EXTRACTION OF CAFFEINE FROM SPENT COFFEE GROUNDS USING IONIC LIQUIDS

Submitted in fulfilment of the requirements for the degree of Master of Engineering in the Department of Chemical Engineering, Faculty of Engineering and the Built Environment at the Durban University of Technology

Green Engineering & Sustainability Research group

By

Nikita Singh (21518142)

2022

SUPERVISOR: _____ Date: 16 June 2022

Prof. M. Chetty

CO-SUPERVISOR: _____ Date: 14/06/2022

Prof. N. Deenadayalu

DECLARATION

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N. Singh

ABSTRACT

Coffee is the most popular beverage consumed and the second-highest commodity in the world, after crude oil. In 2018, a total of 9,5 million metric tons of coffee were produced globally. This in turn generated 6 million tons of waste coffee grounds. In South Africa alone, it is estimated that approximately 100 million cups of coffee are brewed a year, resulting in 3000 tonnes of waste produced, of which 93% ends up in landfill sites (Lombard, 2021). This abundant waste source has shown promising potential for reusing, recycling, or converting the waste into valuable products like biofuels, fertilizers, animal feed, high-value chemicals, cosmetics and pharmaceutical products such as caffeine for medicinal purposes. Besides coffee being one of the most important agricultural commodities in the world, coffee is also one of the most valuable primary products in world trade. Coffee is also the central and popular activity of many cultures. The most popular reason for the consumption of coffee is its refreshing properties. Large quantities of this waste pose threats to the environment as it is a source of severe contamination and serious health problems. To avoid this catastrophe of the coffee waste, spent coffee grounds can be utilised to generate valuable products. The long-term usage of fossil fuels depletes the finite supply and contributes to greenhouse gas (GHG) and exhaust emissions. The global economic and environmental crisis related to the usage of fossil fuels and the fast depletion of natural resources has raised much awareness and need to find alternate strategies for cleaner and greener energy and chemical products needed for recycling waste has risen drastically. The use of biomass and other lignocellulosic material to produce bio-fuels and other high value products show promising results. Using lignocellulosic material has attracted considerable amounts of attention due its renewable nature and being abundantly available. Lignocellulosic material is used for sustainable development in the world. In this study caffeine extraction is a promising solution for sustainable development, where biomass is valorised. The characterisation of spent coffee grounds (SCGs) using Technical Association of the Pulp and Paper (TAPPI) methods was carried out. The effect of temperature, reaction time and solid-to-liquid loading ratio on the yield of caffeine extracted from spent coffee grounds was investigated. Simultaneously, the best extraction solvent between the (i) ionic liquid (IL) 1-ethyl-3-methylimidazodium chloride (98%), (ii) dichloromethane and (iii) water was determined. Variation of the parameters were established using the Box-Behnken design of experiment (DOE) methodology which varied the (i) temperature (88-120 degrees Celsius), (ii) reaction time (15-35 minutes) and (iii) solid-to-liquid loading ratio (20 g/10-25 mL). For the extraction

process, both the conventional method and green method (IL and water) were investigated. The conventional method includes using dichloromethane as the extraction solvent, whereas the green method makes use of the ionic liquid 1-ethyl-3-methylimidazolim chloride and water as the extraction solvents. Extraction was carried out in a Parr pressure reactor where solid-liquid extraction occurs. High performance liquid chromatography (HPLC) was used to quantify the yield of extracted caffeine. Recrystallization of the highest caffeine yield was carried out and thereafter analysed using Scanning Electron Microscopy (SEM), Transition Electron Microscopy (TEM), Energy Dispersive Spectroscopy (EDS) and Differential Scanning Calorimetry (DSC). The maximum yield of caffeine was obtained at the optimum conditions of 120 °C for 25 minutes using 25 mL volume of extracting solvent. The caffeine extracted from 1-ethyl-3-methylimidazolium, water and dichloromethane was 726.22mg/L, 646.33mg/L and 566.12mg/L respectively. Alternatively stated as 1-ethyl-3-methylimidazolium chloride, water and dichloromethane extracted 0.00363 g caffeine / 1 g SCG, 0.00323 g caffeine / 1 g SCG and 0.00283 g caffeine / 1 g SCG respectively.

SEM images of the spent coffee grounds prior to extraction displayed a dense morphological chain-like structure, with large lumps present. The structure was tightly bonded together and appeared rough. After extraction using each solvent, the SEM micrographs were analysed. Extractions done with the IL demonstrated full degradation. The structure was loose, multiple open pores on the surface with a smooth and thin appearance. The water extractions appeared almost same to that of the IL, but slightly thicker. Lastly, extractions using DCM appeared to be unsuccessful as the SCG attempted to be broken but were still together. The surface had no open pores, rather an oil coated layer covering the spent coffee grounds.

EDS results from 99% pure caffeine standard was compared against the caffeine extracted by all three extraction solvents. Pure caffeine appeared clean, properly formed, big separate particles and distinctive shapes. The caffeine extracted using IL was similar to the structure, crystallinity and appearance of the pure caffeine. Caffeine extracted by water were in long shards, but not fully individual/separated. The caffeine extracted by DCM appeared less crystalline, much smaller in size and more compact. DSC compared the melting points of the pure caffeine standard to those caffeine samples extracted by different solvents, thus providing the purity of the extracted caffeine. The standard caffeine sample had a melting point of 233. 55 ºC equalling 99 % pure. The melting points of 226. 52 ºC; 212. 28 ºC and 200 ºC were obtained for IL, water and DCM respectively. Purity obtained were 96 %, 90 % and 85 % per respective extraction solvent.

DEDICATION

This research study is first dedicated to my Mother, Father and the Almighty God and thereafter to all those who are enthusiastic about gaining knowledge in the field of coffee, spent coffee grounds, renewable energy, environmental pollution and all those aiming to assist in the medicinal field.

ACKNOWLEDGEMENTS

In the name of the Almighty, Shri Sathya Sai Baba, I give thanks for the mercy, compassion, guidance and strength provided to me during all the struggles faced and through the entire duration of compiling this research thesis.

Thereafter, I would like to extend my deepest gratitude to my supervisor Dr Manimagalay Chetty and co-supervisor Prof Nirmala Deenadayalu for their constant willingness, sharing of extensive knowledge and overseeing my entire research project. Their advice, comments, weekly meetings and mentoring support enabled me to produce the best level of research work and learn how to be a better researcher, thus I am grateful.

Secondly, I take the opportunity to appreciate and salute all of the students in Dr Chetty's Green Engineering and Sustainability group for their motivation, valuable comments, sharing of knowledge and their help which I believe benefitted me abundantly. Jeremiah Adedeji and Edward Armah skills, knowledge and willingness are highly commendable.

To the laboratory technician Jaafar Mohammed Bux and PhD student Lethiwe Mthembu, I appreciate all the efforts made to assist me in the laboratory and always willing to go the extra mile. Also, I would like to thank the HPLC technician Devrani Naicker and SEM technician Miranda Waldron (UCT) for their willingness to assist, mentor and provide expert advice during my analysis. Thanks to Dr Avinash Singh for his ability to observe problems and provide his opinions with various views due to his extensive and vast knowledge.

To both my parents, I thank you for constantly being there for me through this stressful yet enjoyable time. The motivation, academic guidance provided and the trust you had in me pushed me to greater heights.

To all my friends, colleagues and family, my gratitude is sent for helping me get through my thesis during the global pandemic with which we were challenged.

I can confidently say that because of all this, I personally have gained a lot of knowledge throughout my study period and I am now a better equipped individual for the challenges which lay ahead for me.

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ABBREVIATIONS

[EMIM][Cl]: 1-ethyl-3-methylimidazolium chloride

ANOVA: Analysis of variance

DCM: Dichloromethane

DOE: Design of experiment

DSC: Differential Scanning Calorimetry

DTG: Differential thermal gravimetric

DUT: Durban University of Technology

EDS: Energy dispersive X-ray Spectroscopy

GHG: Greenhouse gases

HPLC: High Performance Liquid Chromatography

ICO: International coffee organisation

IL: Ionic Liquid

LB: Lignocellulosic biomass

LLE: Liquid-Liquid extraction

SACEC: South African Chemical Engineering Congress

SBP: Sustainable Bio Energy and Processes conference

SCA: Speciality Coffee Association

SCG: Spent coffee grounds

SEM: Scanning Electron Microscopy

SLE: Solid-Liquid Extraction

TAPPI: Technical Association of the Pulp and Paper Industry

TEM: Transmission electron microscopy

TG: Thermo gravimetric

PREFACE

Research outputs

Conference participation:

Singh, N., Chetty, M. and Deenadayalu, N. 2021. Valorisation of spent coffee grounds. South African Chemical Engineering Congress (SACEC 2021) Virtual conference, 20th – 22nd September 2021, South Africa

Singh, N., Chetty, M. and Deenadayalu, N. Extraction of Caffeine from spent coffee grounds. 1st Sustainable Bioenergy and Processes conference, 13th – 15th December 2021, Cape Town, South Africa.

In progress:

Singh, N., Chetty, M. and Deenadayalu, N. 2022. Extraction of caffeine from spent coffee grounds using ionic liquids. CHISA 2022 conference, Journal of Chemical Engineering Technology, 22nd – 25th August 2022, Prague.

Co-author for book chapter

Armah, E. K. et al., 2022. Resource reclamation for biogas and other energy resources. In: *Biogas- Basics, intergrated approaches and case studies.* England: IntecOpen, pp. 851-897.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Various considerations of the worldwide economic and environmental issues associated with non-renewable resources and extensive use of daily required products, created the urgent need to search for suitable alternatives to produce much needed high value products. Increasing population growth, industrial growth and human consumption has led to the exponential growth of waste generation. Expanding on the wide variety of natural resources which exist, research has been redirecting interests towards the area of biomass and lignocellulosic waste materials. Due to its renewable nature and abundance, it has attracted considerable attention as an alternative energy source and feedstock.

Dating back to the 9th century, an Ethiopian goat herder named Kaldi had made a significant and unusual discovery of hyper and energetic goats on his farmyard. After multiple investigations, it was finally concluded that after his goats consumed the cherry like substance, their behavioural patterns had changed. Investigations had then concluded that this cherry was the coffee bean, an energising substance which turned into the second most consumed beverage in the world (Stone, 2022). This accidental discovery of coffee traced back to the high plateau of Abyssinia, Ethiopia.

Coffee was traditionally developed as a colonial crop having 11 million hectares of the world's farmland dedicated to coffee cultivation. Usually the traditional methods of coffee production involved the planting of trees under shaded canopy areas of trees. This created a valuable habitat for animals and insects, as well as prevents top soil erosion. The industrial scale processing of coffee involves the conversion of the raw plant fruit into the finished coffee. The overall process generates a large quantity of solid waste (by-products) and waste water. The solid wastes include by-products from cultivation and preparation (spent coffee grounds), by-products of coffee fruit (coffee cherry) and bean processing (coffee husk, peel and pulp). The waste water is generated during the several steps of washing processes.

1.2 Problem statement

Coffee being the most consumed beverage in the world, estimates to 2.25 billion cups of coffee consumed worldwide every day (Perkins, 2022). Grown in over 70 countries, coffee bean usage estimates to over 16 billion pounds each year. Since the coffee bean is only used once during the production of coffee and thereafter disposed, it contributes to large amounts of waste build up.

Irrespective of coffee being the most valuable traded commodity after petroleum, coffee processing presents major sources of waste build up and pollution. These waste sources and ecological impacts results in large amounts of pollutants released into the rivers, waterways, dumpsites, and atmosphere. In order to eradicate this potential threat, it is important to design a feasible yet highly effective method of processing the waste into valuable products. Consequently, these waste residues and by-products can potentially be used as raw materials for other major processes and utilizations depending on the type of coffee grounds namely; coffee husks, coffee pulp, coffee silverskin or spent coffee grounds (SCGs). This waste source can be transformed into various useful products such as fuel pellets, briquettes, fertiliser manure, source of natural antioxidants, biodiesel production, composting as well as a biomaterial in the pharmaceutical industry. Caffeine is a valuable, useful and much needed product in the health, food and medicinal field that can be obtained from waste coffee. Finding an alternate use for coffee grounds is of importance because its toxic character will have dangerous and harmful effects on the environment. Also, at the same time of job creation, valuable products with great monetary value can be created to generate revenue, reduce waste build ups and most importantly produce high quality caffeine for the food and medicinal industry.

1.3 Aim and objectives of the study

This study aims to evaluate the best extraction solvent with the highest yield and purity of caffeine from spent coffee grounds using three different extracting solvents and green methods.

To achieve the aim of this study, the following objectives were carried out:

i. Characterisation of spent coffee grounds.
ii. The effect of varying solid-solvent loading ratio, reaction temperature and steeping/reaction time on the yield of caffeine extracted.
iii. Process optimisation for the caffeine extraction using design of experiment (DOE), with optimal model design of the extraction process.
iv. Cost analysis and feasibility study.

1.4 Importance and significance of study

Globally, huge amounts of waste are generated on a daily basis. These waste sources include municipal waste, food and beverage waste and industrial waste. Disposing these wastes by dumping them directly in landfills is very harmful because they are toxic and pose as serious threat to the environment. A large contribution to the food and beverage wastes is the restaurants and beverage shops.

The harvesting, preparation, and production of coffee produce significantly high volumes of waste known as SCG. SCG waste is an unavoidable by-product of coffee consumption. According to the Speciality Coffee Association (SCA), the golden ratio for coffee production is 14 g per 250 mL cup. This amount may seem small and insignificant individually, but not when billions of cups are consumed every single day, 2.25 billion cups of coffee are consumed worldwide on a daily basis, with an estimated 14 g of fresh coffee grounds going into each cup totals to 381,000 tonnes of coffee brewed every year, resulting in an 500,000 tonnes of wet spent coffee grounds waste (Bio-bean, Ltd, 2019).

We generally assume that it is safe to discard these SCGs together with our other organic wastes, but that is not the case. SCG has significant impacts on the environment when being placed in landfill sites. They start decomposing three months later and polluting the environment. Besides this, SCG contains oils and other compositional compounds which will make the soil more acidic. This will create acidic leachate (liquid) which will damage the surrounding soil. In addition to this catastrophe, the decomposition of the SCG also generates

greenhouse gases, which is a major environmental threat and health hazard. Decomposing SCG emit methane which is 34 times more potent than carbon dioxide over a 100-year period. Furthermore, greenhouse gases contribute to climate change and by releasing methane gases into the atmosphere increases the global warming effect (Global, 2018).

Currently, most countries repurpose their coffee waste to manufacture agricultural products like fertilizers; nonetheless, 75 % of SCG still end up in landfill sites. Other serious environmental problems include pollution of rivers, drainage clogging, unpleasant smells and pests.

Spent coffee grounds have high nutrient potential both in terms of organic compounds and composting as well as in terms of energy recovery and the production of the new materials. Creation of high-value products from waste coffee leads to many environmental, social and financial benefits.

Currently, coffee is the most popular beverage consumed in the world. It is the second most important commodity in the word, that after crude oil. In 2018, a total of 9, 5 million metric tons of coffee were produced globally (John, 2022). This in turn generated 6 million tons of waste coffee grounds. This greatly abundant waste source has shown promising potential when reusing, recycling or converting the waste into valuable products like biofuels, fertilisers, animal feed, high value chemicals, pharmaceutical products and cosmetics.

This research study aims to evaluate the feasibility of extracting caffeine from SCG waste for application in the medical field, energy creation and alertness, stimulants, anti-depressants, and over-the-counter medication production. The study will also determine the optimized conditions for caffeine extraction. This will also distinguish how much waste will be reduced from the overall coffee production steps.

This study is therefore of importance as spent coffee grounds can be converted into value added products and at the same time generate revenue for the country, reduce waste build up in landfill sites, reduce environmental pollution, minimize health risks and damage to the ecosystems. By doing this, more business opportunities are created which will help to reduce the current unemployment rate in South Africa.

This study aims to optimise the extraction method for caffeine from spent coffee grounds using various extracting solvents and green methods. On completion of this project, it will

also be possible to distinguish the more feasible, sustainable, and cost-effective method together with the extraction yield and purity.

1.5 Thesis structure / outline

Chapter 1: Overview of the background of the study taking into full consideration the amount of spent coffee ground waste generated throughout the world on a daily basis. It also describes the dire need, urgency and strong motivation behind the study of extracting caffeine from abundantly available waste products.

Chapter 2: Literature review of work completed by previous researchers regarding the characterisation of spent coffee grounds, pre-treatment methods, extraction and purification methods of caffeine. The literature also focussed on the parameters affecting the overall process such as; particle size, brewing time, pressure, temperature and solid-to-solvent loading ratio.

Chapter 3: Materials and full methodological processes that are used to extract caffeine collect data and information, equipment used to extract, purify and analyse data. Detailed methods and explanations are given to how each research objective will be fulfilled.

Chapter 4: Results and discussion of the experimental findings and analysis of all the obtained data.

Chapter 5: Cost analysis and feasibility study of the overall process. A full comparison between green methods and conventional methods will be represented.

Chapter 6: Presentation of general observations throughout the study, conclusions of the study and recommendations for further research will be stated.

Bibliography to present a list of references used to conduct the study.

Appendices presenting all data relevant to the study and any material used to achieve the objective of the study.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Coffee is a perennial plant from *coffea* genus belonging to the *Rubiaceae* family. Although over 103 different species are recognized, only two are responsible for world trade, namely *Arabica* and *Canephora*. *Arabica* coffee constitutes to more than 60 % of coffee that is commercialized in the international market for its exceptional organoleptic properties. Arabica coffee is widely preferred due to the wide variety of organic and chemical compounds responsible for the sensory quality and stimuli to one's nervous system. This ensures that coffee remains of great importance worldwide. Although the agricultural value of coffee is high, it also contributes to 80 % of its total volume in agro-waste.

Coffee has three main characteristics features namely aroma, taste and acidity and is derived from over 1500 various chemical substances, 850 volatile and 700 soluble (Blinova, et al., 2017). After the coffee is extracted in water, majority of the hydrophobic compounds (oils, lipids, fatty acids, triglyceride, insoluble carbohydrates (cellulose, indigestible sugars, sugars) remain in the coffee grounds. However, some of the oils, structural lipids and aroma producing compounds are also found in the brewed coffee (Girotto, 2018).

The industrial process of coffee production involves converting raw fruit from the plant into finished coffee. This process generates solid wastes and contaminated waste water from several necessary washing steps. This water has a high carbon load which is a threat to the environmental ecosystem. The main solid by product from preparation and cultivation of the coffee is spend coffee grounds, the by-products of the coffee fruit (coffee cherry) and the by-product of the bean processing (pulp, husks and pulp).

The sustainable harvesting methods indicate great potential great potential and will scientifically reduce the waste build-up. Figure 2.1 compared the amount of greenhouse gases (GHGs) released based on the use of various feedstock materials (Moyer, 2017).

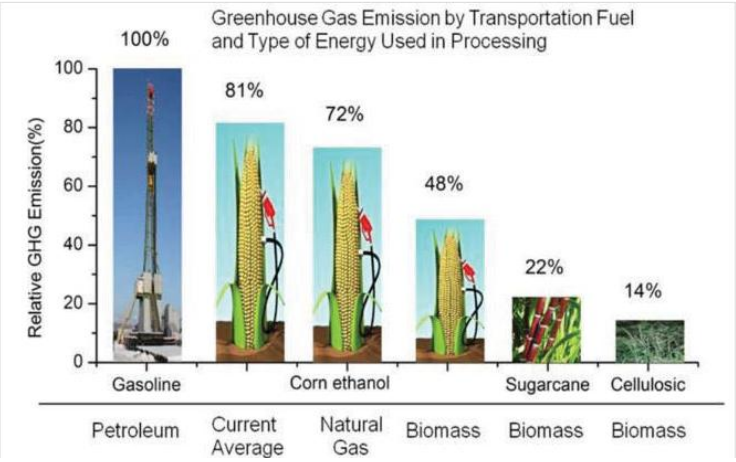


Figure 2.1: Alternative sources for bio processing (Moyer, 2017)

2.2 Origin and availability of coffee

Coffee consumption is an addictive daily routine for billions of people around the world. For households, restaurants, cafeterias, hotels, and industries to make use of coffee, coffee harvesting, processing, production, and brewing produces a significant amount of waste by-products that cannot be avoided. There is a sharp increase in the amount of coffee that is being consumed in recent years. This directly affects the amounts of waste generated and at the same time, the amount of waste that ends up in landfill sites. These daily human activities generate large amounts of waste which if not released to the environment correctly without adequate treatment, will result in environmental pollution, health hazards, and adding to the carbon footprint (Alejandro, *et al.*, 2016).

Arabs were the first people to consume coffee regularly and started the primary cultivation of the coffee plant; hence they are considered as the pioneer in the establishment of coffee crops. Later, coffee spread to Medina, Mecca, Syria, Aden and Cairo slowly covering the entire Muslim world by 1510, and Turkey by 1554 (National Coffee Association of U.S.A., 2020). Coffee was then introduced in America in 1718 starting with the Dutch colony followed by the French. In the 1730s, the British introduced coffee into Jamaica who later spread it to the entire continent. Historically, coffee was introduced to Columbia via Venezuelan border by an individual traveller who carried a coffee plant. Thus, the first crops that were initially cultivated appeared from Columba and belonged to the *Typica* variety of coffee. At the end of 1920, the second coffee variety "Colombia" had been cultivated from Caturrra. In Columbia, mainly Arabica coffee is cultivated because of its acceptance in the national and international market. The Arabica variety is low or tall plants and has red or

yellow fruits which are cultivated in greater proportions. Of the world's coffee production, 80% is Arabica species which are cultivated mainly in Brazil, Columbia, India, Africa, Kenya and Ethiopia. The remaining 20% corresponds to the *canephora* species which are cultivated in Africa, Brazil and Indonesia with differentiating properties such as rust resistance and caffeine content (Adjay, 2019).

The first commercial production of coffee was recorded in 1835, where 2560 sacks of coffee were exported from Cucuta. The National Federation of Coffee Growers (NFCC) was enthusiastic to promote the development of Colombian coffee. This included the purchasing, storing, exporting, and advising coffee growers from different regions of the country. To date, coffee is cultivated in 560000 farms which totals to 948 000 hectares of land, providing over 2,5 million jobs (Adjay, 2019).

Table 2.1 lists the different varieties of coffee together with their descriptions and properties.

Table 2.1: Coffee varieties and distinctive properties

Variety of Coffee	**Descriptive property**
Typica	Arabica or national coffee. Coffee trees appear very tall with bronze/reddish elongated leaves. Trees are susceptible to rust. Harvesting yield is at a greater percentage than Caturra and Bourbon varieties. Trees are planted in density of 2500 per hectare.
Bourbon	Tree appears much more branched in structure therefore producing 30% greater yield than Typica species. Leaves and buds appear green and round nut are susceptible to rust. Trees are planted in density of 2500 per hectare.
Tabi	This is derived from a hybrid of Typica and Bourbon specie. This bean is excellent for supreme coffee production and appears 80% larger. However, the trees are still susceptible to rust. Trees are planted in density of 3000 per hectare.
Caturra	These coffee beans rounder in shape and are produced in lesser quantities than Bourbon and Typica. Caturra behaves well in the coffee industry with a planting density of 10000 trees per hectare.
Colombia	These coffee buds appear bronze in colour and are durable to rust attack. They are produced in equal or greater amounts than Cattura and have the quality similar to Arabica coffee.

Table 2.2 demonstrates that the Arabica variety has the highest caffeine content. The least caffeine content is found in the Caturra variety.

Table 2.2: List of coffee variants and its compositional analysis

Coffee variety	Fibre (%)	Lipids(%)	Proteins(%)	Caffeine (%)	Chlorogenic acids(%)	Ash(%)
Bourbon	21.75	15.27	13.90	1.15	7.37	3.78
Caturra	18.85	13.98	14.79	1.13	6.97	3.39
Colombia yellow fuit	18.45	13.07	14.45	1.16	7.55	3.49
Columbia red fruit	16.69	14.27	13.92	1.19	7.42	3.52
Typica	18.71	13.99	14.59	1.20	6.66	3.43
Arabica	16.223	11.32	15.98	2.32	7.03	3.88
Robusta	15.553	11.42	15.66	2.10	8.08	3.96

2.3 Composition of the coffee bean

Genus Coffea is the generic term for coffee, which also belongs to a group of flowering plants in the *Rubiaceae* family. The cultivated seeds of the coffee plants are known as the coffee bean. These beans are cultivated and harvested throughout the world for human consumption. (Murthy and Madhava Naidu, 2012).

A coffee tree can take up to five years to bear its first coffee beans. The two most important types of coffee in international trade are *Coffea Arabica L* and *Coffea Canephora P,* more commonly known as Arabica and Robusta respectively.

The "*cherry"* consists of two main parts known as the *pericarp* and the *seed.*

The pericarp composes of three outermost layers of fruit, namely:

i. The skin (exocarp)
ii. The mucilage (mesocarp)
iii. Parchment (endocarp)

During the processing of coffee, the pulp refers to a composition of the mucilage and the skin. This is a constant waste stream in the post-harvesting period. Surrounding the coffee seed is a thin layer known as the parchment or hull. In the middle of the cherry, known as the heart are two seeds. Figure 2.2 illustrates the deconstruction of each coffee seed compromising of a silver skin, endosperm and an embryo (Murthy and Madhava Naidu, 2012).

Figure 2.2: Deconstruction and composition of the coffee bean (Blinova *et al*, 2019)

The Endosperm is a tissue like membrane of the bean. This layer consists of holocellulose, sugars, lignin, proteins, oils, bioactives (including chlorogenic acids, trigonelline, nicotinic acid, caffeine) alkaloids and minerals.

The endosperm layer is a crucial layer as it is responsible for the flavour and its complex determinants such as taste and aroma. Many external factors cause these characteristics to vary such as environmental factors, origin, climate, altitude, fertilizer, processing methods and brewing techniques.

The silver skin is a thin skin surrounding the endosperm and is lost during the roasting process. It is given of as a waste residue known as chaff.

The coffee tree produces berries which are referred to as the coffee cherry. The outer red colour in figure 2.3 indicated the ripeness of the berry and whether it is ready for harvesting. The cluster fruits are harvested when ripe and rich red in colour. The skin of the coffee cherry (exocarp) is thick and very bitter in taste. The fruit beneath is the mesocarp (pulp) which is extremely sweet and has a texture of that of a grape. Thereafter, is the parenchyma, a slime like honey layer, serving as protection of the bean. The beans are covered by a parchment-like envelope called the endocarp, also protecting the blue-green coffee beans. The beans also have another protection layer called the spermoderm or silverskin (Blinova *et al*, 2019)

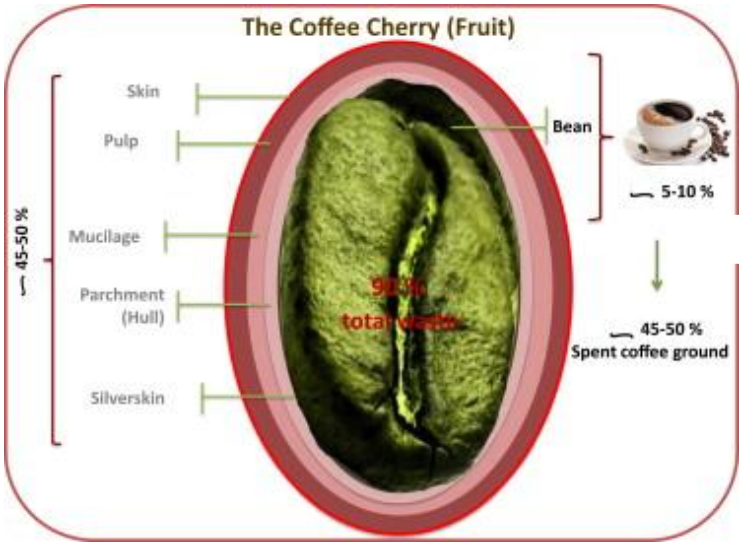


Figure 2.3: Layers present in the coffee cherry (Blinova *et al*, 2019)

The overall coffee production consists of a sequence of technological processes being:

- → Planting: the seeds are planted in large beds during wet seasons. The reason for this is the soil remains moist while the roots are fully established and strong for the duration of the growing period.
- → Harvesting of the coffee cherries: harvest period depends totally on the variety of coffee bean. However, on average 3 to 4 years is the average time period before a harvest takes place.
- → Cherry processing: is done in one of three methods namely dry, wet or semi-dry processing. All three methods aim for the same goal, which is to remove all the fleshy part of the coffee cherry.

→ Drying of the coffee beans: if the coffee cherries underwent wet processing methods, it is utmost importance where the pulped and fermented beans are now dried to an approximate 11% moisture content for ideal storage conditions.

→ Milling of the beans:

→ Exporting the coffee beans:

→ Tasting of the coffee beans: the harvested coffee is repeatedly tasted for quality and taste.

→ Roasting the coffee beans: this process transforms the green coffee beans into aromatic brown beans which are immediately cooled by air or water.

→ Grinding the coffee: the grinding of the beans is done to reduce the preparation time of the coffee. The finer the grind, the quicker the coffee is prepared.

→ Brewing of coffee

2.4 Life cycle of coffee

Figure 2.4 shows the post harvesting steps which contribute to the major generation of solid residues, which are spent coffee grounds, coffee cherries, coffee husk, peel and pulp. The washing steps lead to large amounts of contaminated water high in carbon load.

The method chosen for coffee processing will generate different by-products.

Pre-roasting coffee by-products:

- Dry/unwashed processing: coffee cherry husks (dried skin, pulp, parchment)
- Semi dry / wet processing: coffee pulp
- Post roasted coffee: coffee silverskin, spent coffee grounds.

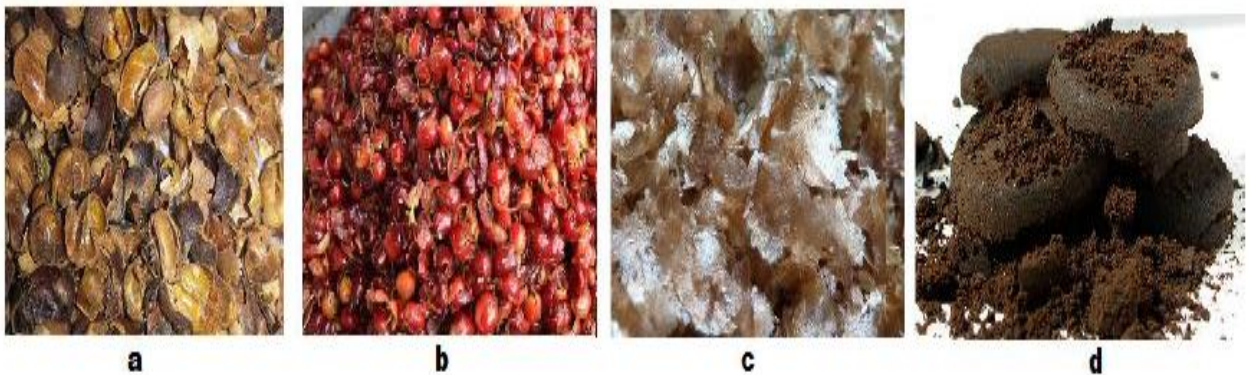


Figure 2.4: Waste generation at each stage of coffee processing (Blinova *et al,* 2019)

a) Coffee cherry husk

The main by-product of the/unwashed process is cherry husk, composed of dried skin, pulp and parchment. It makes up 12 percent of the actual cherry on a dry basis. One ton of berry produces 0.18 tons of husks and simultaneously are able produce 150-200 kg of green coffee. Coffee husks composes of 58-85 % carbohydrates, 8-11 % protein, 0.5-3 % lipids together with minor amounts of bioactives such as caffeine, chlorogenic acids and tannins. (Cruz *et al*, 2014)

b) Coffee pulp

This is the first by-product using wet or semi-dry method and accounts for 29% of the dry weight of the cherry. For every ton of green coffee produced, 500 kg coffee pulp is produced. This pulp consists of the exocarp (outerskin) and the mesocarp (fleshy portion). The pulp is rich in protein, carbohydrates, fat and minerals. Considerable amounts of tannins, polyphenols and caffeine exist within the pulp composition.

c) Coffee silverskin

Frequently referred to as *chaff*, this waste is produced and released during roasting. It has a very low mass and makes up 4.2% of the actual coffee bean. Silverskin has less impact on the environment and is rich in soluble dietary fibre, antioxidant capacity and phenolic compounds.

d) Spent coffee grounds

After the coffee brewing process, the waste generated is spent coffee grounds. Usually brews are prepared from Arabica coffee or Arabica/Robusta blends which are produced in different geographic origins, and being available in different variations. Spent coffee grounds can be those obtained from homemade coffee or from the industries and cafeterias.

The ratio of ground coffee to spent coffee grounds is extremely high and explains the high need and importance to reuse this waste and produce valuable products. From literature, it is said that for every 1 g of ground coffee used, 0.91 g of spent coffee ground is produced.

2.5 Current processes of coffee production

Coffee production is a complex 10 step technological processes, and this is represented in figure 2.5.

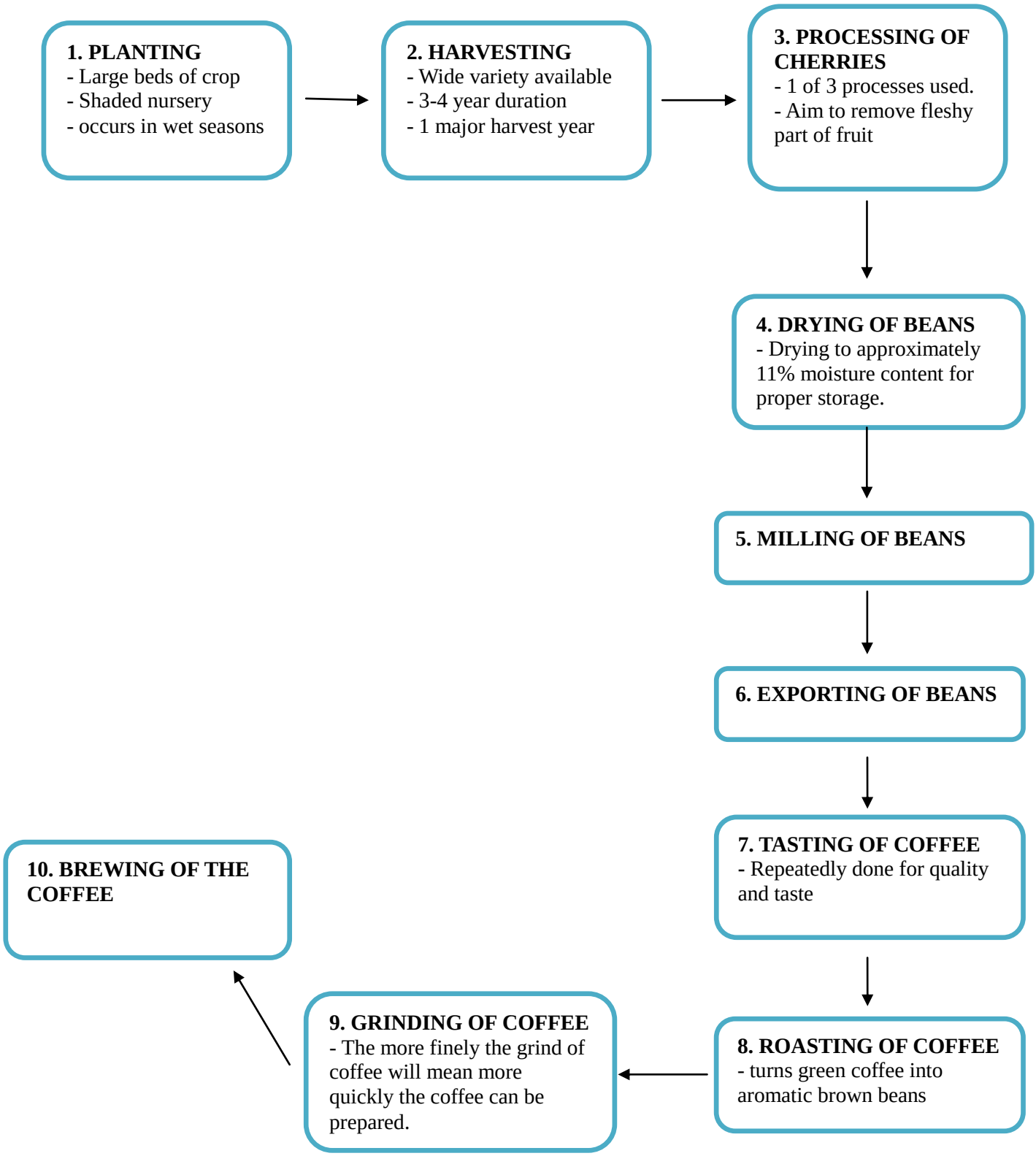


Figure 2.5: Process flow route from seed to beverage

2.6 Types of harvesting

There are currently two types of harvesting methods which exist, namely manual harvesting and mechanical harvesting. These methods are self-chosen before the processing period and are crucial to the final quality of the coffee produced.

Manual harvesting: is a process carried out by hand where selective picking is done. Figure 2.6 illustrates the suitability and selection of coffee beans based on colour and the appearance of the cherry used as basic indicators of ripeness and readiness for harvest. This stripping method removes cherries from the branches in any random order depending on external appearance only.

Mechanical harvesting: this process is large scaled where machinery equipment is often used. This machinery is typically on wheels or hand held. These machines use shakers or vibrations which initiate cherry fall. The disadvantage of this process is that beans containing physical defects, overripe and under ripe beans are also harvested. These defected beans bestow unwanted qualities in the final beverage, which then gives of a harsh, green or even alcoholic taste. From this, it is justified why a separation process is crucial post harvesting.

