distillation experiments were conducted at three levels of biomass comminution; no reduction, moderately reduced, and highly reduced. The three reduction sizes were distilled at two steam input flowrates: low flow at ˜12g/min, and high flow at ˜18g/min. In total there was 14 experiments, conducted at 6 parameter sets; no reduction low flow (NR-LF, 3 serials), no reduction high flow (NR-HF, 3 serials), moderately reduced low flow (MR-LF, 3 serials), moderately reduce high flow (MR-HF, 3 serials), highly reduced low flow (HR-LF, 1 serial), highly reduced high flow (HR-HF, 1 serial).

The oil was separated from the collected condensate samples by gravity and centrifugal separation, all oil and condensate collected were quantified by mass. An experimental serial from each parameter point (NR-LF, NR-HF, MR-LF, MR-HF, HR-LF, and HR-HF) was chosen for chemical analysis for tasmanone content. The samples chosen for chemical analysis were prepared at the engineering laboratories and chemical analysis was conducted at the JCU-AAC.

5.2.4 Solvent Extraction from Biomass

Solvent extraction was conducted on *E. cloeziana* biomass in order to determine the tasmanone concentration per weight of biomass and establish a theoretical maximum yield of tasmanone. The solvent extraction was conducted in triplicate to confirm the tasmanone concentration. After size distribution was determined, while collecting a distillation sample as described in Section 5.2.2, a 25g sample of biomass was collected using the same size distributions as the 800g distillation sample. For solvent extraction ethyl acetate was chosen due to its efficacy in solvating ketonic compounds [115]. The 25g biomass samples were pulverised using a food processor prior to solvent extraction. The extraction was conducted by mixing with 150ml of ethyl acetate for 24 hours. The ethyl acetate portion was separated and de-watered with sodium sulphate, which was removed with any small particulates, by vacuum filtration. The ethyl acetate was removed from the product using a Heidolph rotary evaporator under reduced pressure. The oil was analysed for tasmanone content by GC-FID at the JCU-AAC.

5.2.5 Chemical Analysis

Chemical analysis for tasmanone content was conducted at JCU-AAC as detailed in Section 3.4. These analyses were conducted in order to establish a theoretical maximum yield of tasmanone from *E. cloeziana* biomass, and to analyse the effect of comminution and steam input flowrate on the tasmanone content of oil extracted by steam distillation.

5.3 Results and Discussion

5.3.1 Particle Size Reduction and Calculation

Sieve analysis was conducted on uncut biomass samples to find the particle size that would be used for the no reduction biomass. Using Equation 5.1 the average surface mean diameter of uncut biomass was calculated as 5.57mm. Using the surface mean diameter of 5.57mm as the no reduction experiments, surface mean diameter to be used for the moderate reduction experiments was determined to be ˜2.78mm, and the surface mean diameter to be used for the high reduction experiments was determined to be ˜1.39mm.

As the biomass was comminuted to the lowest surface diameter there was noticeable effects of the plant morphology. The biomass was seen to break down into fibrous particles that would interlock with surrounding particles, this could form “fluffy” agglomerates that would make sieving more challenging. The packing of the biomass into the distillation chamber can be seen

in Figure 5-2. The highly reduced biomass (Figure 5-2B) is more consistent and pack more uniformly, reducing the total volume required within the distillation chamber.

Figure 5-2: Biomass packing in distillation chamber. A) Uncut biomass, B) High reducion biomass.

The size fractions for all experiments are shown in Table 5-1, this represents the size fractions after comminution was carried out to reduce the mean surface diameter to the desired sizes of ˜2.78mm for moderate reduction and ˜1.39mm for high reduction. The size fractions are expressed by the “fraction midpoint” which is the average of the aperture size of the sieve that the biomass rests on and the aperture size of the sieve above where the biomass rests. Table 5-1 also displays the mean surface diameter for each trial, the average mean surface diameters for each reduction size was calculated to be; NR 5.57 ± 0.45mm, MR 2.91 ± 0.09mm, HR 1.55mm. The size distributions are shown in Figure 5-3, this figure shows a clear distinction in the biomass size between the three different reduction parameters.

Table 5-1: Particle size fractions determined by sieving and calculated mean surface diameter for each trial.

Trial	Parameters	Particle size fraction midpoint [mm]					Mean Surface Diameter [mm]
		1.18	3.56	7.13	14.25	28.25	
		Biomass Fraction					
1	NR-LF	0.09	0.10	0.25	0.40	0.16	5.83
2	NR-LF	0.11	0.12	0.34	0.27	0.16	4.99
3	NR-LF	0.07	0.12	0.29	0.31	0.22	6.29
4	NR-HF	0.11	0.14	0.27	0.38	0.12	5.05
5	NR-HF	0.09	0.11	0.27	0.32	0.21	5.74
6	NR-HF	0.09	0.12	0.28	0.34	0.17	5.56
7	MR-HF	0.26	0.27	0.47	nil	nil	2.76
8	MR-LF	0.25	0.24	0.51	nil	nil	2.84
9	MR-HF	0.20	0.30	0.50	nil	nil	3.06
10	MR-LF	0.24	0.25	0.51	nil	nil	2.90
11	MR-LF	0.24	0.27	0.50	nil	nil	2.89
12	MR-HF	0.22	0.30	0.49	nil	nil	2.97
13	HR-LF	0.64	0.36	nil	nil	nil	1.55
14	HR-HF	0.64	0.36	nil	nil	nil	1.55

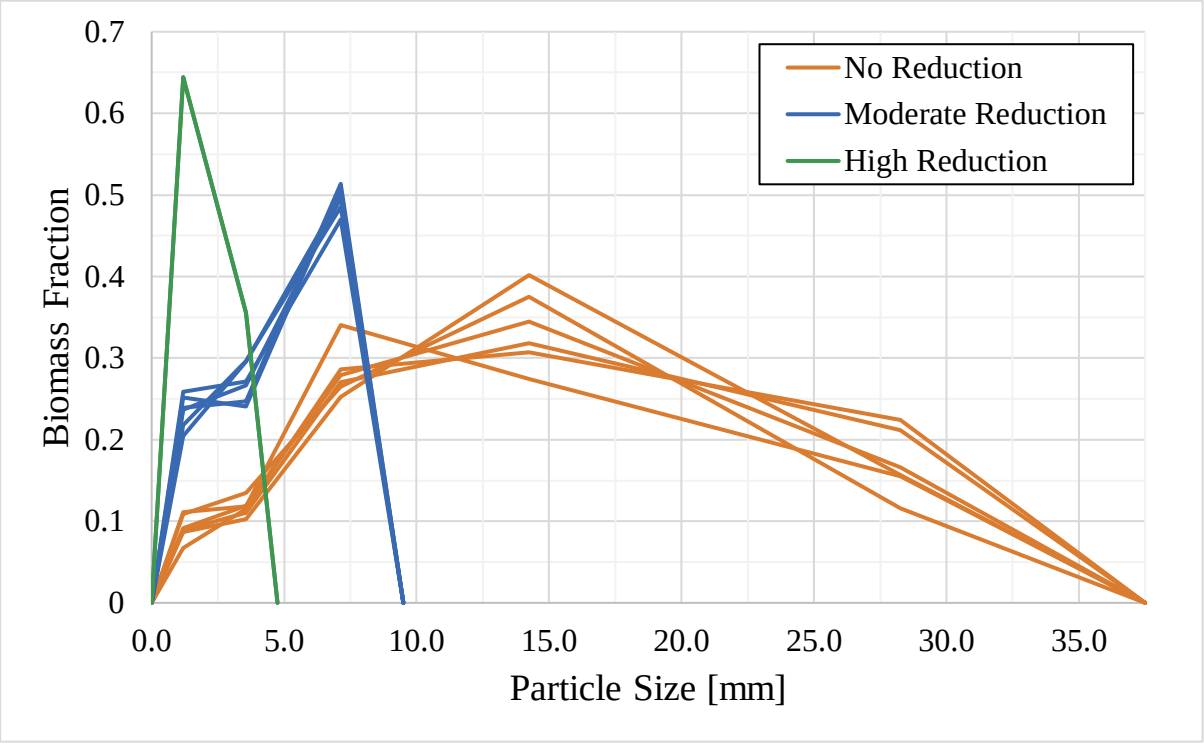

Figure 5-3: Size distribution of distillation biomass used in comminution experiments.

5.3.2 Surface Area Calculation

E. cloeziana Leaf surface area analysis was conducted using imageJ software, with thickness measurements conducted with electronic callipers and weight measurements done with electronic scales. The thickness across the base, centre, and top of the leaves were measured and the average leaf thickness was calculated to be 0.26mm. The volume of each leaf was calculated by multiplying the thickness by the measured leaf surface area, and the density was calculated by division of the leaf mass by the calculated volume. These measurements were conducted on 4 leaves, the results of which is shown in Table 5-2.

Table 5-2: Leaf dimensions

Leaf	Mass [g]	Volume [mm^3]	Density [mg/mm^3]
1	0.0956	154.56	0.000619
2	0.1915	279.24	0.000686
3	0.0852	125.45	0.000679
4	0.0989	161.90	0.000611

From Table 5-2, the average density of *E. cloeziana* leaves was calculated to be 6.49×10^{-4} g/mm^3. Although the biomass contained impurities from weeds, and woody material, the biomass samples were found to be made up of approximately 90% *E. cloeziana* leaves. The density of the *E. cloeziana* leaves was assumed to be the density of the biomass samples for the purpose of calculations during this experiment. From a visual inspection of the biomass, it was determined that most of the plant material is consistent with a cylindrical shape, where its sphericity for each size increment was determined with equation 5.3. With the sphericity determined, equation 5.2 was used to calculate the specific surface area, which was further used to determine the interfacial surface area of each sample. The specific surface area, and the calculated surface area for each sample is shown in Table 5-3. The calculated surface area for each of the size reduction categories is; NR 3.97 ± 0.04m^2, MR 5.54 ± 0.08m^2, and HR 7.94m^2. With the reduction in particle size for each category the overall contact surface area is significantly increased. If there is any significance in the oil yield for each size category, there will be evidence to suggest whether size reduction had correlation with Qcide yield.