Figure 2.6: Selection and harvesting of coffee beans (Moskowi, 2011)

2.7 Coffee brewing

After roasting, the beverage is prepared (brewed) by a brewing process. This process basically requires the addition of hot water to the appropriately sized ground coffee bean. Typical brewing methods which exist involve the use of pressure (espresso), infusion (French-press) or gravity filtration (filter coffee). The coffee bean grind size, solid water ratio, temperature and extraction all influence the final brew composition and taste.

During the brewing process, only certain components found within the coffee is extracted. Therefore, numerous secondary metabolites are left behind. These include carbohydrates, lipids, minerals and many other chemical compounds which can be valorised from the spent coffee grounds or exhaust coffee residues.

2.8 Spent Coffee Grounds

On completion of the coffee bean brewing process, the waste residue which is left behind is known as spent coffee grounds. It is composed of large amounts of organic compounds such as fatty acids, amino acids, polyphenols, minerals and polysaccharides which justify its valorisation. From the average SCG composition in figure 2.6, carbohydrates are the most abundant organic compound in spent coffee grounds, accounting for 45% of its composition and least being ash at 1,5%. Spent coffee grounds production is continuously increasing hence the extraction of specific components for processing higher value products needs to be developed. There is an urgent need for practical and innovative ideas to use SCG as it is an abundant low-cost biomass source which has great potential. Valorisation of SCG will contribute to sustainability in the coffee industry. (Ballesteros, et al., 2014)

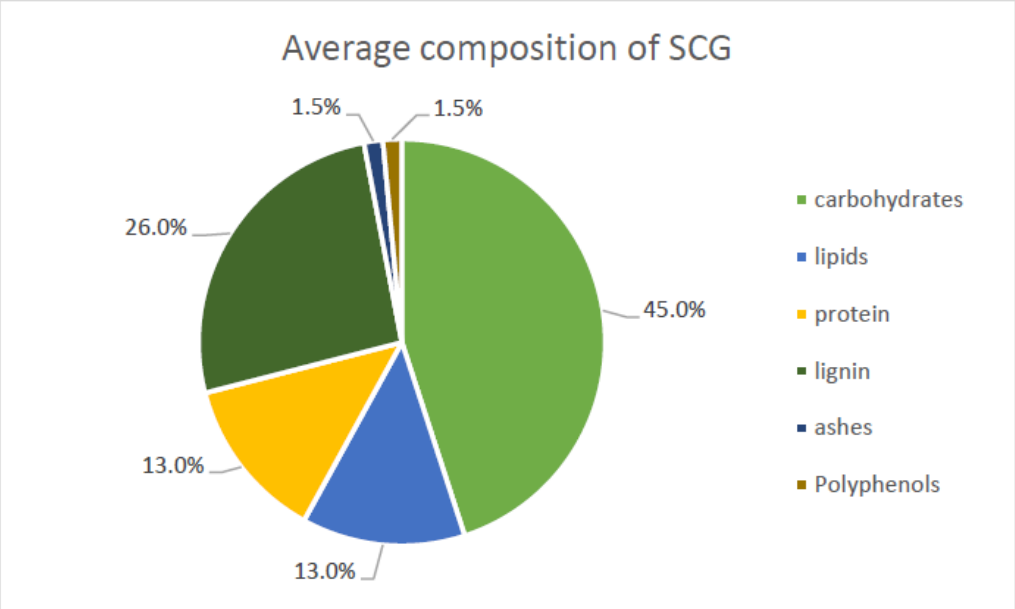


Figure 2.6: Average composition of spent coffee grounds (Girroto *et al*, 2018)

2.9 Source of Spent Coffee Grounds

SCGs are the solid wastes and residue obtained during the manufacturing and brewing process of fresh coffee beverages. (Cruz *et al.* 2012). Spent coffee grounds are obtained as an after product of brewing and preparation of coffee. Usually, spent coffee grounds are regarded as waste and are discarded or composted, however it is hazardous when just released into the environment. Illustrated in table 2.3, SCGs are composed of lignocellulosic material which can be broken down and digested by enzymes, chemicals or a pre-treatment step. The pre-treatment step is necessary because of the of lignin content present in spent coffee grounds.

Table 2.3: Spent coffee grounds composition approximation (Ballesteros, et al., 2014)

Composite	**Percentage (%)**
Cellulose	12.4
Hemicellulose	39.1
Arabinose	3.6
Mannose	19.07
Galactose	16.43
Lignin	23.9
Fat	2.29
Protein	17.44
Dietary fibres	60.46

Advantages of using biomass as an alternate source of chemicals and fuels:

1. Biomass is a reliable renewable source of energy, derived from processing animal and plant waste.
2. Biomass is an always widely available renewable source of energy and will never be depleted.
3. Heavily reduces the usage/ dependence on fossil fuels.
4. Alternative and sustainable source of chemicals, fuels, and materials.
5. Biomass is carbon neutral.
6. Cheaper sources of energy and promotes profitability.
7. Biomass are usually waste products and are less expensive.
8. Reduction of major amounts of garbage/waste in landfill sites.

9. Biomass can be found almost everywhere and also produced locally.
10. Used to create a variety of high, middle and lower value products.
11. Waste by-products can also be utilized into other products.
12. SCG contain large amounts of organic compounds which justify its valorisation.
13. Numerous health and environmental benefits.
14. Renewable energy sources lead to job creation.
15. Biomass valorisation can be carried out using green process.

Challenges faced during the valorization of spent coffee grounds:

1. Pollutants are still released from burning biomass
2. Extraction process is expensive
3. Continuous availability of feedstock material is not guaranteed. (seasonal availability)
4. Raw biomass transportation costs are costly due to the quantity used
5. Releases pollutant gases into the atmosphere
6. Storage of biomass requires large area of spaces
7. Lower efficiency levels
8. Not always a viable option
9. Start-up cost on large scale production is costly. Financial viability is not certain

2.10 Primary products from spent coffee grounds

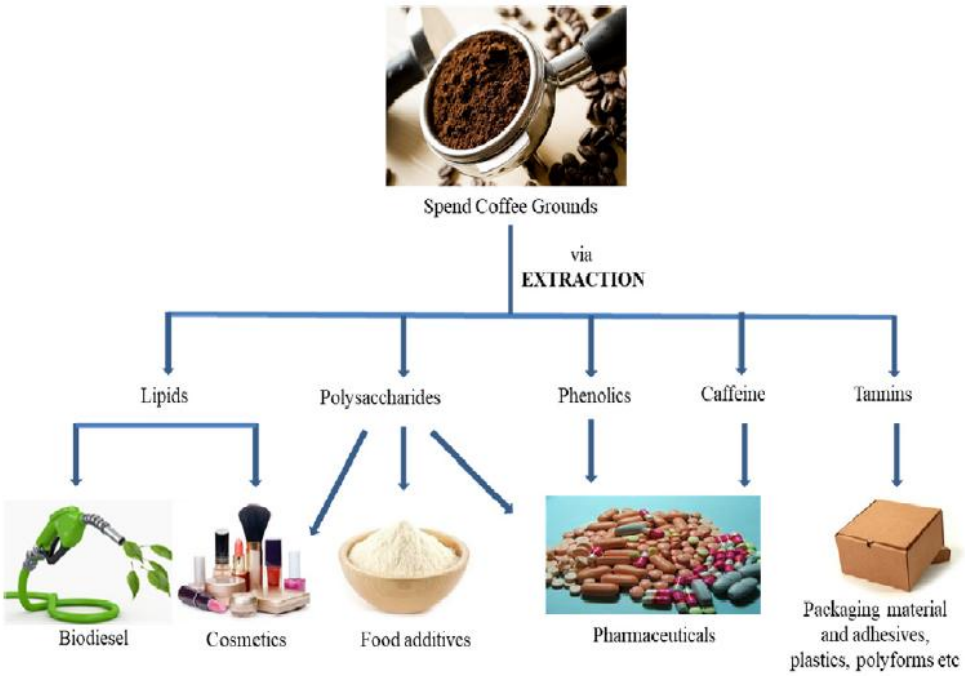


Figure 2.7: Primary products obtained from spent coffee grounds (Caetano *et al*, 2017)

Coffee is a natural source compromised of crude fibre (hemicellulose, cellulose, lignin, polysaccharides, oligosaccharides and monosaccharides), lipids (triacylglycerides, free fatty acids and sterols), nitrogenous compounds (proteins, peptides free amino acids, melanoidins) and minerals. Coffee also contains minor quantities of biological active species namely alkaloids (caffeine, trigonelline) and diterpenes (cafestol, trigonelline), polyphenols (chlorogenic acids) which are responsible for the observed antioxidant activity in coffee brews. However, the composition of the coffee varies along the process chain.

Therefore, the extraction and characteristics of SCG is required to identify and isolate individual components found within the matrix of SCGs. SCGs is considered as a pertinent lignocellulosic feedstock. Considerable quantities of bioactive chemicals found in spent coffee grounds are suitable for valorisation within pharmaceutical, nutraceutical, food and chemical industries (Zabaniotou & Kamaterou, 2019).

2.10.1 Carbohydrates and fibre

Spent coffee grounds contains cellulose, hemicellulose and lignin, however the composition of lignin varies significantly depending on the brewing process. The hemicelluloses have fractions of mannose, galactose and arabinose monomers, SCGs also contains minor quantities of sugar breakdown products such as 5-hydroxymethylfurfural, furfural and acetic acids. Galactomannans and type II arabinogalactans are the two main types of polysaccharides in spent coffee grounds. Galactomannans has a high molecular weight of β-1-4 hydroxyl linkages and contains various C6 linked galactose side chains associated to the cellulose matrix. Arabinogalactans have higher molecular weight β-1-3 linkages with side chains of arabinose and galactose residue. Because of this high degree of branching within the matrix, it contributes to lower thermal stability and lower recovery of monomers during acid hydrolysis. (Abraham *et al*, 2011)

2.10.2 Hemicellulose

Hemicellulose is a highly heterogeneous branched polysaccharide composed of pentose, acetic acids and hexoses. This branched structure makes it susceptible to enzyme and chemical attack. Hemicellulose and lignin molecules are usually attached via hydrogen and covalent bonds. These can be broken by using various pre-treatment techniques.

2.10.3 Cellulose

The polysaccharide with a chemical formula $(C_6H_{10}O_5)_n$ is known as cellulose (Figure 2.8). The cellulose molecule appears as a linearly shaped polymer having the ability to form insoluble crystalline bonds of 36 chains. These chains are strong and highly resistant to enzymatic hydrolysis. The bonds within the molecule are held together by β-1-4 glycosidic bonds, therefore forming many inter- and intra- molecular bonds.

Since the surface area of the molecules are so small, a prior soaking or wetting method will be highly beneficial.

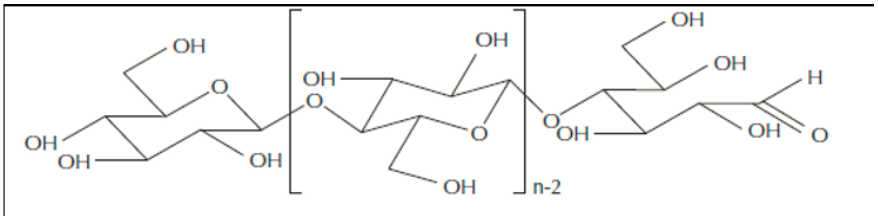


Figure 2.8: Structure of cellulose

2.10.4 Lignin

Lignin is a complex network containing many different molecular weights. This phenolic polymer is composed of various alcohols with different aromatic ring structures. The monomers are bonded together by alkyl-alkyl, alkyl-aryl and aryl-aryl bonding (Figure 2.9). The monomer unit of lignin are coniferyl, syringe and coumaryl.

Lignin bonds to cellulose and hemicellulose. Due to this tight complex bonding, the energy dense molecule is difficult to degrade during the process of biomass degradation. Lignin presence hinders enzymatic attack and swelling of fibres within the molecule. Before enzymatic hydrolysis, a delignification process can be carried out to increase the rate of the reaction during enzymatic hydrolysis (Yu, et al., 2011).

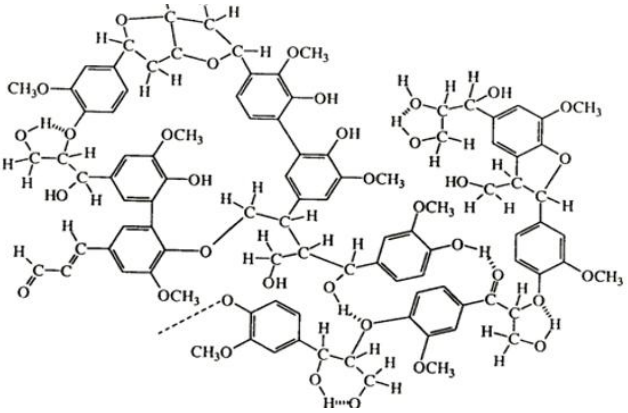


Figure 2.9: Structure of lignin

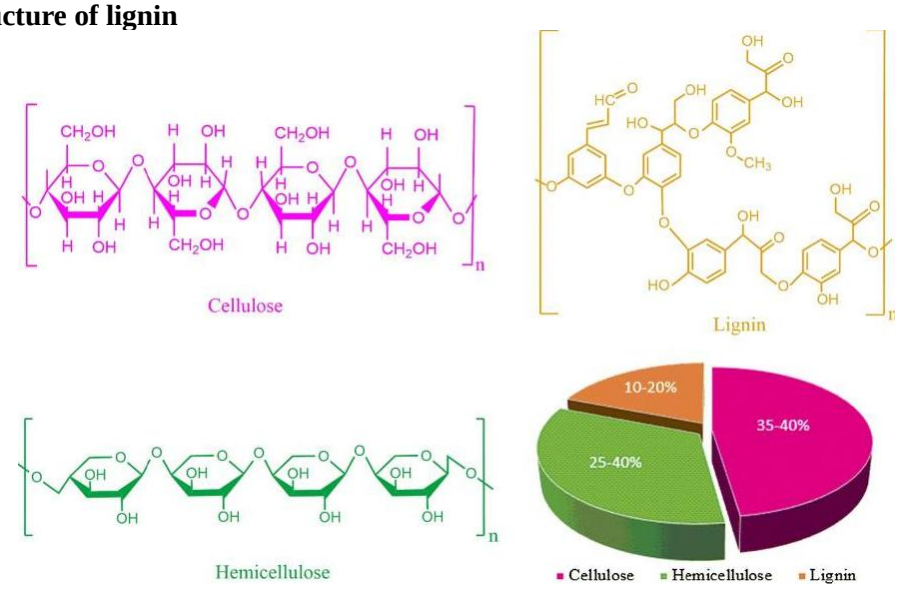


Figure 2.10: Composition (%) of cellulose, hemicellulose and lignin present in SCG without a pre-treatment step (Francesta *et al*, 2019)

2.10.5 Lipids content within spent coffee grounds

The general coffee oil yield from SCG contributes to 10-15 wt% of the dry SCG, and contains 80-90 wt% of glycerol and free fatty acids (FFA). The oil extracted from SCGs consists of triglycerides, diglycerides and monoglycerides. Free fatty acids and unsaponifiable compounds, namely diterpenes, sterols and tocopherols. The oil yield from SCG extraction varies depending on the extraction regime, feedstock type and the brewing techniques used.

2.10.6 Sterols, tocopherols and diterpenes

During transesterification, the unsaponifiable fraction of spent coffee grounds which is usually isolated contains sterols, diterpenes, phytosterols and tocopherols. Diterpenes are esterified together with fatty acids in small amounts. Therefore, due to the bioactivity of these minor components, green extraction methods and the various uses of co-solvents are employed (Massaya, et al., 2019). Diterpenes are directly isolated from the saponified SCG by either extraction or saponification of the SCG oil.

2.10.7 Protein content in spent coffee grounds

Cruz *et al.* (2012), has shown that characterised spent coffee grounds amino acids were present in low amounts of 6.7-16.9 w/w % .

2.10.8 Bioactive components

Phenolic compounds which are found in spent coffee grounds are compromised of melanoids, protocatechivc acids (PCA), chlorogenic acids and tannis. Together with these are nitrogenous compounds such as caffeine and trigonelline. These minor components have great pharmacological value since it has positive effects such as antioxidants, anti-aging, anti-microbial, anti-diabetic, anti-cancer, anti-fungal and anti-allergic activity.

2.11 Secondary product classes from spent coffee grounds

2.11.1 Lactic acid

Hudeckova *et al.* (2018) study had shown that reducing sugars from spent coffee grounds hydrolysates (SCGH) derived from dilute acid hydrolysis to culture lactic acid producing bacteria, 25.69 g/L of lactic acid was produced using SCGH substrate The SCGH underwent cellulase hydrolysis before batch inoculation to find that there was a presence of antimicrobial 5-HMF, levulinic acid, polyphenol components. Lactic acid is a high value molecule having its main use as a monomer in producing the biodegradable polymer known as polylactic acid (PLA).

2.11.2 Poly-(3-hydroxybutryate) (PBH)

Oils produced from spent coffee grounds can be used as a fermentation broth for the production of Poly-(3-hydroxybutryate), a biodegradable alternative to polypropylene and polyethylene. The undesirable high free fatty acid content found in SCG oil during biodiesel production is found to be beneficial in the accumulation of PBH.

2.11.3 Anaerobic digestion

Spent coffee grounds is utilised during anaerobic digestion (AD) whereby gas yields of 0.54 m3kg^{-1} (56–63 % methane) of methane are produced (Lane 1983). The co-digestion of food waste and spent coffee grounds has a cumulative potential for biomethane production.

2.11.4 Triglyceride oils

After dilute acid hydrolysis and enzymatic saccharification pre-treatment of the spent coffee grounds, sugars are released. Oleaginous yeasts are then added to the defatted spent coffee grounds for the enhanced production of triglycerides and ammonia. The fatty acid compositions are amendable for the production of biodiesel.

2.11.5 Bioethanol

Saccharamyces cerevisiae is used during the bioconversion of spent coffee ground derived sugars into bioethanol. According to (Kwon *et al.* 2013), higher alcohols such as isobutnol, isoamylic alcohols and esters also appeared in minor quantities.

2.11.6 Biodiesel

During the production of biodiesel from alkali or acid transesterification of fatty acid triglycerides and alcohols, glycerol is simultaneously produced. The physiochemical

properties of the biodiesel produced are influenced by the fatty acid methyl ester (FAME) composition. The direct applicability of the spent coffee grounds for biodiesel production is dependent on the free fatty acids which include linoleic acid, palmitic acid, oleic acid and stearic acids. The major issues affecting the commercial widespread use of biodiesel is the poor oxidative capacity due to the autoxidation of unsaturated fatty acids (UFA). A higher paraffin fuel has been derived from coffee oils using the hydrogenation/deoxygenation process.

2.11.7 Fertilisers

Despite the initial mineralisation, and high amounts nitrogen and phosphorus released into the soil with the direct use of spent coffee grounds. Hardgrove and Livesley, (2016) has shown much better growth and yield results when used as a fertiliser. Phytotoxins, caffeine, tannins and chlorogenic acids found in spent coffee grounds are beneficial attributes during the composting and vermicomposting process. Emmanuel *et al.* (2017) stated that tomatoes, pepper plants and lettuce had grown up to 16% better in yield. After usage of SCG fertiliser, organic rich compounds such as nitrogen, carbon, potassium, phosphorus and sodium concentrations had increased in the produce grown, justifying the use of spent coffee grounds as a nutrient-rich fertiliser source.

2.11.8 Animal feed

Due to the dry nature of spent coffee grounds, much disregard for the use of animal feed has been shown. Using the feed as a ruminant source also observed that it appeared to have a low digestibility rate and a negative effect on the metabolism system. This can be improved and adjusted by a microbial pre-treatment step which will increase the digestion, energy content, palatability, crude protein and utilisation for livestock. However, an increase in production cost is then added which negates against the economic value of the actual animal feed (Seo *et al.*, 2015).

2.11.9 Antioxidant formulations

Research by Mussatto *et al.* (2014) investigated the antioxidant activity of bioactive materials obtained from spent coffee grounds. These antioxidants are incorporated into cosmetic products, make-up or skin care products. These biomaterials are advantageous and appear effective in the skin care industry to assist in the anti-aging,

anti-wrinkle, moisture renewal and skin lightening applications (Monente *et al.*, 2014).

2.11.10 Biosorbents

Spent coffee grounds appear as natural absorbents due to their polyphenolic properties together with its lactose and amino components. The matrix composition of spent coffee grounds itself can be further exploited by thermochemical processes to be used for contaminant uptake. The thermochemical processing will alter the surface area and porosity of spent coffee grounds and the biochar products. Spent coffee grounds can be utilized for decontamination processes such as the removal of dyes, antibiotics and heavy metals which are present in waste water or other effluent streams. An advantage of such material is that it can repeatedly be used with a minor drop in overall performance (Sánchez-Vioque *et al.* 2013).

2.11.11 Biodegradable films for packaging food

Incorporating the rich polysaccharide extracts from spent coffee grounds showed that it enhanced the barrier of the carboxymethyl cellulose films. These films can be used for packaging or wrapping. Alkali pre-treatment or autohydrolysis attributed to the film's greater opacity. Ballesteros *et al.* (2018) demonstrated the efficacy of using spent coffee grounds extract to improve materials used for food packaging, which included better coloration, thermal stability, tensile strength and reduction in water vapour permeability.

2.11.12 Construction materials

Spent coffee grounds are used to stabilise activated ash and subgrade construction materials. Increasing the portions of spent coffee grounds used have been reported to reduce the thermal conductivity of bricks by up to 50% and also bestow excellent insulation properties relative to the plaster composite materials. (Valesco *et al.*, 2016)

2.11.13 Energy storage devices

Pyrolysed spent coffee grounds and poly vinylidene fluoride (PVDF) binder together with carbon black material in carbonaceous lithium battery resulted in a anode material with high capacity retention. Considerable amounts of current density were measured signalling the development of high performance sustainable energy storage devices. such as flow batteries, solar panels, fuel cells, power banks and supercapacitors (Luna-Lama *et al.* 2019).

2.11.14 Bio-oils derived using pyrolysis

The thermal decomposition of biomass material in the absence of oxygen and below the temperature of 500 degrees Celsius is known as pyrolysis. Biomass which undergo pyrolysis produce condensable liquids such as pyrolytic oil or biocrude oil together with non-condensable gases in the form of a residue, biochar. Bio-oils applications include fuels for boilers, engines and turbines for heat and power generation (Massaya et al, 2019).

2.11.15 Hydrothermal converted products

Spent coffee grounds with 50-60% moisture content are suitable feedstock for hydrothermal processes. The energy expenditure is a major barrier against implanting pyrolysis for wet biomass. However, the resulting biomass breaks down into biocrude oil which compromises of both a liquid aqueous phase and a solid phase. This solid phase residue is the hydrochar and biochar which are used for solid fuel pellets, absorbants, decontaminates and as catalysts (Massaya et al, 2019).

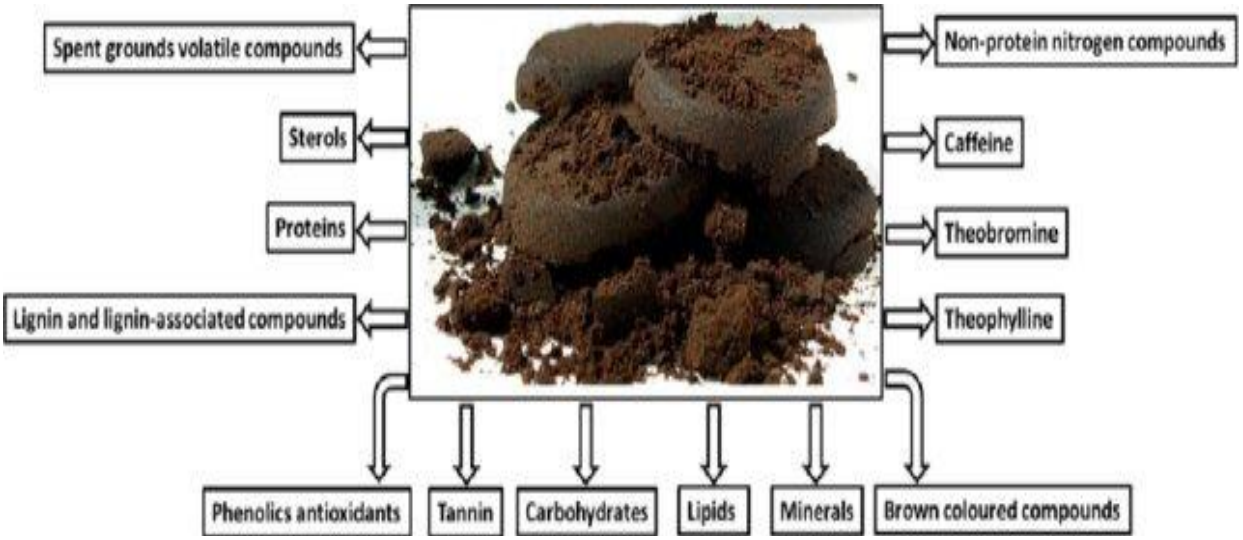


Figure 2.11: Secondary products attainable from SCG (Massaya et al, 2019)

2.12 Spent coffee grounds as a feedstock in biorefineries

Industries where biomass materials are converted into energy, chemicals or other high value products through a series of conversion technologies and methods are known as biorefineries. Biorefineries main goal is to maximise the highest value achievable from the feedstock material through the isolation of high, medium and low value materials. Examples of spent coffee grounds biorefineries start by separating the starting material into bioactives and/or carbohydrates. Downstream processing then includes biotechnological conversions and conversion of sugars to bioethanol, lactic acid and other molecules. The lipid fractions from spent coffee grounds are used with the oil in the production of biodiesel and glycerol. Glycerol can be further converted into biohydrogen through steam reforming. Figure 2.12 depicts the chain of materials which can be produced along the refining process. Spent coffee grounds are able to undergo conversions into solid, liquid and gaseous liquid fuels, composite materials, energy and substrates. Figure 2.12 and 2.13 displays all the possibilities of producing low, medium and high value products uisng various processing routes.

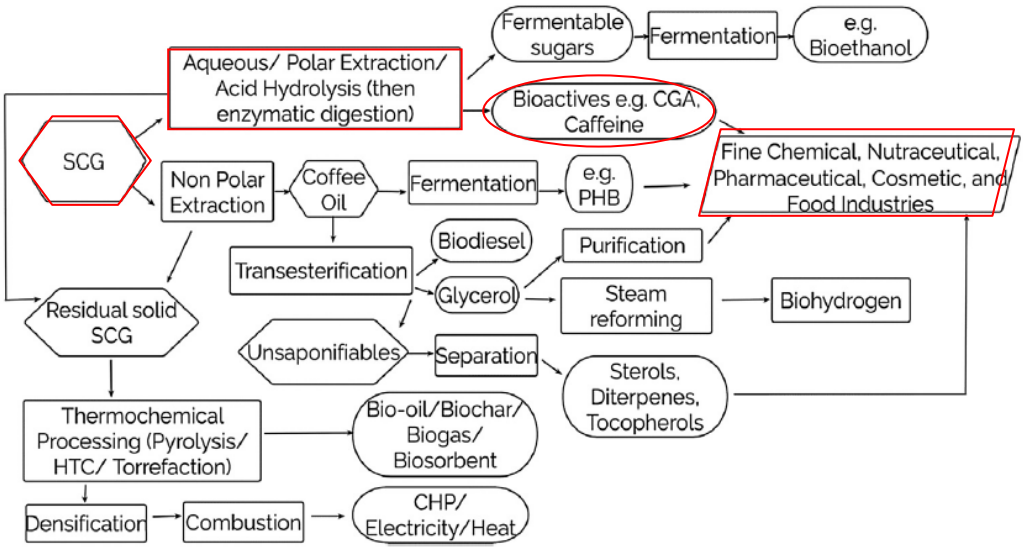


Figure 2.12: Conceptualisation of spent coffee grounds biorefinery (Massaya *et al.* 2019)

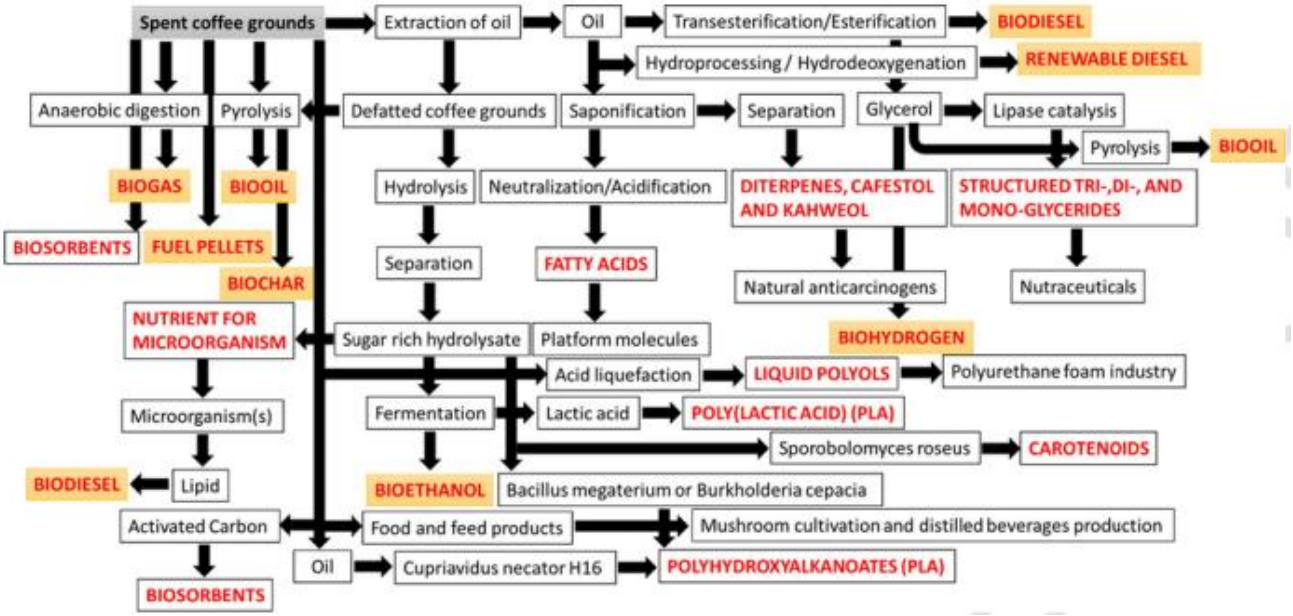


Figure 2.13: Variety of products attainable from spent coffee grounds (Karmee, 2018)

Table 2.4: By-product of coffee and its uses

By-product of coffee	**Process adapted**	**Final use**
Fresh pulp	Dried	Biocomponents
	Chopped	Animal feed
	Silage	Animal feed/ vermicomposting
	Fermentation	Ethanol
	Hydrolysis and fermentation	Ethanol
	Pre-tretment with Mycotypa sp.	Biogas
Fresh and dried pulp	Wash, drying, blend and freeze extracts with hot water	Extracted biocomponents , antioxidats activites, bacterial inhibitory activities
	Mixed together with solvents	Antioxidant activity and biocomponents
	Co-digestion and biomethanization	Biogas
Husk	Supercritical CO_2	Caffeine extraction
	Combustion in furnace and fluidized bed combustion	Pyrolysis
Silverskin	Subcritical water (25-270°C)	Antioxidant activities and caffiene extraction
	Batch culture fermentaion	Prebiotic potential

	Solis-state fermentation	Extract rich in chlorogenic acids
Parchment	Extraction using water	Biocomponents and antioxidant activity
	Varied temperture with ethanol extraction	Biocomponents and antioxidant activity
	Hydrolysis and fermentation	Ethanol
	Hydrolysis and biomethanization	Biogas
	Microwave and traditional heating	Pyrolysis

2.13 Spent coffee grounds considered as a biomass

SCG valorisation is extremely crucial since the amount of waste is generated in extremely high amounts. The SCG waste shows great potential as a feedstock for various high-value products. According to Girotto *et al.* (2018), spent coffee grounds contain carbohydrates, proteins, and many other rich chemical components which were not extracted from the coffee bean when brewed. These can be used for a wide range of alternative options such as food additives, pharmaceutical products, caffeine, bio-sorbents and bio-fuels.

To achieve a long-term sustainable objective, valorisation of SCG needs to go beyond just fertilizer, livestock feed, composts, pharmaceutical products, cosmetics and biofuels, but also focuses on sustainable strategies to reduce the impact on natural resources, waste generation, and optimise energy usage. It is therefore necessary for a fundamental shift in resource and environmental management. By doing this it will result in multiple benefits such as maximizing nutrient recovery, producing beneficial products, reducing the carbon footprint, and valorise SCG (Bounocore et al, 2018).

2.14 Lignocellulosic biomass

Lignocellulosic material has displayed the most promising characteristics of feedstock as a natural, abundantly available and renewable resource that can potentially provide long term sustainable goods supply. The demand for green chemistry together with the increasing cost of materials and the amount of greenhouse emissions have created the dire need to explore cheaper and environmentally friendly bio-materials. Some production systems pose threats and concern against the food and feed supplies. However, the recent bio-material production is more focussed to be derived from inedible sections of food crops or food waste. Utilization of this waste aims for the low-cost production from lignocellulosic or agro-industrial waste

materials such as wood, straw, fruit and vegetable waste, wheat straw, rice straw, corn cobs, sugar cane bagasse, paper waste and coffee waste (Olani, 2018).

These lignocellulosic and high-energy materials have one common problem whereby they are required to undergo a pre-treatment step for the removal of lignin. Alkali pre-treatment is substantially expensive and adds on to the overall production cost and also contributes to the environmental challenge. This poses as the largest bottle-neck problem and hinders the large-scale commercial production. There is a need to develop a cheap and effective delignification process for the lignocellulosic biomass materials to maximise lignin removal, minimise the loss of hexoses and pentose and reduce the overall cost of the process.

Advanced research has proved that bio-delignification and enzymatic delignification is very useful in pre-treatment and easily replaces the chemical pre-treatment including acid, alkali or steam explosions.

2.15 Lignocellulosic biomass components

Lignocellulosic biomass (LB) materials are a renewably abundant resource obtained from plants matter composed of polysaccharides (cellulose and hemicelluloses) and lignin. Lignocellulosic biomass has a high potential as a substitute to fossil fuel resources to produce second-generation biofuels and other biomaterials and chemicals without compromising global food security. One of the major problems experienced with LB valorisation is the resistance to enzymatic hydrolysis caused by the heterogeneous multi-scale structure present in plant cell walls which are strongly interconnected and very difficult to dissociate. Other factors posing limitations are structural factors like cellulose specific surface areas, cellulose composition, degree of polymerization, pore size and volume. Chemical factors include the lignin content, hemicellulose and acetyl groups. Besides the lignocellulosic biomass being composed of the three polymers cellulose, hemicellulose and lignin, other small components like acetyl groups, minerals, phenolic substituents are also present. These polymers appear as complex three-dimensional structures with various degrees of composition. (Iqbal & Kamal, 2012) Over time lignocelluloses has evolved to resist degradation and the recalcitrance from the crystallinity of cellulose, hydrophobicity of lignin and the encapsulation of cellulose. Cellulose is the major component found in lignocellulosic biomass. The cellulose molecule is a chain of disaccharide cellobiose, which is a structure that consists of extensive intermolecular and intramolecular hydrogen bonding networks. Having almost of the organic

carbon in the biosphere present in the form of cellulose, it is of paramount importance to convert the cellulose into fuels and valuable chemicals.

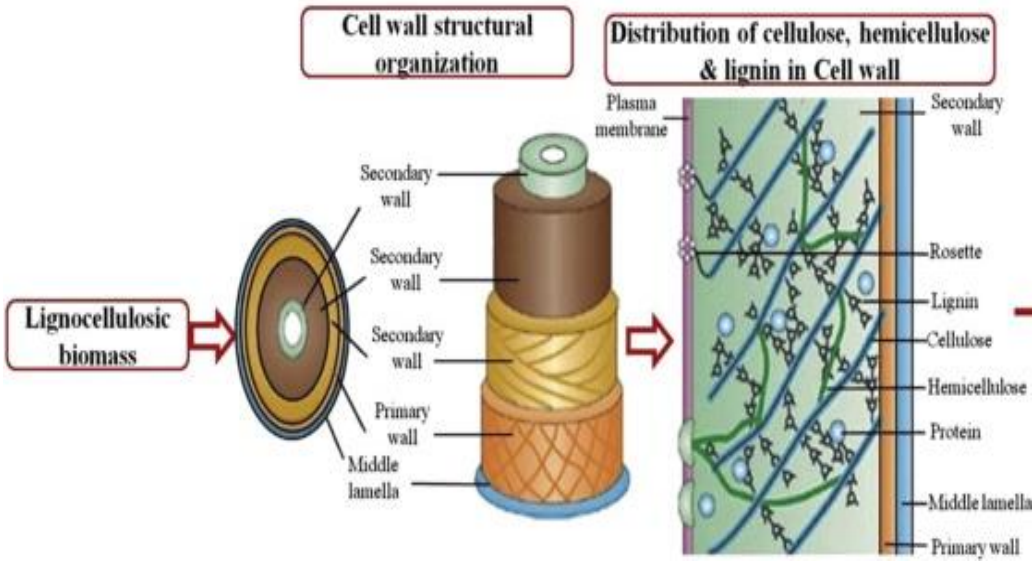


Figure 2.14: Lignocellulosic biomass structure organization (Sheng et al, 2021)

Hemicellulose is the second most abundant polymer but unlike cellulose, hemicellulose has a random amorphous structure composed of several heteropolymers like galactomannan, xylan, arabinoxylen, glucomannan and xyloglucan. The heteropolymers found in hemicellose consists of different 5-carbon and 6-carbon monosaccharide units, pentose (xylose,arabinose), hexoses (mannose, glucose, galactose) and acetylated sugars.

Hemicellulose is found imbedded in the plant cell walls forming a complex network of bonds which provide structural length by cross-linking the cellulose fibres with lignin (Figure 2.15).

Lignin is a three-dimensional polymer consisting of several phenylpropanoid units. This is glue like characteristic which provides strength to plant tissue and fibres, stiffness to the cell wall and resistance against pesticides and pathogens.

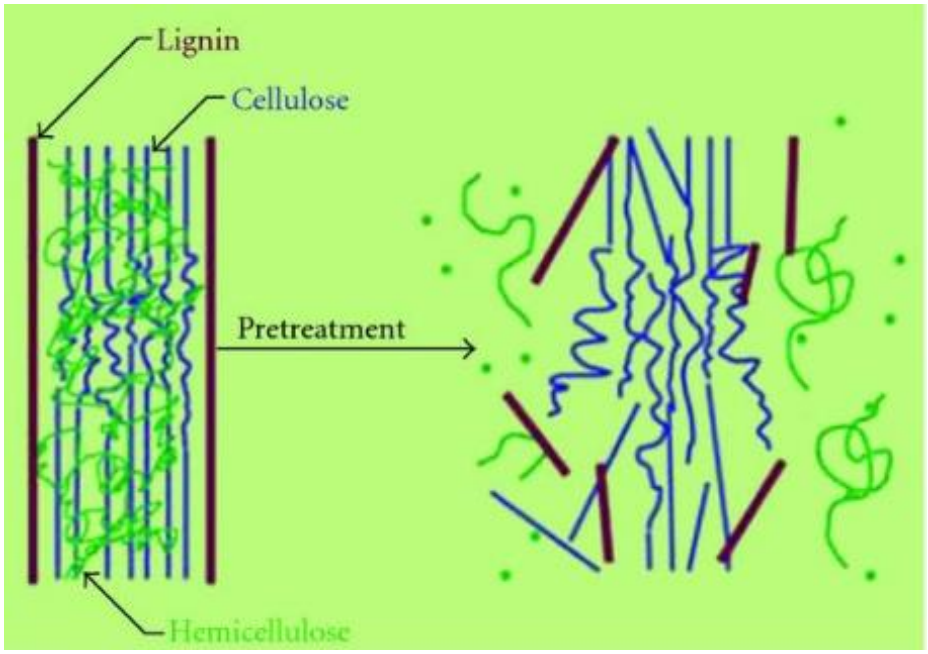


Figure 2.15: Pre-treatment demonstration of lignocellulosic biomass materials (Xiao *et al*, 2012)

2.16 Pre-treatment methods of lignocellulosic biomass

Lignocellulosic biomass composes of a variety of feedstock raw materials which appear in abundance and are renewable. Majority of the materials compositions are constituted by carbohydrates macromolecules namely hemicellulose, cellulose and lignin. Between this biomacromolecules are several covalent and non-covalent bonding making up an intricate, complex and rigid structure. Deconstruction of the lignocellulosic biomass makes these fractions easier for the transformation into large commodities including energy, chemicals, and materials in the biorefinery. The conventional pre-treatments of the lignocelluloses materials main limitations are the excessively high costs, dissatisfactory efficiencies and non-versatile uses. The use of ionic liquids in biomass processing is relatively recent however depicts many structural changes in the regenerated biomass such as the reduction in cellulose crystallinity and the lignin content. Lopes, *et al.,* (2013) showed that these findings provided served ILs as a tool on biomass pre-treatment's and advantageous uses of their specific properties over the conventional pre-treatment processes and still achieve the purity and efficiency equal to or superior to the classical pre-treatment methods.

This review study also critically studied the dissolution and changes of biomass materials when using ionic liquids as well as the influence of several crucial parameters. Valuable products can be generated by processing the cellulose, hemicellulose and lignin fractions

within the biorefinery process which is depicted in the schematic representation in figure 2.6 . (Gschwend, et al., 2016)

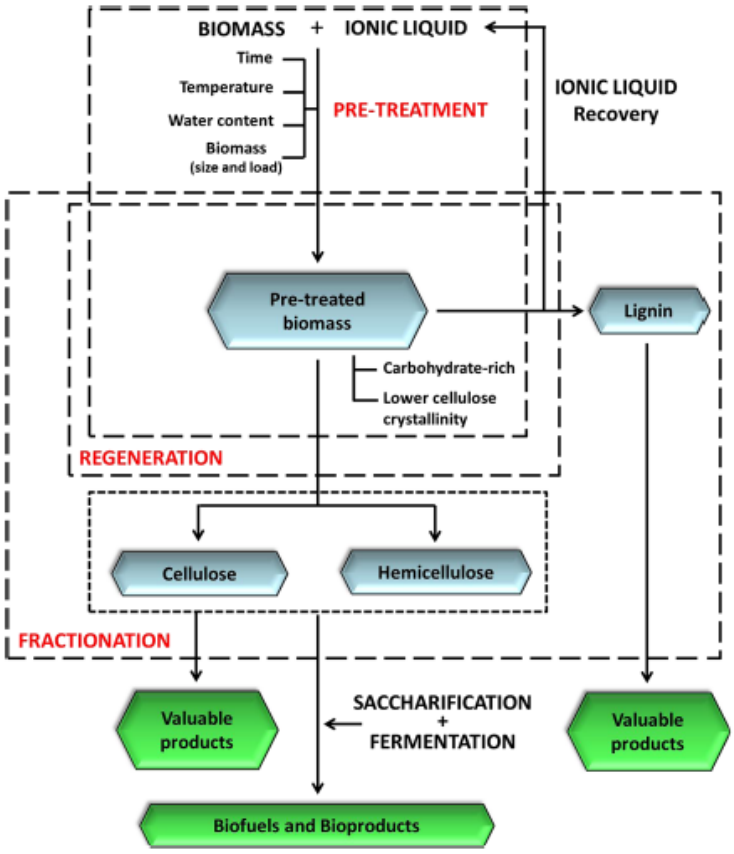


Figure 2.16: Pre-treatment methods prior to extraction (Xiao *et al*, 2012)

Table 2.5: Method and effect of pre-treatment on biomass

Method of pre-treatment	Description and effect of pre-treatment
Physical: ➢ Milling ➢ Microwave ➢ Pyrolysis	Physical methods of biomass prior to any other form of pre-treatment is primarily carried out to reduce the particle size of the organic matter with the intention to increase surface area and decrease the crystallinity and degree of polymerization. (Rajendran *et al.,* 2017). Performing the subsequent physical pre-treatment, the process after becomes more effective and easier. (Chen *et al.*, 2017). Physical methods are eco-friendly and seldom produce any toxic materials and by-products. A major factor linked to physical treatment is the high energy consumption depending on the type of biomass (Shirkavand *et al.*, 2016).
Chemical: ➢ Dilute acid hydrolysis ➢ Mild alkali pre-treatment ➢ Ionic Liquids ➢ Deep eutic/ natural solvents	This type of chemical reaction is used to alter the structure of lignocellulosic materials by the use of acids, alkalines, ionic liquids (ILs), oxidising agents or organosolv treatments all depending on the specific aim of the process.
Physiochemical: ➢ Steam explosion ➢ Ammonia based ➢ Oxidative pre-treatment	Physiochemical pre-treatment makes use of both physical and chemical processes to ensure the digestibility of the lignocellulosic material undergoing pre-treatment. Therefore, this could be higher cost depending on the type and quantity of biomass.
Biological: ➢ Fungi ➢ Bacterial ➢ Enzymatic hydrolysis	Biological pre-treatment is a low cost and eco-friendly techniques used for biomass treatment. This method is promising as no inhibitors are formed during the process and lesser energy is consumed. (Sindhu *et al.,* 2016; Bhatia *et al.,* 2017b). Enzymes that are used in degradation of lignin are best suited for such applications as they are capable of degrading cellulose, hemicelluloses, and lignin. Biological pre-treatment is not only used for lignin removal, but also for removal of specific components such as antimicrobial substances (Wan and Li. 2012).

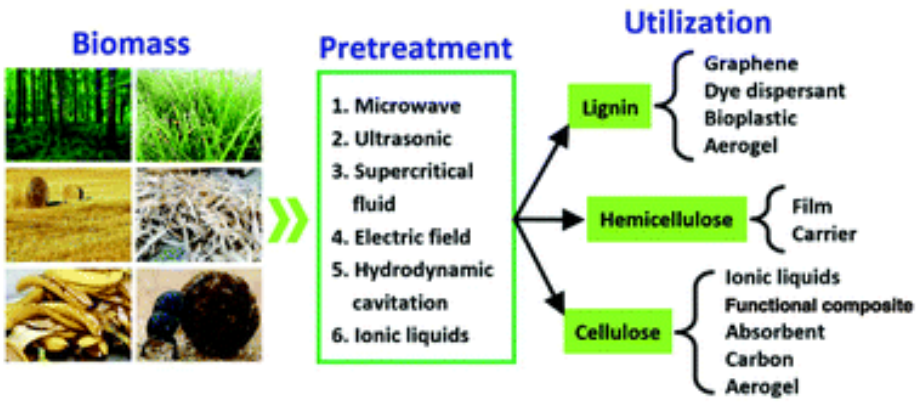


Figure 2.17: Pre-treatment methods prior to extraction (Liu et al, 2019)

2.17 Effect of pre-treatment

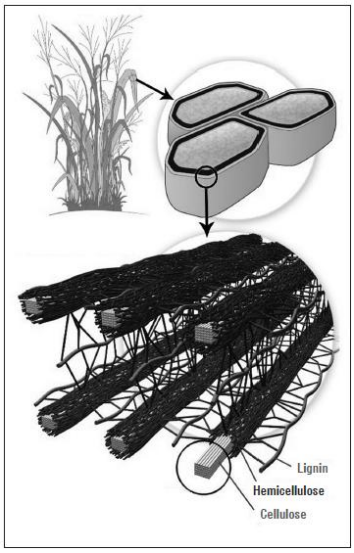


Figure 2.18: Effect of pre-treatment on biomass materials (Liu et al, 2019)

Studies on the selective and total dissolution of lignocellulosic biomass in ionic liquids have stimulated much interest in the use of ILs for pre-treatment. Majority of the interest of ILs are towards 1-ethyl-3-methylimidazolium chloride or [EMIM], 1-allyl-3-methylimidazolium or [AMIM] and 1-butyl-3- methylimidazolium or [BMIM] cations. The selection of ILs that has extensively been studied is shown in table 2.5 and table 2.9. Presently, the 1,3-dialkylimidazolium acetates, especially [EMIM] [CH3COO], are amongst the most widely investigated ionic liquids.

2.18 Literature investigated for study

According to the International Coffee Organization (ICO) in 2016, coffee consumption exceeded 9.3 billion kilograms in the world. Caffeine represents the main coffee bioactive compound and this pure alkaloid is extracted significantly in the espresso beverage, however a large amount of caffeine still remains back in the spent coffee grounds. Loizzo, (2019) reported after analysis the content of caffeine from spent coffee grounds range between 6-11.5 mg/g of waste. An amount of 200mg of caffeine and 10mg of chlorogenic acids were extracted from 100g of spent coffee grounds if the material was freeze-dried before extraction. The same study has shown that Robusta extracted coffee grounds had approximately double the amount of caffeine content compared to Arabica coffee waste. However, it is known that all recoveries of bioactive compounds are solely dependent on the extraction procedure followed. This study has also shown that caffeine concentrations range from 0.734 to 41.3 mg from spent coffee ground wastes using the soxhlet, ultrasound or supercritical CO_2 fluid extraction methods.

Over the past years, the conventional methods of extraction such as soxhlet, solvent extraction, heat reflux and supercritical fluids have presented many shortcomings such as low extration effieciency, long precedures, high energy requirements, toxicity and non selectivity. New techinques demonstrated the use of ionic liquids have been projected as the best solvent for separation and extraction of bioactive compounds from various biomass materials. The biomolecules appear in complex forms such as protiens, nucleic acids, antibodies, enzymes and even smaller antibodies like alkaloids, eessential oils, vitamins, amino acids, fats, phenolic acids and carotenoids. Ullah, *et al.*, (2018) had studied the use of ionic liquids for the extraction and purification of these bioactive compounds such as terpenoids and phenolics. In this study ionic liquids were used as solvents, co-solvents and other supporting materials in the separation process. Liquid-based extraction procedures were reported as ionic liquid-based solid-liquid extraction and liquid-liquid extraction. In this study, aqueous ionic liquids, pure ionic liquid solvents and a mixture of ionic liquid solvents together with organic solvents were addressed. It is evident from this study that ILs mixtures with organic solvents together with water can be directly applied to the solid-liquid extraction process of biomaterials from biomass plant matter (Mussatto, et al., 2011).

2.19 Trends in caffeine intake and exposure

Observing from the trends of caffeine intake assessed using a 24 hour information system over time, using data from 2001 to 2010. Mostly all of the age group categories displayed flat regression lines over time meaning that the caffeine intake has remained relatively stable over the past decade. Age groups 2-11 years and 35-39 years show a slight regression suggesting slightly lower intake of caffeine. According to the study by Pray, *et al.*, (2014), the caffeine consumption per day amongst the sample population (N=17,387), the mean intake ranged from about 25 mg amongst 2-11 year olds to more than 200 mg in older adults. Amongst the daily consumption of consumers (N=13,923) ranged from 50mg in 2-11 year olds to more than 250mg in the 50-59 year olds age group. Looking at the 90th percentiles of intakes, the daily consumption of 50mg between young children (2-11 year olds) and 100mg amongst the adolescence kids (12-17 year olds). Looking at *figure 2.19*, the highest caffeine consuming age group occurred within the (50-59 years old) having a 450mg/day. Pray *et al.* noted that both the mean and 90th percentile, caffeine intake is highly age dependant. The lowest intake occurred in children while the highest recorded were in 50-59 year old adults.

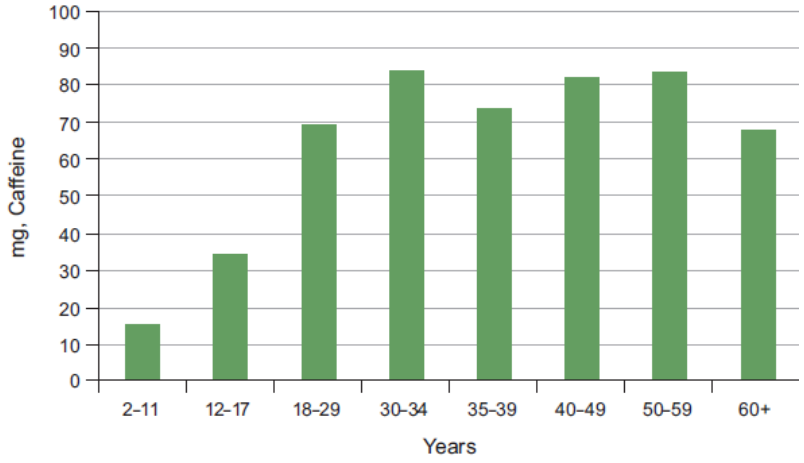


Figure 2.19: Trends of caffeine exposure and intake per 24hour cycle (Pray et al, 2014)

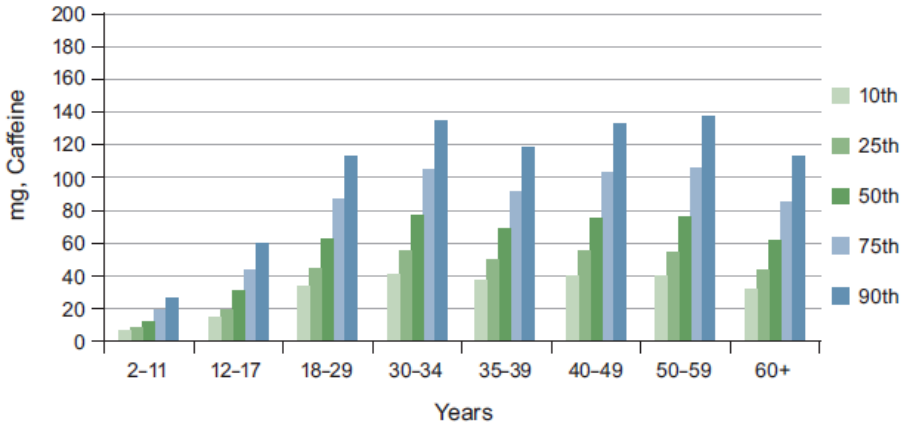


Figure 2.20: Caffeine consumption trends per age categories (Pray et al, 2014)

The caffeine content found in green coffee beans is 0.58%-1.7% for the Arabica variety and 1.16%-3.27% found in Robusta variety. (Bota, et al., 2015)

During the decaffeination process of raw unroasted coffee beans, common methods used for the removal of caffeine makes use of solvents such as dichloromethane, acetone, chloroform and water. The common method is the direct extraction of the bean with a solvent and water followed by an organic solvent. Besides caffeine, other compounds are also extracted. According to Rostango & Meireles (2015), the extracted caffeine should be in a state of 70-85% purity.

2.20 Factors affecting extraction of caffeine from spent coffee grounds

Coffee being the most popular beverage in the world, it is prepared using several methods. The two most common forms are boiled coffee (brewed) and pressurised coffee (espresso). Brewed coffee and espresso coffee possess extremely different and peculiar aroma profile, which clearly demonstrates the importance of the coffee brewing process and overall product quality. More importantly, the extraction mechanism used plays a crucial role against the bioactive compounds. The differences in the composition of coffee brew are the chlorogenic acids and caffeine content resulting from the various procedures used during the preparation.

2.20.1 Preparation of boiled coffee

Brewing coffee was the earliest method used for brewing coffee prepared from roasting, grinding and pounding coffee beans into a fine powder, thereafter adding hot water to a pot and bringing to a boil for a short duration of time. This method produces a strong blend of coffee with a thin layer of foam on the surface, and sediments (not for drinking consumption) settled at the bottom. The popular method used for brewing coffee is by steeping the beans in a mechanical device such as a French press (cylindrical vessel) or coffee plunger. Using this plunger, a circular filter fitted tightly is plunged down from the top through all the liquid content, forcing the grounds the grounds to the bottom. As soon as the coffee grounds are in direct contact with the water, most free active substances from the coffee beans are released into the beverage making it stronger.

Another common brewing technique used is the drip method, where boiling water is poured into a container with a perforated base above the filter, varying the steeping time to allow for the coffee to drip through into a container below. The filter method is similar to the drip method, often used in automatic coffee machines, where hot water flows down the filter containing the coffee resulting in the brewed coffee streaming into the container below.

2.20.2 Preparation of pressurised coffee

A wide spreading method used to produce pressurised coffee is prepared from roasted and ground coffee bean together with pressures of 7 bar , using a machine induced during percolation of limited hot water through ground coffee for a short time producing a very concentrated foamy drink. Espresso is one of the strongest tasting types of coffee with distinctive flavours and cream, with a layer of emulsified oil in the form of colloidal foam over the liquid.

Presented data values (Desbrow, et al., 2004) indicate the mean and range of caffeine per serving of beverage (mg caffeine/250 mL) from coffee using various preparation methods (Table 2.6). According to Desbrow *et al.*, the espresso coffee type contains the highest caffeine content of 97 mg/250 mL beverage. The drip method compared to the espresso is almost half the caffeine content.

Table 2.6: Caffeine content (mg) per 250 mL serving of coffee beverage (Desbrow, *et al.*, 2004)

	Coffee type	**N**	**Mean**	**SD**	**Min**	**Max**
Independent testing						
Gold Coast 2005	Espresso	97	106	38	25	214
Food standard agency 2004	All ground coffee	52	105	60	15	254
FSANZ 2002	Espresso	10	91	31	54	167
Stravric *et al.* (1988)	Drip/filtered	46	78	19	52	156
Recently published summary papers						
Knight *et al.* (2004)	Brewed	-	85	-	-	-
Mandel (2002)	Espresso	-	62	-	40	116
Harland (2000)	Espresso	-	35	-	-	-
Barone and Roberts (1996)	Drip	-	78	-	37	148

There are various factors which can be taken into consideration which affect the overall coffee brewing process. Different factors are adjusted according to the end goal of the brewing process, being taste, strength and aroma.

Table 2.7: Influencing factors on caffeine content present

Factor of interest	**Possible impact of the caffeine content**
Specie/type of coffee	Robusta coffee genetically possesses more caffeine than Arabica coffee.
Brewing time	Not a decisive factor
Temperature of water used	Caffeine highest solubility occurs at 100°C. Temperature and extraction have a directly proportional relationship
Pressure	Not a decisive factor, as high water pressures does not increase the caffeine extraction.
Roasting of coffee beans	Possibility of increase in caffeine loss during the roasting process.
Grinding degree	The degree of grinding is closely related to the amount of surface area allowed for extraction.
Type of water	This does not affect the caffeine extraction but may affect the taste, aroma and flavours.
Coffee-to-water ratio	One of the greatest influences in the content of caffeine in the brew.
Volume of coffee solution	Various brewing methods have different volumes, which affects the overall caffeine content in the brewed solution.
Origin of coffee beans	Climatic and environmental factors may have a small influence on quality of coffee produced.
Light exposure	This can have a positive effect of the caffeine content on the coffee beans.
Methods of growing	The use of nitrogen-rich fertilises can increase the amount of caffeine present in the beans itself.

2.21 Factors of interest for this particular study

2.21.1 Particle size of coffee grinds

Reducing the particle size of coffee grind through milling or grinding has shown to increase the volatile and non-volatile compounds during coffee brewing (Akiyana *et al.* 2015). Grinding the coffee grounds into finer particles increases the surface area for contact during the extraction process, which then increases the permeability of chemicals and substances into the water. According to Cordoba *et al.* (2019), grinding has a critical impact on the extraction yield of coffee.

2.21.2 Impact of brewing/ reaction time

Brewing time solely depends on the method used for brewing due to different qualities of coffee being obtained after a period of time (Nhan & Phu, 2012). Impact of brewing time is a decisive factor in influencing caffeine content which is explained below. The shortest brewing time is a distinctive property in espresso machines (3 times for 13 seconds or 42 seconds) opposing, the longest cold brewing time of 282 or 420 minutes. According to the study by Ludwig et al (2014), the highest caffeine concentration of 7,908 g/mL whereas the lowest value for the cold brew was recorded by Rao *et al.* (2020) was 1,036 g / mL. Considering brewing time only as a variable affecting caffeine extraction, despite shorter brewing times, the coffee made in the coffee machine during shorter times had a significantly higher caffeine content compared to that of the cold brew coffee. However, the brews differ from each other in terms of other parameters used. Differences in caffeine content can also be caused by the amount of coffee bean used during brewing and volume of water used. It can be assumed that the difference in caffeine content was affected by other factors like coffee variety, degree of grind, temperature, pressures and so forth. Judging from all the above research studies, it is evident that high caffeine yields can be achieved from shorter brewing times, if other conditions like temperature and pressures are met (Olechno, et al., 2021).

2.21.3 Impact of pressure

Pressure is one of the factors which can make a significant difference in coffee brews and their corresponding caffeine content. Caprioli *et al.* (2015) studied the effect of different pressures (7, 9, 11 bar) on the caffeine content of both Arabica and Robusta coffee. Maximum caffeine yields results were obtained at 7 bar and a water temperature of 92 °C: 10.303 g/L.

2.21.4 Impact of temperature

When water flows through the coffee grinds, many bioactive substances are released into the brew. The temperature of water has a significant impact on the content of caffeine released into the coffee solution due to the fact that caffeine is moderately soluble in water at 20°C (1.46 mg/mL). The solubility of caffeine increases at 80°C to 180 mg/ mL and reaches its peak at 100°C (670 mg/mL). It can be assumed that with lower temperatures, the extraction of caffeine is slowed down.

Espresso prepared in coffee machines make used of temperatures between 92 °C and 94 °C and pressure of 1 bar. Caprioli *et al.* (2014) investigated the effect of temperature on caffeine extraction during brewing and noticed an increase in temperature (88 °C to 92 °C) led to an increase in caffeine contents released. Increasing the temperature to 98°c showed that less caffeine was extracted regardless of the pressured used. Seeing from this, the authors concluded that the best conditions for caffeine extraction for espresso coffee was at 92 degrees Celsius and 1 bar. Salamanca *et al.* (2017) showed that lowering the temperature from 93°C to 88°C contributed to the reduction of caffeine extraction. The results recorded by Rao *et al.* (2020) and Angeloni *et al.* (2016) confirm that the lower temperatures slows down the extraction yield of caffeine in a brew as their studies both used the same amounts of coffee and water and the same types of coffee.

2.21.5 Solid-liquid loading ratio

According to the study by Bi *et al.*,(2010) who studied the decaffienation proves of coffee bean waste by solid-liquid extraction, showed that the extraction of caffeine increased with an increasing solid/solvent ratio, which is obvious that the solid-solvent tario is useful to improve the extraction yields. Increasing the solvents increases the dissolution of the bioactive components into the solution. The theory of increasing the volume of solvent to allow more contact with the solid material, hence releasing higher caffiene yields. According to Bi et al, the solid-solvent ratio of 1:5 to 1:60 were analysied, concluding that 1:20 is sufficient to reach a high extraction effieicncy.

2.22 Solvent selection for extraction process

Water is almost always one of the liquids present in liquid-liquid extraction process, however the choice of solvent choice is quite diverse. Any good solvent is required to fulfil five essential properties namely:

1. Solvent should be immiscible with the other solvent (usually water).
2. Solvent should have a relatively low boiling point so that it can be easily removed from the compound after the extraction process.
3. Solvent should extract very little to none of the impurities or compounds present in the mixture.
4. Solvent should be non-toxic, readily available, non-reactive and cost effective.
5. Solvent should have a high solubility for the organic compounds.

According to the study by (Shinde & Shinde, 2017), the following solvents were tested for the extraction of caffeine from coffee.

Table 2.8: Percent extraction achieved using various solvents

Solvent of choice for decaffeination process	**Descriptive properties of solvent**	**% extraction**
1.Ethyl Acetate	Removes caffeine together with little flavouring compounds. Mildly toxic	96-98 %
2.Water	Removes caffeine and little flavouring components Non-toxic solvent	94-96 %
3.Dichloromethane	Removes caffeine and little flavour components. Highly toxic	94-96 %
4.Supercritical CO_2	Selectively removes caffeine and little flavour compounds. Very expensive solvent	96-98 %
5.Acetone	Removes caffeine and a little of flavouring compounds Mildly toxic solvent	96-98 %

2.23 Quantification of caffeine from spent coffee grounds.

Various analytical methods exist which are able to quantify caffeine, however the International organisation for Standardisation (ISO) has recommended two method for caffeine content determination in coffee samples. The ISO 4052 determines the caffeine content using UV spectrophotometry and ISO 20481 estimated the caffeine content through high performance liquid chromatography (HPLC). In this study (Gopinandhan, 2014) compared twenty two coffee samples following both ISO methods to test the proficiency of both methods. Results obtained from the ISO 20481 (HPLC method) were significantly higher than those of ISO 4052 in all 22 samples. The standard deviation of results ranged from 0.07 to 0.71% on dry matter. The HPLC method therefore seems to be simple, rapid and realistic in the quantification of caffeine.

Quantification of caffeine was carried out using the Agilent 1200 HPLC system. (Shang, et al., 2017). This process makes use of a reverse phase Prevail C18 analytical column of dimensions 250 x 4.6mm internal diameter and 5µm particle size. The mobile phases used were water and acetonitrile with flow rate of 1 mL/min. A gradient program was performed using 105 acetonitrile for 10 minutes and thereafter increased from 10% to 40% in 20 minutes and lastly to 100% after a further 10 minutes. The chromatogram for caffeine was recorded at 280nm. The calibration curve was established in the range of 0.5-500µg/mL for the quantification of caffeine.

2.24 Caffeine

2.24.1 Overview of caffeine

For centuries caffeine has been part of innumerable cultures. According to researcher Bisht & Sisodia, (2010), caffeine is considered as "*a wonder gift to medicinal science".* Caffeine is the most frequently ingested pharmacological active substance in the world found naturally occurring in the more than 60 plants including tea leaves, cola nuts, cocoa pods and coffee beans. This naturally occurring chemical stimulant is also found in leaves, seeds, fruit and plant species which belong to a group of compounds called trimethylxanthine.

According to the World Health Organisation (WHO), caffeine has become the most widely used and legal drug in the world. It was first isolated in 1820 from commonly consumed products like coffee, tea, soda and over the counter prescription drugs. Caffeine is usually produced commercially by a decaffeinating coffee process. Caffeine occurs naturally in tea leaves, coffee beans, nuts, maté leaves and guarana plants. Caffeine is the defence mechanism in plants due to its antifungal effect and sterilant against certain insects. Caffeine is further processed into items such as sodas, cosmetics and pharmaceutical products that allow us to enjoy our daily lives

During the coffee brewing process, caffeine is released into the boiling water together with tannins. Tannins are responsible to give the coffee its rich and dark colour. However, caffeine is soluble in water but only to a certain extent of 1g per 46mL of water. Therefore, we can gather that an excess amount of caffeine is remainder in the already brewed, waste coffee beans. (Chiang, et al., 2018)

Caffeine IUPAC name 1, 3, 7-trimethylpurine-2, 6-dione appears as a white crystalline powder with a melting point range of 234-236.5°C. Caffeine is an alkaloid (organic molecule containing nitrogen) in the purines group, which has pharmacological effects on humans and animals. It has a bitter taste and can be used as a sharp flavouring additive as well. The chemical and physical structures of caffeine are shown in figure 2.21.

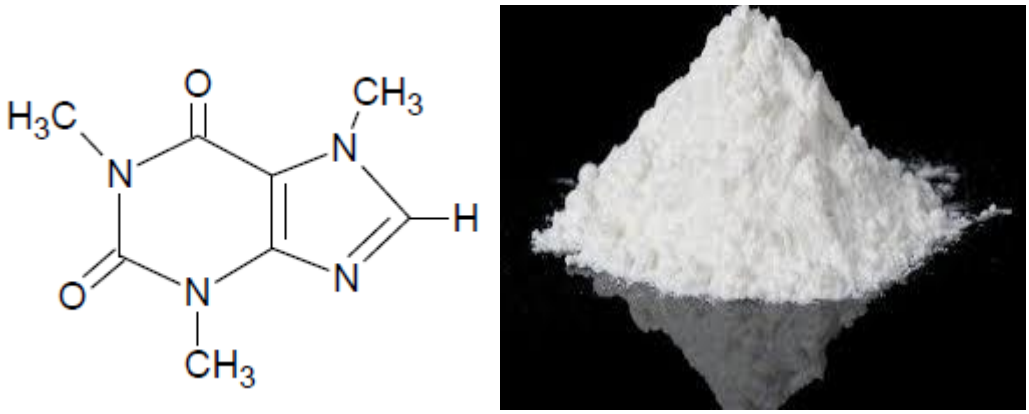


Figure 2.21: Molecular structure and physical appearance of pure caffeine

0.24.2 Properties of caffeine

Systematic name	1,3,7-trimethyl-1H-purine-2,6(3H,7H)-Dione
Alternative name	1.3,7-trimethylxanthine & 1,3,7-trimethyl-2,6-dioxopurine
Molecular formula	$C_8H_{10}N_4O_2$
Melting point	238°C
Molecular mass	194.19g/mole
Solubility in water	slightly soluble

2.24.3 How does caffeine work?

Caffeine works as a stimulant to the central nervous system (CNS), cardiac heart muscles, respiratory system, and centres that control blood pressure. To first time consumers, caffeine may raise blood pressure but will not have an effect on people who consume it all the time. Caffeine also acts as the "water pill" where it increases urine flow.

2.24.4 Uses of caffeine

Caffeine has a wide variety of uses, some of which are:

- Caffeine is used as a stimulant for the central nervous system, cardiac muscles in the body and the respiratory tract. (Whitsett, et al., 1984)
- Caffeine helps to protect brain cells, therefore lowering the risk of developing certain diseases such as Parkinson's disease.

- Caffeine also acts as a diuretic. This is a form of assistance in the medical field where an excess amount of water needs to be removed from the body.
- Regular cups of coffee stimulate the gallbladder and at the same time reduce the risk of gallstones.
- Caffeine has the ability to improve one's mind. Consumption of caffeine improves an individual's sense of wellbeing, energy and sociability.
- Increases the levels of concentration, therefore making it possible to perform a variety of cognitive tasks more quickly. Caffeine is a type of drug which promotes alertness.
- Sport athletes are able to train, exercise and perform for much longer durations because the exhaustion levels are minimised during the consumption of caffeine.
- Caffeine consumption also affects the utilization of glycogen in the human body. This also assists athletes, as muscle pain is extensively reduced.
- Medication containing caffeine makes the blood vessels in the body constrict. This will in turn make the body to absorb the medication more quickly and have a faster effect of the medication.
- Constricting of the blood vessels help to reduce pain such as headaches.
- Caffeine helps to prevent diabetes as it stimulates the muscles in the body to burn fat and sugar more efficiently.
- Caffeine has been proved to act as an antimicrobial agent (Bhakar & Yadav, 2015).
- Caffeine stimulant ability helps to prevent many other diseases and health problems such as Parkinson's, Alzheimer's and asthma relief.
- The antioxidants present in caffeine helps to stabilize any free radicals in the body. This will assist in slowing down the aging process, skin shrinking and lethargic nature of the body.
- Coffee reduces inflammation which may help to prevent certain heart related illnesses.
- Caffeine consumption is also used in weight loss.
- Creams containing caffeine are used to reduce redness, skin rashes and itching in dermatitis.

A 10 grams quantity of caffeine is considered the lethal dose of caffeine. However, the amount of caffeine varies depending on what is actually consumed, serving size and preparation method. A portion of chocolate may contain 5 mg of caffeine, compared to an energy drink which could contain as much as 160 mg or even pain medication which contains amounts of caffeine as high as 200 mg. The recommended daily consumption of caffeine is 200 to 300 mg, which equates to two to four cups of brewed coffee. This recommended amount is considered safe for adults. If individuals consume between 500 mg to 600 mg of caffeine on a daily basis, then like any other drug, the over consumption of caffeine can lead to severe side effects (Chagule *et al,* 2019)

2.24.5 Over consumption/ side effects of caffeine

- Headaches, muscle cramps, tremors.
- Over consumption of caffeine cause insomnia and prevent sleep. This alters sleep patterns which lead to fatigue.
- Caffeine consumption raises blood pressure, therefore over consumption will pose as a risk of cardiovascular diseases or strokes.
- It can also amplify stress levels as stress hormones are elevated during this consumption period.
- Because of the rapid contractions, gastrointestinal problems may be caused. This includes abdominal pains, diarrhoea, etc.
- Over consumption or the daily consumption of caffeine can prevent or inhibit the absorption of some nutrients and this could possibly lead to nutrition deficiencies.
- Serious male problems like possible risk to urinary infections and prostate problems and female problems such as miscarriages, infertility and hot flashes can be caused with an over dosage of caffeine.
- Excessive amounts of caffeine consumption decreases bone mineral density, which can eventually lead to osteoporosis.
- Caffeine is a diuretic and can cause dehydration.

2.25 Ionic liquids

2.25.1 Background

Even though biomass is considered as a fuel source that is renewable, available in abundance and environmentally friendly, it still is of great interest to produce green energy and bio-products from lignocellulosic materials (Xiao, et al., 2018). The commonly used conventional organic solvents are required to be replaced by solvent which are less toxic, less flammable and less polluting since these are just a few of the major challenges the chemical industry faces. Ionic liquids are often sometimes called Green Solvents and are closely related to Green Chemistry. The main reason for this statement is that many ionic liquids have a negligible vapour pressure, making them non-flammable, cannot be inhaled and less volatile. The aforementioned characteristics make ionic liquids much safer and environmentally suitable compared to that of the conventional volatile organic compounds (VOCs). All ionic liquids are not intrinsically green, however they can be made green and their use in specific processes lead to improvements that comply with the principles of green chemistry and engineering. ILs can be the vehicle to drive design processes which comply to the 12 principles of Green Chemistry and Engineering (Anastas *et al.*, 2012).

The US Environmental Protection Agency (US EPA) has defined Green Engineering as the design, commercialisation and the use of products and/or processes which are feasible and economical, while reducing the amount of pollution generated and minimising the risk to human health and the environment both during production and consumption. Sine ionic liquids have low vapour pressures; it can be easily recycled as the reaction medium.

Taneja, *et al.* (2015) and Swatloski, *et al.* (2002) put forward the use of ionic liquids for the use of a solvent or pre-treatment step of the LB material. Hence the prospect for the extraction of caffeine using ionic liquids was adapted. ILs are relatively new in the regards to solvents with a melting point of less than 100°C, compromising of both cations and anions. The cations in general are organic viz. imidazolium while the anions include both organic and inorganic ions (Yoo *et al.*, 2017). Both cations and anions play a significant role in solubilising the cellulose and lignin, interrupting the intra- and inter molecular hydrogen bonding. Since ILs are of adjustable nature of their cations and anions on which the properties of their IL depend on, they have been described as green solvents. After extraction processes, most ionic liquids are recoverable and reusable which is advantageous in the overall feasibility of the application used (Chen *et al.*, 2017).