Table 5-3: Experimental parameters for each trial with calculated particle size and surface area.

Trial	Temperature [°C]	Steam Input Flowrate [g/min]	Ds [mm]	SSA [m2/kg]	SA [m2]
No Biomass Reduction (NR)					
1	124.7	12.73	5.83	4.85	3.88
2	124.4	12.58	4.99	5.19	4.16
3	125.1	12.66	6.29	4.71	3.77
4	124.2	17.90	5.05	5.17	4.13
5	125.2	17.83	5.74	4.87	3.89
6	124.9	18.13	5.56	4.95	3.96
Moderate Biomass Reduction (MR)					
7	125.2	18.44	2.76	7.09	5.67
8	125.5	12.04	2.84	6.99	5.59
9	124.9	18.54	3.06	6.75	5.40
10	125.0	11.62	2.90	6.91	5.53
11	125.0	12.21	2.89	6.93	5.54
12	124.7	18.27	2.97	6.85	5.48
High Biomass Reduction (HR)					
13	125.1	11.91	1.55	9.92	7.93
14	124.8	18.61	1.55	9.92	7.94

5.3.3 Tasmanone Content of Biomass

Oil extracted through ethyl acetate maceration was isolated using a rotary evaporator and analysed using GC-FID at the JCU-AAC. Three solvent extractions were conducted and analysed, the results of these are shown in Table 5-4. Equation 3.5 and Equation 3.6 were used to calculate the theoretical maximum yield of tasmanone in the *E. cloeziana* biomass ($Yt_{b\ max}$) and the theoretical maximum yield of tasmanone from a distillation of an 800g *E. cloeziana* biomass sample ($Yt_{D\ max}$). The calculated mean and standard deviation of the tasmanone yield from biomass was 19.15 ± 2.05 $mg_{tasmanone}/g_{biomass}$, or 1.92 ± 0.20 $wt\%_{biomass}$. The theoretical maximum yield of tasmanone from a distillation of an 800g *E. cloeziana* biomass sample was calculated to be 15.32 ± 0.86 grams.

Table 5-4: Results of GC-FID analysis on oil extracted from *E. cloeziana* biomass by ethyl acetate maceration.

Sample	$m_{b\ solvent\ extraction}$ [g]	$m_{extract}$ [g]	$C_{extract\ GC}$ [mg/ml]	$C_{t\ GC}$ [mg/ml]	$Yt_{b\ max}$ [mg/g]
1	25.00	1.5630	10.38	3.2765	19.74
2	25.00	1.7466	10.18	3.1055	21.32
3	25.00	1.3537	10.10	3.0592	16.40
Mean and Standard Deviation					19.15 ± 2.05

This analysis was conducted on *E. cloeziana* biomass collected in July from a single growing region in North Queensland. This analysis of *E. cloeziana* biomass approximated a tasmanone content that differed from that which was calculated in Section 4.4.2. The difference in tasmanone content indicates that there is a possibility of variable tasmanone content that would require further investigation. Three possibilities for this variability are: 1) Seasonal variation in tasmanone content causing *E. cloeziana* to have higher concentration in July compared to November; 2) Variations in specific growing area within *E. cloeziana* plantations/individual trees caused by age, coppice level, fire ecology, and cultivation conditions; and 3) Variation in efficacy of solvent extraction. In order to assess the accuracy of the tasmanone-biomass concentration, extended seasonal, conditional, and solvent extraction analysis would be required.

5.3.4 Distillation Oil Yield

The parameters of each experiment are shown in Table 5-3, all experiments were conducted with a distillation temperature of ˜125°C. There were 6 unique parameter sets with a number of experiments conducted for each parameter set: NR-LF 3 serials, NR-HF 3 serials, MR-LF 3 serials, MR-HF 3 serials, HR-LF 1 serial, HR-HF 1 serial. The oil extracted from distillation experiments is shown in Appendix C, the results are shown as a mean and the standard deviation of experiments conducted at each parameter point specified in Table 5-3.

The oil yields from the low flow experiments are shown in Figure 5-4. For the NR and MR experiments the largest amount of oil was collected during the first two hours, after which the individual yield declines rapidly until the 4th hour, where the decline becomes more gradual. The largest individual yield was seen in the HR sample in the second hour of extraction (0.41 Wt%$_{biomass}$/hour), this distillation showed increased extraction during the first 3 hours then declined rapidly in the 4th hour to become comparable to the NR and MR experiments.

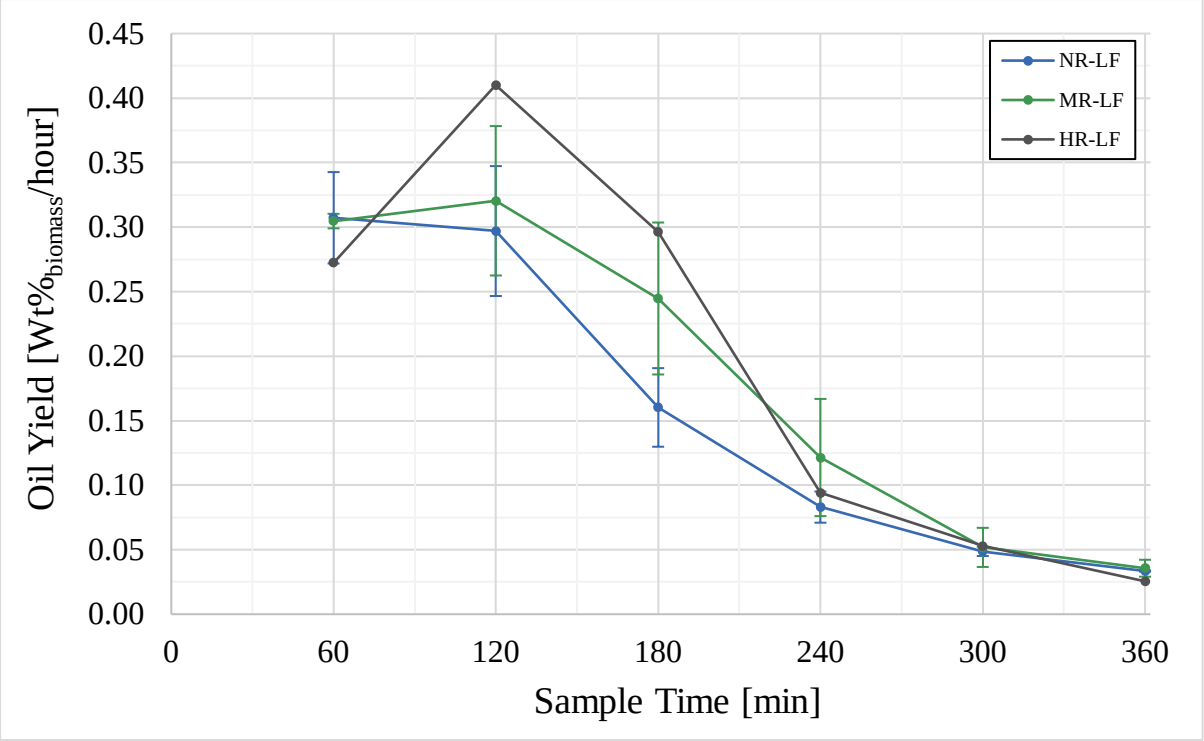

Figure 5-4: Sample oil yield for low flow experiments, displayed in wt% of the biomass per hour, showing mean and standard deviation of all LF experiments.

The accumulation of Qcide oil over the low flow experimental period is shown in Figure 5-5. This plot shows that there was very little change in the total oil yield after the 4th hour of the low flow experiments. After the first 4 hours the accumulated oil yields were 0.85 $Wt\%_{biomass}$ (NR-LF), 0.99 $Wt\%_{biomass}$ (MR-LF), and 1.07 $Wt\%_{biomass}$ (HR-LF); which represented 91.2% (NR-LF), 91.9% (MR-LF), and 93.2% (HR-LF) of the yield that was achieved over the full 6 hours of distillation. Given the comparative amount of oil that is extracted in the first 4 hours of distillation, it is inefficient to continue low flow distillation at 125°C longer than 4 hours. When comparing the oil accumulation in the different particle sizes, there is increasing accumulation with reduced particle size; however, these increases are only statistically significant when comparing the HR experiments to the NR, or the HR to the MR from the 3rd hours of accumulation. The difference in yield between particle sizes is visually apparent from the 3rd hour of distillation, at this point the MR experiments represent a 13.8% increase in yield compared to the NR and the HR represent a 12.5% increase compared to the MR.