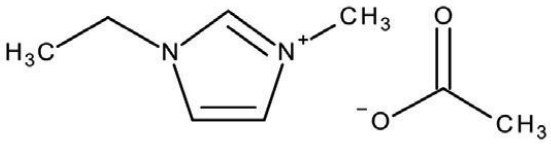
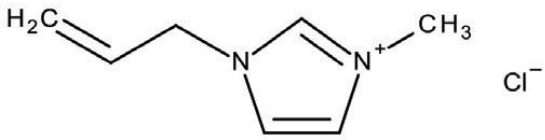
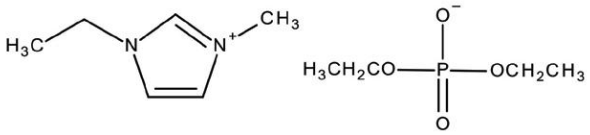
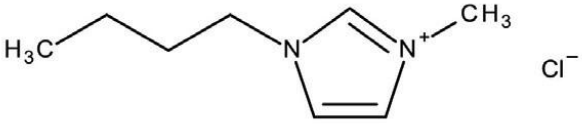
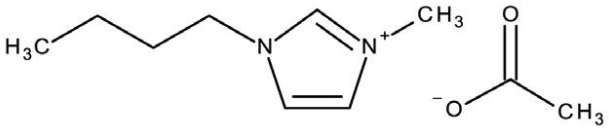
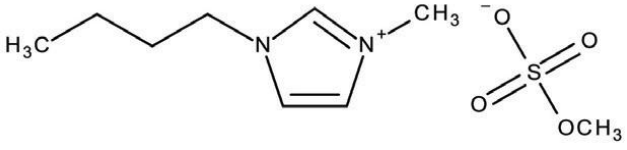
2.25.2 Advantages and disadvantages of the use of ionic liquids.

Table 2.9: Advantages and disadvantages of the use of Ionic liquids

Advantages		**Disadvantages**	
1. Large variety of solvents available	Up to 10^{18} possible types of combinations available	**1. Cost/Pricing**	Ionic liquids are 5-20 times higher in price compared to conventional solvents.
2. Ability to altered	Ionic liquids are easily amendable in nature and can vary functional groups or alkyl chain length.	**2. Viscosity**	Pure Ionic liquids often have a high viscosity.
3. Vapour pressure	Almost negligible vapour pressure at normal conditions, hence no emissions released into the atmosphere.	**3. Vapour pressure**	Having such low vapour pressure affects/hinders distillation processes difficult during solvent separation.
4. Flammability	Non-flammable and therefore less risk	**4. Synthesis**	Many situations require multi-step synthesis which is expensive.
5. Detachability	Compounds can be easily separated using their vapour pressures.	**5. Sustainability**	Ionic liquids have a green nature however they are non-sustainable and non-biodegradable.
6. Stability	Ionic liquids are stable against a wide range of temperature and electrochemical decomposition	**6. Hygroscopic tendency**	Many ionic liquids tend to be moisture sensitive
		7. Corrosive	Ionic liquids have significant high corrosiveness; hence require special containers for storage in industrial processes.

2.25.3 Ionic liquid: 1-ethyl-3-methylimidazolium chloride [EMIM][Cl]

Table 2.10: Ionic liquid names and their correspsonding structure

Name of Ionic Liquid/ Abbreviation	Structure
1-ethyl-3-methylimidazolium chloride [EMIM][Cl]	
1-ethyl-3-methylimidazolium chloride acetate [EMIM][CH_3COO]	
1-allyl-3-methylimidazolium chloride [AMIM][Cl]	
1-ethyl-3- methylimidazolium diethylphosphate [EMIM][DEP]	
1-butyl-3- methylimidazolium chloride [BMIM][Cl]	
1-butyl-3- methylimidazolium acetate [BMIM][CH3COO]	
1-butyl-3- methylimidazolium methylsulfate [BMIM][MeSO4]	

The ionic liquid 1-ethyl-3-methylimidazolium chloride or [EMIM][Cl] is a commonly used ionic liquid in biomass and cellulose processing. The cation is composed of a five-member ring with two nitrogen atoms and three carbon atoms. It is a derivative of imidazole with ethyl and methyl groups substituting the two nitrogen atoms.

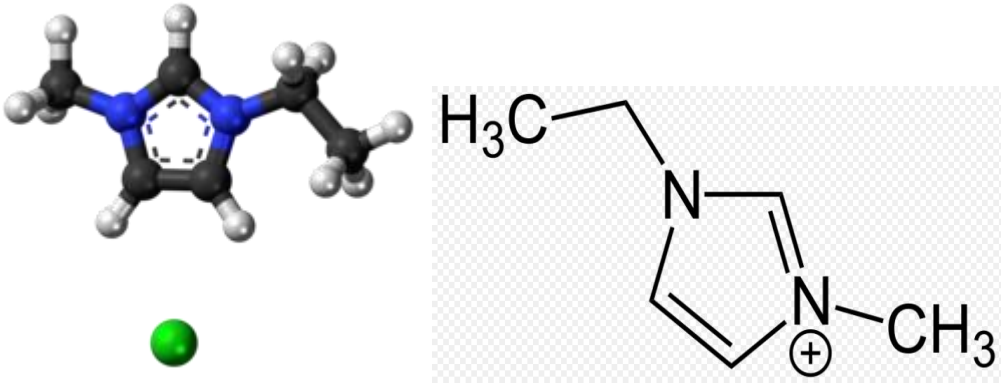


Figure 2.22: Molecular and structural configuration of 1-ethly-3-methylimidazolium

2.26 Insight on processes and equipment used for this study

2.26.1 Extraction using various extraction solvents to obtain pure solid caffeine

Extraction is a physical process whereby a compound (or mixture of compounds) is transferred from one initial phase to another. Extraction occurs every time coffee is brewed where the water-soluble components exit the solid phase (coffee beans) and enter the liquid phase (hot water). This is solid-liquid extraction. (Chaugule, et al., 2019).

When the coffee beans are brewed, not all organic materials are removed. Organic solvents can be used to extract the organic components from the waste coffee beans. This process is known as liquid-liquid extraction. It allows the isolation of single components present in a mixture. The physical process which rules liquid-liquid extraction (LLE) is solvent-solvent partitioning, or the distribution of solvents between a pair of solvents. When two solutions vary in densities, a two-layer system is obtained. Hence, the organic solvents have limited solubility in water leaving the less dense solvent at the upper layer and the denser solvent, being water at the bottom.

Caffeine can be recovered from the organic phase by evaporation of the solvent. The evaporation will yield the caffeine content. However, this will be crude caffeine and not pure caffeine. The crude caffeine obtained from the organic phase prior to purification will be process further to form crystals of pure white caffeine. The crude caffeine extract contains impurities which were not removed in the aqueous layer. After concentration of the organic phase using a rotary evaporator, a brown waste layer is present at the bottom of the flask which requires further purification. Increasing the volume of the organic solvent, it automatically will be expected to increase the percent recovery of the caffeine. This is due to more solute being extracted into the larger organic phase.

Caffeine has a higher affinity for certain organic solvents compared to that of water at room temperature. Therefore, it can be isolated using simple liquid-liquid extraction. In liquid-liquid (solvent-water) extraction, caffeine will be separated from water-soluble substances. After removing traces of water, the low-boiling point solvent is evaporated to yield crude organic extract. After further purification by sublimation, white caffeine crystals are obtained.

Figure 2.23 below shows the extraction procedure to follow in order to extract caffeine: (Palleros *et al.*, 2010)

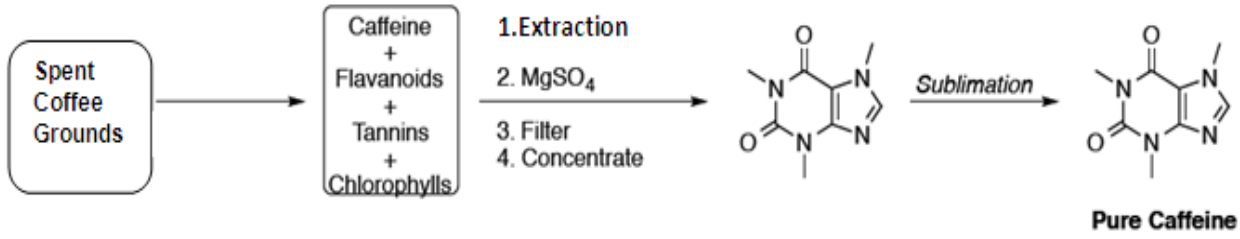


Figure 2.23: Overall scheme for caffeine extraction

The organic extraction solvent dichloromethane or ionic liquid 1-ethyl-3-methyl-imidizodium will be used to extract caffeine from the aqueous extract. The solvent-water mixture can be easily separated based on variation in densities, as the extracting solvents are much denser than water. Excess residue water is separated by draining out the extracting solvents using a separating funnel (Palleros *et al.*, 2010).

Using the conventional method, also known as the decaffeination process of spent coffee grounds, many physical processes are implemented. The physical steps included crucial steps prior to the final stage. These steps included: preparation of the solution; solid-liquid extraction; liquid-liquid extraction; drying; filtration; concentration and purification.

2.26.2 Liquid-liquid extraction

During the separation between two liquids, it is important to distinguish whether the aqueous layer is above or below the organic layer when in the separatory funnel, as this distinguishes which layer is kept and discarded respectively. Immiscible liquids/solvents will stack on the top of each other depending on the differences in densities. Lower density solutions will settle at the top and the denser solution at the bottom. (Postu, 2013)

Using liquid-liquid extraction method separates metal complexes or various compounds based on their relative solubilities in two different immiscible liquids. These immiscible liquids are usually one polar (water) and one non-polar (organic solvent).

The overall net transfer of species from one liquid phase to another liquid phase generally occurs from aqueous to organic, the chemical potential drives the process until the overall process is complete, leaving the overall system more stable (lower free energy). The solvent

that is enriched in solute(s) is known as the extract, while the feed solution that is depleted in the solute (s) is called raffinate.

This extraction method is widely used for the separation of organic compounds from a mixture or compound. The chosen solvent has the capability to dissolve one or more compounds into the solvent. These dissolved compounds in the solvent makes up the extract.

In order to extract the caffeine from waste coffee, first solid-liquid extraction is compulsory to get the solid natural products into the solvent. Thereafter, boiling is done with the addition of sodium carbonate as a base. This then allows the tannins to be separated from the solution using vacuum filtration. The vacuum filtration is able to separate the caffeine from the organic layer. The organic layer is kept for evaporation based on the boiling points of the extraction solvent content, using a rotary-evaporator Thereafter the raw crude caffeine is turned into pure white caffeine is obtained.

Boiling/steeping of the spent coffee grounds allows the tannins and caffeine to dissolve in the water. If no base is added, the tannins will also be extracted into the solvent. The base converts the tannins into their respective sodium salts. And being ionic, these salts are not soluble in the solvents. Therefore, they remain in the aqueous phase during extraction, allowing more pure caffeine to be extracted.

2.26.3 Solid-Liquid extraction

Many methods are used to separate a mixture containing solids and liquids. If the solids settle well at the bottom, the liquid can be easily decanted. Small sized solid particles tend to from cloudy/milky emulsions which have to be centrifuged or passed through micro scaled filter paper. Common laboratory solid-liquid separation techniques are gravity filtration or suction filtration (vacuum filtration). Gravity filtration if the pouring of a solid-liquid mixture through a funnel containing filter paper, allowing liquids to pass through and solids to collect on the filter paper. Suction filtration has a similar approach; however differs in the use of a vacuum pump below the funnel to pull the liquid through.

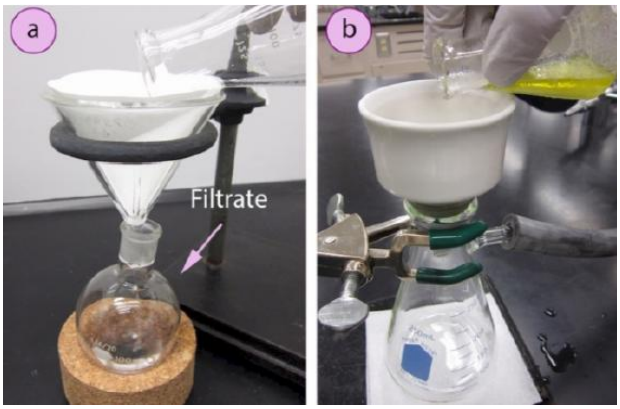


Figure 2.24: Filtration apparatus used for the separation of solid SCG and filtrate

Gravity filtration and suction filtration both have pros and cons which help decide which method to use. The main indicator of which filtration to use is whether the solid or filtrate (liquid which passes through the filter paper) should be retained. Gravity filtration is commonly used when the filtrate is retained, whereas suction filtration is used when the solid is retained. Suction filtration has the potential to suck through small solids through the filter paper which can contaminate the filtrate. Suction filtration is preferred only when the solids are retained as gravity filtration is less efficient in removing all residual liquid from the solids through the filter paper. When filtering hot solutions, it is essential that the solution filters quickly before it cools down in the funnel with the fluted filter paper. By folding the paper with a greater number of bends, the surface area is increased for quicker filtration. The folds assist in creating a space between filter paper and the funnel, allowing for the displacement of air to exit more easily as the liquid is filtered.

Table 2.11: Advantages and disadvantages of various filtration

Advantageous of suction/vacuum filtration	**Disadvantageous of suction/vacuum filtration**
Much faster/quicker compared to gravity filtration(<1minute with a good seal)	The force applied during suction can pull through fine particles hindering the quality of the filtrate. Most efficient for large crystal/particles.
More efficient in removing residual liquids , hence a purer solid	Loss of material to the filter paper and filtrate is significant, therefore only recommended for microscale work.

CHAPTER 3

THEORY AND EXPERIMENTAL

3.1 Introduction

Investigating spent coffee grounds as a primary source makes it necessary to know the compositional analytics of the material itself. This compositional analysis is referred to as characterisation. This is critical for the basic understanding of the feedstock material which is being used to effectively produce fuels and chemicals. This characterisation method was carried out using Technical Association of the Pulp and Paper Industry (TAPPI) method (Akdogan, 2022). These standard test methods utilize testing procedures for evaluating, measuring, describing or scientific investigations of substances.

The proposed experimental methodology framework used for the extraction of caffeine from spent coffee grounds was based on the research by Pradnya Ingle et.al, (2019). This chosen guideline was selected due to its simplicity, equipment availability, green methods, sustainability and the overall feasibility of the process. A great advantage of using this guideline is the high yield of the extracted caffeine from spent coffee grounds. The spent coffee grounds were obtained from local shops, takeaways and restaurants around Durban, Kwa-Zulu Natal as well as higher grade imported coffee which were obtained during a business fair.

The following procedure was undertaken for all samples

Step 1: Drying of the spent coffee grounds samples sample at 105°C, to attain a moisture content of 10% after drying.

Step 2: Characterisation of spent coffee grounds using TAPPI methods.

Step 3: Extraction of caffeine from spent coffee grounds. Optimization of extraction conditions such as reaction time, extraction solvent and temperature.

Step 4: Analysis of the purity and yield of caffeine obtained.

Step 5: Cost analysis and feasibility study of the overall process.

The study used quantitative research approach to achieve its objectives. The proposed experimental methodology framework used for the extraction of caffeine from spent coffee grounds is based on Pradnya Ingle et al, (2019). This chosen procedure was selected due to its simplicity, equipment availability, green methods, sustainability and the overall feasibility. A great advantage of using this guideline is the expected yield of the extraction of caffeine from spent coffee grounds. For the experimental work, the spent coffee grounds were obtained from local shops, takeaways and restaurants around Durban, Kwa-Zulu Natal.

3.2 Characterisation of spent coffee grounds.

Characterisation of spent coffee grounds using TAPPI methods. Characterisation will be carried out following the TAPPI methods which involves elemental analysis, ash content, mineral composition and summative composition (Caetano, *et al.*, 2012). TAPPI characterisation involves ash content, moisture content and particle size deformation of the spent coffee grounds.

The methodology for the determination of lignin and carbohydrate content is included in this chapter for research purposes and further studies, however for this specific study, lignin determination and carbohydrate content are excluded as it has no significant impact on caffeine extraction. Caffeine is relatively hydrophobic due to its weakly hydrating faces and the water molecules not as strongly localised as other complex solutes such as xylose, guanidinium and pyridine which are required for further extraction of products (Tavagnacco, et al., 2011).

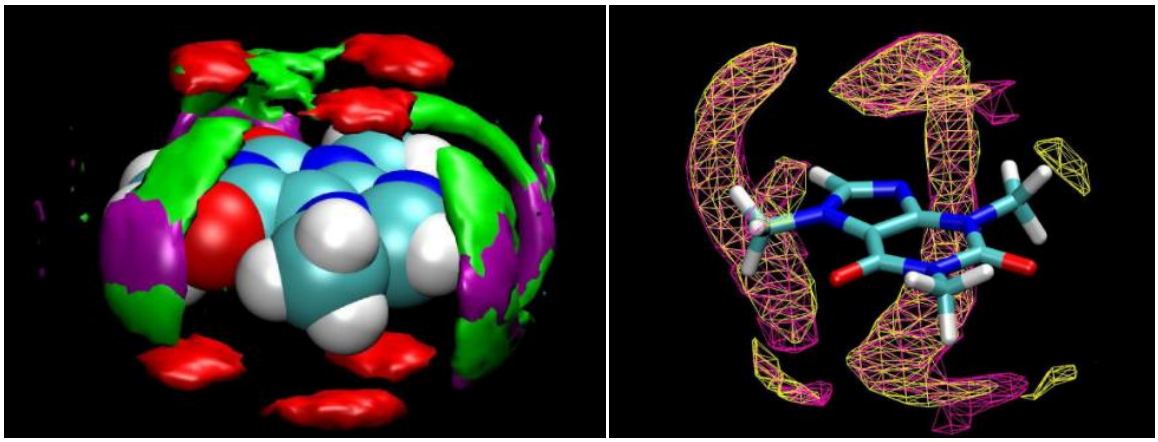


Figure 3.1: Caffeine molecules and molecular dynamics appearing in coffee with other present substances (Tavagnacco, et al., 2011)

3.2.1 Moisture content of spent coffee grounds

A predominant characteristic of biomass materials is their hydroscopic tendency. They tend to absorb various amounts of moisture from the surrounding atmosphere, therefore containing different amounts of moisture.

The National Renewable Energy Laboratory (NREL) procedure will be used to describe the ratio of solids to moisture in the spent coffee grounds material. A predominant characteristic of biomass materials are their hygroscopic tendency. They tend to absorb various amounts of moisture from the surrounding atmosphere, therefore containing different amounts of moisture. By heating the SCG to a temperature of 105 °C, the water is evaporated leaving behind all solid content, which could not be vaporized. The measurement of moisture content will indicate the amount of water and other volatile components that vaporized at 105 °C.

Materials required: Spent coffee grounds, weighing dish, analytical balance, and oven

Equal weights of spent coffee grounds was be weighted, ranging from 1g to 5g. Sample preferably should contain 10% moisture after drying. The weight of the empty weighing dish, the weight of the weighing dish plus the sample before drying and the weight of the weighing dish and sample after drying was recorded before further processing.

The total solid percentage on a 105°C dry weight basis will be calculated using the equation 3.1.

Procedure: 1. Five weighing dishes were labeled No.1-5, weighted and recorded respectively.

2. Using one dish weighting dish per coffee ground sample, samples weights of 1g to 5g of spend coffee grounds were weighted and recorded.

3. All 5 samples were then inserted into the oven at 105°C.

4. On removal of the samples, each dish were weighted and recorded.

$$\%\ \boldsymbol{Moisture\ content} = \frac{W_2 - W_1}{W_2 - W_T} \times 100 \quad \text{..... Equation 3.1: Moisture content equation}$$

W_2 = weight of weighing dish and biomass sample prior to drying, grams

W_1 = weight of weighing dish and biomass sample after drying, grams

W_T = tare weight of empty weighing dish, grams

3.2.2 Ash content in spent coffee grounds

Materials required: crucibles, platinum, porcelain or silica 50-100ml with covers, furnace

After dry oxidation, a residue which is known as ash remains. This procedure takes samples of 1g to 5g in a furnace at temperatures of 525°C ± 25°C. Results obtained are in relation to 105°C oven dry weight (ODW) of sample, and are found using equation 3.2.

$$\% \boldsymbol{Ash} = \frac{\boldsymbol{A}}{\boldsymbol{B}} \times \mathbf{100} \text{ ... ...}$$ **Equation 3.2: Ash content equation**

A = weight of ash, grams

B = weight of biomass sample (moisture-free), grams

3.2.3 Particle size of the biomass material

Figure 3.2: Sieve shaker apparatus

To determine the particle size of spent coffee grounds, samples were taken using laboratory sieves of different sizes apparatus. This included 600µm, 425µm, 355µm, 250µm, 150µm and 75µm.

The various screens will be mounted in the vibrating sieve shaker, operating at 50rpm for the duration of 25 minutes. This sieve shaker apparatus is clearly indicated in the image below.

Each individual sieve weight is recorded prior to adding the spent coffee ground content. The sieves weight is recorded prior and after the 25 minute shaking period.

This is done in order to determine the estimated mass of the particle retained in each sieve. To establish the percentage of the spent coffee grounds particles of a specific range, the following formula is used:

$$\% \boldsymbol{P} = \left(\frac{\boldsymbol{M_2 - M_1}}{\boldsymbol{M_{Total}}}\right) \times \mathbf{100}$$ **.....Equation 3.3: Particle size determination equation**

Where:

P = percentage of a particular particle size

M_2 = weight of the sieve after the shaking period

M_1 = weight of the sieve prior to shaking

M_{total} = total mass of the spent coffee grounds used

The average particle size diameter of each spent coffee grounds sample is determined using the following equation:

$$\boldsymbol{D} = \frac{\sum_{i=1}^{n}\left[\frac{S \times P}{100}\right]}{n}$$ **.....Equation 3.4: Average particle size diameter equation**

Where:

D = average particle diameter size

S = sieve aperture size

P = percentage of particles in sieve tray

n = number of sieve tray used

3.3 Extraction method

3.3.1 Introduction

Prior to the extraction process, matrixes of experimental runs were generated using Design of Experiments (DOE) from the Design-Expert 13 software. DOE provided a systematic method for determining the relationship between the (A) reaction time (15-35 minutes) , (B) reaction temperature (88 ℃ - 120 ℃) and (C) solid-to-solvent loading ratio(5g SCG/10-25 mL solvent) affecting the overall extraction process. The DOE established the cause-and-effect relationship between experimental variables which is necessary in order to optimise the caffeine extraction. For this research study, standard Quadratic design Response Surface was selected. Box-Behnken design type with three factors generated runs coded low, medium and high from the standard numeric inputted. Build information and factor ranges can be seen in table 3.1 below.

Table 3.1: Build information and factors used during Design of Experiment

Build Information

File Version	11.1.0.1		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	17.00
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	4.00		

Factors

Factor	Name	Units	Type	SubType	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Reaction Time		Numeric	Continuous	15.00	35.00	-1 ↔ 15.00	+1 ↔ 35.00	25.00	7.07
B	Reaction Temperature		Numeric	Continuous	88.00	120.00	-1 ↔ 88.00	+1 ↔ 120.00	104.00	11.31
C	S/L Ratio		Numeric	Continuous	10.00	25.00	-1 ↔ 10.00	+1 ↔ 25.00	17.50	5.30

The DOE matrix varied reaction times of 15 – 35 minutes, reaction temperatures between 88 – 120 °C and solid-to-solvent ratio ranges of 10 mL to 25 mL, providing various combinations of the investigated parameters. The generated DOE matrix was used for each extracting solvent.

During the preparation of the solution, 5g of spent coffee grounds and 1.5g of base (sodium carbonate powder) is added to the Teflon cup. The $Ca(OH)_2$ will react with the tannins in the coffee grounds to initiate precipitation forming calcium salts and tannins. After the solvent amount is added to the cup, the remainder volume is filled with boiling distilled water, mixed with a rod to prevent lumps and thereafter allowed to stand for 5-7 minutes. This steeping

process serves as a pre-treatment step prior to going into the Parr reactor vessel, making the biomass more readily reactable. On completion of the reaction time, the solids are filtered using course (Whatman 4) filter paper and a 250 mL filter flask. The filtrate obtained is used for liquid-liquid extraction to obtain the organic layer which contains the caffeine. Two organic layers become present once the organic solvents are added to the separation funnel. The solvent amount is the value obtained from the DOE matrix. The separation funnel is set up on a ring stand and once the organic layer is obtained, it is kept for evaporation to evaporate all the extraction solvents present n the solution. A 2 mL sample is kept aside for HPLC analysis. Thereafter, evaporation is completed, yellow crude is left behind. This raw crude is recrystallized using ethanol, into pure white powdered caffeine which is the analysed for its purity.

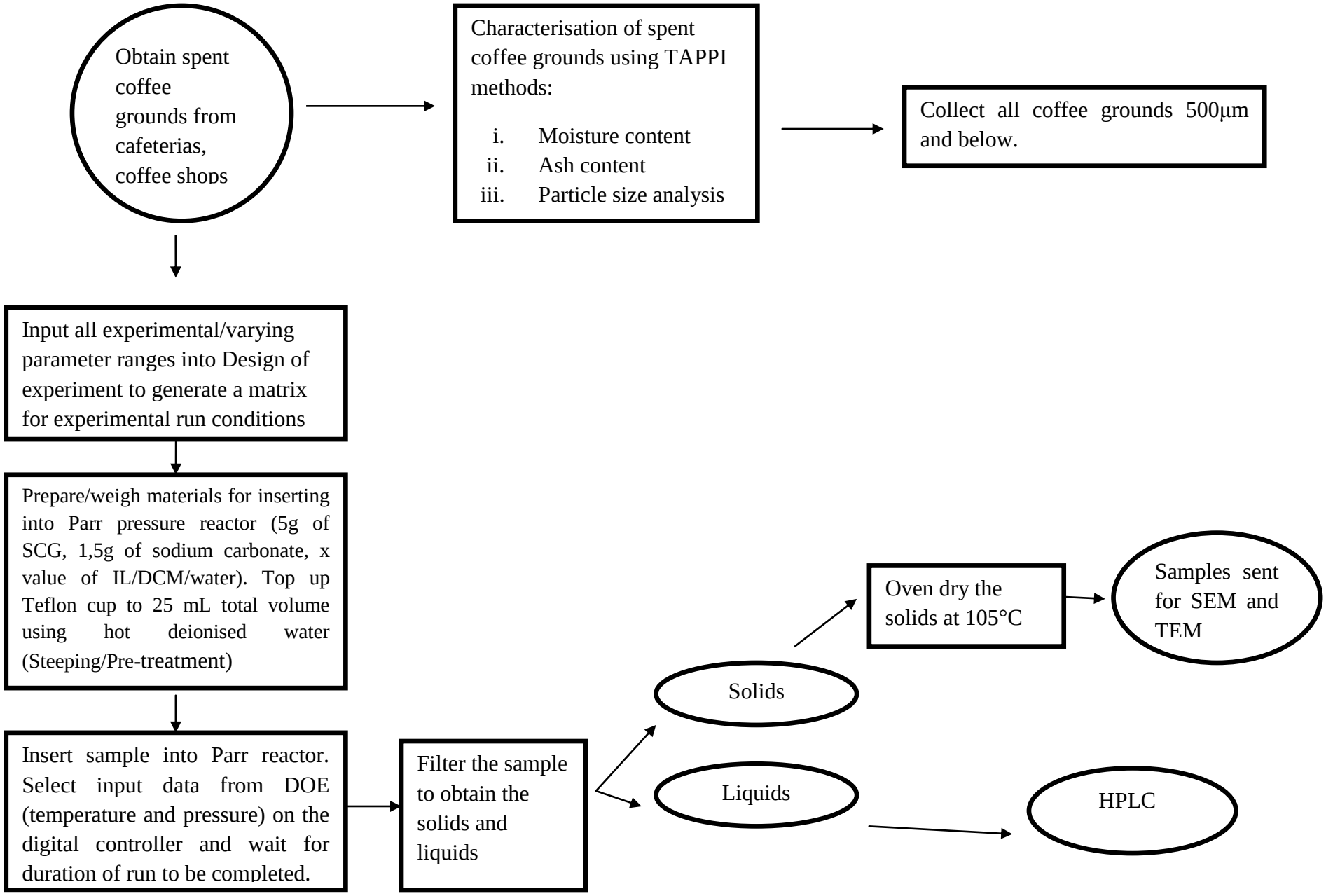
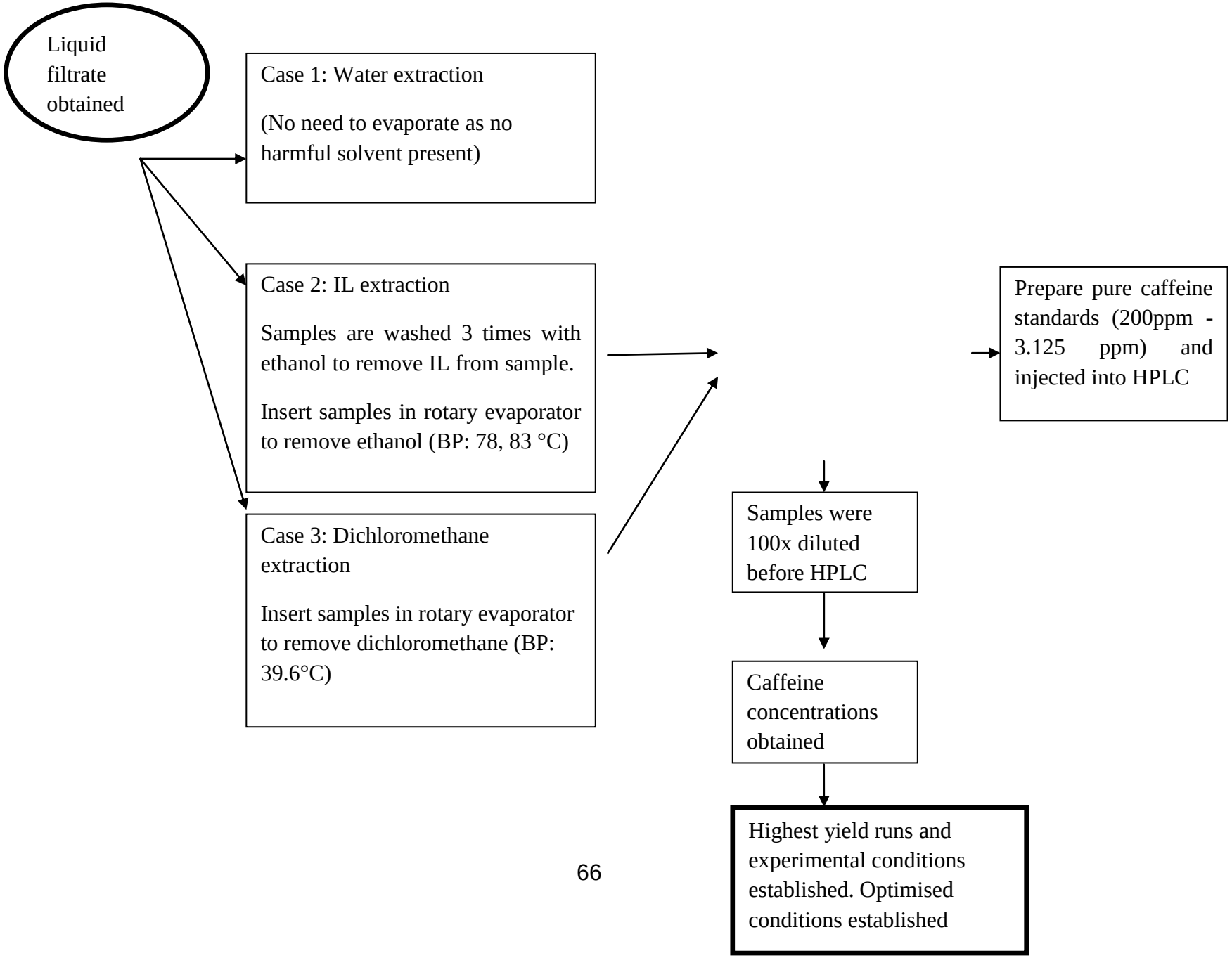
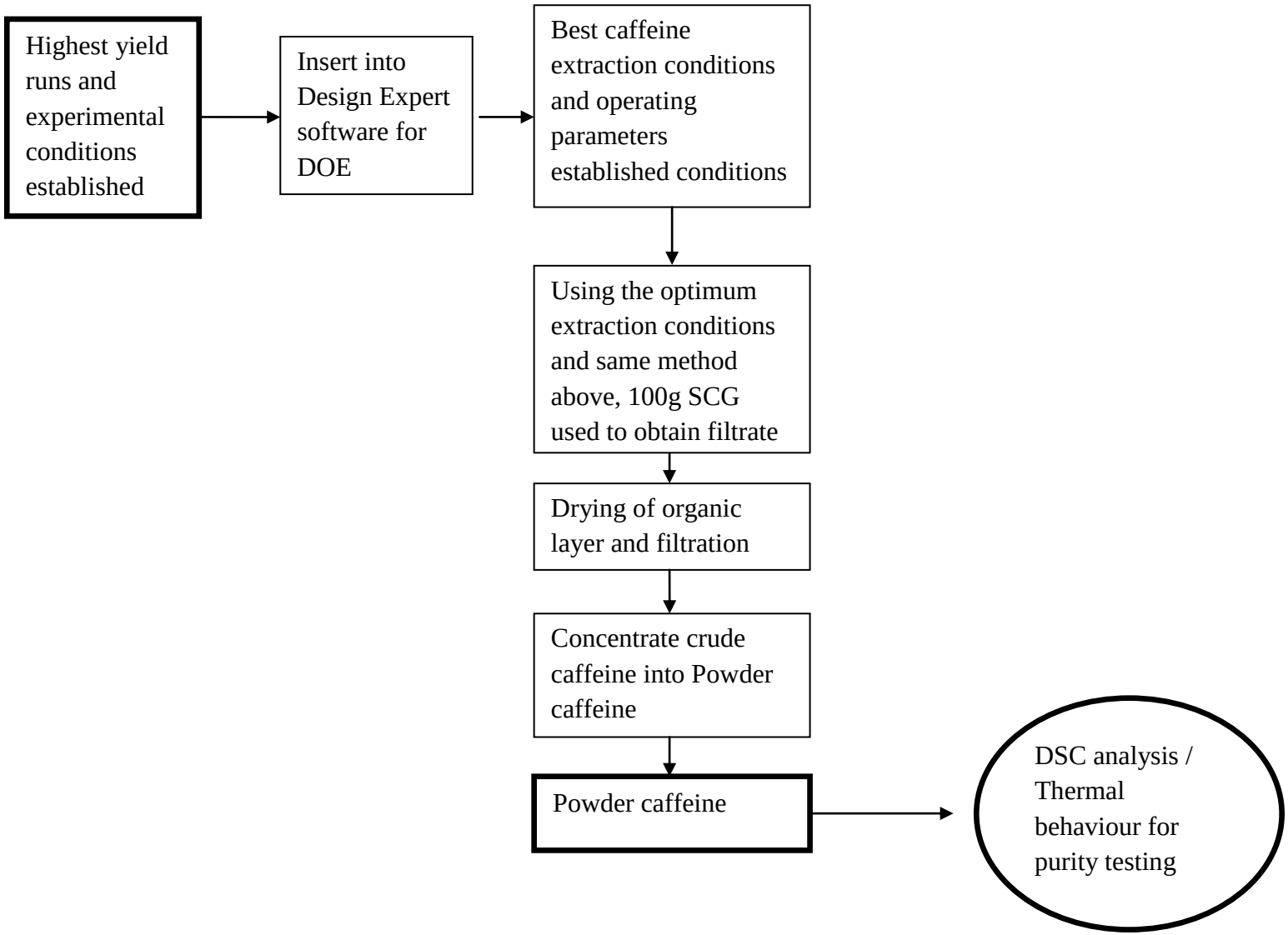


Figure 3.3: Block flow diagram of overall methodology, process and analysis

3.1.1.1


Liquid filtrate obtained
Case 1: Water extraction
(No need to evaporate as no harmful solvent present)
Case 2: IL extraction
Samples are washed 3 times with ethanol to remove IL from sample.
Insert samples in rotary evaporator to remove ethanol (BP: 78, 83 °C)
Case 3: Dichloromethane extraction
Insert samples in rotary evaporator to remove dichloromethane (BP: 39.6°C)
Prepare pure caffeine standards (200ppm - 3.125 ppm) and injected into HPLC
Samples were 100x diluted before HPLC
Caffeine concentrations obtained
Highest yield runs and experimental conditions established. Optimised conditions established


Highest yield runs and experimental conditions established
Insert into Design Expert software for DOE
Best caffeine extraction conditions and operating parameters established conditions
Using the optimum extraction conditions and same method above, 100g SCG used to obtain filtrate
Drying of organic layer and filtration
Concentrate crude caffeine into Powder caffeine
Powder caffeine
DSC analysis / Thermal behaviour for purity testing

3.3.2 Part A: Extraction

The Parr pressure reactor (Parr, USA) allows reactions to occur under much higher conditions of temperature (500°C) and pressure (5000psi). The Parr pressure reactor (Figure 3.4) consists of a 50mL Teflon cup where 5g spent coffee ground and the extracting solvent (mL) are inserted. The Teflon cup is placed inside the vessel with a stirring component. The desired temperature, pressure and time are selected on the digital reactor controller. Nitrogen gas is used for pressure build up within the reactor at a set 7 bar pressure limit was used through all runs. Once the reaction time is complete, the reactor is cooled down to room temperature before removing the sample.

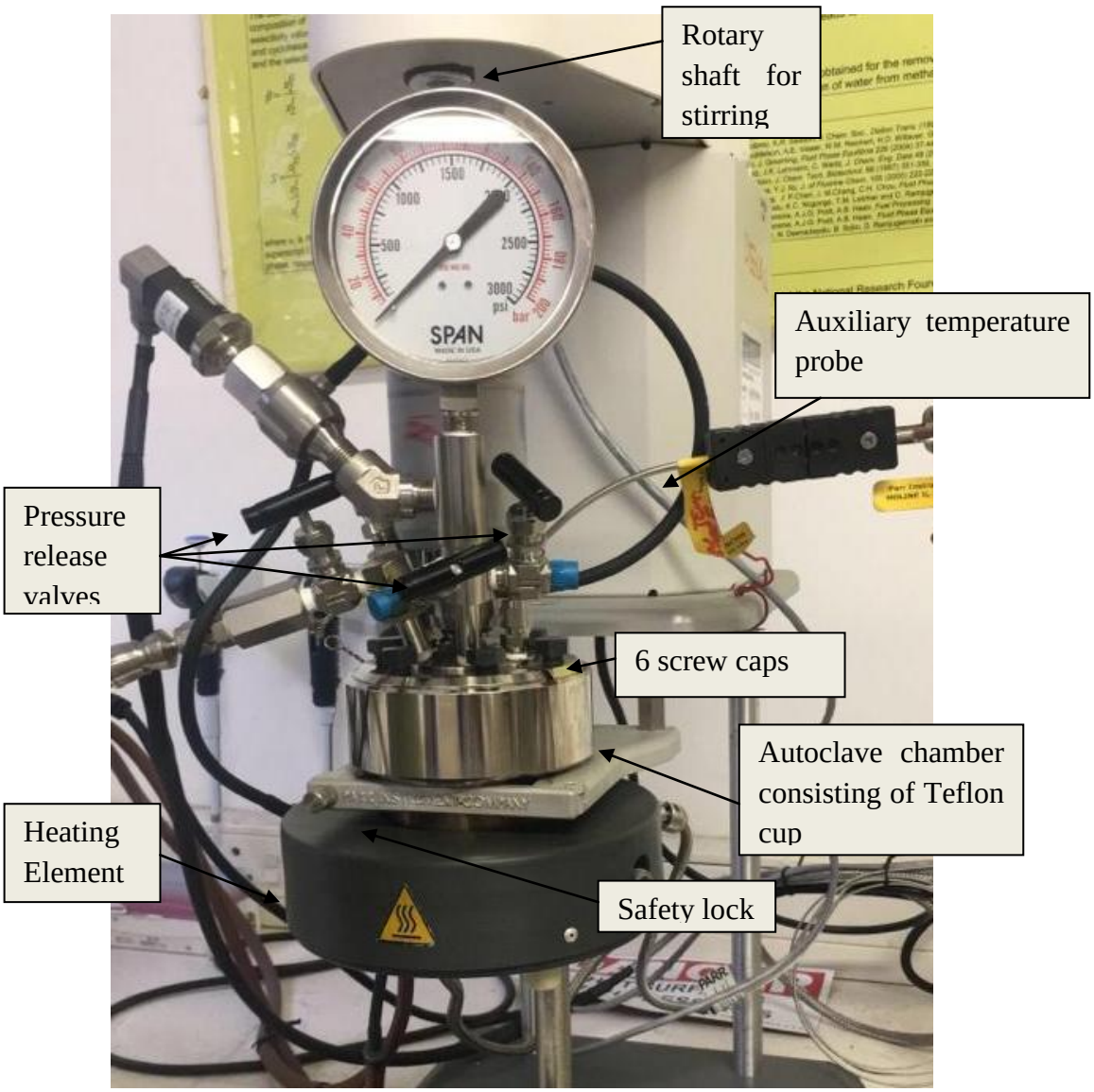


Figure 3.3: Parr pressure reactor

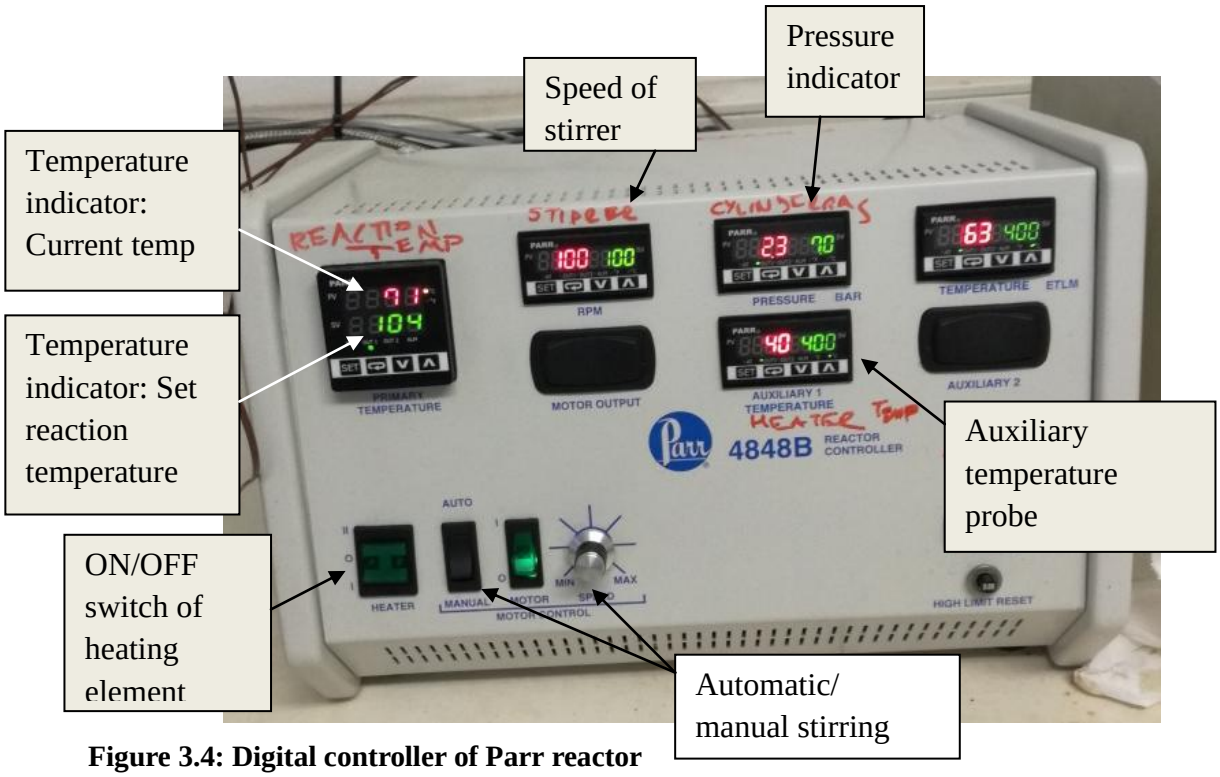


Figure 3.4: Digital controller of Parr reactor

The generated Design of experiments had to be scaled down to allow the contents of the cup to not exceed 25 mL, compared to the initial DOE matrix (Appendices: Table 7.1), the amount of the solvent used was quarter of the initial DOE matrix. The solid spent coffee grounds used for each experimental run was 5g while only changing the volume of solvent accordingly. Preparation of the solution included weighing of solid materials was done using an analytical balance whilst liquid volumes were weighted out using a pipette and volumetric cylinder.

Once all materials have been gathered, the polytetrafluoroethylene (PTFE) liner cup can be filled. To open the vessel, the stirrer at the top is unscrewed allowing the vessel to be removed and placed on the bench top. Thereafter, the 6 screw caps on the outer band of the vessel were loosened using a spanner. The outer band of the vessel was also removed together with the cone shaped pointed screw. Once this was done, the head of the cylinder is free and able to be opened. By carefully lifting the head of the cylinder without damaging the stirring shaft and internal components, the PTFE liner can be removed from the vessel and the prepared sample was inserted (Spent coffee grounds, 1,5 g sodium bicarbonate and the corresponding volume of solvent).

After the sample is inserted into the PTFE liner cup having a maximum volume of 25mL, the head of the vessel is connected to the base by tightening the 6 screw caps to prevent any leakages of the reactants from within the vessel. After the 6 screw caps are tightened, the

protection ring is inserted over the vessel to secure the vessel onto the stand using the magnetic head. Once the vessel is secured in place, secure the safety lock. Connect the stirrer string to the head and observe if the stirrer moves. Thereafter, the heating band was moved up to the vessel by pressing the two claps. All valves on the reactor vessel must be closed tightly to ensure there are no gas leaks. The water bath was then switched on to circulate water to the reactor. Lastly, the gas was switched on by opening the valves anticlockwise located on the cylindrical tank. The pressure gauge is located at the head of the reactor vessel which indicated the pressure on the digital meter.

The reactor control switch located on the back of the controller was switched on. The temperature on the primary temperature control using the SET button and the ^ and v buttons. The reactor switch is set on low (I) or (II) settings depending on the reactor temperature. Low temperatures are selected for temperatures 175ºC and below. The stirrer is set to automatic position after the PTFE set-up is connected. The vessel is left to now build up temperature. Once the reaction temperature is selected and the vessel built up temperature, autotuning of the set temperature must be done to maintain the set point temperature and prevents the reaction from heating beyond the set point temperature. At 50°C, press the set button on the primary controller. The screen will display AT at the top and OFF at the bottom. Press ^ to change off to on, and the display will blink Press set again to confirm. The blinking light indicates that the heating block is regulating the heat supply to the reactor.

Once the upper value on the primary temperature block found on the digital meter reaches the desired reaction temperature, a timer is set for the reaction temperature using a stopwatch. Once the duration is completed, the heating band is slid down from the vessel and the vessel is allowed to cool down to room temperature before opening it. Once the vessel temperature reaches below 50 ºC, the gas release valves are opened to discharge any internal pressure. The vessel can now be carried out and the 6 screw caps loosened, allowing the head of the vessel to be removed and the PTFE cup with the contents can be removed.

The content of the PTFE cup is then decanted into a volumetric beaker using a funnel and filter paper.

Once the extractions are completed and the solutions filtered, it is ready for LLE in order to achieve the final powder caffeine.

3.3.3 Part B: Purification of extracted caffiene by sublimation

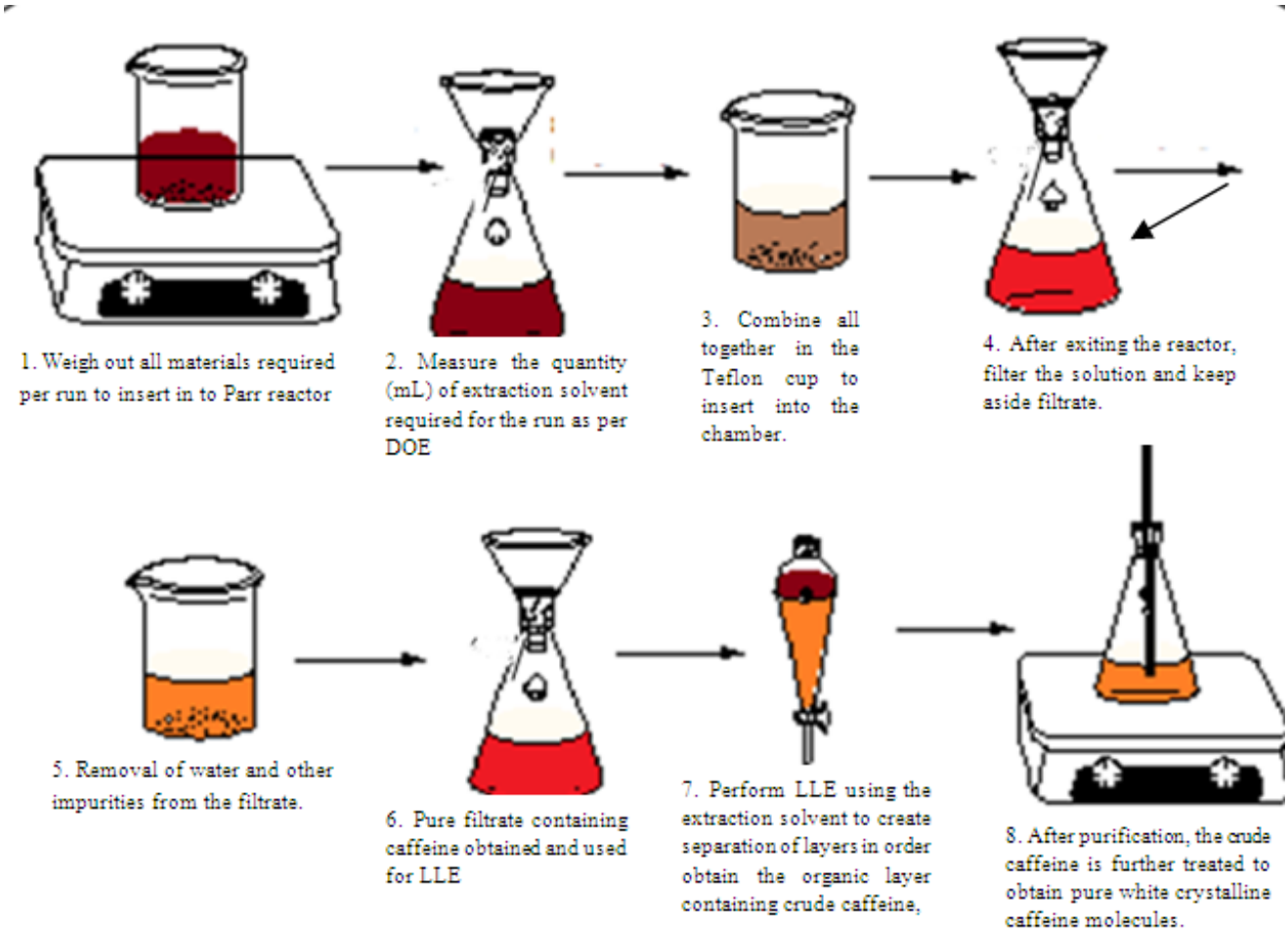


Figure 3.5: Schemtic of steps followed during experimental runs

The crude extract is purified via sublimation process to yield pure caffeine. After concentration of the crude caffeine, scrape off as much crude extract as possible from the round bottom flask into a clean, dry Petri-dish. Place the Petri-dish on a hot plate and cover with a stack of 3-4 disks of filter paper (Whatman 1). Over the filter paper, place a 250mL beaker/flask with 30-50mL of water. This helped hold the filter paper in place and served as a cooling mechanism. Keep the hot plate to low-medium heating. After a few minutes, the side of the Petri-dish displayed white vapours of caffeine. The white gas formation indicated solids sublimes. Let sublimation occur for 5-8 minutes, before switching of the heat. Allow the beaker cooled down completely to room temperature. Do not remove immediately from the hot plate as removing prematurely will cause caffeine vapours to escape. Scrape off all the purified caffeine from the filter paper onto a pre-weighted filter paper.

Figure 3.6: (a) Coffee solution prior LLE; (b) Two visible density layers; (c) Organic extract; (d) Crude Caffeine

a) Extraction with dichloromethane and 1-ethyl-3-methylimidizodium

On completion of the extraction and filtration step, the solution is cooled down using an ice-water bath. 5 g of sodium chloride and 0,25 g of calcium hydroxide is added to the filtrate. This step is performed in a fume hood/cupboard. This will salt out the water from the caffeine. The water will be attracted to the salt making the caffeine more free. Adding the sodium chloride decreases the miscibility of the aqueous and organic layers. It also helps in avoiding the formation of unwanted emulsions. To avoid this problem, the separating funnel was not shaken vigorously. Invert and rotate gently, several times for 5 minutes while opening and closing the vent to release build-up of unwanted pressure. On completion of rotating the separation funnel, place the funnel on a ring stand and wash the extracted solution with the extraction solvent (Figure 3.7a).

b) Separation of both layers

Supporting the separating funnel on a ring stand, two layers are clearly visible (Figure 3.7b). Carefully remove the stopper. If the layers are not clearly visible, add 10 mL more of extracting solvent to the flask and swirl gently to fully break down the emulsion. Open the stopcock carefully and collect bottom layer in 125mL Erlenmeyer flask. Cap the flask with a cork or rubber stopper. Some emulsion may be present in the organic phase and wash with 25mL of 10% aqueous NaCl solution. The remaining aqueous portion is collected in a flask labelled aqueous extract, and discarded at the end of the experiment.

c) Drying the organic extract

The organic layer is treated with a drying agent, anhydrous magnesium sulphate ($MgSO_4$). $MgSO_4$ has a dual purpose to remove water and break down any emulsions present. Add a generous scoop of anhydrous magnesium sulphate to the organic flask and swirl. It could be necessary to add more, depending on the amount of water in the sample. Water molecules present will soak up the $MgSO_4$ and been visible as white clumps at the bottom of the flask. When sufficient $MgSO_4$ is added, flakes will form around the globe resembling a snow globe (Figure 3.7c). This is an indication that enough $MgSO_4$ is added to the flask. Cover the flask with a cork stopper. Leave aside for 10 minutes, with occasional stirring.

d) Set up the filtration system

Filter the organic phase using a small piece of cotton wool, loosely packed at the neck of the glass funnel. Using a glass rod, apply pressure gently on the cotton wool to secure it in place at the neck of the funnel during filtration. Collect the filtrate in a pre-weighted 100mL round bottom flask. Rinse the $MgSO_4$ with 2mL fresh methylene chloride. The filtrate obtained must be clear, without any traces of water or magnesium sulphate. If water molecules are present, transfer the organic solution back to the Erlenmeyer flask and repeat the drying system with 1-2g of anhydrous magnesium sulphate. If $MgSO_4$ filters through the cotton plug, re-filter the solution using a larger piece of cotton.

e) Concentrate

Removal of the solvent was done using a rotary evaporator (Figure 3.8) equipped with a luke warm water bath connected to a cold tap. Dichloromethane will evaporate at 39.6°C leaving the crude caffeine behind. (Murray, 1995) (Hampp, 1996) (Barbaro, 2000). For removal of the ionic liquid (1-ethyl-3-methylimidazodium), the sample was first washed thrice with ethanol, thereafter evaporated to remove all the solvent present.

Figure 3.7: Rotary evaporator used for evaporation of solvent

Figure 3.8: Pure caffeine obtained after sublimation of crude caffeine

3.4 Analytical methods

3.4.1 Introduction

Besides quantification of the caffeine present in the extracted samples, the purity of the extracted caffeine and the surface structure was also analysed. (Belay, et al., 2007)**.** Quantitative and qualitative analysis will be performed using the following instruments.

3.4.2 High Performance Liquid Chromatography (HPLC)

The method used for the quantification of caffeine was High Performance Liquid Chromatography (HPLC). HPLC is most popular because it is non-destructive and unlike gas chromatography may be applied to thermally liable compounds. It is a very sensitive technique which incorporates a wide range of detective methods. HPLC can easily detect compounds that do not usually provide adequate detector response and due to this wide applicability, HPLC as a separation method is a highly valuable tool. Using the HPLC, the retention time and relative peak area of the extracted caffeine was determined. The amount/yield of caffeine extracted for the different experimental runs were determined from the relative peak area on the chromatograms and calibration curve (Figure 3.14 and Appendices figure 7.7).

The HPLC analysis was carried out with a HPLC Shimadzu system. A reverse phase column Germin 5µ C18 (outer diameter 5µm, inner diameter 4.6µm and length 150mm) was used at 25°C. The sample injection was 2mL. The chromatographic separation was performed using an isocratic elution with a mixture of 0.1% (w) of acetic acid in water (solvent A) and acetonitrile (solvent B). A constant flow of solvent of 0.5 mL/min was used. A/B ratio of 90/10 (v/v) during 30 min was applied. Detection was accomplished with a UV/Visible diode detector at a wavelength of 272 nm as indicated on the spectrum in figure 3.15.

Figure 3.9: HPLC apparatus used during caffeine quantification

This analytical technique is a form of column chromatography that pumps a sample mixture or analyte in a solvent at a high pressure though a column containing stationary packing material. The injected sample is moved through the equipment using a carrier stream that is either helium or nitrogen. The HPLC set up composes of a mobile phase, injector, pump, column and detector. Standard are prepared and thereafter the standard compositions or concentrations are selected to resemble the ones in lignocellulosic biomass or the extracted material. The standards and sample are then measured by the HPLC.

Advantages of this analysis test are that HPLC is able to separate and identify any compounds that are present in the sample. It involves the separation, identification and quantification of components that are dissolved in a mixture or solution. HPLC is so accurate that it can identify concentrations as low as parts per trillion. Because of this versatility of HPLC, the use is implemented in industry and scientific purposes for various fields such as pharmaceutical, environmental, forensics and chemical fields. (Rodrigues, et al., 2007)

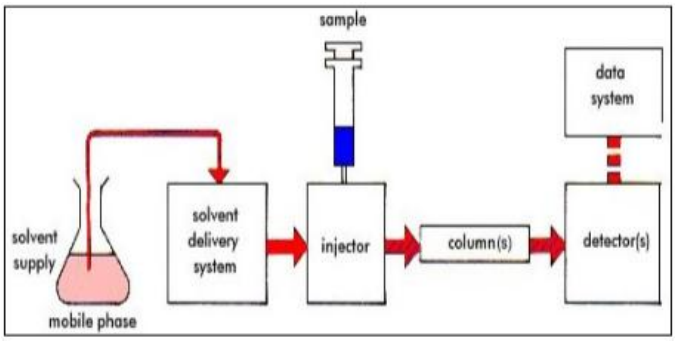


Figure 3.10: Typical schematic representation of an HPLC system (Czaplicki et al, 2013)

HPLC analysis of all extracted samples was carried out using a Shimadzu System equipped with a reverse phase column (Gemini 5µ C18110A, 150mm x 4.6mm ID) used at 25°C. The sample injection used was 2 mL. Chromatographic separation was carried out using an isocratic elution of a mixture composing of 0.1 % (w/w) acetic acid in water (solvent A) and acetonitrile (solvent B). Solvent A had a constant flow of 1.50 ml/min^{-1} with a A: B ratio of 90:10 (v/v) during the 15-minute period time. Detection of the caffeine was then accomplished using a UV/Visible diode at a wavelength of 272nm. A wavelength of 272nm was selected as the maximum height (peak of standard) due to the clear peak that was detected on the spectrum (Figure 3.13) and indicated in the area when the various standards were inserted into the HPLC. Figure 3.12 indicates the peak of the standard and the peak of the injected sample. This is an example of how the concentration peaks are demonstrated during HPLC analysis. The retention time of the pure standard caffeine and the extracted caffeine were almost similar, which confirms the identity of the caffeine present.

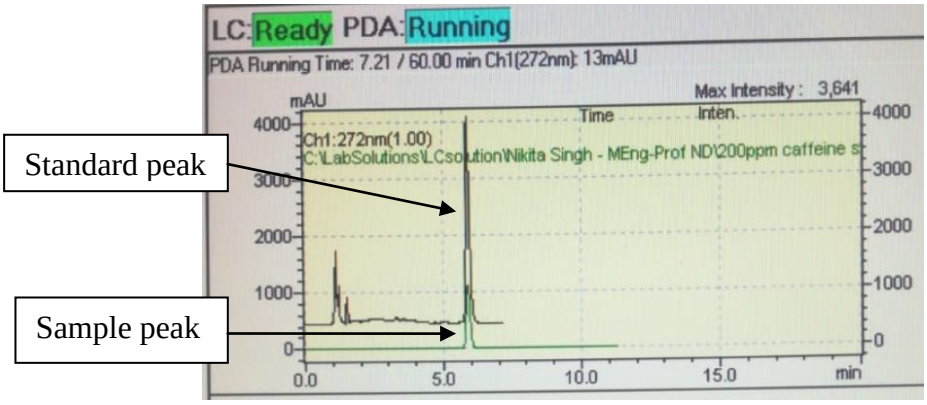


Figure 3.11: Example of Chromatograph generated during HPLC analysis, comparing injected sample (lower peak) against the caffeine standard (upper peak)

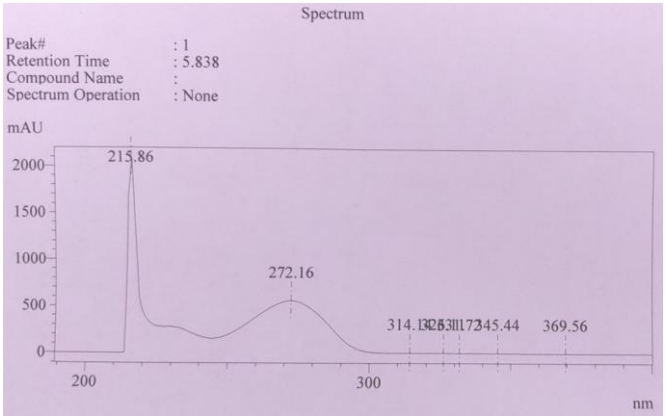


Figure 3.12: HPLC spectrum used when selecting the wavelength for caffeine evaluation peak

3.4.3 Scanning electron microscopy (SEM)

Scanning electron microscopy uses a microscope that produces images of a sample by scanning the sample with a focussing beam of electrons. This produces magnified images for analysis. Scanning electron microscopy is very effective in micro analysis of solid organic material. (Giron & Goldbronn, 2007). The electrons in the beam interact with atoms from the sample producing various signals containing information about the surface topography, micro structure, chemistry and composition of the sample (Chiang, *et al.*, 2018). For the analysis of this study, the Tescan MIRA3 RISE SEM instrument was used (Figure 3.14). The Tescan MIRA 3, installed in 2017 has a high-resolution Field emission SEM, with low kV imaging, low vacuum performance, Electron beam Lithography, electron backscattter diffraction (EBSD) detector and Raman spectroscopy. The EBDS detector is used to perform quantitative microstructure analysis on nanometre scale as small as 40μm.

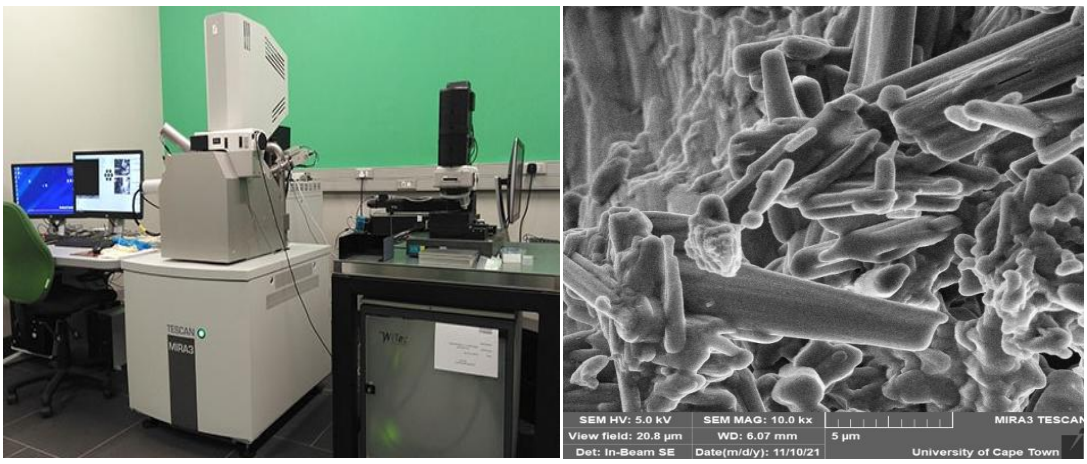


Figure 3.13: (Right) Scanning electron microscopy apparatus used; Images produced by SEM analysis

3.4.4 Transmission Electron Microscopy (TEM)

Transmission electron microscopy allows electrons to pass through an analysis sample and transmit an ultrathin sample to a detector below to provide near atomic resolution images. TEM are suitable for high end work and observes small details such as individual atoms at molecular levels, structural information, biological (proteins, vesicles, viruses, bacteria, DNA, inorganic material etc) and material sciences (nanoparticles, polymers, particles, polymers, etc) all at the highest resolution. TEM is advantageous as it is able to provide information about internal structures which SEM cannot provide. For this study the FEI F20 CRYO FEGTEM equipment was used (Figure 3.15).

Figure 3.14: Transmission Electron Microscopy apparatus used

3.4.5 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry is a thermo analytical technique which was developed by Watson and O'Neill in 1962. DSC makes use of the difference in heat required to raise the temperature of a sample and the reference standard and is measured as a function of temperature. Both samples are maintained at the same temperature thorough the analysis period. The DSC sample holder temperature increases linearly as a function of time while directly measuring the energy and heat capacity. Using the heat-flux DSC, the change in heat flows are calculated by integrating the $\Delta T_{reference}$ curve. DSC enables the measuring of the transition stage such as glass transition, melting points and crystallization. Weight amounts of 2.5 – 4 mg of caffeine sample and the standard caffeine were placed on a sample holder with temperature integrated sensors. The samples were maintained at the same heating rate of 5°C/minute to 300 °C/minute for a period of time. Each sample was inserted into the sample holder under nitrogen flow of 50 mL/min.

For this study, differential scanning calorimetry is used for purity analysis based on thermodynamic phase diagrams to analyse the rate of the melting points of the extracted caffeine sample and the reference caffeine standard. It is a common method used for the purity testing of pharmaceutical products. The difference in the depression of the melting point between the sample and standard is caused by the presence of impurity. DSC is attractive for global assessment of eutectic impurities and pharmaceutical development due to stability assessment, purity profile, fast analysis time and the small amount of sample necessary.

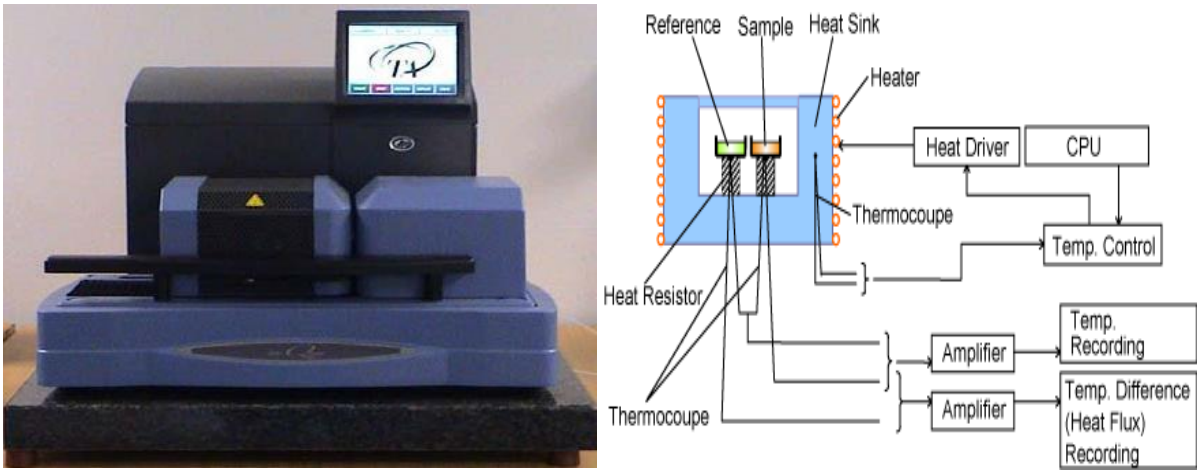


Figure 3.15: Differential Scanning Calorimetry apparatus with typical schematic system

3.4.6. Energy dispersive X-ray spectroscopy (EDS)

EDS is a common non-destructive X-ray technique that is used for elemental analysis of the compositions of nanomaterials. This electron microscopic technique used SEM and TEM to find out the relationship between composition, microstructure and elemental spectra (Anake *et al.*, 2016). This is significant technique for the determination of compositional, physical and chemical properties of biosynthesized nanomaterials, i.e. caffeine. EDS analysis is widely used on large scale to gather data on elemental composition and the presence of any impurities.

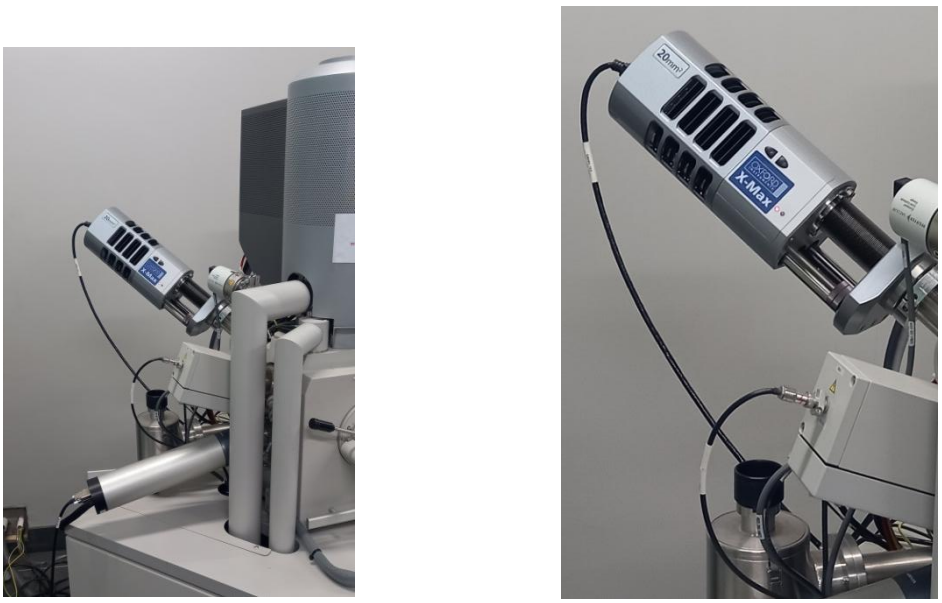


Figure 3.16: Energy dispersive X-ray spectroscopy equipment

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

In this chapter the results of the characterisation of spent coffee grounds, quantification of the extracted caffeine extracted by the conventional extraction method using dichloromethane and the green extraction method using water or the ionic liquid 1-ethyl-3-methylimidazolium chloride are presented and discussed. Scanning electron microscopy, energy dispersive X-ray spectroscopy and transmission electron microscopy images are also presented indicating surface structure, topography, particle size and composition. Differential scanning calorimetry indicated the difference in purity of the extracted caffeine from waste coffee against the pure standard.

4.2 Characterisation of spent coffee grounds using the Technical Association of the Pulp and Paper Industry (TAPPI) method

The TAPPI method for the characterisation of spent coffee grounds, moisture content, ash content and particle size of the coffee grounds were established.

4.2.1 Moisture content of spent coffee grounds

Moisture content of the spent coffee grounds were established using equation 3.1 to have approximately 61-62% moisture content. Kang, *et al*., (2017) had established similar results off 55% moisture content from freshly brewed coffee beans after brewing. Table 4.1 gives the moisture content found in the spent coffee grounds used from the 1 g to 5 g sample sizes.

Table 4.1: Raw data and calculations of moisture content characterisation

Sample weight (g)	**W2**	**W1**	**WT**	**Moisture content**
	Weight of weighing dish and biomass sample prior to drying (g)	Weight of weighing dish and biomass sample after drying (g)	Tare weight of empty weighing dish (g)	$= \frac{W_2 - W_1}{W_2 - W_T} \times 100$
1	24.7560	24.1351	23.7542	61.97
2	28.5410	27.2908	26.5355	62.33
3	23.1730	21.3148	20.1710	61.89
4	25.0236	22.5431	21.0236	62.01
5	26.0276	22.9478	21.0200	61.50

4.2.2 Ash content of spent coffee grounds

Using equation 3.2, the ash content present in the spent coffee grounds used is 2.4 - 4.7 %. The ash remains were left behind once exiting the furnace as seen in figure 4.1 below. Table 4.2 illustrates the raw data obtained and the use of equation 3.2 to calculate the ash content per 1 g sample to 5 g sample sizes.

Figure 0.1: Furnace used for determination of Ash content in SCG

Table 4.2: Raw data and calculations of ash content characterisation

Empty cup(g)	**Cup with sample(g)**	**Cup with sample(g)**	**A**	**B**	**Ash content (%)**
	Before Furnace	After Furnace	Weight of ash (g)	Weight of moisture free biomass sample (g)	$\%\,Ash = \frac{A}{B} \times 100$
28.29	29.32	28.337	0.0470	1	4.70
28.23	30.26	28.31	0.0800	2	4.00
28.43	31.49	28.56	0.1300	3	4.33
28.43	32.45	28.57	0.1400	4	3.50
28.89	33.95	29.01	0.1200	5	2.41

4.2.3 Particle size distribution of spent coffee grounds

Using equation 3.4, the particle size diameter of 47.7 % spent coffee grounds was 425 μm. This particle size is well within the range of the optimum extraction size particles required for the caffeine extraction. According to Akiyana *et al.* (2015) and Cordoba *et al.* (2019), in section 2.21.1, 500 μm particle sizes and below allow for the full absorption/release of materials and compositions due to the increased surface area. From the particle size distribution, it is evident that 17,4 % of the particles exceed the optimum size conditions, while the other 82,6 % of the biomass require no processing or prior grinding before the extraction.

Table 4.3: Particle size analysis

Tray size (μm)	**M1 Mass of sieve tray (g)**	**M2 Mass of tray after shaker (g)**	**Particle mass (g)**	**% of Particular particle size**
600	318	379	61	17.4
425	310	477	167	47.7
355	286	358	72	20.6
250	290	321	31	8.9
150	272	292	20	5.7
75	272	273	1	0.3
Bottom pan	350	350	0	0.0
Sample size (g)	350			

4.3 Extraction of caffeine from spent coffee grounds using water as the extraction solvent and the Parr reactor.

4.3.1 Extraction of caffeine using water as the solvent, for the duration of 15 minutes.

Table 4.4: Responce obtained from DOE using water as the extraction solvent

Std	Run	Factor 1 A:Reaction Time	Factor 2 B:Reaction Tem...	Factor 3 C:S/L Ratio	Response 1 Response
9	1	25	88	10	542.723
12	2	25	120	25	646.34
15	3	25	104	17.5	263.83
6	4	35	104	10	398.216
11	5	25	88	25	566.314
4	6	35	120	17.5	285.011
16	7	25	104	17.5	421.649
2	8	35	88	17.5	348.03
17	9	25	104	17.5	405.095
13	10	25	104	17.5	387.927
8	11	35	104	25	422.432
7	12	15	104	25	333.265
14	13	25	104	17.5	385.848
5	14	15	104	10	392.644
10	15	25	120	10	576.593
1	16	15	88	17.5	337.718
3	17	15	120	17.5	272.801

At a reaction temperature of 88 ºC, caffeine concentration of 333, 26 mg/L was obtained. Taking the reaction pass boiling point to 104 ºC with the solvent volumes varying from 10 mL and 25 mL, yields of 392,64 mg/L and 337, 72 mg/L was obtained. A larger volume of 25 mL solvent extracted lower concentrations compared to the 10 mL solvent which extracted the highest caffeine concentration during the 15 minute extraction period. At 120 ºC reaction temperature the lowest caffeine yield of 272, 80 mg/L caffeine concentration was obtained. From this reaction time we can see that 15 minutes reaction time using water as the extraction solvent extracted very low concentrations of caffeine.