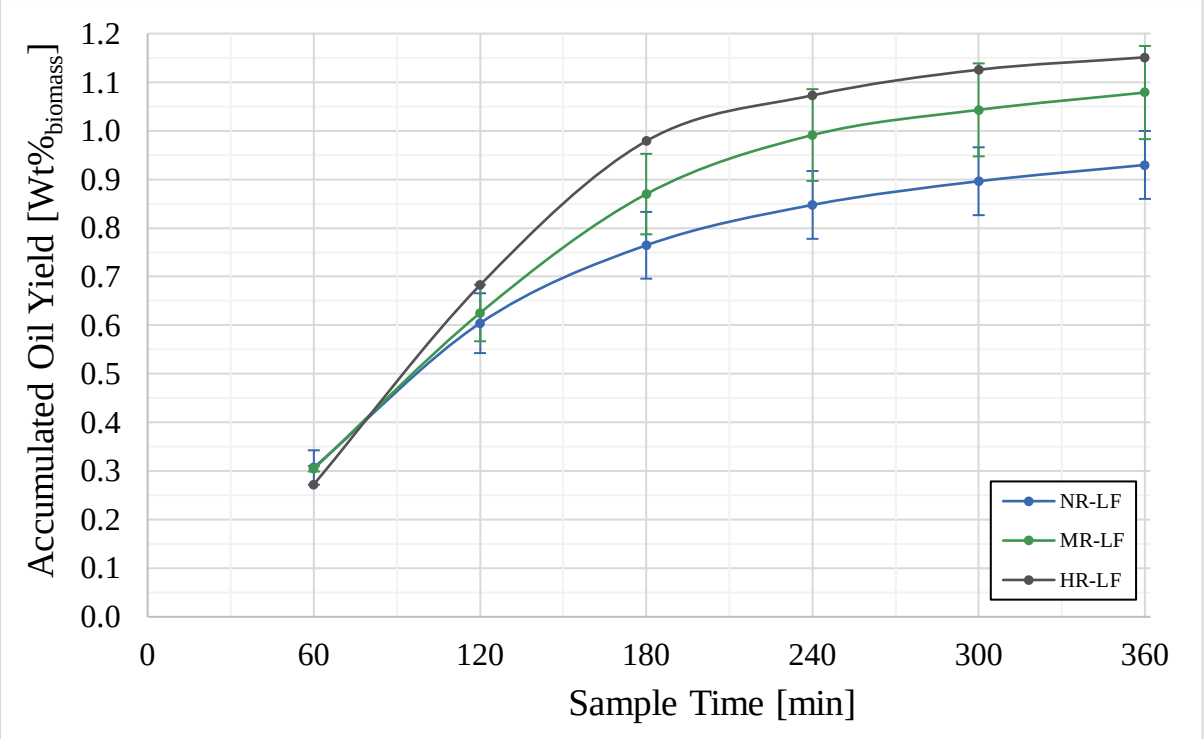

Figure 5-5: Total accumulated oil from samples in low flow experiments, displayed in wt% of the biomass, showing mean and standard deviation of all LF experiments.

The oil yields from the high flow experiments are shown in Figure 5-6. These experiments show the highest individual oil yields in the first hour of the experiments (NR-HF 0.55 ± 0.02 $Wt\%_{biomass}$/hour, MR-HF 0.61 ± 0.04 $Wt\%_{biomass}$/hour, HR-HF 0.60 $Wt\%_{biomass}$/hour), after which the yields decreased in an exponential fashion over the 6 hour experimental period. Although the yield of each experiment declined in a similar way, the yield decline was slightly lower in the HR-HF experiment, and slightly higher in the MR-HF experiment.

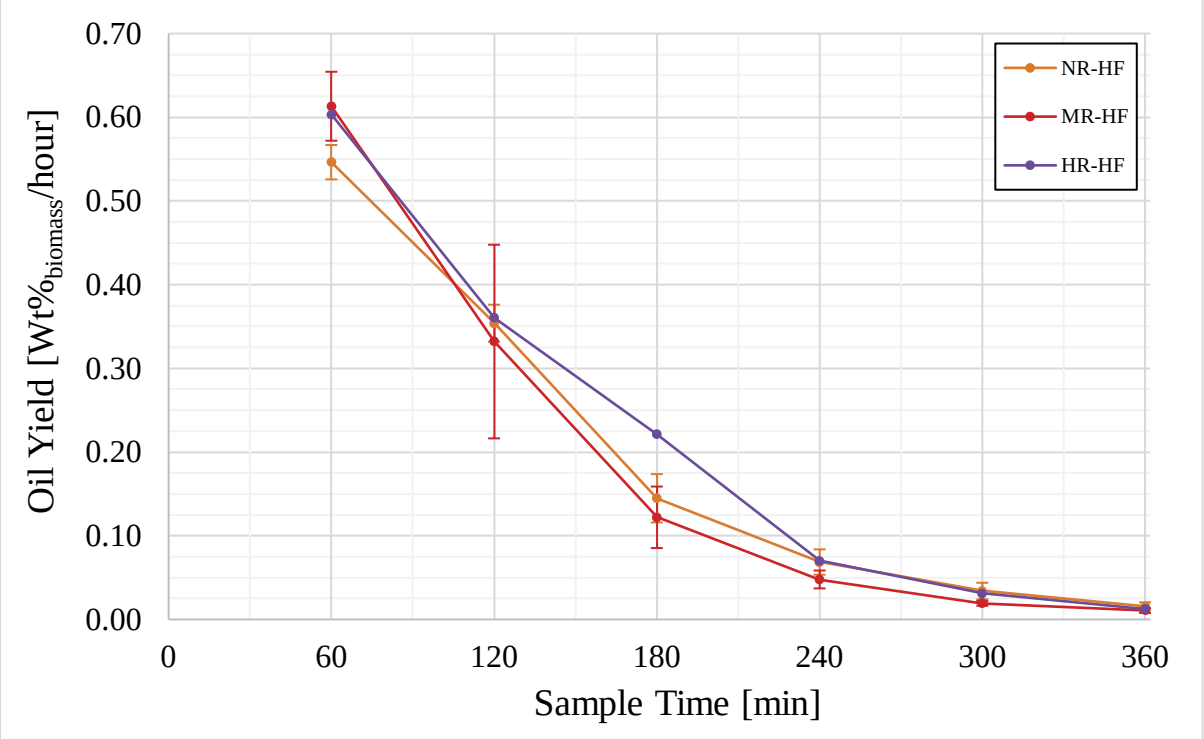

Figure 5-6: Sample oil yield for high flow experiments, displayed in wt% of the biomass per hour, showing mean and standard deviation of all HF experiments.

The accumulation of Qcide oil over the high flow experimental period is shown in Figure 5-7. This accumulation plot shows that there is little change to the total amount of oil accumulated by high flow experiments after the 3rd hour of distillation. After the first 3 hours the accumulated oil yields were 1.05 $Wt\%_{biomass}$ (NR-HF), 1.07 $Wt\%_{biomass}$ (MR-HF), and 1.19 $Wt\%_{biomass}$ (HR-HF); which represented 89.83% (NR-HF), 93.21% (MR-HF), and 91.24% (HR-HF) of the yield that was achieved over the full 6 hours of distillation. Given the comparative amount of oil that is extracted in the first 3 hours of distillation, it is inefficient to continue high flow distillation at 125°C longer than 3 hours. When comparing the oil accumulation in the different particle sizes, there is no significant difference between the oil accumulated from the NR-HF and the MR-HF experiments. There is an increase in the accumulation for the HR experiment due to the slightly higher initial yield and slower decline in oil yield in the 3rd and 4th hours of the experiment; however, the increased oil yield is only statistically significant when comparing the HR to the NR experiments. After 3 hours of distillation the accumulated oil yield of the HR experiment is 11.0% greater than the MR experiments and 13.4% greater compared to the NR experiments.

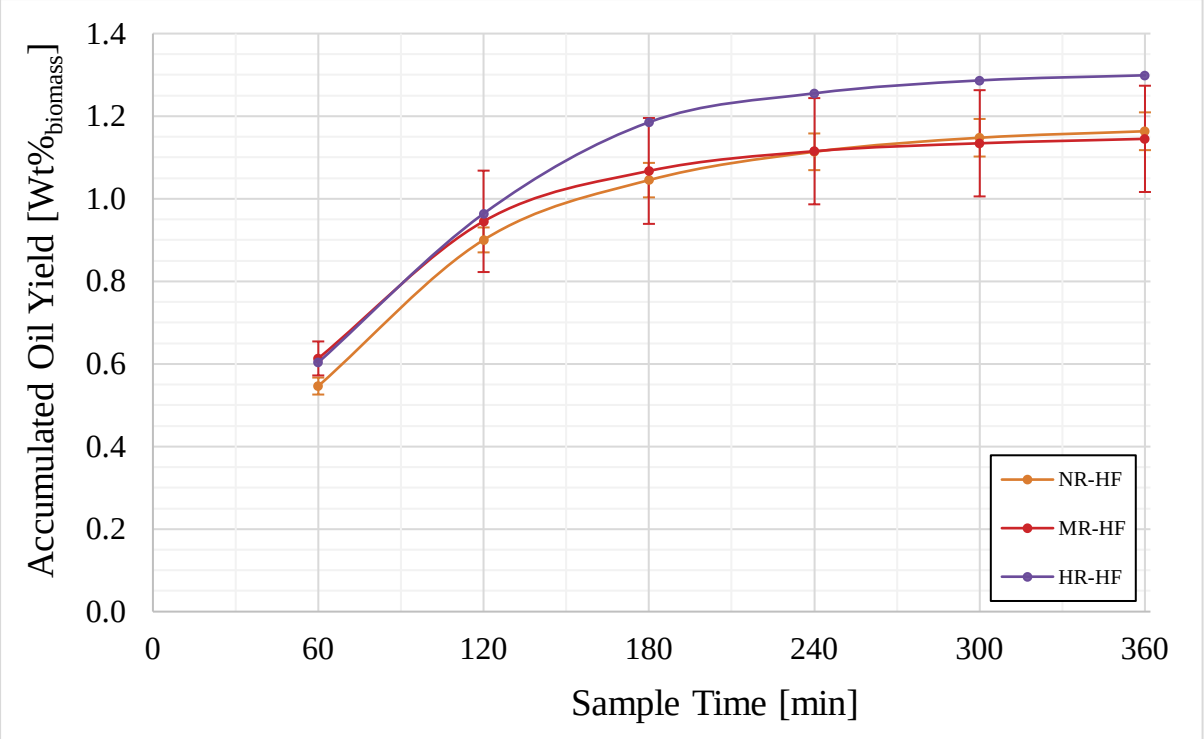

Figure 5-7: Total accumulated oil from samples in high flow experiments, displayed in wt% of the biomass, showing mean and standard deviation of all HF experiments.

There is a noticeable difference in accumulated oil between the low flowrate and high flowrate experiments, with all the high flow experiments extracting greater amounts of oil than the low flow counterpart in the same size category. When looking at the accumulated oil yield by the high flow experiments after 3 hours (NR-HF 1.05 $Wt\%_{biomass}$, MR-HF 1.07 $Wt\%_{biomass}$, and HR-HF 1.19 $Wt\%_{biomass}$); it should be noted that of the low flow counterparts, the MR-LF takes 6 hours to achieve a similar amount (1.08 $Wt\%_{biomass}$), and the HR-LF takes 4 hours (1.07 $Wt\%_{biomass}$). This is a clear indication that the extraction rate and quantity of Qcide oil is increased by increasing the steam input flowrate, regardless of biomass particle size. At this stage while there does appear to be an increase in the oil yield when smaller biomass particle size is used, the effects of this is seen to a greater degree in low flowrate distillations, and the increase is not always significant.

While the particle size does increase the oil yield, the observed effect is overshadowed by the much more dramatic effect that higher flowrate has on the increase of oil yield, with the LF experiments all consistently yielding less oil than the HF at all comminution levels. Another consideration for the size reduction experiments is the mixed results in the moderately reduced biomass experiments. When comparing the NR-HF and MR-HF experiments (Figure 5-6), it can be seen that the MR-HF experiment only extracts more oil than the NR-HF experiment in the first hour. From the second hour of distillation the individual oil yields drops below the

NR-HF experiments, but the accumulated oil yield of the NR-HF experiment does not surpass that of the MR-HF until the 5th hour of distillation. For the high flow experiments, the greatest extraction efficiency seems to occur after 3 hours of distillation, at this time the accumulated oil yield for MR-HF is 1.07 ± 0.13 $Wt\%_{biomass}$, and the accumulated oil yield for NR-HF is 1.05 ± 0.04 $Wt\%_{biomass}$. Taken to the full six hours of distillation, the accumulated oil yield for MR-HF is 1.15 ± 0.13 $Wt\%_{biomass}$, and the accumulated oil yield for NR-HF is 1.16 ± 0.05 $Wt\%_{biomass}$. Comparing accumulated oil yield of the different particle size experiments at 3 hour and 6 hours: NR→MR after 3 hours results in a 13.8% increase for low steam flow and a 2.1% increase for high steam flow. NR→MR after 6 hours results in a 16.0% increase for low steam flow and a 1.6% decrease for high steam flow. MR→HR after 3 hours results in a 12.5% increase for low steam flow and a 11.1% increase for high steam flow. MR→HR after 6 hours results in a 6.7% increase for low steam flow and a 13.4% increase for high steam flow. The results show an increase in the Qcide oil yield when reducing the surface mean diameter of *E. cloeziana* biomass; however, these results were not always statistically significantly, and the greatest significance is seen when comparing the extremely reduced particle size of ˜1.55mm to the largest particle size.

5.3.5 Chemical Composition of Distillation Oil

GC-FID was conducted at the JCU-AAC on a selected array of oil samples, chosen to be representative of each parameter set in these experiments. All oil samples collected from the selected trials were analysed by GC-FID to investigate the tasmanone content of extracted oil. The experiments chosen for GC-FID were trials 2, 4, 10, 12, 13, and 14. These trials were found to be the best representative runs in accordance with the target steam input flow rate, distillation temperature, and particle size distribution. The tasmanone concentration for all analysed samples is shown in Figure 5-8. All samples were shown to have a tasmanone concentration greater than 81.75%.

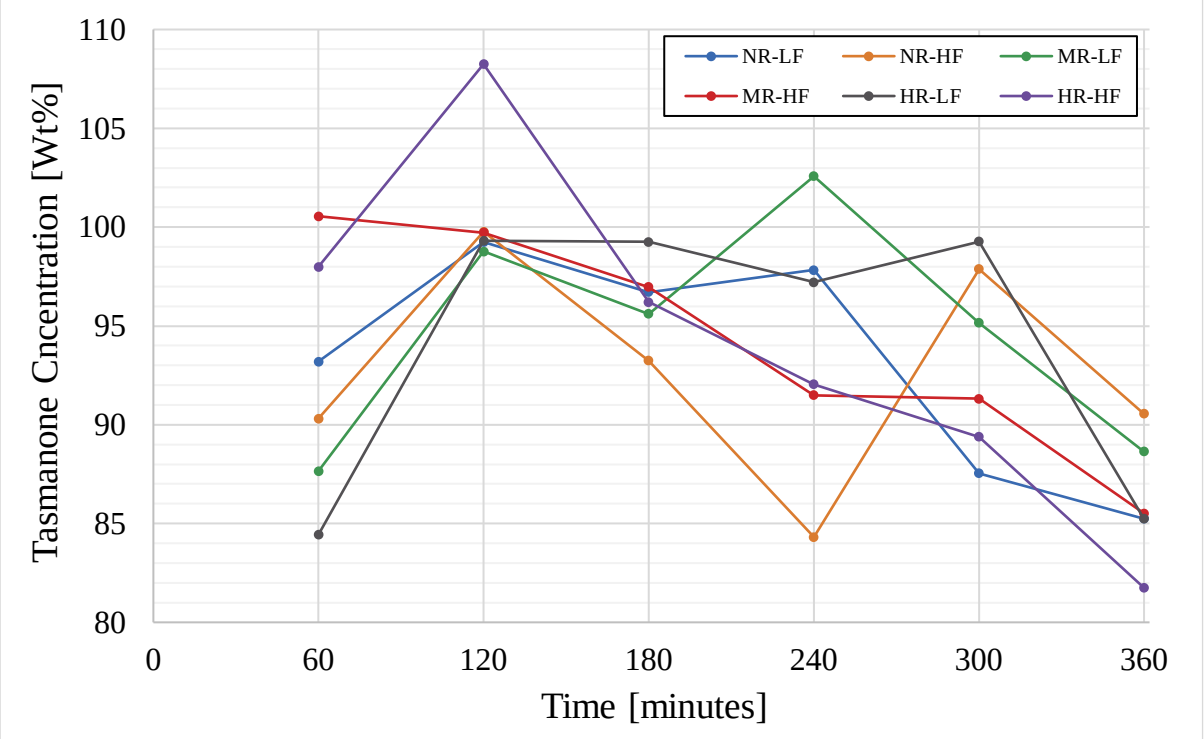

Figure 5-8: GC-FID analysis of oil collected during distillation experiments. Tasmanone concentratiom in Wt% of the sample.

The general trend for all trials is that of an early peak in tasmanone concentration followed by a decline over the total experiment time. There is a trend of lower tasmanone concentration in the first hour of the experiment, even though the total oil extraction may be considerably high (Figure 5-4 and Figure 5-6). The tasmanone concentration is lower at this time period due to the presence of lighter oil components present in the *E. cloeziana* biomass. These lighter components are more volatile than tasmanone, and are more readily permeated through the leaf structure. An example of the high early concentrations of lighter components can be seen in oil analyses shown in Chapter 4.

When looking at the high flowrates against the low flowrates (Figure 5-9), the tasmanone concentration for the high flow experiments seems to peak earlier than the low flow experiments. The tasmanone concentration of the HF experiments peak in the second hour before declining over the experimental period. The low flow experiments also show increased concentration in the second hour, but the concentration plateaus until the 4th hour, after which the concentration declines over the experimental period. While the data for the two flowrates does not show a significant difference in the tasmanone concentrations (except in the samples collected during the 4th hour of distillation), there are some slight differences that could indicate that high flowrate would reduce the time required to extraction tasmanone from *E. cloeziana* biomass.