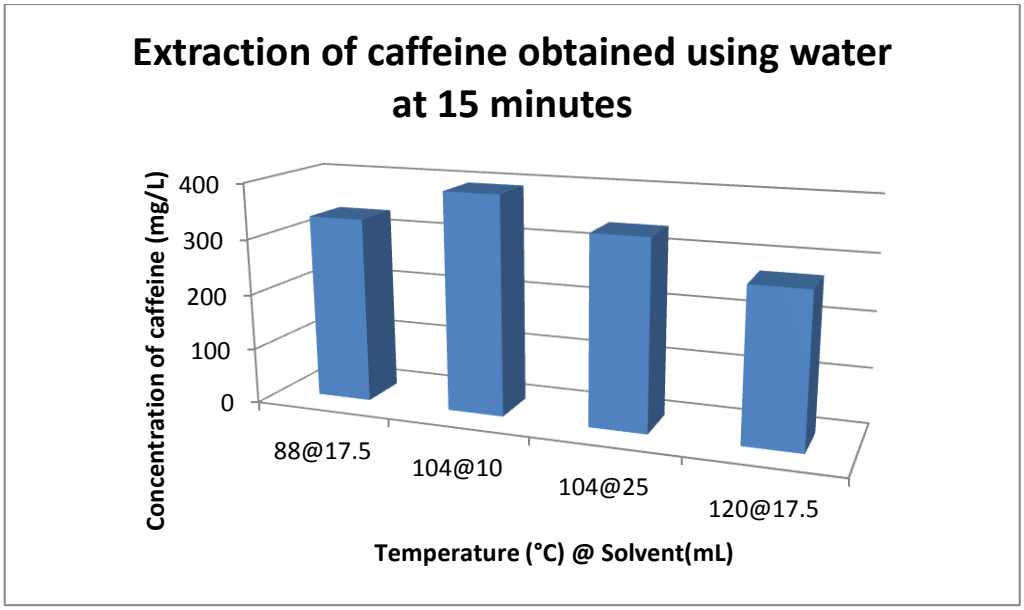


Figure 0.2: Graph indicating the yield of caffeine extraction at various temperatures during a 15 minute period, using water as extraction solvent.

4.3.2 Extraction of caffeine using water as the solvent, for time duration of 25 minutes

The 25 minutes reaction time and lowest reaction temperature of 88 ºC showed significantly higher concentrations of 542,72 mg/L and 566,31 mg/L respectively. Testing repeatability at 104 ºC the concentrations of 385,84 mg/L to 421,65 mg/L which are within close proximity as and this is due to the combination and variance of coffee grounds waste. At the highest temperature of 120 ºC, there is the highest concentration of 646 mg/L. For the 120 ºC extractions, 576,59mg/L and 646,34 mg/L with 10 mL and 25mL solvent volume respectively of extracted caffeine was obtained. Since the lowest reaction time had extracted very low caffeine concentrations, a longer time frame of 25 minutes was analysed. This was higher concentrations achieved and the highest thus far using water as an extraction solvent.

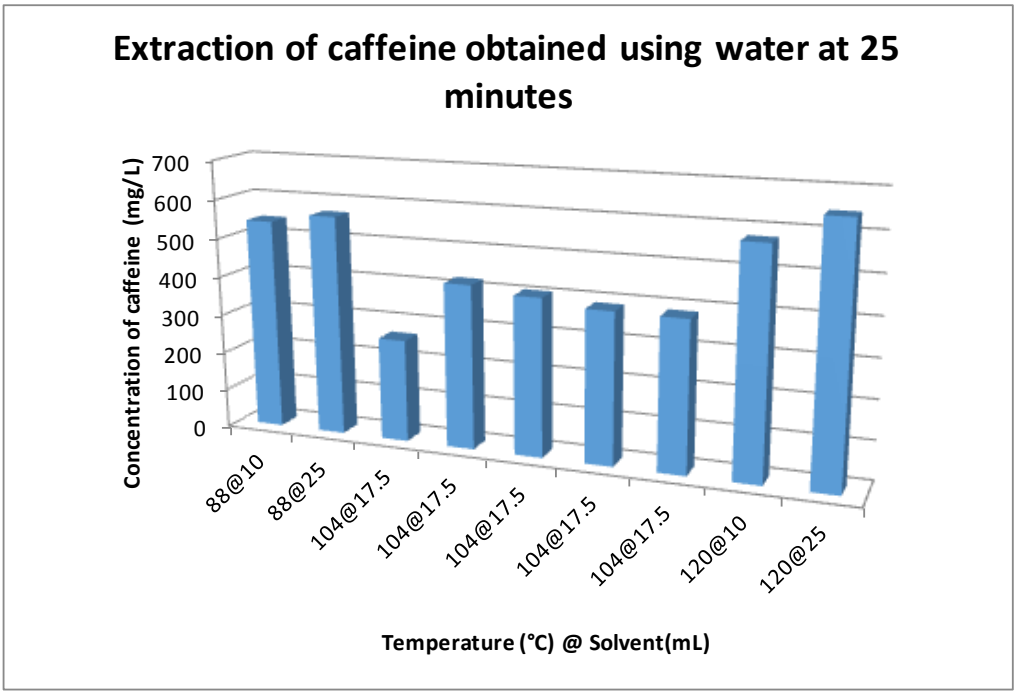


Figure 0.3: Graph indicating the yield of caffeine extraction at various temperatures during a 25 minute period according to DOE, using water as extraction solvent.

4.3.3 Extraction of caffeine using water as the solvent, for time duration of 35 minutes.

Using the longest reaction time of 35 minutes achieved concentrations much lower than what was achieved at 25 minutes. Using 88 ºC as the reaction temperature, 348, 08 mg/L caffeine concentration was achieved which is almost equal to the concentrations achieved at 15 minutes and temperature of 88 ºC. Even though a longer reaction time was used, there was no significant difference visible at the low temperature. Increasing the temperature to 104 ºC, did give a slight increase to 398,22 mg/L and 422,43 mg/L in the yields achieved. However, this is still lower than results at 25 minutes. At 35 minutes reaction time did not achieve the best extraction yields assuming that the water was evaporating during the extraction process.

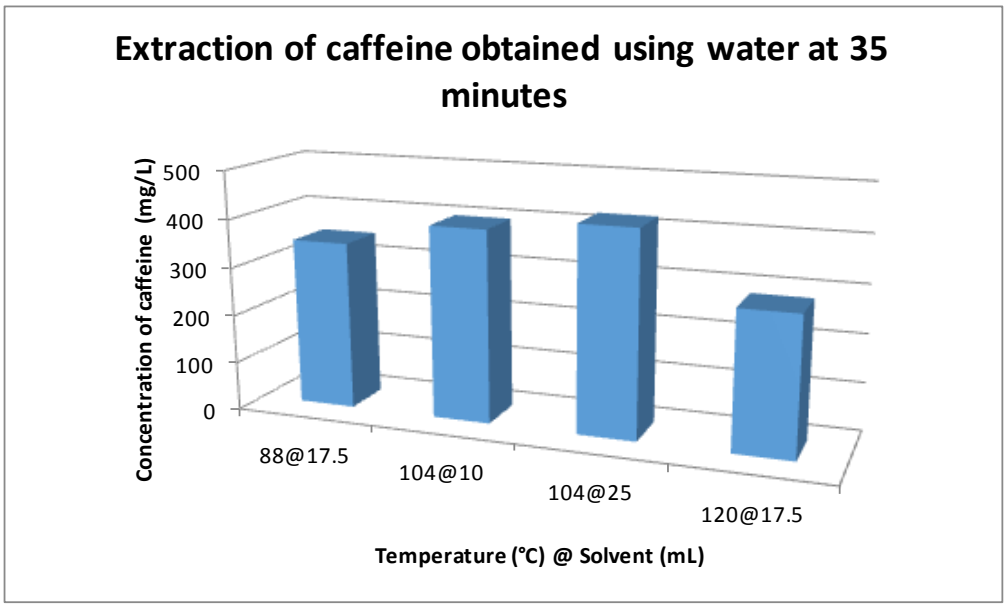


Figure 0.4: Graph indicating the yield of caffeine extraction at various temperatures during a 35 minute period, using water as extraction solvent.

4.4 Extraction of caffeine from spent coffee grounds using 1-ethyl-3-methylimidazolium chloride as the extraction solvent in the Parr reactor.

4.4.1 Extraction of caffeine using 1-ethyl-3-methylimidazolium chloride as the solvent for the duration of 15 minutes.

Table 4.5:Responce obtained from DOE using 1-ethyl-3-methylimidazolium as the extraction solvent

Std	Run	Factor 1 A:Reaction Time	Factor 2 B:Reaction Tem...	Factor 3 C:S/L Ratio	Response 1 Response
9	1	25	88	10	609.801
12	2	25	120	25	726.224
15	3	25	104	17.5	296.438
6	4	35	104	10	447.434
11	5	25	88	25	636.307
4	6	35	120	17.5	320.237
16	7	25	104	17.5	473.763
2	8	35	88	17.5	391.045
17	9	25	104	17.5	455.163
13	10	25	104	17.5	435.873
8	11	35	104	25	474.643
7	12	15	104	25	374.455
14	13	25	104	17.5	433.537
5	14	15	104	10	441.173
10	15	25	120	10	647.858
1	16	15	88	17.5	379.459
3	17	15	120	17.5	306.518

Investigating the minimum extraction time using [EMIM][Cl], first an average solvent volume of 17,5 mL was selected at the lowest reaction temperature of 88ºC. At the temperature of 88 ℃ , 374,45 mg of caffeine/L was extracted. Raising the temperature to 104 ºC and decreasing the solvent volume to 10 mL yielded a higher caffeine content of 441,17 mg/L, indicating that the increasing in temperature beyond the boiling point of solvent was able to extract more caffeine even though the solvent amount was reduced. Increasing the solvent increases the rate of reaction due to more reacting molecules or ions present. Seeing that the caffeine yield increased at 104 ºC, the temperature was maintained but adjustment of the solvent volume was raised to 25 mL. The caffeine yield did not increase, rather decreased to 379,46 mg/L almost equal to the 374, 45 mg/L yield of caffeine obtained at 88 ºC with 17,5 mL solvent volume, showing that the higher volume of solvent used does not mean higher yields of product obtained. At the temperature of 120 ºC, and 17,5 mL of solvent, a lower yield of 306,52 mg/L was obtained . For the reaction time of 15 minute scenario, the

highest yield (441.17 mg/L) were obtained using the lowest solvent volume of 10 mL at 104 ^{o}C. .

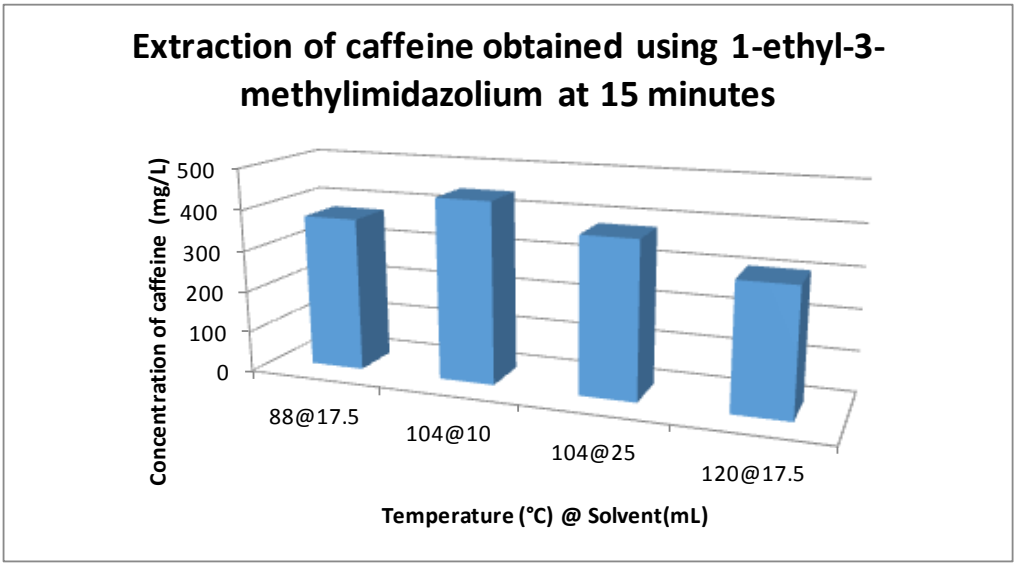


Figure 0.5: Graph indicating the yield of caffeine extraction at various temperatures during a 15 minute period, using 1-ethyl-3-methylimidazolim as extraction solvent.

4.4.2 Extraction of caffeine using 1-ethyl-3-methylimidazolium chloride as the solvent, for time duration of 25 minutes.

At the lowest extraction temperature of 88 ºC and the lowest solvent volume of 10 mL yielded 609,80 mg/L compared to 374,45 mg/L during the 15 minute reaction time. Increasing the solvent volume to 25mL, 636,31 mg/L of caffeine was obtained. This clearly indicates that using 25 minutes allowed the solvent more time to react with the caffeine molecules and ions present, maximising its extraction. Using 104 ºC with average solvent volume of 17,5 mL obtained yields of 435 mg/L which is lower than yields obtained from 88 ºC. Repeatability was tested using the 104 ºC and 17,5 mL solvent volume. A higher temperature of 120 ºC for a longer duration of 25 minutes with 10 mL and 25 mL solvent solution, yielded 647,86 mg/L and 726,22 mg/L respectively. This condition achieved the highest caffeine yield thus far using [EMIM][Cl].

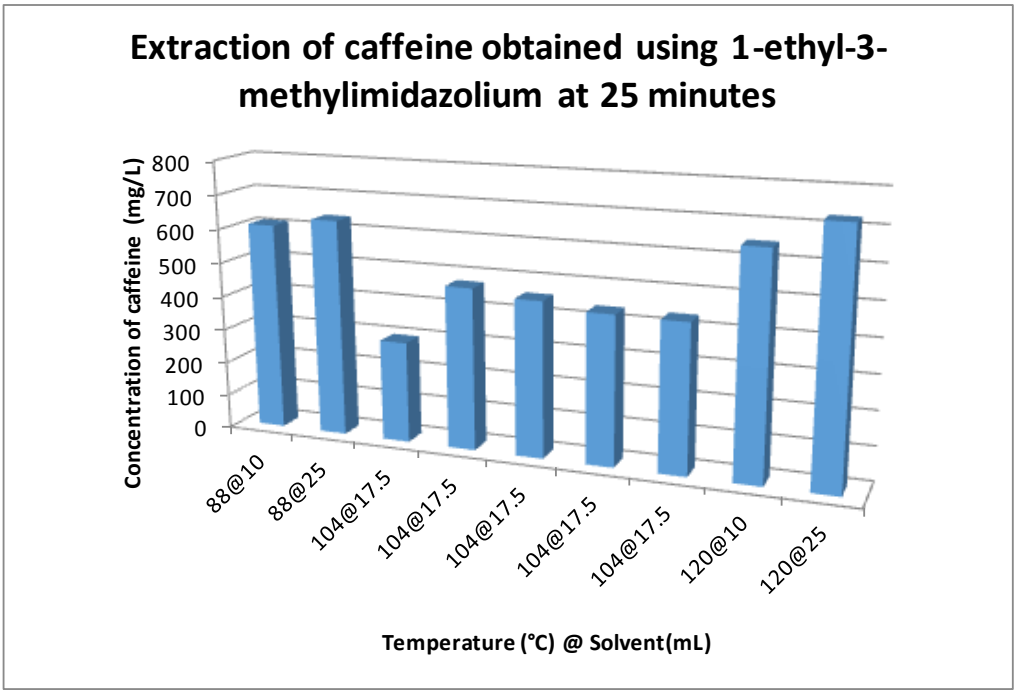


Figure 0.6: Graph indicating the yield of caffeine extraction at various temperatures during a 25 minute period, using 1-ethyl-3-imidazolium as extraction solvent.

4.4.3 Extraction of caffeine using 1-ethyl-3-methylimidazolium as the solvent, for time duration of 35 minutes.

At the longest reaction time of 35 minutes together with the lowest reaction temperature of 88 ºC and the highest temperature of 120 ºC, extracted caffeine was 391.04 mg/L and 320 mg/L, respectively with solvent of 17.5 mL. This amount of caffeine extracted was equivalent to that of the 15 minute reaction time and temperatures. For the 35 minutes reaction time, the highest yield of 474,43 mg caffeine /L was obtained during the run of 104 ºC with 25 mL solvent. However this is still lower than that obtained for the temperatures at 25 minutes.

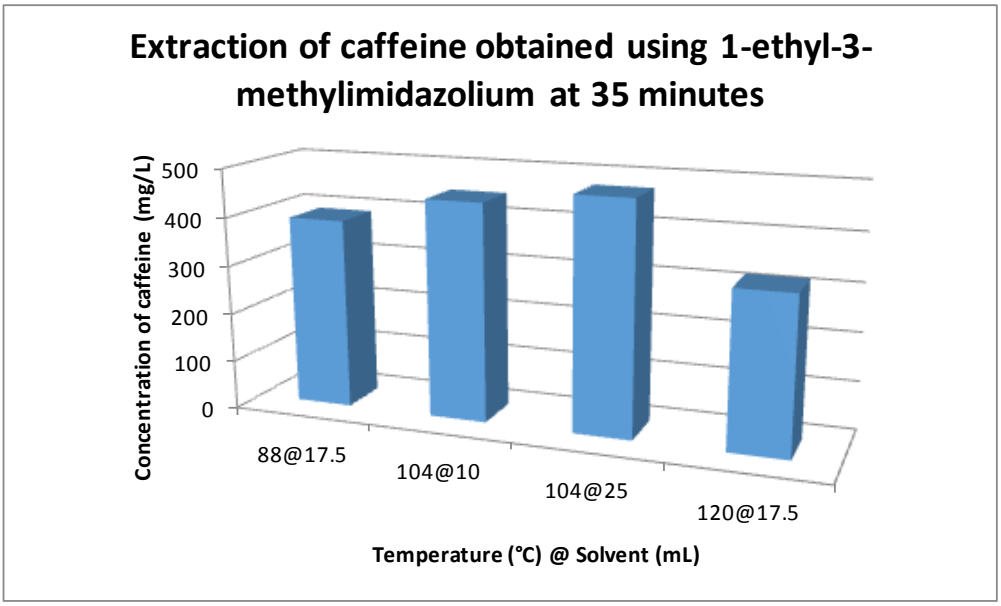


Figure 0.7: Graph indicating the yield of caffeine extraction at various temperatures during a 35 minute period, using 1-ethyl-3-methylimidazolium as extraction solvent.

4.5 Extraction of caffeine from spent coffee grounds using dichloromethane (DCM) as the extraction solvent.

Table 4.6: Responce obtained from DOE using dichloromethane as the extraction solvent

Std	Run	Factor 1 A:Reaction Time	Factor 2 B:Reaction Tem...	Factor 3 C:S/L Ratio	Response 1 Response
9	1	25	88	10	513.618
12	2	25	120	25	566.123
15	3	25	104	17.5	602.85
6	4	35	104	10	636.061
11	5	25	88	25	673.046
4	6	35	120	17.5	565.295
16	7	25	104	17.5	634.313
2	8	35	88	17.5	496.686
17	9	25	104	17.5	606.67
13	10	25	104	17.5	609.028
8	11	35	104	25	703.299
7	12	15	104	25	416.464
14	13	25	104	17.5	604.892
5	14	15	104	10	316.847
10	15	25	120	10	530.128
1	16	15	88	17.5	439.705
3	17	15	120	17.5	440.458

4.5.1 Extraction of caffeine using dichloromethane as the solvent, for time duration of 15 minutes.

Using dichloromethane at the shortest reaction time of 15 minutes , at 88 ºC and the highest solvent volume of 25 mL, the caffeine yield obtained was 439,70 mg/L. Increasing the temperature to 104ºC with the solvent volume of 10 mL and 17,5 mL, achieved caffeine yields of 316,85 mg/L and 416,46 mg/L, respectively. These conditions both with varied solvent amounts and higher temperature still achieved lower yields to that of the 88 ºC obtaining 439,70 mg/L. Increasing the reaction temperature to 120 ºC and an average solvent volume of 17,5 mL achieved 440,45 mg/L. This yield result is similar to that from 88 ºC and was almost equal and had no significant difference. Therefore, the highest yield obtained using 15 minutes reaction time was at 88 ºC yielding 439,70 mg/L.

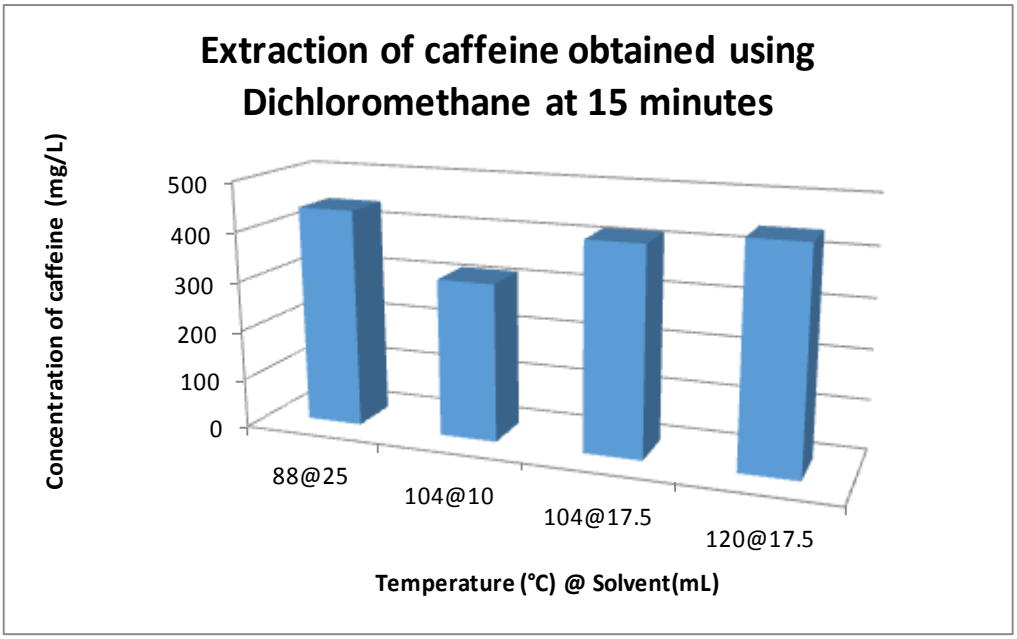


Figure 0.8: Graph indicating the yield of caffeine extraction at various temperatures during a 15 minute period, using dichloromethane as extraction solvent.

4.5.2 Extraction of caffeine using dichloromethane as the solvent, for time duration of 25 minutes

At a reaction time of 25 minutes at the lowest temperature of 88 ºC and the lowest and highest solvent volume of 10 mL and 25 mL respectively yielded results of 513,62 mg/L and 673,05 mg/L , respectively. This is a significant increase from the results obtained during the 15 minutes reaction times. Increasing the temperature further to 104 ºC and an average solvent volume of 17,5 mL, while also testing repeatability also as indicated on figure 4.10, gave results of 600 mg/L. This is lower than the results obtained at 88 ºC and 25 mL. At the highest temperature of 120 ºC, at the highest and lowest solvent volumes of 10 mL and 25 mL yielded results of 530,13 mg/L and 566,12 mg/L, respectively. These conditions also yielded lower caffeine concentrations than the 88 ºC extractions. It is evident to say that dichloromethane extracted the highest caffeine concentration at the temperature of 88 ºC.

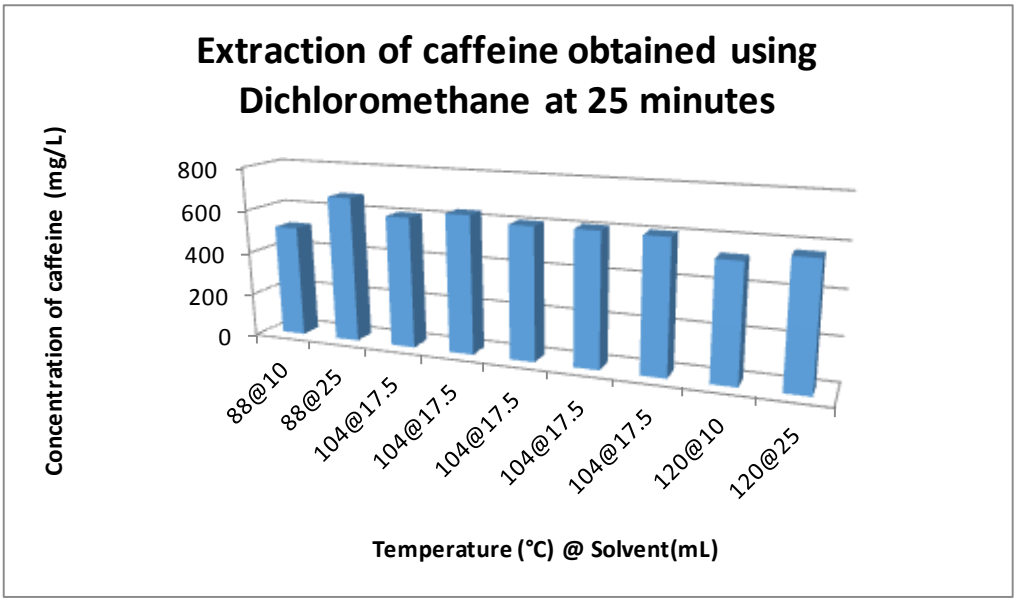


Figure 0.9: Graph indicating the yield of caffeine extraction at various temperatures during a 25 minute period, using dichloromethane as extraction solvent.

4.5.3 Extraction of caffeine using dichloromethane as the solvent, for time duration of 35 minutes.

At a longer reaction time of 35 minutes at the lowest temperature of 88 ºC caffeine yield was 496,68 mg/L almost equivalent to what was achieved at 15 minutes operating conditions. However, increasing the reaction temperature slightly higher to 104 ºC with 10 mL and 25 mL solvent volumes, increased the caffeine yield significantly to 636,06 mg/L and 703,30 mg/L. The highest temperature of 120 ºC obtained 565,29 mg/L caffeine concentrations which was in line with the results obtained from extractions at 25 minutes. Therefore it is observed that the optimum conditions at 35 minutes using dichloromethane was at 104 ºC at 25 mL solvent volume.

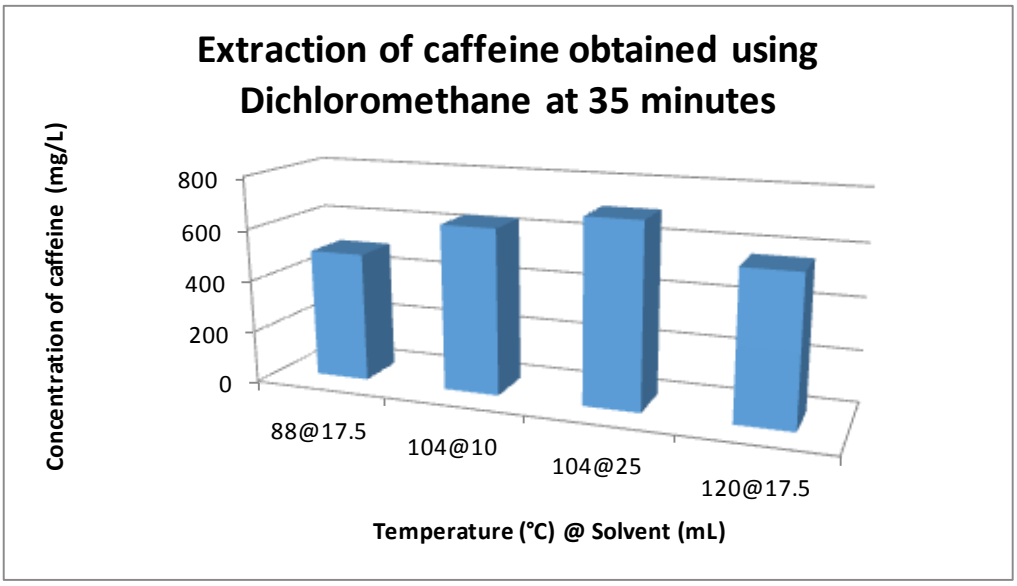


Figure 0.10: Graph indicating the yield of caffeine extraction at various temperatures during a 35 minute period, using dichloromethane as extraction solvent.

4.6 Cumulative results of extraction of caffeine

At 88°C with the shortest extraction time (15 minutes) and the average amount of solvent (17,5 mL), 333.26 mg/L – 439.70 mg/L of caffeine was extracted using water or dichloromethane, respectively. Keeping the temperature at 88 °C and increasing the time to 25 minutes at the lowest and highest solvent of 10mL and 25mL with a higher reaction time increased the extraction yield of caffeine to 513,62 mg/L - 609,80 mg/L. To test the effect of the solid: liquid loading ratio at a reaction time of 25 minutes, 25 mL solvent a higher caffeine yield of 566.31 mg/L – 673.04 mg/L was obtained. The caffeine yield increased as time increased from 15 minutes to 25 minutes. When a higher amount of solvent was used at a shorter duration, the amount of caffeine did not increase. In order to analyse the effect of the reaction time, the 88°C temperature is kept constant while increasing the reaction time to 35 minutes while keeping the solid: liquid loading ratio to the average 17,5 mL Under these conditions, the caffeine yield was significantly lower than the 25 minutes batch time and almost equal to that of the 15 minutes extraction conditions and yield. This indicated that reaction time is crucial in achieving the highest yield of caffeine possible. It also proves that longer reaction time does not necessarily mean larger extraction yields. At 88 °C, for 25 minutes and 25 mL, the highest caffeine content achieved was 673 mg/L using dichloromethane; 636 mg/L using 1-ethyl-3-methylimidazolium chloride and 576 mg/L using water (Figure 4.11).

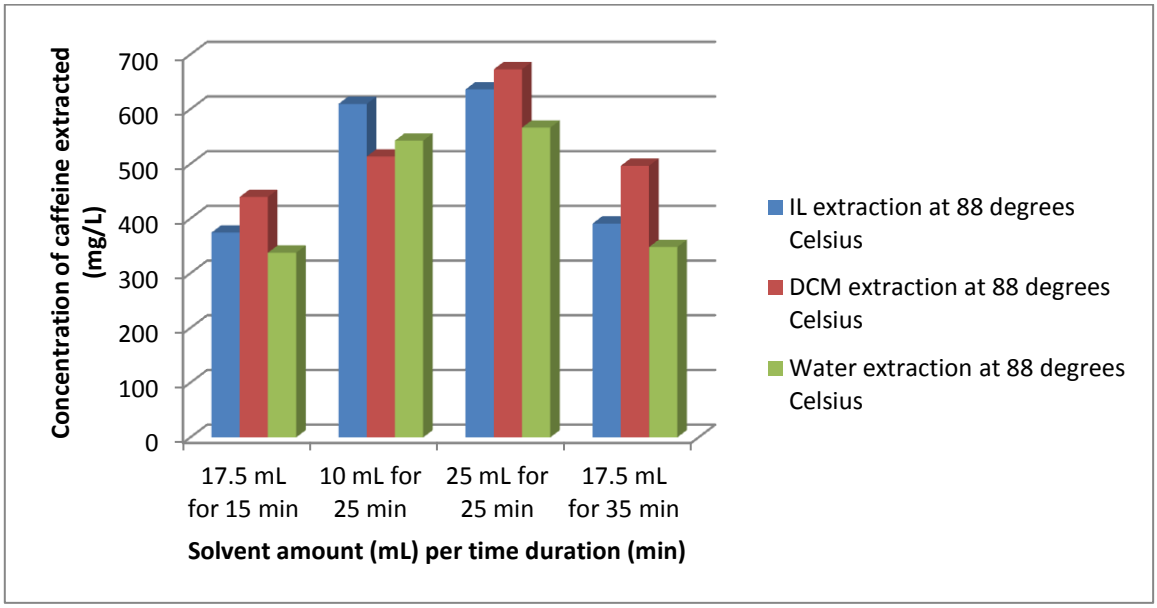


Figure 0.11: Cumulative extractions results obtained at 88 degrees Celsius using various solid-solvent ratio and time durations.

Increasing the reaction temperature to 120 °C, the shortest reaction time of 15 minutes with both the lowest and highest volume of solvent (10 mL and 25 mL) achieved a caffeine range of 272.80 mg/L – 441,17 mg/L. This was a lower yield to what was achieved at 88 °C for 15 minutes and minimum 10 mL solvent usage.

Using the same temperature of 104°C but increasing the time duration to 25 minutes, the caffeine yield did increase slightly to 455 mg/L – 606.66 mg/L. However, this still did not exceed the value of the lower temperature (88 °C) experimental conditions. Increasing the reaction time further to 35 minutes at 104 °C, still achieved the same extraction possible at lower temperatures and time. It can be seen that 104 °C was not efficient in all the extraction conditions to achieve the expected yield. (Figure 4.13)

Knowing that the boiling point of water is 100 °C, it would be expected to achieve more of the caffeine content after this point. A higher temperature point of 120 °C was considered to analyse the effect on the caffeine extraction.

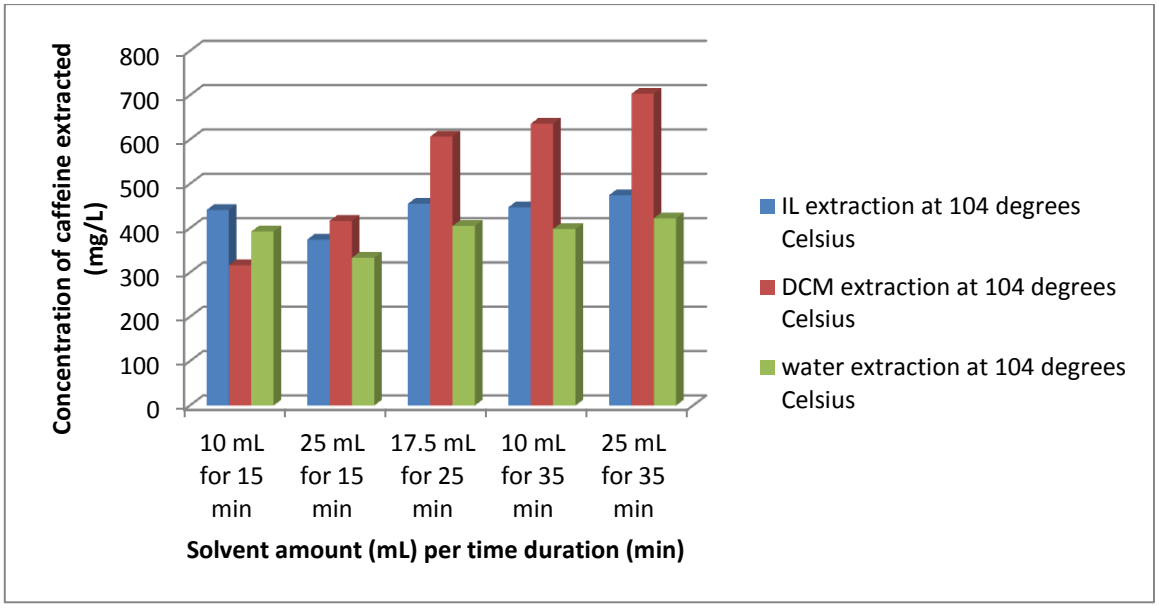


Figure 0.12: Cumulative extractions results obtained at 104 degrees Celsius using various solid-solvent ratio and time durations.

At 120°C reaction temperature, minimum extraction solvent volume (10mL) is tested and yielded very low extraction results, actually lower than that what was achieved at 88°C. From this analysis, it is possible to state that the solvent evaporated at the high temperature before being able to react with the spent coffee grounds. Also, the temperature was too high in such a short duration (15 minutes) to actually start the reaction fully.

Increasing the reaction time to 25 minutes as this is the time where most caffeine was achieved in both the 88°C and 104°C, showed much greater results as well at 120°C. At 120°C for minimum and maximum solvent volume of 10mL and 25mL respectively, the highest caffeine yield of 530.12mg/L – 726,22mg/L was obtained. This range of caffeine yield obtained was obtained using 120°C tested using both minimum and maximum solvent volume. It is evident that this was the best condition for the caffeine extraction. From figure 4.14, 120°C for 25 minutes at 10mL solvent ratio, 1-ethyl-3-methylimidazolium chloride extracted the highest caffeine yield of 647,86 mg/L, followed by water and dichloromethane having extracted 576,53 mg/L and 530,13 mg/L respectively.

Increasing the solvent amount to 25 mL, all the extraction yields increased as well, still having the ionic liquid 1-ethyl-3-methylimidazolium extracting the highest yield of 726,22 mg/L, followed by water and dichloromethane at 646,33 mg/L and 566,12 mg/L respectively.