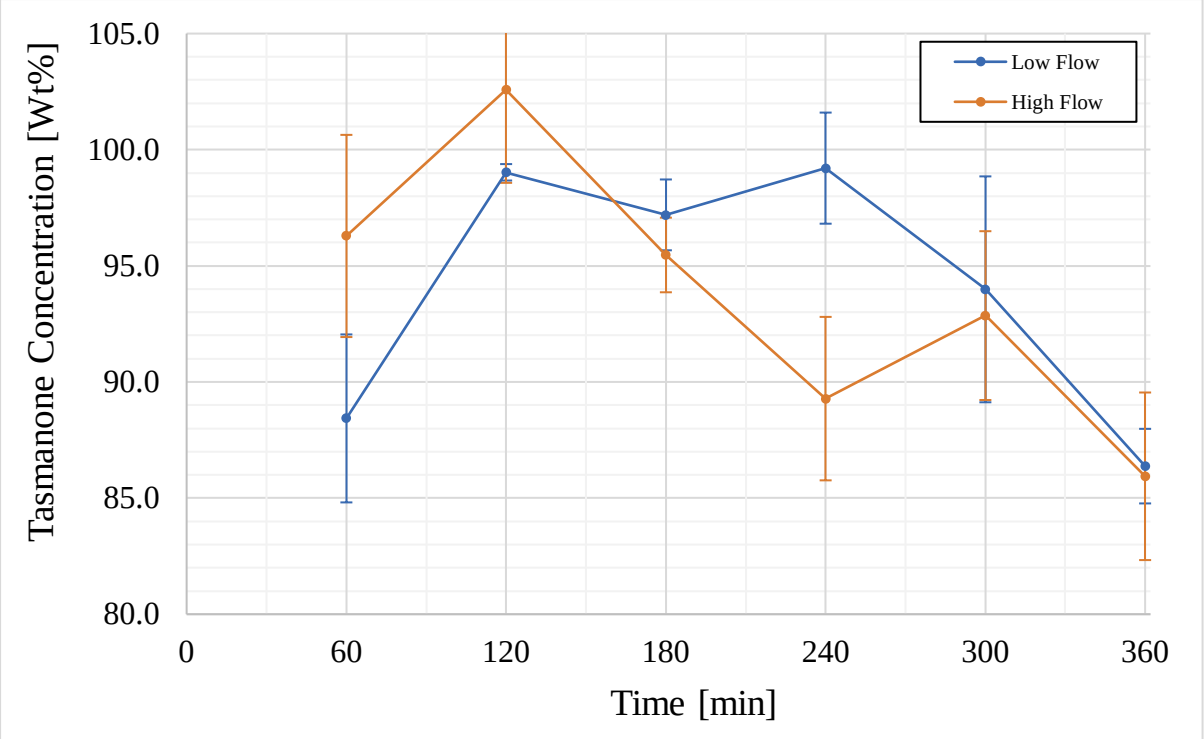

Figure 5-9: Mean and standard deviation of GC-FID tasmanone analysis results for low flow and high flow experiments.

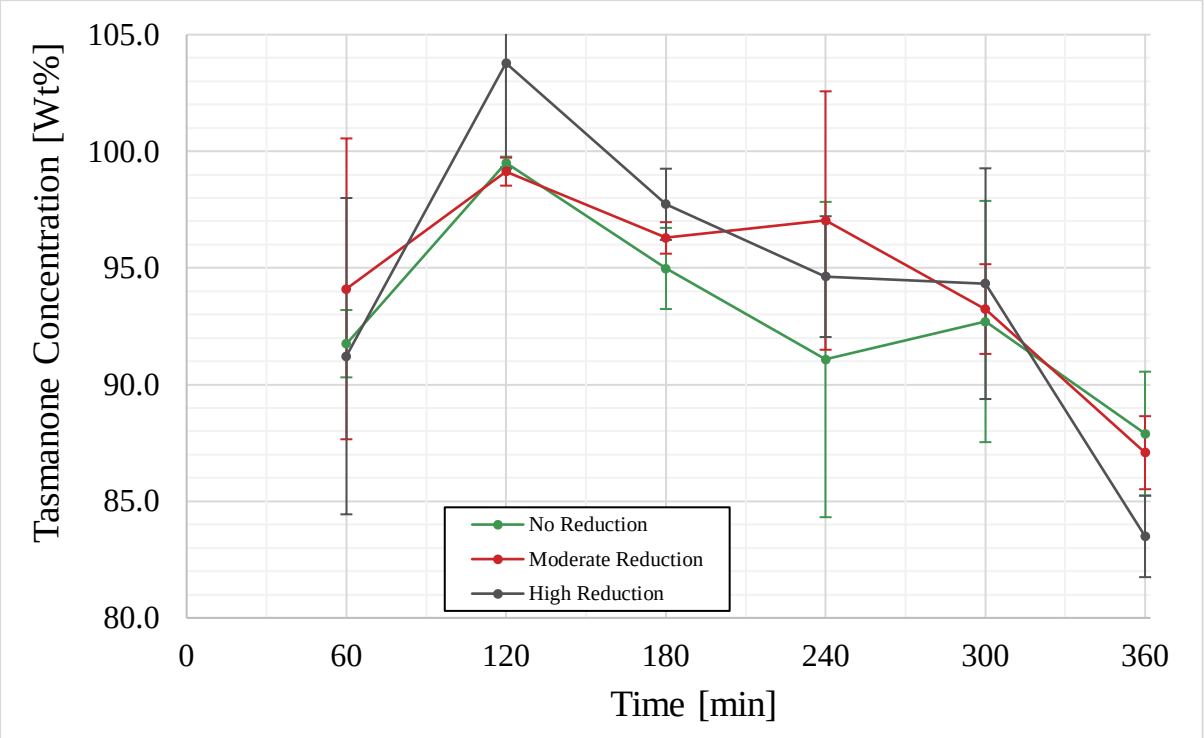

Figure 5-10: Mean and standard deviation of GC-FID tasmanone analysis results for NR, MR, and HR experiments.

When looking at the different size categories together (Figure 5-10), all experiments appear to follow a similar trend. The tasmanone concentration for each size category peaks in the second hour before declining over the experimental period, with all size categories showing

~88.25% tasmanone concentration. There is no significant difference in the tasmanone concentration for oil distilled from different particle size biomass.

5.3.6 Tasmanone Yield of Distillation Oil

The maximum theoretical yield of tasmanone from the biomass samples was calculated in Section 5.3.3; this was calculated to be 19.15 ± 2.05 $mg_{tasmanone}/g_{biomass}$, or 15.32 ± 0.86g of tasmanone for a distillation of 800g biomass. Using the distillation oil yield shown in Section 5.3.4 and the tasmanone content calculated in Section 5.3.5, the accumulated yield of tasmanone during each experiment was calculated. Figure 4-12 shows the yield of tasmanone during each experiment set, as a percentage of the theoretical maximum ($Yt_{D\ max}$). As there is not a dramatic difference in the tasmanone concentration between the individual experiments, the tasmanone yield follows the trends seen in Section 5.3.4. However, as the tasmanone concentration of the oil declines in the later distillation stages, the apparent inefficiency of the later extraction is more pronounced in the tasmanone yield. The oil yields established that the greatest extraction efficiency occurs during the first three hours of distillation, which represents an important time parameter for industrial extraction of this tasmanone-rich oil. After 3 hours of distillation, the tasmanone yield is; 38.43% for NR-LF, 51.25% for NR-HF, 42.64% for MR-LF, 55.66% for MR-HF, 48.63% for HR-LF, and 62.37% for HR-HF. Looking at the full 6 hours of distillation results in a tasmanone yield of; 46.39% for NR-LF, 56.76% for NR-HF, 53.37% for MR-LF, 59.34% for MR-HF, 57.45% for HR-LF, and 67.73% for HR-HF. As discussed in Section 4.4.4, there is likely a considerable amount of tasmanone that was removed in purge water, which would explain why the tasmanone yield is considerably lower than theoretical maximum yield.

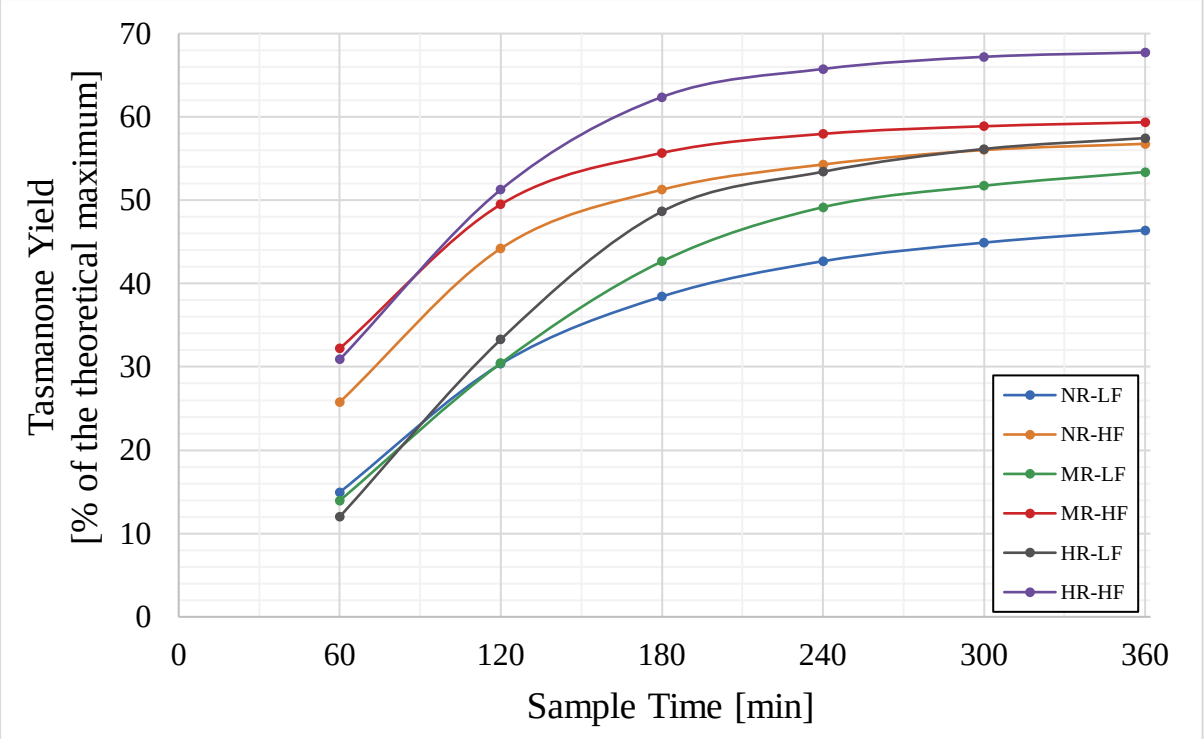

Figure 5-11: Accumulated tasmanone yield from experiments, displayed in % of the maximum theoretical yield ($Yt_{D\ max}$).

5.4 Conclusion

During this study, 14 experiments were conducted to examine the effect of comminution and steam input flowrate on the Qcide oil extraction from *E. cloeziana* biomass by steam distillation. The experiments were conducted at two steam input flowrates, and at three levels of biomass comminution. The steam input flowrates were; low flow of ˜12g/min and high flow of ˜18g/min. The mean surface diameter at the three levels were; no reduction at 5.57 ± 0.45mm, moderate reduction at 2.91 ± 0.09mm, and high reduction at 1.55mm (two samples). The 14 experiments were broken up into 6 unique parameter sets; NR-LF 3 serials, NR-HF 3 serials, MR-LF 3 serials, MR-HF 3 serials, HR-LF 1 serial, HR-HF 1 serial.

These experiments show that the rate of Qcide extraction is dependent on the steam input flowrate for all tested particle sizes, and that the most efficient extraction occurs in the first 3 hours of distillation. During the 3 hours of distillation, the oil yield of the HF experiments was consistently and significantly higher than the LF experiments of the same mean surface diameter; NR-HF 1.05 ± 0.04 $Wt\%_{biomass}$, NR-LF 0.76 ± 0.07 $Wt\%_{biomass}$, MR-HF 1.07 ± 0.13 $Wt\%_{biomass}$, MR-LF 0.87 ± 0.08 $Wt\%_{biomass}$, HR-HF 1.19 $Wt\%_{biomass}$, and HR-LF 0.98 $Wt\%_{biomass}$. Increased flowrate resulted in the oil yield being increased by 36.72% for the NR experiments, 22.71% increase for the MR experiments, and a 21.09% increase in the HR experiments.

The investigation into the effect of comminution led to mixed results regarding the relationship between surface mean diameter and oil yield. For each flowrate there was a general trend of increased yield with decreasing mean surface diameter; however, this trend did not hold true for every size reduction. The size reduction from NR to MR showed decreased oil yield in the later stages of distillation resulting in a 1.6% decline in total oil accumulation over 6 hours. At both flowrates the size reduction from NR to MR resulted in no significant difference in the accumulated oil for the duration of the experiments; however, there was a clear increase when observing the more extreme HR results. At both flowrates, the HR accumulated yields were significantly higher than the NR distillations from the 3rd hour. There was no significant difference between the HR and the MR distillation yields.

GC-FID analysis of oil extracted by distillation found that the tasmanone concentration varied at each hour of the experiments. The analysis showed no significant difference in oil tasmanone concentration that can be linked to the distillation steam flowrate or biomass particle size. While there is some indication that increased steam input flowrate could accelerate the extraction of tasmanone, the observations were minor.

GC-FID analysis was conducted on *E. cloeziana* biomass to analyse the tasmanone content of whole biomass as extracted during industrial operations. The calculated tasmanone yield from *E. cloeziana* biomass was 19.15 ± 2.05 $mg_{tasmanone}/g_{biomass}$, or 1.92 ± 0.20 $wt\%_{biomass}$.

6 Conclusions and Recommendations

This study investigated several parameters relating to steam distillation conditions for the optimal extraction of Qcide oil, with high tasmanone content from *Eucalyptus cloeziana* biomass. The investigations were conducted through two series of experiments; **Chapter 4: Effect of Distillation Temperature and Steam Input Flowrate on the Extraction of Qcide Oil**, and **Chapter 5: Effect of Comminution and Steam Input Flowrate on the Extraction of Qcide Oil**.

6.1 Conclusions

6.1.1 Distillation Temperature

Temperature experiments were conducted at three temperatures: Low Temperature (˜105ºC), Medium Temperature (˜125ºC), and High Temperature (˜145ºC). The temperature experiments were conducted with biomass taken directly from the industrial harvester (uncut biomass) with low steam input flowrate (˜12g/min) and high steam input flowrate (˜18g/min). The investigation found that for higher flowrates the extracted yield of Qcide oil is significantly higher in higher temperature experiments, this holds true for the accumulated yield for all time points in the 6-hour extraction. For the lower flowrate experiments the accumulated oil yield was always greater under higher temperature distillation, but the results were not significant for all time points. The results show a clear trend of increasing Qcide oil and tasmanone yield with increasing temperature, up to 150 ºC.

At low steam input flowrate, the effect of increasing distillation temperature on the mean yields was:

1. Low Temperature to Moderate Temperature; 45.40% increase in oil yield after 3 hours of distillation, 35.16% increase in oil yield after 6 hours of distillation.
2. Moderate Temperature to High Temperature; 21.59% increase in oil yield after 3 hours of distillation, 11.75% increase in oil yield after 6 hours of distillation.
3. Low Temperature to High Temperature; 76.80% increase in oil yield after 3 hours of distillation, 51.05% increase in oil yield after 6 hours of distillation.

At high steam input flowrate, the effect of increasing distillation temperature on the experimental mean yields was:

4. Low Temperature to Moderate Temperature; 27.41% increase in oil yield after 3 hours of distillation, 17.44% increase in oil yield after 6 hours of distillation.
5. Moderate Temperature to High Temperature; 21.38% increase in oil yield after 3 hours of distillation, 19.00% increase in oil yield after 6 hours of distillation.
6. Low Temperature to High Temperature; 54.66% increase in oil yield after 3 hours of distillation, 39.75% increase in oil yield after 6 hours of distillation.

Oil extracted during temperature experiments were subjected to detailed GC analysis to quantify any effects of elevated temperature on the composition of the oil. The detailed results are not shown in this thesis, due to the nature of commercial confidentiality; however, summarised versions of the analyses are shown in **4.4.5 Chemical Composition of Distillation Oil**. The analyses show that while there was some variation in the tasmanone content of the experiments, this did not seem to be linked with the distillation temperature. When looking at the sum of all triketones (tasmanone and all non-tasmanone triketone), all experiments appear to have a similar composition and composition changes over distillation time.

6.1.2 Steam Input Flowrate

All experiments conducted during this thesis operated at either low steam input flowrate (˜12g/min), or high steam input flowrate (˜18g/min). During these experiments it was seen that increasing the steam flowrate had the effect of not only increasing the overall oil yield, but also increased the rate of extraction. Experiments run at high flowrate were shown to reach a high level of extraction efficiency an hour earlier than the low flow counterparts, and in some cases reached a point of apparent biomass exhaustion before the 6-hour distillation endpoint.

During the temperature experiments (Chapter 4) the oil yields of the High Flow experiments were always greater than that of the Low Flow experiments; for the Low Temperature experiments the High Flow yield was significantly higher for the entire distillation, for the Medium Temperature experiments the High Flow yield was significantly higher for the first 240 minutes of distillation, and for the High Temperature experiments the High Flow yield was significantly higher for the entire distillation. During the temperature experiments, the effect of increasing the steam input flowrate on the experimental yields was;

1. Low Temperature Low Flow to Low Temperature High Flow; 54.16% increase in oil yield and 65.84% increase in tasmanone yield after 3 hours of distillation, 31.19% increase in oil yield and 41.62% increase in tasmanone yield after 6 hours of distillation.

2. Medium Temperature Low Flow to Medium Temperature High Flow; 35.08% increase in oil yield and 40.00% increase in tasmanone yield after 3 hours of distillation, 13.98% increase in oil yield and 18.14% increase in tasmanone yield after 6 hours of distillation.
3. High Temperature Low Flow to High Temperature High Flow; 34.85% increase in oil yield and 30.72% increase in tasmanone yield after 3 hours of distillation, 21.38% increase in oil yield and 17.13% increase in tasmanone yield after 6 hours of distillation.

During the comminution experiments (Chapter 5) the comparative results between the high and low flowrates were comparable to what was seen in the medium temperature experiments from Chapter 4. This is not unexpected as the analysis of the comminution experiments found that the yields were affected by flowrate to a higher degree than comminution. In the comminution experiments the yield was always greater in the High Flow experiments compared to the Low Flow experiments; for the No Reduction experiments the High Flow yield was significantly higher for the entire distillation, for the Medium Reduction experiments the High Flow yield was significantly higher for the first 2 hours of distillation, and for the High Reductions experiments there was not enough experiments conducted to comment on the significance of results. During the comminution experiments, the effect of increasing the steam input flowrate on the experimental yields was;

1. No Reduction Low Flow to No Reduction High Flow; 36.72% increase in oil yield and 33.37% increase in tasmanone yield after 3 hours of distillation, 25.15% increase in oil yield and 22.35% increase in tasmanone yield after 6 hours of distillation.
2. Medium Reduction Low Flow to Medium Reduction High Flow; 22.71% increase in oil yield and 30.53% increase in tasmanone yield after 3 hours of distillation, 6.15% increase in oil yield and 11.19% increase in tasmanone yield after 6 hours of distillation.
3. High Reduction Low Flow to High Reduction High Flow; 21.09% increase in oil yield and 28.25% increase in tasmanone yield after 3 hours of distillation, 12.84% increase in oil yield and 17.90% increase in tasmanone yield after 6 hours of distillation.

During all experiments it was seen that increasing steam input flowrate increased the overall tasmanone oil yields and the rate of oil extraction. That is, increasing the steam flowrate always resulted in positive results for oil and tasmanone extraction.