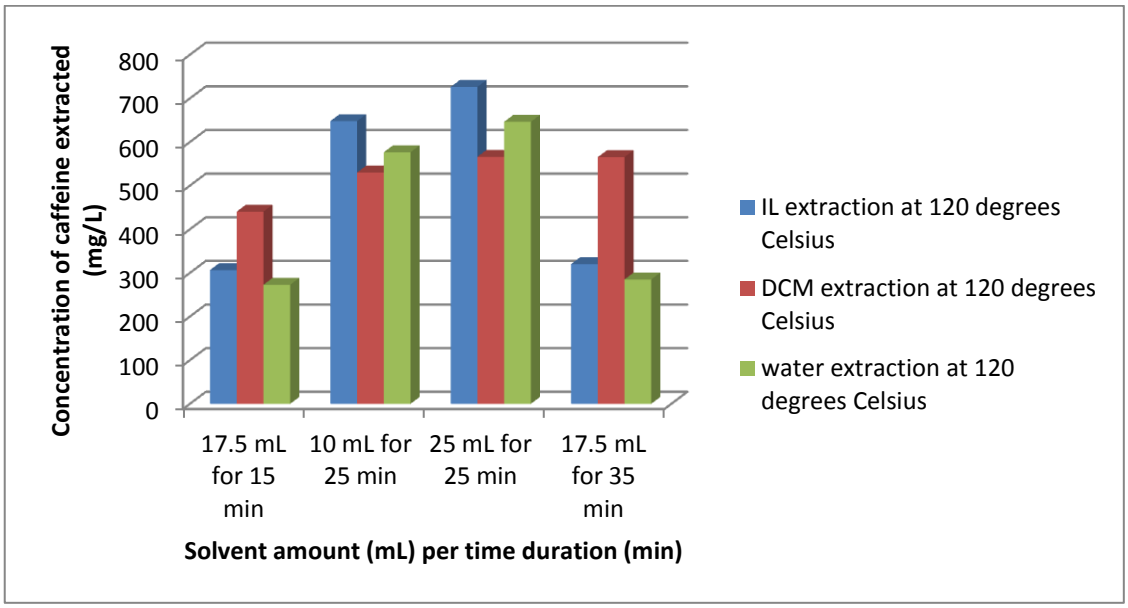


Figure 0.13: Cumulative extractions results obtained at 120 degrees Celsius using various solid-solvent ratio and time durations.

The highest reaction time produced the highest yields of caffeine obtained. A reaction time of 25 minutes extracted yields of 566,12 mg/L to 726,22 mg/L which achieved almost 60% overall recovery of caffeine from spent coffee grounds. Using SEM analysis, figure 4.23 illustrates the pure caffeine standard alongside which is the extracted caffeine sample. The averages particle size of pure caffeine standard is 9.61 µm and the extracted caffeine having an average particle size of 6.37 µm. It can be seen that the commercially bought pure caffeine standard appeared more crystalline when compared to the extracted caffeine. This could be due to other impurities and water molecules present and therefore not allowing the large crystals to form through completely. After obtaining the crude caffeine, more purification is necessary to make the crystals more porous and amorphous.

4.7 Effect of input parameters on the yield of caffeine extracted.

4.7.1 Effect of reaction time

Table 4.7: Effect of reaction time on extracted caffeine yields

Sample	Reaction time(min)	IL Concentration mg/1000mL	DCM extraction Concentration mg/L	Water extraction Concentration mg/1000mL
Run 1	25	609.8007968	513.6180286	542.7227092
Run 2	25	726.2241941	566.1225263	646.3395328
Run 3	25	296.4378848	602.8501089	263.8297175
Run 4	35	447.4339008	636.0609791	398.2161717
Run 5	25	636.3074973	673.0461131	566.3136726
Run 6	35	320.2372329	565.2949531	285.0111373
Run 7	25	473.7631293	634.3130385	421.6491851
Run 8	35	391.0449113	496.6859199	348.029971
Run 9	25	455.1629844	606.6698229	405.0950561
Run10	25	435.8728721	609.0275542	387.9268562
Run 11	35	474.6432452	703.29893	422.4324882
Run 12	15	374.4549076	416.46435	333.2648678
Run 13	25	433.536762	604.8915822	385.8477182
Run 14	15	441.1734879	316.8468895	392.6444042
Run 15	25	647.8576603	530.1278288	576.5933176
Run 16	15	379.4585295	439.7045734	337.7180913
Run 17	15	306.5175661	440.4582899	272.8006338

Analysing the effect of reaction time with each solvent used for the extraction of caffeine justifies the results discussed in section 4.3, 4.4 and 4.5. Using the ionic liquid, the highest caffeine yield was obtained at 25 minutes, whereas the higher time duration of 35 minutes yielded results as low and equivalent to the 15 minute duration extraction. It was also noticed that the 15 minute experimental runs using the ionic liquid produced a very lightly coloured solution after exiting the Parr reactor. The solution appeared weak and filtered very easily compared to that of the 25 minute experimental run. Observing the solution obtained from the 25 minute run, the solution was extremely dark and thicker, also having an increase in filtration time. The 35 minute solution obtained from the reactor appeared almost black of burnt nature. The solution was also very difficult to filter as it appeared thick and required an anti-solvent, ethanol to dilute the solution for easier filtration. From this it is evident that the IL optimum reaction time of 25 minutes was more than sufficient to interact with the components and release the bioactive components.

Observing the extraction solvent dichloromethane, the potential of the solvent differed too that if the IL. The highest yield caffeine was obtained at 35 minutes using dichloromethane compared to 25 minutes using the IL. The solutions obtained from the 15 minutes and 25 minutes produced lower yields of caffeine and also appeared much lighter in colour after filtration. However, the challenge experienced when using dichloromethane as the extraction solvent is that the solution became oily and thick. The 15 minutes duration using DCM has a thin oil film over the solution compared to the 35 minute solution that had a thick oily film making the filtration process difficult and slower.

Lastly, the last extraction solvent water had more similar observations and visual appearances to that of the IL. Using water as the extraction solvent was simple and no unusual or unexpected experiences occurred. The solutions appeared lighter at 15 minutes and darkest at 25 minutes corresponding to the lowest and highest caffeine yield obtained respectively. During the 35 minutes experimental runs, the solution obtained from the Parr reactor decreased in quantity compared to that of the other reaction times. This could be due to evaporation of the water within the cylindrical chamber; therefore, insufficient solvent was present to react with all the spent coffee grounds.

4.7.2 Effect of reaction temperature

Table 4.8: Effect of reaction temperature on extracted caffeine yields

Sample	Reaction Temperature (°C)	IL Concentration mg/1000mL	DCM extraction Concentration mg/L	Water extraction Concentration mg/1000mL
Run 1	88	609.80	513.62	542.72
Run 2	120	726.22	566.12	646.34
Run 3	104	296.44	602.85	263.83
Run 4	104	447.43	636.06	398.22
Run 5	88	636.31	673.05	566.31
Run 6	120	320.24	565.29	285.01
Run 7	104	473.76	634.31	421.65
Run 8	88	391.04	496.69	348.03
Run 9	104	455.16	606.67	405.10
Run10	104	435.87	609.03	387.93
Run 11	104	474.64	703.30	422.43
Run 12	104	374.45	416.46	333.26
Run 13	104	433.54	604.89	385.85
Run 14	104	441.17	316.85	392.64
Run 15	120	647.86	530.13	576.59
Run 16	88	379.46	439.70	337.72
Run 17	120	306.52	440.46	272.80

Observing the effect of reaction temperature independently on the yield of caffeine obtained did not show any clear trend or relationship. Using the ionic liquid showed that at the lowest and highest temperature of 88 °C and 120 °C obtained the highest caffeine yield, compared to that of the middle temperature of 104°C having a lower caffeine yield of both. Whereas, with DCM, the lower temperatures of 88 °C and 104 °C extracted higher caffeine yields compared to 120 °C. We can state that this specific solvent worked best at lower temperatures, and possibly got hindered as well as evaporated at too high temperatures. It is possible that the solvent volume denatured at 120 °C, hence the low caffeine concentration obtained. Observations with water as the extraction solvent, was again in similar trends to the IL. Water was able to extract most caffeine at 88 °C and 120 °C, and produced the lowest yields at 104°C. This explains why most caffeine remains behind in the usual home brewing or percolation, as most coffee making processes occur at 100 °C leaving behind majority of the bioactive components.

4.7.3 Effect of extraction solvent and solid-solvent ratio

Table 4.9: Effect of extraction solvent and solid-solvent ratio on extracted caffeine yields

Sample	Solid/Liquid loading ratio	IL Concentration mg/1000mL	DCM extraction Concentration mg/L	Water extraction Concentration mg/1000mL
Run 1	10	609.80	513.62	542.72
Run 2	17.5	726.22	566.12	646.34
Run 3	10	296.44	602.85	263.83
Run 4	10	447.43	636.06	398.22
Run 5	25	636.31	673.05	566.31
Run 6	17.5	320.24	565.29	285.01
Run 7	17.5	473.76	634.31	421.65
Run 8	17.5	391.04	496.69	348.03
Run 9	17.5	455.16	606.67	405.10
Run10	17.5	435.87	609.03	387.93
Run 11	25	474.64	703.30	422.43
Run 12	25	374.45	416.46	333.26
Run 13	25	433.54	604.89	385.85
Run 14	25	441.17	316.85	392.64
Run 15	17.5	647.86	530.13	576.59
Run 16	10	379.46	439.70	337.72
Run 17	10	306.52	440.46	272.80

A clear trend cannot be distinguished unless compared to that with temperature as well, due to the solvent only being activated at a specific temperature. 10 mL of solvent at 88 °C and 10 mL at 104 °C obtained caffeine concentrations with significant values. However, it can be stated that the IL extracted the highest caffeine concentrations. Table 4.6 above displays the highest caffeine content extracted per each volume used. Looking at the IL, 609.80 mg/L – 726 mg/L was obtained. Dichloromethane and water have extraction ranges of 634.06 mg/L-703.29 mg/L and 542.72 mg/L – 646.34 mg/L respectively.

4.8 Interaction of parameters on the extraction yield of caffeine obtained.

Table 4.10: Parameter interaction of best extraction runs data

Run	Reaction time (min)	Reaction Temp (°C)	Solid-solvent (mL)	Solvent used	Caffeine yield obtained (mg/L)
2	25	120	25	IL	726.22
				DCM	566.12
				Water	646.33
5	25	88	25	IL	636.30
				DCM	673.04
				Water	566.31
11	35	104	25	IL	474.64
				DCM	703.29
				Water	422.43

Observing the separate trends and relationships between the parameters of interest; i.e. reaction time, reaction temperature and the solid-solvent loading ratio, table 4.10 was composed depicting the best extraction runs based on the caffeine yield with the corresponding temperatures. Using the individual data obtained from tables 4.4, 4.5 and 4.6, it can be concluded that runs 2, 5 and 11 are the best achieving runs with the experimental conditions. Each run established had different operating temperatures with two runs having the same operating time of 25 minutes and three runs having the same solid-solvent ratio of 25mL. Run 11 however, only high yield using dichloromethane, compared to runs 2 and 5 which have high yields of caffeine produced for each solvent at the respective operating conditions.

4.9 Optimal conditions for the enhanced extraction of caffeine from spent coffee grounds

Runs 2 and 5 are considered the best operating conditions for caffeine extraction from spent coffee grounds. It is clear that run 2 extracted higher caffeine concentrations for both IL and water compared to run 5. This concludes that a reaction time of 25 minutes, 120 ℃ and 25 mL of solvent extracted the highest caffeine content. At the optimised reaction conditions, IL extracted 726.22 mg/L and water extracted 646.33 mg/L of caffeine from spent coffee grounds.

Table 4.11: Final optimum conditions and yield results of caffeine experimental runs

Run	Reaction time (min)	Reaction Temp (°C)	Solid-solvent (mL)	Solvent used	Caffeine yield obtained (mg/L)
2	25	120	25	IL	726.22
				DCM	566.12
				Water	646.33
5	25	88	25	IL	636.30
				DCM	673.04
				Water	566.31

4.10 Scanning electron microscopy and EDS analysis results

Figure 4.14 and figure 4.15 indicates spend coffee grounds before the extraction process. From the SEM (Figure 4.14a), the chemical compositions, functional properties and the structural characteristics that allow their reutilization can be evaluated. SCG differ in morphology and crystallinity due to the mixture and variety of coffee, however they do have high porosity. Images 4.14 (a) and (b), revealed the dense morphological structure of spent coffee grounds. Black patches indicate the amount of nitrogen absorbed by spent coffee grounds were low indicating they have poorly developed mesoporosity less than 12μm. The structure of the coffee grounds magnified 5000 times in the EDS (Figure 4.14 (b)) explains why pre-treatment is necessary for partial or full degradation of the complex cellulose and lignin matrix present within spent coffee grounds. Chain-like fibre structures and large lumps are present in the spent coffee grounds which hinder the full extraction; hence the rough structure and tight bonds require to be broken down for the release of their full contents.

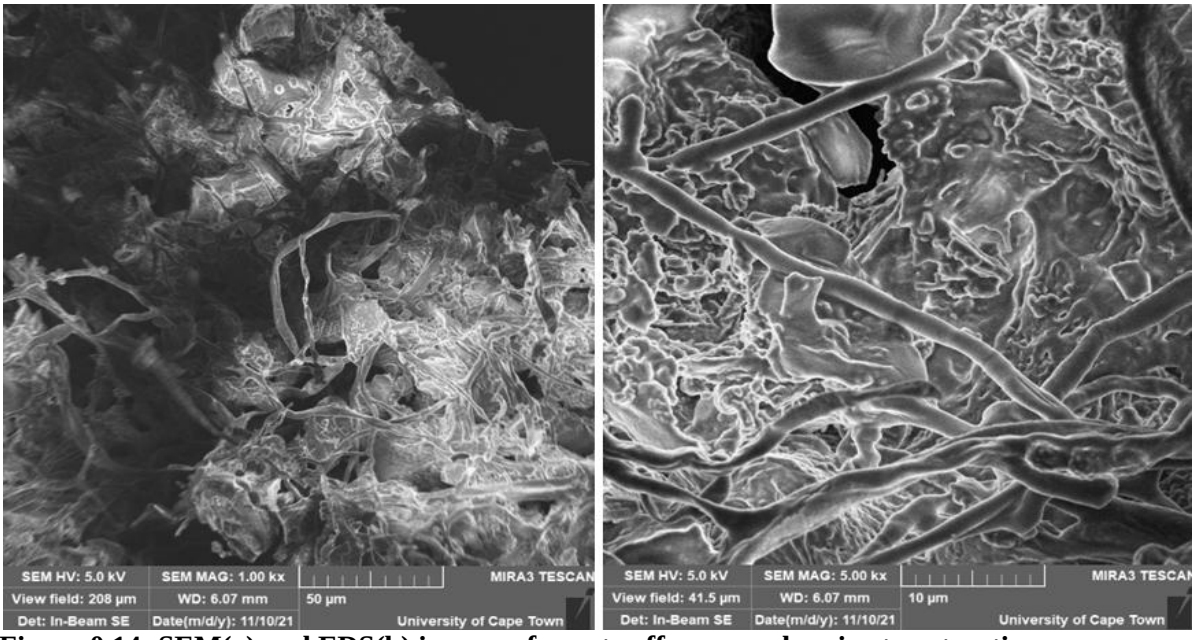


Figure 0.14: SEM(a) and EDS(b) images of spent coffee grounds prior to extraction process.

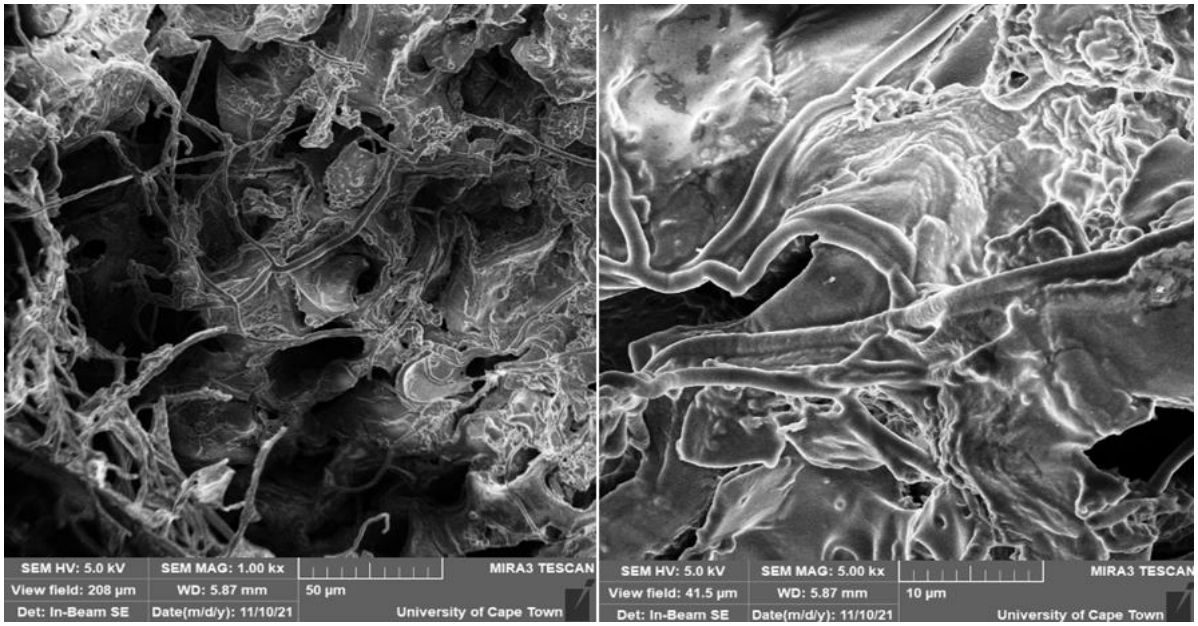


Figure 0.15: Spent coffee grounds SEM (a) and EDS (b) structural visibility before extraction.

Figure 4.16 represents the SEM and EDS images of experimental run number 14. It is evident from the images that the extraction solvent used was capable of breaking down the initial rough structure of the spent coffee ground (Figure 4.14 and 4.15), creating more open pores after the release of the bioactive caffeine. Figure 4.17 shows that the solvent did not successfully break down the SCG structure and extract a lot of the caffeine present, as the structure still appears quiet compact with very few open pores. From figure 4.17(b), the joined structure and thickness of the coffee ground still appears to be approximately 3,3µm. Judging from this, we can state that the degradation rate of the material was low, hence the low yield value of caffeine obtained.

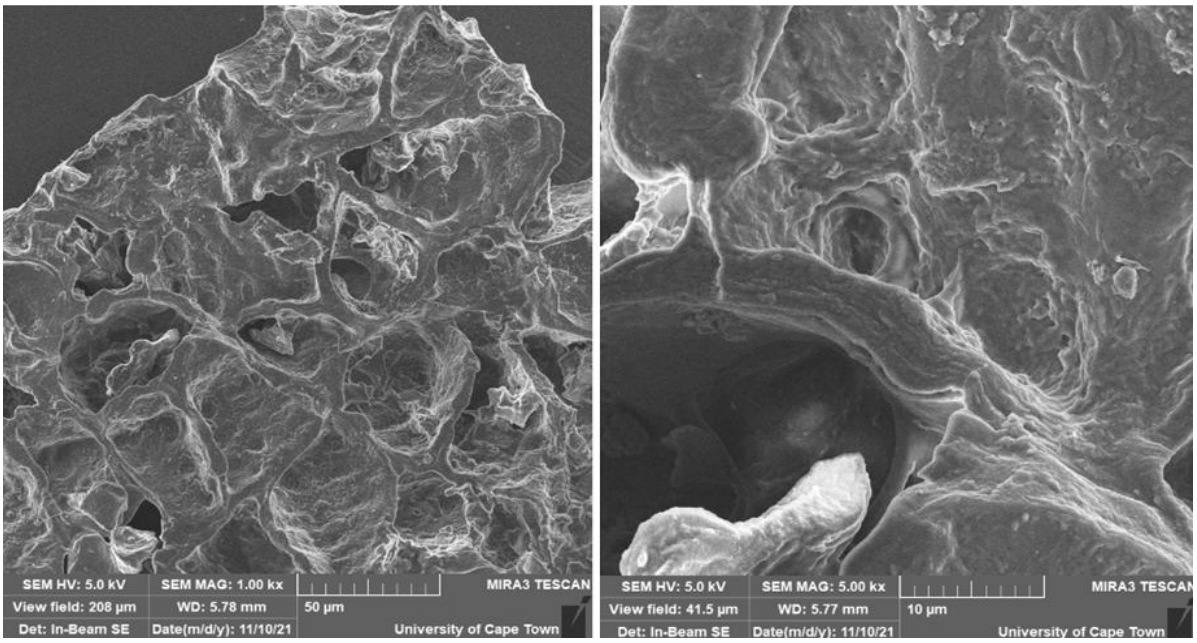


Figure 0.16: SEM (a) and EDS (b) of spent coffee grounds after 15 minute extraction at 104 °C and 10 mL solid-solvent ratio (IL).

In comparison to experimental run 14, run 16 which underwent conditions of lower reaction temperature and higher solvent ratio at 88 °C and 17,5 mL [EMIM][Cl] respectively; but for the same 15 minute time duration. Figure 4.17(b) clearly shows the effect that a higher solvent ratio had on the degradation of the coffee grounds. The coffee structure now appears looser and degraded indicating the solvent reacted more intensely with the spent coffee grounds, extracting more caffeine. More open pores are also visible on the surface of the material, increasing their surface area which allowed better extraction. Besides the separation of the particles, the thickness of the coffee ground is much thinner of approximately 2,5µm. The caffeine concentration achieved from this experimental run appeared was higher validating the above statements.

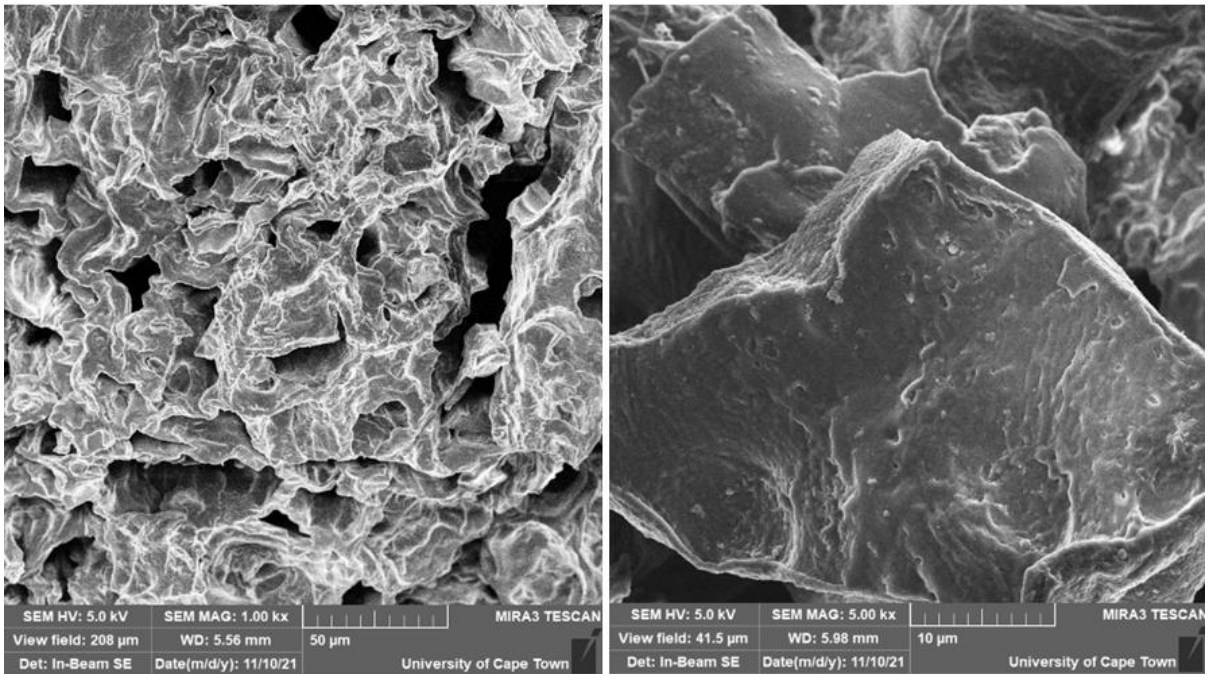


Figure 0.17: SEM (a) and EDS (b) of spent coffee grounds after 15 minute extraction at 88°C and 17, 5mL solid-solvent ratio (IL).

Figure 4.18 are the SEM (a) and EDS (b) for run 17, having a 15-minute reaction time, the 120°C reaction temperature and solvent loading of 17,5 mL The images display what effect a higher temperature has in the overall reaction. In this instance, the particle appears as it has been altered and attempted to be broken, but was not successful as no open pores or loosely packed structures are visible. Figure 4.19 (b) indicated that the high temperatures and solvent were able to dissolve and thin the spent coffee grounds, but was unable to actually separate and remove the caffeine from within the particles.

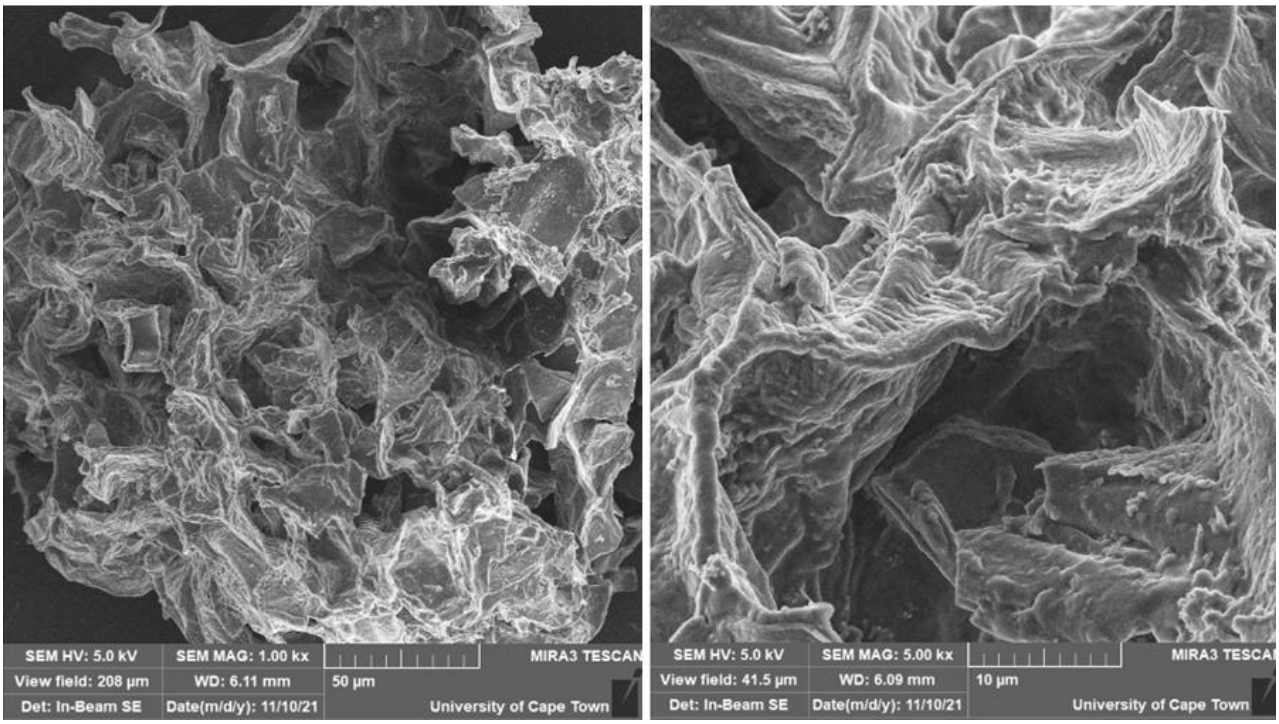


Figure 0.18: SEM (a) and EDS (b) of spent coffee grounds after 15 minute extraction at 120°C and 17,5mL solid-solvent ratio (IL).

Using the best extraction conditions of 25 minutes reaction time, 120°C reaction temperature and 25 mL of extraction solvent, established in section 4.9, the effect of solvent choice used i.e. 1-ethyl-3-methylimidazolium chloride, water or dichloromethane can be seen in figure 4.19, 4.20 and 4.21 respectfully. Figure 4.19 shows the effect 1-ethyl-3-methylimidazolium has on the spent coffee ground particles. It can be seen that the ionic liquid fully degraded the individual particles and hence allowed the maximum amount of caffeine to be released. More open pores on the surface structure indicated that the coffee grounds were fully exposed to the operating conditions. Figure 4.19 (a) shows the thinness of the coffee particle. The solvent was able to react fully and penetrate the sample within the allowed time.

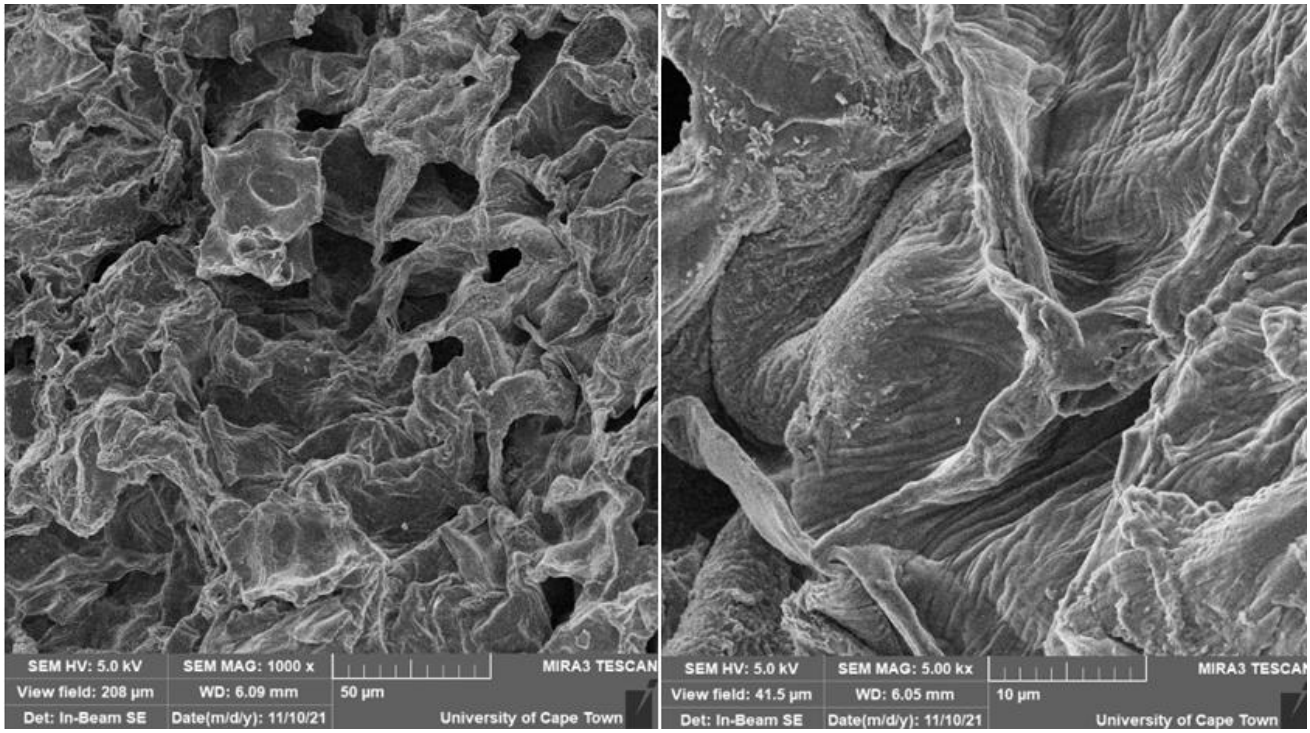


Figure 0.19: SEM (a) and EDS (b) of spent coffee grounds after 25 minute extraction at 120°C and 25mL solid-solvent ratio (IL).

Using water as the extraction solvent, it can be seen from figure 4.20 that there is no significant difference from the imaged produced when using the IL as the extraction solvent. Using the best established conditions as stated above, the water achieved almost the same effect on the spent coffee grounds to that of the ionic liquid. Multiple pores are visible on the surface of the material; hence maximum caffeine was released from the sample. The material thickness is more than half lower of what was achieved during the 15 minutes. This smooth texture of the material indicated that the sample was homogenised thoroughly with the solvent allowing the caffeine to be extracted.

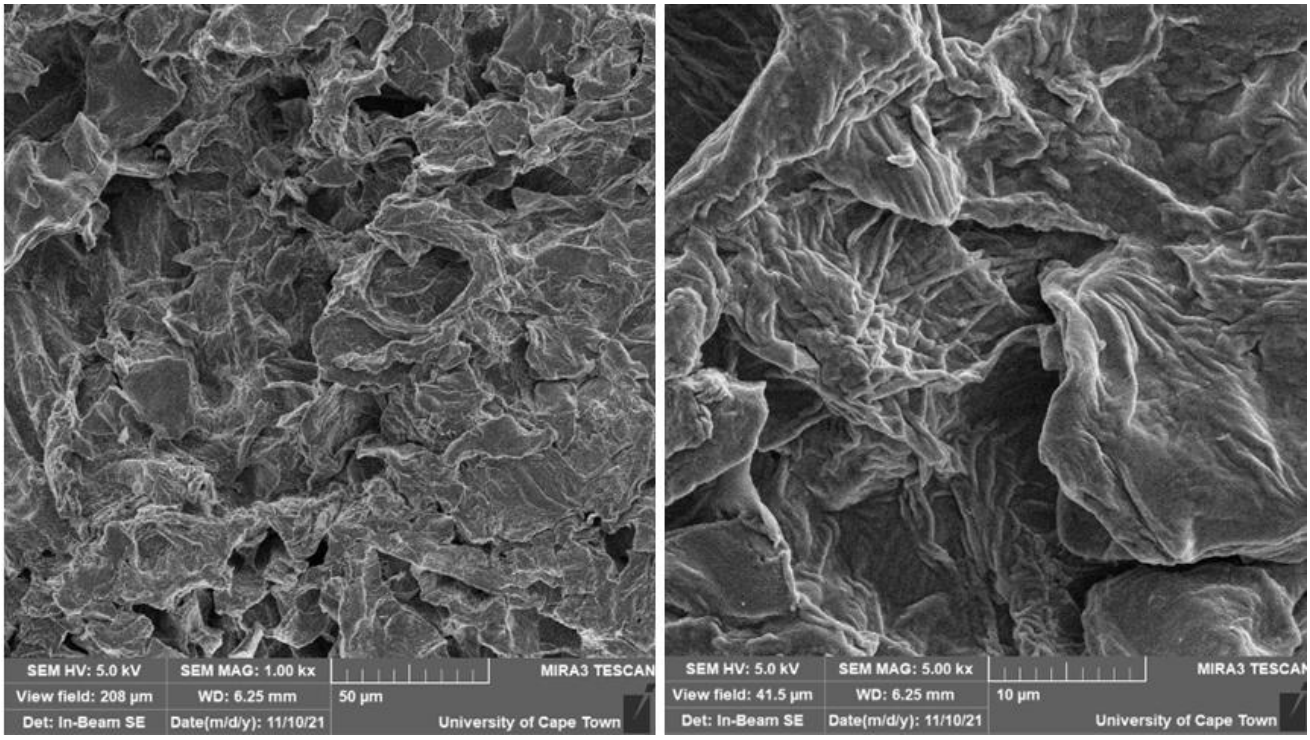


Figure 0.20: SEM (a) and EDS (b) of spent coffee grounds after 25 minute extraction at 120°C and 25mL solid-solvent ratio (water).

Investing the effect that the third extraction solvent, dichloromethane had on the spent coffee grounds indicates that the strength was not as high compared to that of the IL and water. Observing from figure 4.21(b), the extraction sample was oily (black patches) after completion which could have possibly held back the caffeine from being released. The solvent had formed a slurry rather than a solution. This statement was noted after the extractions with dichloromethane as the extract appeared much darker in colour with an oil residue, with an increase in filtration time. The dichloromethane extracted the lowest yield of caffeine, indicating it did not thoroughly penetrate the particles, but only surround the exterior surface of the particles. Figure 4.22 on the right depicts the particles individually, surface covered in an oil-like substance coat which hindered the full extraction of caffeine. The extraction solvent was unable to fully disintegrate the spent coffee grounds allowing the full release of the bioactives and caffeine.

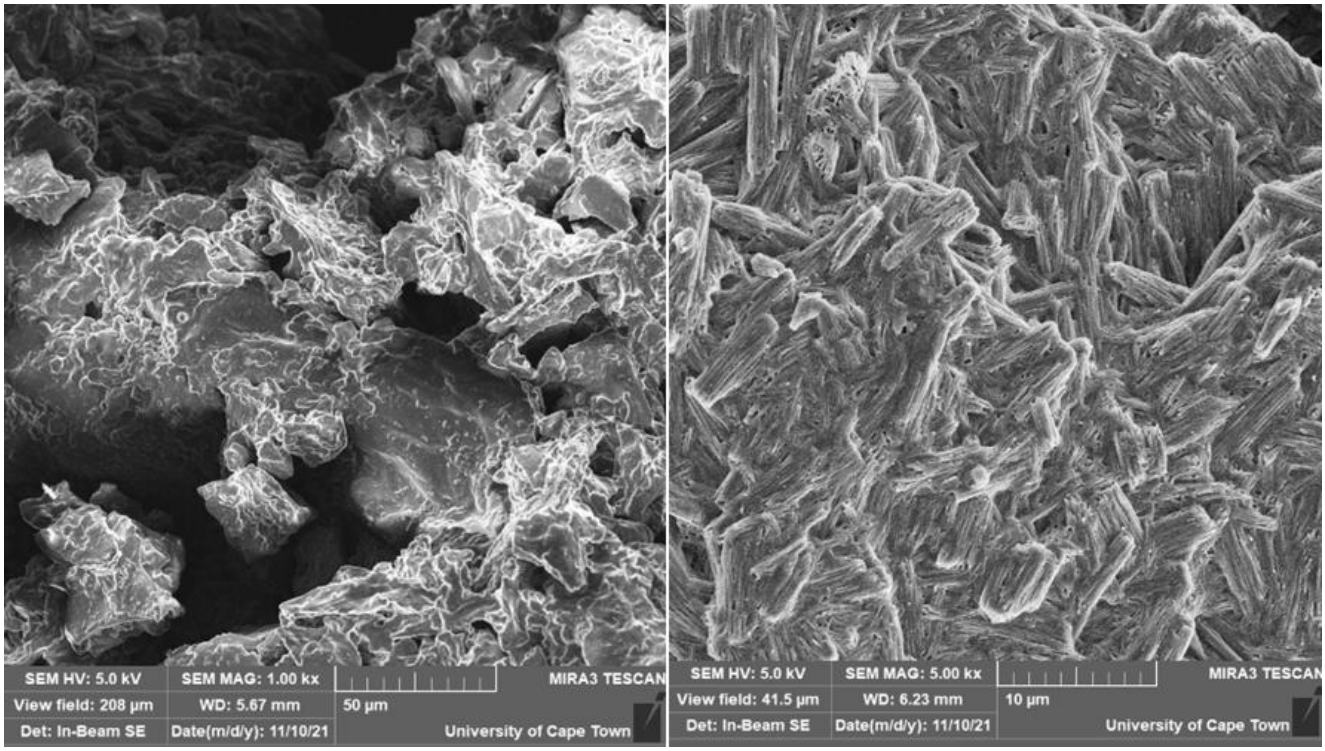


Figure 0.21: SEM (a) and EDS (b) of spent coffee grounds after 25 minute extraction at 120°C and 25mL solid-solvent ratio (Dichloromethane).