6.1.3 Biomass Particle Size

Biomass particle size experiments were conducted at three levels of comminution; No Reduction (surface mean diameter ˜5.57mm), Moderate Reduction (surface mean diameter ˜2.78mm), and High Reduction (surface mean diameter ˜1.39mm). There were no observed detrimental effects of biomass size at any of the tested levels of comminution, such as steam channelling or poor steam penetration. The comminution experiments were conducted at ˜125ºC with low steam input flowrate (˜12g/min) and high steam input flowrate (˜18g/min). The investigation into the effect of comminution led to mixed results in regard to the relationship between surface mean diameter and oil yield. For each flowrate the size reduction from NR to MR (and from MR to HR) resulted in no significant difference in the accumulated oil for the duration of the experiments; however, there was a clear increase when observing the more extreme HR results. At both flowrates the HR accumulated yields were significantly higher than the NR distillations from the 3rd hour.

At low steam input flowrate, the effect of increasing comminution (decreasing surface mean diameter) on the experimental mean yields was:

1. No Reduction to Moderate Reduction; 13.78% increase in oil yield and 10.96% increase in tasmanone yield after 3 hours of distillation, 16.03% increase in oil yield and 15.05% increase in tasmanone yield after 6 hours of distillation.
2. Moderate Reduction to High Reduction; 12.54% increase in oil yield and 14.04% increase in tasmanone yield after 3 hours of distillation, 6.72% increase in oil yield and 7.64% increase in tasmanone yield after 6 hours of distillation.
3. No Reduction to High Reduction; 28.05% increase in oil yield and 26.55% increase in tasmanone yield after 3 hours of distillation, 23.83% increase in oil yield and 23.84% increase in tasmanone yield after 6 hours of distillation.

At high steam input flowrate, the effect of increasing comminution (decreasing surface mean diameter) was:

4. No Reduction to Moderate Reduction; 2.12% increase in oil yield and 8.61% increase in tasmanone yield after 3 hours of distillation, 1.59% decrease in oil yield and 4.56% increase in tasmanone yield after 6 hours of distillation.
5. Moderate Reduction to High Reduction; 11.05% increase in oil yield and 12.05% increase in tasmanone yield after 3 hours of distillation, 13.45% increase in oil yield and 14.13% increase in tasmanone yield after 6 hours of distillation.
6. No Reduction to High Reduction; 13.41% increase in oil yield and 21.70% increase in tasmanone yield after 3 hours of distillation, 11.65% increase in oil yield and 19.33% increase in tasmanone yield after 6 hours of distillation.

While the results indicate that increasing comminution has a positive effect on the distillation yield of tasmanone rich oil, the results only show a significant increase when comparing the two extremes of NR and HR. Comminution was found to have no significant effect on the tasmanone concentration within the extracted oil. Between the two process variables, the steam input rate always has much more influence on the extraction rate, and oil yield, than comminution.

6.1.4 Tasmanone Content of Biomass

Two solvent extractions were conducted. These extractions were conducted with the sole purpose of examining the tasmanone content of the oil, and to provide a theoretical maximum yield from the biomass. The biomass that was used during these experiments was representative of tree biomass that is harvested during industrial operations. The biomass being in this state means that analysing the tasmanone concentrations of these samples should give the tasmanone concentration of the entire *E. cloeziana* tree.

One solvent extraction was conducted using ethyl acetate as the extraction solvent, this biomass that was harvested on 16th of July 2020. The extraction found that this biomass had a tasmanone concentration of 19.15 ± 1.18 $mg_{tasmanone}/g_{biomass}$. The other solvent extraction was conducted using dichloromethane as the extraction solvent. This biomass was harvested on 22nd of November 2020. The extraction found that this biomass had a tasmanone concentration of 11.52 ± 0.19 $mg_{tasmanone}/g_{biomass}$. The calculated tasmanone concentrations are significantly different and this could have important implications for the harvesting of biomass and the extraction of Qcide. While it is possible that there is a significant difference in the tasmanone solvation of ethyl acetate and dichloromethane, it is unlikely that the variation in tasmanone concentration is solely due to different chemicals used. It is possible that the variation in

tasmanone concentration is due to one, or a combination of, several factors including; coppice level and cycle, seasonal variation in chemical composition, and specific tree ecology.

6.1.5 Condensate Accumulation inside Distillation Vessel

Steam distillation was chosen as the method of extraction due to steam volatility of Qcide, the apparent immiscibility of Qcide with water, and the favourability of steam distillation as an industrial extraction. However, the low heat efficiency, due to the small scale of the equipment, resulted in substantial internal condensate that required purging. The amount of purge required during the experiments varied depending on the temperature and steam flowrate of the particular experiment, with the fraction of steam input that required purging ranging from 15% to 52%. Across 33 experiments the mean and standard deviation of the purged fraction was 31.3 ± 9.6%. One single sample of purge water was analysed, from an experiment conducted at ˜125°C, with ˜18g/minute steam input flowrate, and uncut biomass. The purge water analysis found that the tasmanone content was 3.93 $mg_{tasmanone}/g_{purgewater}$, which is comparable to the concentration of tasmanone in the distillate. The nature of the purge water made the separation of tasmanone-rich oil difficult, which makes the purge water undesirable. This undesirable product should be eliminated by increased heat efficiency in the process, by improving the distillation equipment to reduce heat inefficiency.

6.2 Recommendations

6.2.1 Distillation Temperature

The results from temperature experiments show that increased temperature (up to 150°C) results in increased extraction rate and yield of Qcide oil from biomass. These experiments did not analyse the cost of using steam at temperatures above 100°C, or the cost of commissioning distillation equipment capable of achieving these temperatures. Increased distillation temperatures did not result in any detrimental effects in regard to Qcide composition, but there were some undesirable physical changes observed in the oil extracted at higher temperatures. In order to increase the extraction rate and yield of Qcide oil it is important to raise the temperature as far as practicable. While it is recommended to increase the temperature of commercial extraction of Qcide oil, investigation into the setup and operational viability will be required, which including analyses on:

1. the extent, and effect of, steam-biomass contact on a large scale and at high temperature,

2. the cost of high temperature steam generation against the economic benefits of increased Qcide yield,
3. the fabrication cost of pressurised distillation equipment, and
4. the operational cost of a distillation system at an elevated pressure and temperature (i.e., satellite boiler and high capacity condenser).

6.2.2 Steam Input Flowrate

All experimental results from this investigation show that an increase in the steam input flowrate resulted in significantly increased yields. It is recommended that distillation be operated with elevated steam input flowrate where practical. To assess the commercial viability of increasing the steam input flowrate at an industrial scale, several investigations should be undertaken.

1. Extended flowrate analysis to find extraction effects at a greater range of input flowrates,
2. Analysis of the unit cost of steam input against the unit gain of Qcide oil at a range of input flowrates, and
3. Analysis into the cost and operation of equipment capable of maintaining distillation at high steam input flowrates (i.e., satellite boiler and high capacity condenser).

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Appendix A: Sieve Analysis Data from Temperature and Steam Input Flowrate Experiments

This appendix contains the biomass classification data from experiments investigating the effect of temperature and steam input flowrate on the extraction of Qcide oil from *E. cloeziana* biomass. Classification was conducted using laboratory classification sieves of aperture sizes 19.00mm, 9.50mm, 4.75mm, and 2.36mm. In this analysis any biomass that was larger than 38mm in length was reduced to 38mm. Each sample was comprised of ~950g of *E. cloeziana* biomass which underwent mechanical sieving until there was no observed significant change in fraction mass. The size distribution of each sample is shown in Table A-1.

Table A-1: Sieve analysis data. Mass percentage of biomass on each sieve apature size and geometric mean particle diameter.

Sample	Percentage of sample mass on specific sieve				
	Base pan	2.36mm	4.75mm	9.50mm	19.00mm
1	8.70	10.26	25.21	40.17	15.66
2	11.16	11.81	34.05	27.45	15.54
3	6.73	11.50	28.65	30.71	22.41
4	10.88	13.50	26.53	37.52	11.57
5	8.91	11.03	27.08	31.83	21.16
6	9.13	11.87	27.88	34.48	16.64
7	4.13	11.79	28.35	43.69	12.05
8	5.33	11.45	28.68	36.65	17.89
9	3.41	11.44	27.22	42.27	15.66
10	4.45	11.86	28.99	36.93	17.78
11	3.27	10.40	27.12	39.77	19.44
12	3.76	9.74	28.88	41.83	15.80
13	2.55	9.94	23.43	49.23	14.86
14	3.44	9.46	23.86	42.24	21.00
15	3.56	9.99	25.00	12.16	49.28
16	2.55	11.59	22.98	44.14	18.75
17	1.14	6.94	28.76	51.86	11.31
18	1.56	9.59	26.95	47.68	14.22
19	4.79	5.14	17.85	37.04	35.19
20	1.69	10.74	24.89	44.08	18.61
21*					
22	1.00	6.40	20.71	42.08	29.81
23	5.34	6.10	25.84	34.92	27.80
24,25,26**	4.59	7.71	18.93	31.82	36.95

* no data available for this serial.
** samples from amalgamated biomass sour

Appendix B: Oil Yield Data from Temperature and Steam Input Flowrate Experiments

This appendix contains oil yield data from experiments investigating the effect of temperature and steam input flowrate on the extraction of Qcide oil from *E. cloeziana* biomass. Distillation was conducted at 6 parameter points: low temperature with low steam input flowrate (LT-LF), low temperature with high steam input flowrate (LT-HF), medium temperature with low steam input flowrate (MT-LF), medium temperature with high steam input flowrate (MT-HF), high temperature with low steam input flowrate (HT-LF), and high temperature with high steam input flowrate (HT-HF). The pressure, temperature and steam input flowrate for each parameter point is shown in Table B-1. The mean and standard deviation of the sample yield (Y_n) and the sample concentration (C_n) for each set of experiments is shown in Table B-2, Table B-3, and Table B-4. The sample yield and concentration were calculated as per Section 3.5.

Table B-1: Parameter poins for all temperature and steam input flowrate experiments. Pressure, temperature and steam input flowrate is shown as the mean and standard deviation during the entire distillation experiment.

Test Parameters Code	Number of serials	Pressure [kPa]	Temperature [°C]	Steam Input Flowrate [g/min]
LT-LF	3	7.7 ± 1.8	102.0 ± 0.5	12.1 ± 0.2
LT-HF	3	25.1 ± 3.2	106.1 ± 1.6	19.2 ± 1.5
MT-LF	3	115.3 ± 23.6	121.9 ± 3.5	12.7 ± 0.5
MT-HF	4	125.5 ± 12.8	123.8 ± 2.6	19.6 ± 1.3
HT-LF	3	293.0 ± 32.3	142.3 ± 3.2	12.6 ± 1.1
HT-HF	3	387.0 ± 28.0	149.9 ± 2.7	19.1 ± 2.7

Table B-2: Results of low temperature distilation experiments showing mean and standard deviation.

Distillation Time [minutes]	LT-LF		LT-HF	
	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]
30	0.1299 ± 0.0204	0.2023 ± 0.0278	0.2774 ± 0.0401	0.2639 ± 0.0285
60	0.1395 ± 0.0070	0.2138 ± 0.0032	0.2545 ± 0.0579	0.2253 ± 0.0105
90	0.1249 ± 0.0232	0.1911 ± 0.0262	0.2105 ± 0.0110	0.1730 ± 0.0199
120	0.1048 ± 0.0059	0.1678 ± 0.0154	0.1303 ± 0.0178	0.1134 ± 0.0186
180	0.0907 ± 0.0069	0.1382 ± 0.0059	0.0881 ± 0.0081	0.0725 ± 0.0073
240	0.0538 ± 0.0056	0.0830 ± 0.0058	0.0398 ± 0.0034	0.0333 ± 0.0020
300	0.0367 ± 0.0047	0.0562 ± 0.0053	0.0195 ± 0.0028	0.0161 ± 0.0030
360	0.0222 ± 0.0029	0.0337 ± 0.0036	0.0104 ± 0.0023	0.0088 ± 0.0021

Table B-3: Results of medium temperature distilation experiments showing mean and standard deviation.

Distillation Time [minutes]	MT-LF		MT-HF	
	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]
30	0.1839 ± 0.0588	0.3320 ± 0.0618	0.4409 ± 0.0496	0.5011 ± 0.1619
60	0.2431 ± 0.0506	0.4037 ± 0.0464	0.3633 ± 0.0670	0.3186 ± 0.0391
90	0.2054 ± 0.0574	0.3436 ± 0.0674	0.2273 ± 0.0720	0.1960 ± 0.0488
120	0.1358 ± 0.0578	0.2339 ± 0.0656	0.1471 ± 0.0369	0.1275 ± 0.0285
180	0.1100 ± 0.0266	0.1871 ± 0.0414	0.0766 ± 0.0222	0.0660 ± 0.0168
240	0.0651 ± 0.0332	0.0991 ± 0.0502	0.0172 ± 0.0082	0.0146 ± 0.0065
300	0.0360 ± 0.0177	0.0541 ± 0.0265	0.0098 ± 0.0053	0.0092 ± 0.0051
360	0.0159 ± 0.0079	0.0239 ± 0.0118	0.0025 ± 0.0022	0.0025 ± 0.0022

Table B-4: Results of high temperature distilation experiments showing mean and standard deviation.

Distillation Time [minutes]	HT-LF		HT-HF	
	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]
30	0.2384 ± 0.0350	0.4225 ± 0.0398	0.8905 ± 0.0697	0.6994 ± 0.0758
60	0.3679 ± 0.0179	0.6307 ± 0.0853	0.3998 ± 0.0764	0.4050 ± 0.0585
90	0.2281 ± 0.0526	0.4307 ± 0.0681	0.1556 ± 0.0201	0.1782 ± 0.0362
120	0.1701 ± 0.0140	0.2998 ± 0.0392	0.0814 ± 0.0195	0.0891 ± 0.0243
180	0.0992 ± 0.0132	0.1806 ± 0.0407	0.0475 ± 0.0069	0.0470 ± 0.0068
240	0.0496 ± 0.0181	0.0886 ± 0.0362	0.0163 ± 0.0046	0.0152 ± 0.0036
300	0.0227 ± 0.0133	0.0411 ± 0.0243	0.0029 ± 0.0024	0.0027 ± 0.0022
360	0.0102 ± 0.0066	0.0181 ± 0.0116	nil	nil

Appendix C: Oil Yield Data from Comminution and Steam Input Flowrate Experiments

This appendix contains oil yield data from experiments investigating the effect of comminution and steam input flowrate on the extraction of Qcide oil from *E. cloeziana* biomass. Distillation was conducted at 6 parameter points: no biomass reduction with low steam input flowrate (NR-LF), no biomass reduction with high steam input flowrate (NR-HF), moderately reduced biomass with low steam input flowrate (MR-LF), moderately reduced biomass with high steam input flowrate (MR-HF), highly reduced biomass with low steam input flowrate (HR-LF), and highly reduced biomass with high steam input flowrate (HR-HF). The temperature and steam input flowrate for each experiment is shown in Table D-1. This table also shows the surface mean diameter of the biomass sample calculated by Equation 5.1, as well as the specific surface area (SSA) of the biomass sample and the total approximated surface area (SA) of the biomass sample. The mean and standard deviation of the sample yield (Y_n) and the sample concentration (C_n) for each set of experiments is shown in Table D-2, Table D-3, and Table D-4. The sample yield and concentration were calculated as per Section 3.5.

Table D-1: Experimental parameters for each comminution and steam input flowrate experiment.

Trial	Temperature [°C]	Steam Input Flowrate [g/min]	Ds [mm]	SSA [m2/kg]	SA [m2]
No Biomass Reduction (NR)					
1	124.7	12.73	5.83	4.85	3.88
2	124.4	12.58	4.99	5.19	4.16
3	125.1	12.66	6.29	4.71	3.77
4	124.2	17.90	5.05	5.17	4.13
5	125.2	17.83	5.74	4.87	3.89
6	124.9	18.13	5.56	4.95	3.96
Moderate Biomass Reduction (MR)					
7	125.2	18.44	2.76	7.09	5.67
8	125.5	12.04	2.84	6.99	5.59
9	124.9	18.54	3.06	6.75	5.40
10	125.0	11.62	2.90	6.91	5.53
11	125.0	12.21	2.89	6.93	5.54
12	124.7	18.27	2.97	6.85	5.48

Trial	Temperature [°C]	Steam Input Flowrate [g/min]	Ds [mm]	SSA [m2/kg]	SA [m2]
High Biomass Reduction (HR)					
13	125.1	11.91	1.55	9.92	7.93
14	124.8	18.61	1.55	9.92	7.94

Table D-2: Results of uncut biomass (NR) distilation experiments showing mean and standard deviation.

Distillation Time [minutes]	NR-LF		NR-HF	
	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]
60	0.3072 ± 0.0354	0.5438 ± 0.0386	0.5463 ± 0.0205	0.5868 ± 0.0299
120	0.2969 ± 0.0503	0.5063 ± 0.0293	0.3540 ± 0.0221	0.3801 ± 0.0363
180	0.1602 ± 0.0305	0.2971 ± 0.0499	0.1448 ± 0.0289	0.1544 ± 0.0332
240	0.0830 ± 0.0121	0.1615 ± 0.0291	0.0686 ± 0.0152	0.0726 ± 0.0164
300	0.0487 ± 0.0036	0.0950 ± 0.0105	0.0340 ± 0.0099	0.0367 ± 0.0118
360	0.0335 ± 0.0005	0.0608 ± 0.0081	0.0157 ± 0.0047	0.0153 ± 0.0046

Table D-3: Results of moderately reduced (MR) distilation experiments showing mean and standard deviation.

Distillation Time [minutes]	MR-LF		MR-HF	
	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]
60	0.3047 ± 0.0056	0.5855 ± 0.0125	0.6132 ± 0.0412	0.6032 ± 0.0411
120	0.3204 ± 0.0579	0.7011 ± 0.0479	0.3320 ± 0.1157	0.3429 ± 0.1199
180	0.2447 ± 0.0589	0.5190 ± 0.1727	0.1220 ± 0.0368	0.1237 ± 0.0357
240	0.1214 ± 0.0454	0.2309 ± 0.0944	0.0478 ± 0.0107	0.0483 ± 0.0104
300	0.0518 ± 0.0152	0.1022 ± 0.0334	0.0192 ± 0.0029	0.0196 ± 0.0034
360	0.0356 ± 0.0066	0.0686 ± 0.0139	0.0107 ± 0.0029	0.0108 ± 0.0030

Table D-4: Results of highly reduced (HR) distilation experiments showing mean and standard deviation.

Distillation Time [minutes]	HR-LF		HR-HF	
	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]	Y_n [wt%$_{biomass/hour}$]	C_n [wt%$_{condensate}$]
60	0.2724	0.5899	0.6034	0.6034
120	0.4101	0.7509	0.3606	0.3607
180	0.2963	0.5092	0.2213	0.2154
240	0.0941	0.1734	0.0701	0.0678
300	0.0527	0.1014	0.0313	0.0316
360	0.0255	0.0497	0.0124	0.0123