4.11 EDS analysis for purity testing of extracted caffeine vs standard caffeine

After extraction and evaporation of the crude caffeine, powered crystalline caffeine was obtained. Each caffeine sample extracted using all three different extraction solvents were analysed for particle size and crystallinity, texture and appearance.

After the extraction and sublimation of the crude caffeine, the powdered caffeine was analysed against the pure cafffeine standard. Figure 4.22 compared the pure caffiene (a) against the caffeine extracted using 1-ethyl-3-methylimidazolium (b) ; which appears clean and similar. From the shape and structure of the standard caffeine against the extracted caffeine, it is evident that the particles have the same crstallianlity, structure and almost the same particle size of 10μm and 8,75μm, respectively. Having the same crystallinaity and structure indicates that the entire extraction process was sucessful in obtaining a clean, uncontaminated sample; corrresponding to the results obtianed in section 4.11.

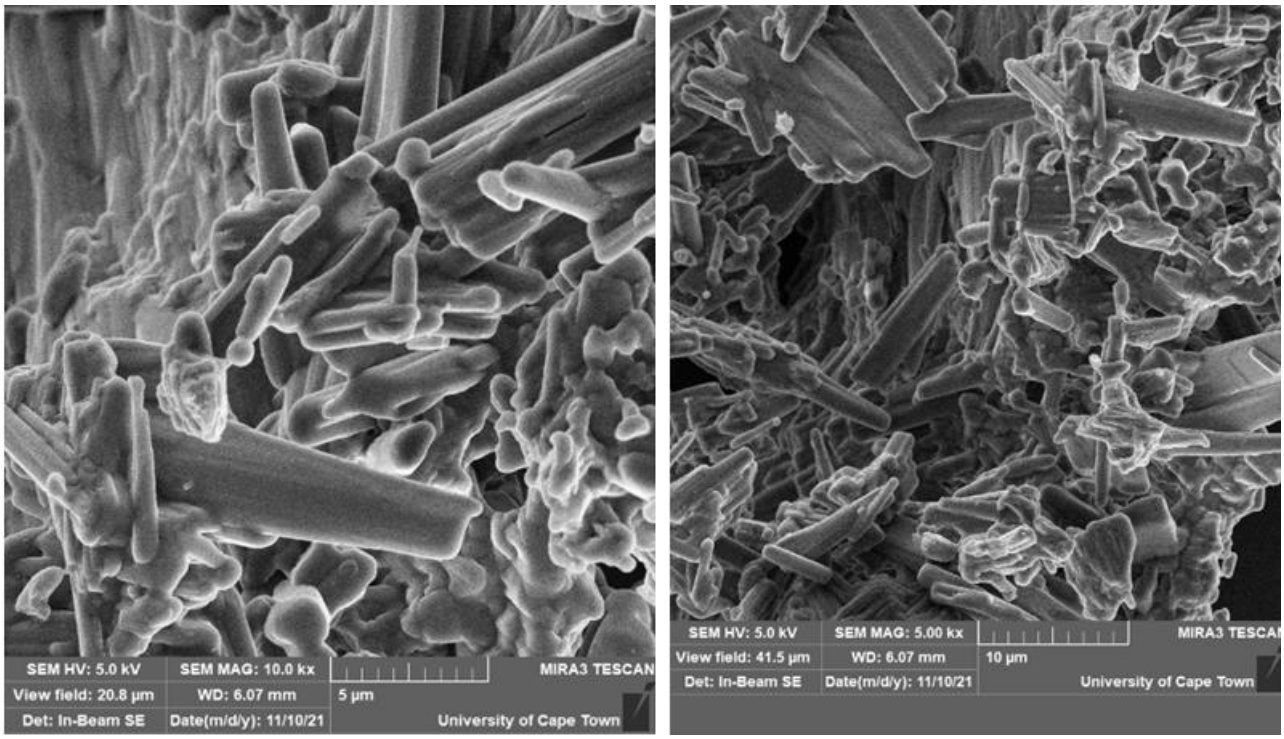


Figure 0.22: EDS of pure caffeine standard (a) and the extracted caffeine samples using 1-ethyl-3-methylimidazolium as the extraction solvent (b)

Comparing the image obtained using the IL during extraction against water, it is shown that the caffeine was released in long shards, but was not able to fully separate (Figure 4.23). Also, oily substances were present surrounding the caffeine molecules which decreased the purity of the caffeine achieved. Assuming that water was not efficient to remove any impurities and contaminants present, hence the lower grade of 90% pure caffeine was achieved. However, even though the purity of caffeine decreased, the yield of caffeine obtained was still high and very close to that achieved from using the IL. Lower grade caffeine can be utilised for other uses such as energy consumption drinks, weaker strength medicine and cheaper food grade products.

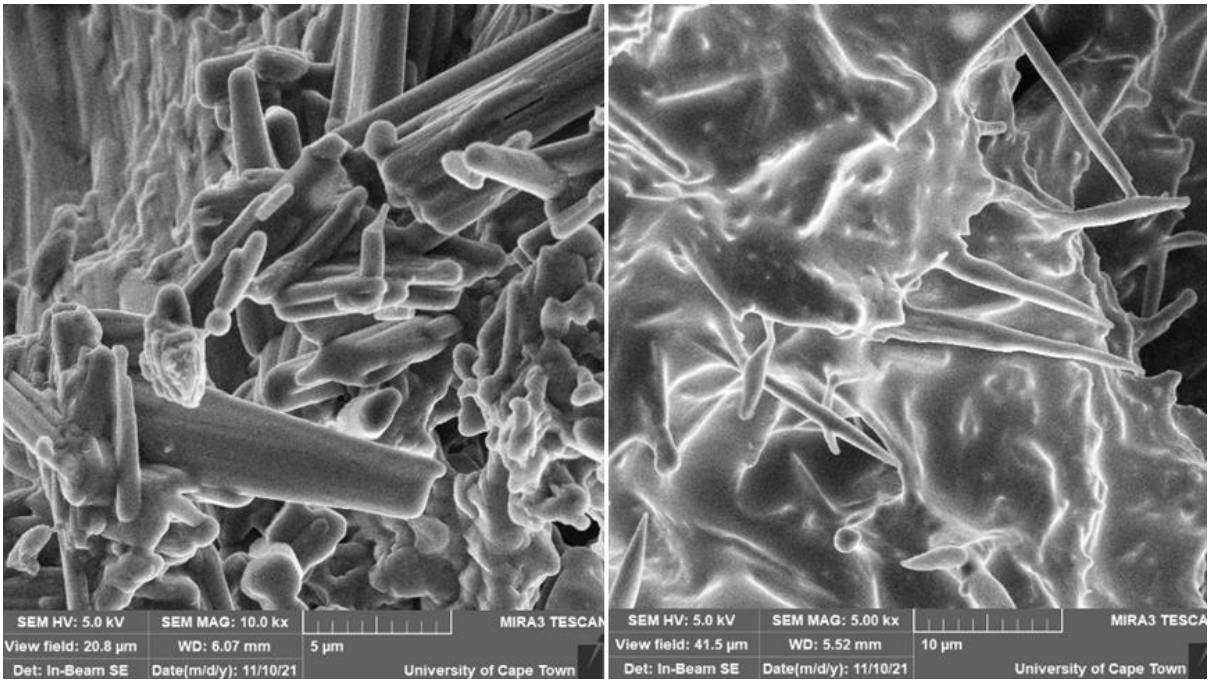


Figure 0.23: SEM and EDS of the extracted caffeine samples using water as the extraction solvent.

The last comparison of the caffeine extracted using dichloromethane, figure 2.24 illustrates that the caffeine was less crystalline. The structures appear very small and compact hence the lower yields obtained. The oily substance released could have possibly hindered the proper formation of caffeine crystals. The dichloromethane did achieve extraction, but at much lower yields and 85% purity of the sample.

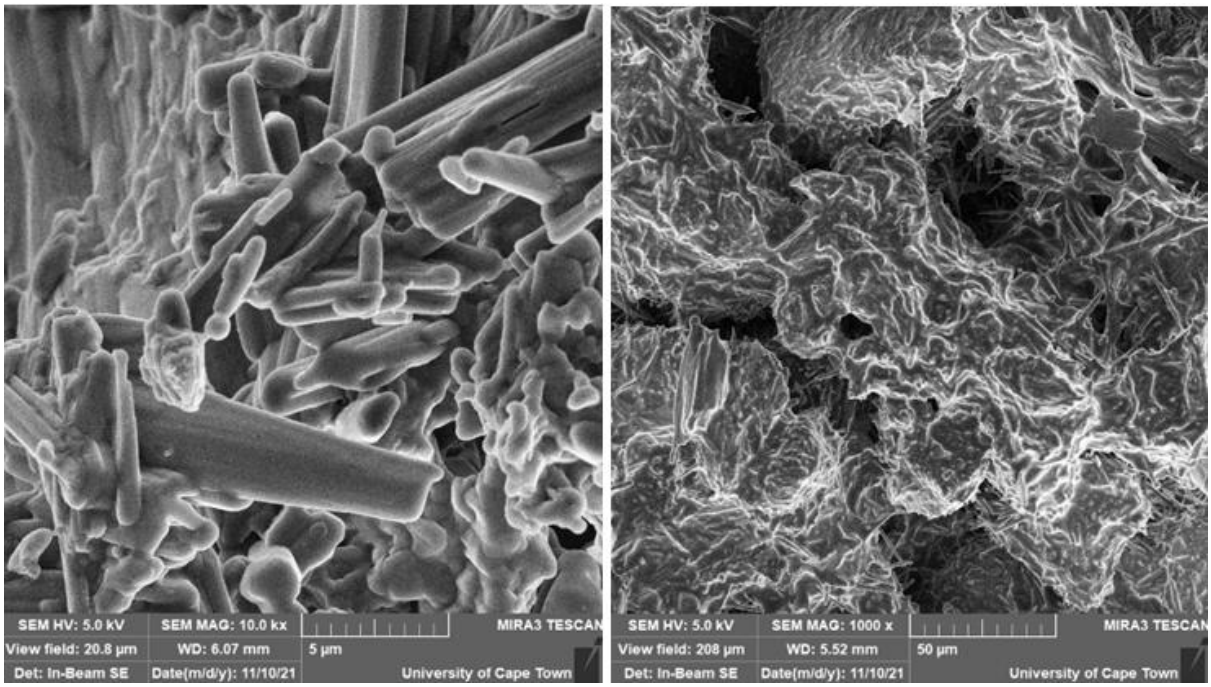


Figure 0.24: SEM and EDS of the extracted caffeine samples using dichloromethane as the extraction solvent.

4.12 Analysis of caffeine purity using Differential scanning Calorimetry (DSC)

4.12.1 Differential scanning Calorimetry results indicating purity of samples

DSC was used to compare the purity of the extracted caffeine sample versus the 99% purity caffeine standard.

The thermo gravimetric (TG) and differential thermal gravimetric (DTG) curves in figure 4.25 illustrate the standard and extracted sample respectively. The TG curve represents the mass of organic matter lost with respect to temperature whereas the DTG curve represents the decomposition of the impurities and constituents of the inserted sample at specific temperature.

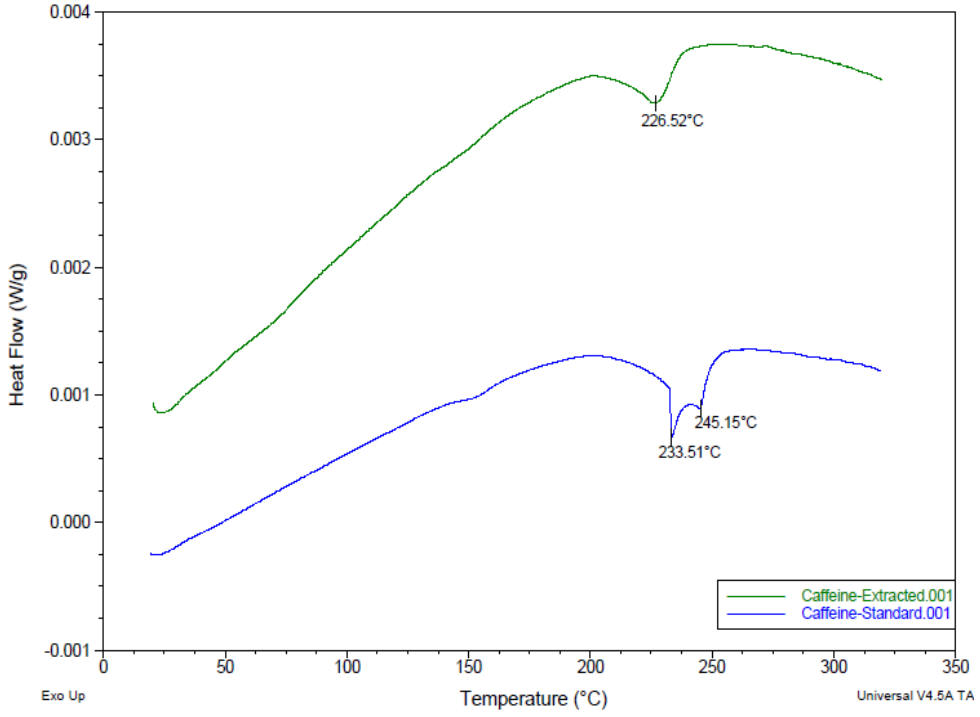


Figure 0.25: TG and DTG curves for caffeine standard (lower) and extracted caffeine (upper)

Figure 4.25 represents the TG (blue) and DTG (green) curves for the extracted caffeine and the pure caffeine standard, respectfully. The upper green line (extracted caffeine sample) showed a weight decrease in the extracted caffeine indicated that other impurities and water molecules were present.

Minimal water molecules were present in the extracted caffeine. Few water molecules desorbed and evaporated and contributed to the actual weight of the sample, hence the weight difference. The pure caffeine standard did not represent any evaporation or weight decrease until 200°C, which indicated that no water was actually present.

According to literature, pure caffeine has a melting point of 234 – 236,5 °C (ChemicalBook, 2021). The blue line in figure 4.26 above, confirms that the 99% pure caffeine sample used has a melting point of 233,51°C.The caffeine samples that were extracted using the IL 1-ethyl-3-methylimidazolium chloride had a melting point of 226,52°C, hence using the cross-multiplication method based on melting points (appendices section 7), we can conclude the extracted caffeine sample had a high purity of 96%, almost pure as the standard sample used. This grade of caffeine is suitable for high end medical uses, pharmaceutical processes and food grade consumption.

The caffeine samples extracted using water as the extraction solvent had a lower melting point of 212,28°C, which achieved a purity of 90%. This grade of caffeine is also used in high end medical processes and food production.

Lastly, the caffeine samples extracted using dichloromethane achieved the lowest melting point and hence the lowest grade of caffeine; of 200,5°C and 85% purity respectively. Caffeine of this grade is used for lower end processes and cheaper food production, such as energy drinks.

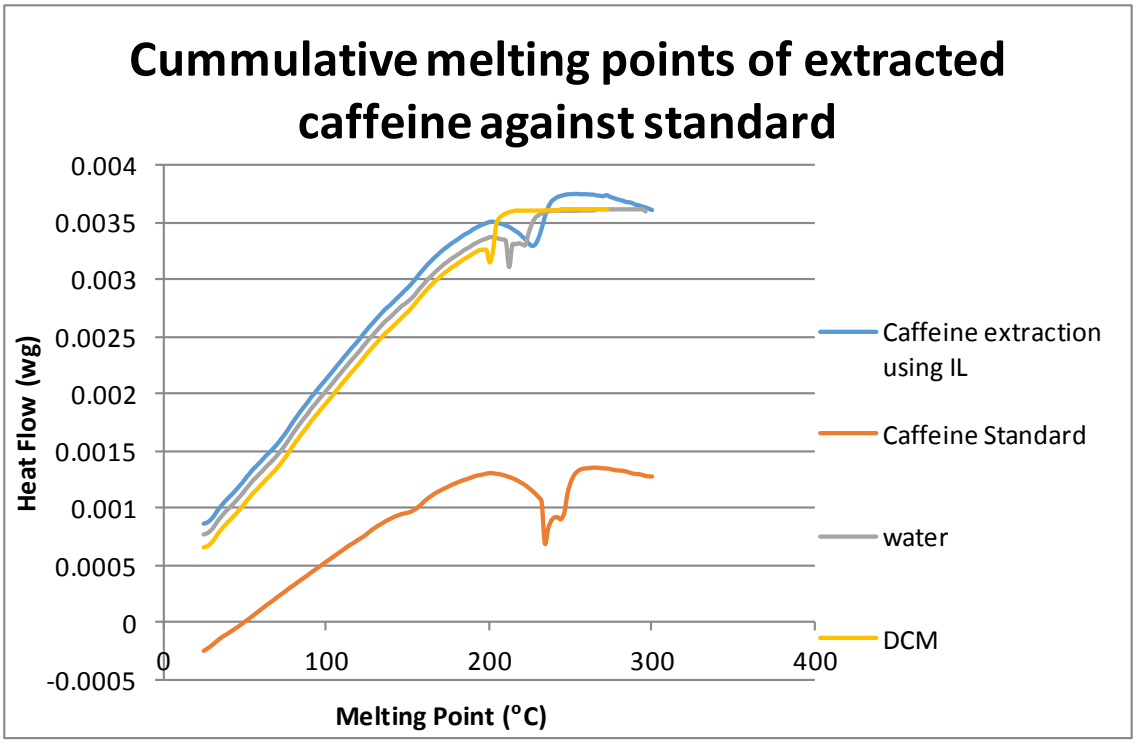


Figure 0.26: Cummulative melting points of extracted caffiene samples using various solvents against the standard caffiene used

Table 4.12: Summary of melting points and caffiene purity achieved using different extraction solvents.

Material source	Melting point (°C)	Percentage purity (%)
Pure Caffeine	234-236,5	100
Caffeine standard purchased	233,51	99
Caffeine extracted by IL	226,52	96
Caffeine extracted by Water	212,28	90
Caffeine extracted by DCM	200,48	85

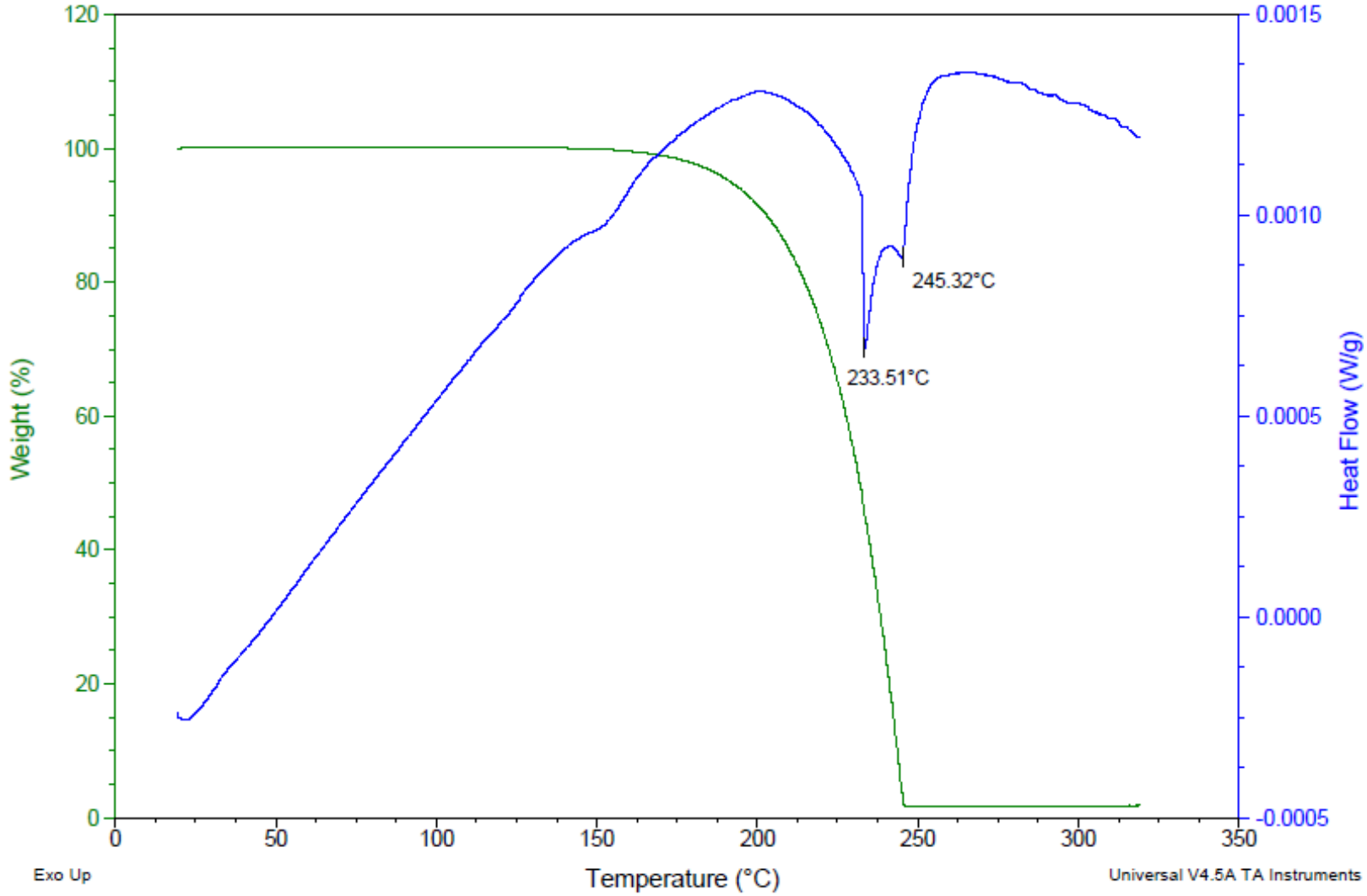


Figure 0.27: TG curve of pure caffeine sample analysed by DSC

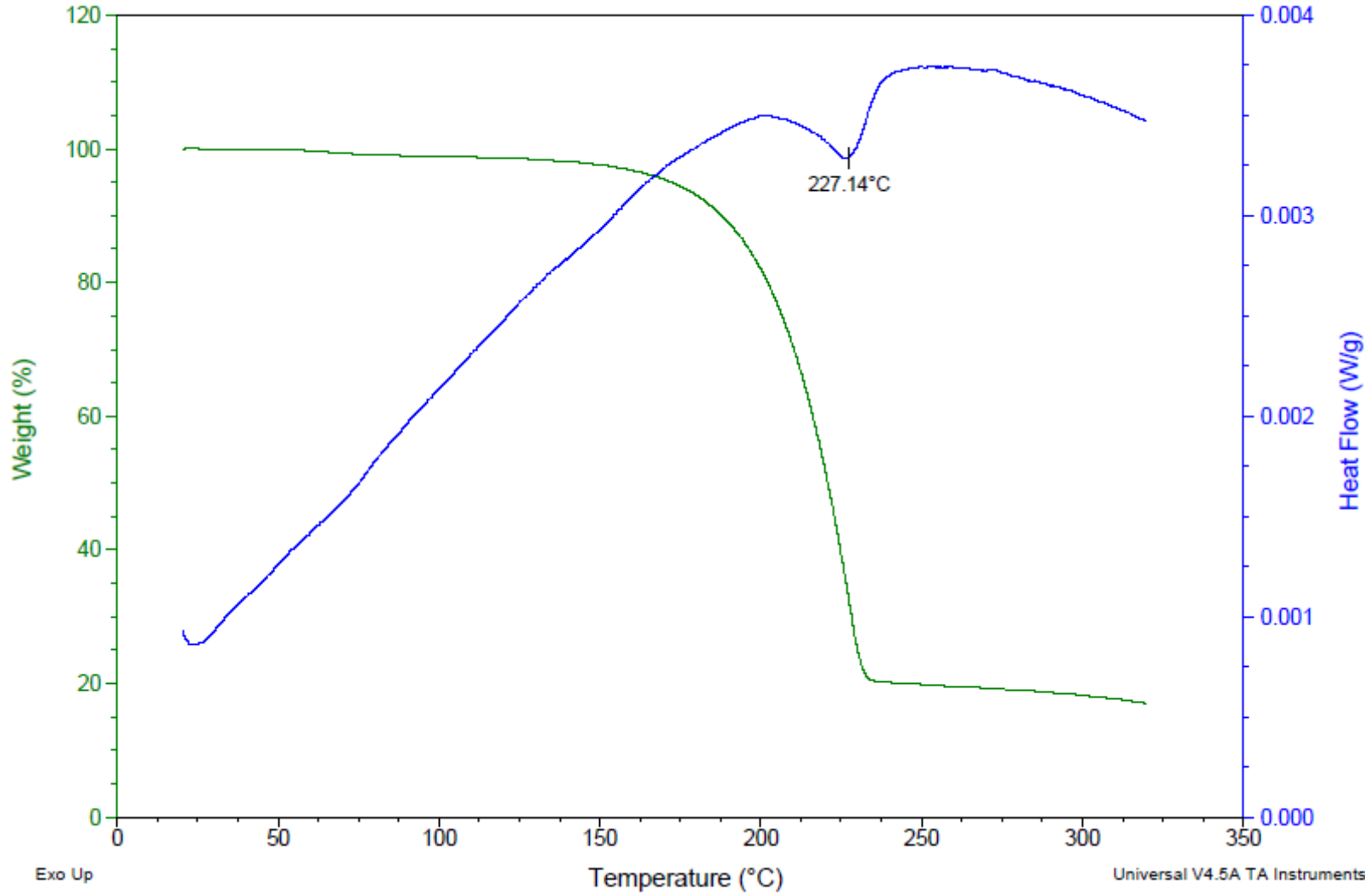


Figure 0.28: DTG curve of extracted caffeine sample analysed by DSC

4.13 TEM analysis of pure caffiene standard against the extracted caffeine samples.

The transmission electron microscopy results provide up-close detailed pictures of the pure caffeine standard at 1µm and 0.5µm shown in figure 4.29. From this the colour appears dark and the thickness of the particle is observed. It is also seen that the particles appear bolder and firm demonstrating the purity of the caffeine sample. As compared to figure 4.30(a) appearing much lighter and thinner in nature, indicating the purity of the caffeine sample extracted using water is lower to the caffeine standard. Figure 4.30(b) displays the TEM of the caffeine sample extracted using 1-ethyl-3-methylimidazolium chloride, appearing darker, thicker and denser indicating the sample is of higher quality as established in section 4.11.1 above. Figure 4.30(b) almost resembles the pure caffeine standard justifying the purity difference existing between both samples.

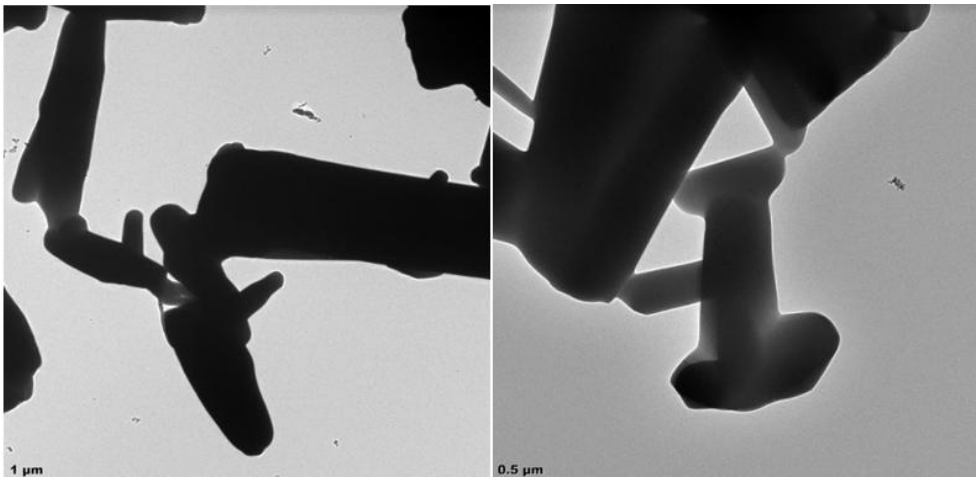


Figure 0.29: TEM images for 99% pure caffeine standard at 1µm and 0.5µm

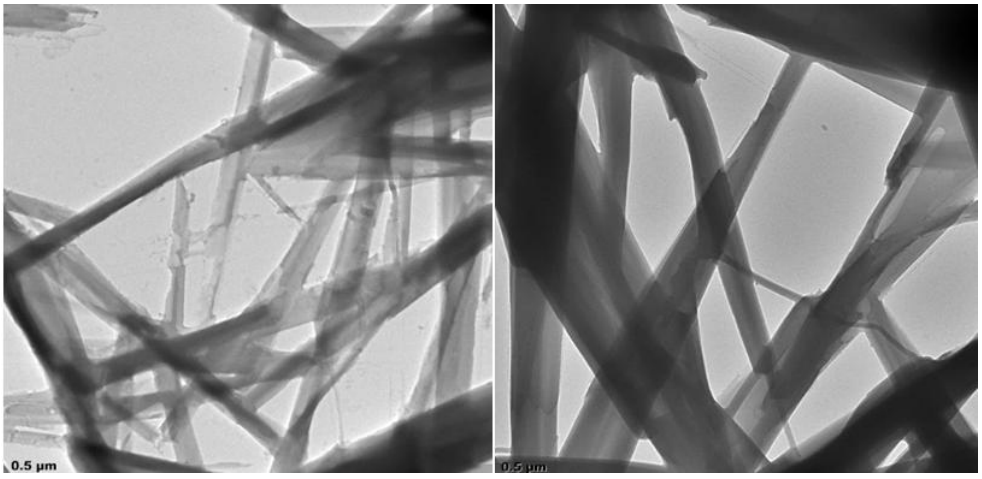


Figure 0.30: TEM images produced for caffeine sample extracted using (a) water and (b) IL at 0.5µm

4.14 Analysis of caffeine experimental results using Design Expert model software

4.14.1 Introduction

This section demonstrates a detailed explanation of the results obtained by Design Expert software. Results include the ANOVA, response surface plots of each factor interaction, diagnostics, model generations and the effect of each factor on the response yield. The DOE results of the caffeine extracted using 1-ethyl-3-methylimidazolium chloride and water is demonstrated in table 7.2 and table 7.4 respectively. Extraction using dichloromethane was excluded from this model development since it was concluded that dichloromethane is not feasible and against the green principles of engineering. Although the extraction using 1-ethyl-3-methylimidazolium chloride was not cost effective in this study, the model was included for research purposes due to the high yield results that were achieved using the ionic liquid and knowing that a recovery method can be established for the ionic liquid.

4.14.2 Caffiene concentration yeild analysis using 1-ethyl-3-methylimidazolium chloride (IL).

The ANOVA model indicates the interaction and the individual sum of squares, p-values and F-vales for each term together with the corresponding effect on the model and lack of fit. Table 4.9 displays the statistical model of the generation for the most optimum response surface model for caffeine-IL extraction experimental study. The model having the highest R^2 and adjusted R^2 was selected. A quadratic model had been selected for the caffeine yield as indicated in table 4.9. The specific model had a standard deviation of 268, 12 and an adjusted R^2 of 0, 0670. As you can see in table, this was the only model with a high R^2 value and non-significant lack of fit. The p value of 0.0019 indicates that the model is suitable.

Table 0.13: Combined model fit ANOVA for model of caffeine yeild using IL

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	2.178E+05	9	24197.82	5.21	0.0203	significant
A-Reaction Time	2169.92	1	2169.92	0.4676	0.5161	not significant
B-Reaction Temperature	31.11	1	31.11	0.0067	0.9370	not significant
C-S/L Ratio	534.06	1	534.06	0.1151	0.7444	not significant
AB	1.14	1	1.14	0.0002	0.9879	not significant
AC	2205.61	1	2205.61	0.4753	0.5127	not significant
BC	672.36	1	672.36	0.1449	0.7147	not significant

A^2	88685.93	1	88685.93	19.11	0.0033	
B^2	23995.01	1	23995.01	5.17	0.0571	not significant
C^2	1.086E+05	1	1.086E+05	23.40	0.0019	
Residual	32482.16	7	4640.31			
Lack of Fit	12657.92	3	4219.31	0.8513	0.5340	not significant
Pure Error	19824.23	4	4956.06			
Cor Total	2.503E+05	16				

The model generated for the caffeine yield was later used to generate the response surface. Equation 4.1 shows the reduced cubic and quadratic model for coded factor while equation 4.2 demonstrates the model for the actual parameters/factors used. Composition of this model is useful when identifying the impact of each factor together with their corresponding factors in the final yield achieved. Equation 4.2 can indicate the individual effects of the components and the combined response depicted by positive and negative signs prior to each model term. Equation 4.1 has the terms A^2, B^2 and C^2 which are of high impact. All positive signs before each variance inflation factors (VIFs) are greater than one which indicates collinearity. The VIFs are less than 10 which indicate the correlation between factors and that the model is reliable. Equation 4.2 in terms of actual factors can be used to make predictions about the response for given levels of each factor studied. This equation is not used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the centre of the design space.

$$\text{Caffeine yield} = +418.95 + 16.47\,A - 1.97\,B + 8.17\,C + 0.5333\,AB + 23.48\,AC + 12.96\,BC - 145.13\,A^2 + 75.49\,B^2 + 160.60\,C^2$$

... (Equation 0.1)

$$\text{Caffeine yield} = +3870.61 + 68.39\,\text{Reaction time} - 63.43332\,\text{Reaction temperature} - 117.90\,\text{S/L Ratio} + 0.0033\,\text{Reaction time} * \text{Reaction temperature} + 0.313093\,\text{Reaction time} * \text{S/L Ratio} + 0.108\,\text{Reaction temperature} * \text{S/L Ratio} - 1.451\,\text{Reaction Time}^2 + 0.2948\,\text{Reaction Temperature}^2 + 2.85515\,\text{S/L Ratio}$$

... (Equation 0.2)

Table 4.13: Fit summary of model interactions

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.9855	0.0656	-0.2173	-0.9587	
2FI	0.9890	0.0353	-0.5641	-3.5582	
Quadratic	**0.0019**	**0.5340**	**0.8702**	**0.0670**	**Suggested**
Cubic	0.5340		0.6831		**Aliased**

The Model F-value of 5.21 implies the model is significant. The analysis of variance of the caffeine ANOVA model showed in table 4.9 above, displays p-values of 0.0033 and 0.0019 for A^2 and C^2 indicating that they are significant. All values are below 0.05 indicating significance of the model to predict experiments accurately using Design Expert 13. The values of A^2, B^2 and C^2 all have p-values less than 0.05 as seen in table 4.9. Similarly, the R^2 value of 0.8702 indicates that good correlation exists between the experimental and predicted values.

The panty plot (figure 4.31) displays the actual versus predicted values showing the degree of alignment of the set data point while figure 4.29(b) demonstrates the equal distribution of data, validating that it a good model.

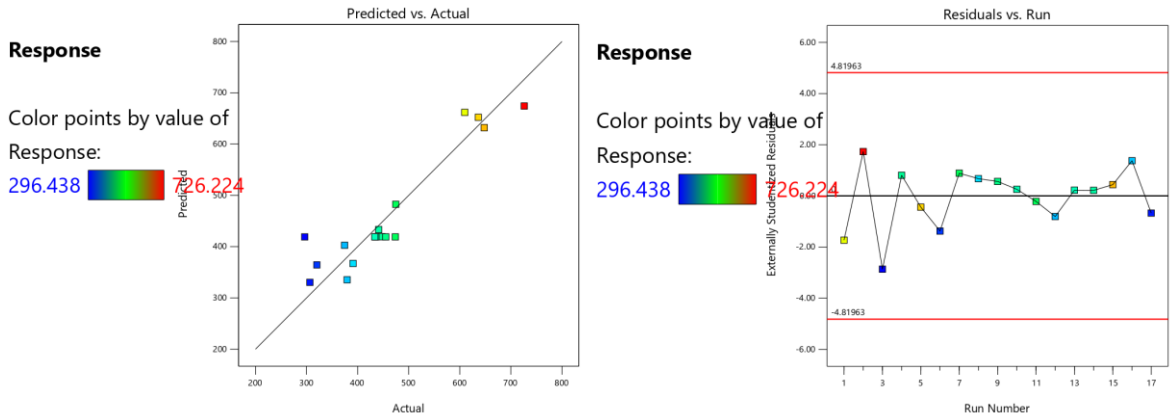


Figure 0.31: Panty plot of predicted vs actual plots(a) and residual vs run(b) of DOE experimental run responce

One of the best ways to evaluate the interaction and effects of the input variables on the output responses is to synthesise a response surface plot as seen in figure 4.30. (Natarajan, Suganthi and Periyanan 2016). In this case, it was done by altering two independent variables within the set experimental range and holding the other at the central point. Effect of temperature-reaction time on caffeine extraction with solid-solvent ratio of 20g/25 mL was conducted.

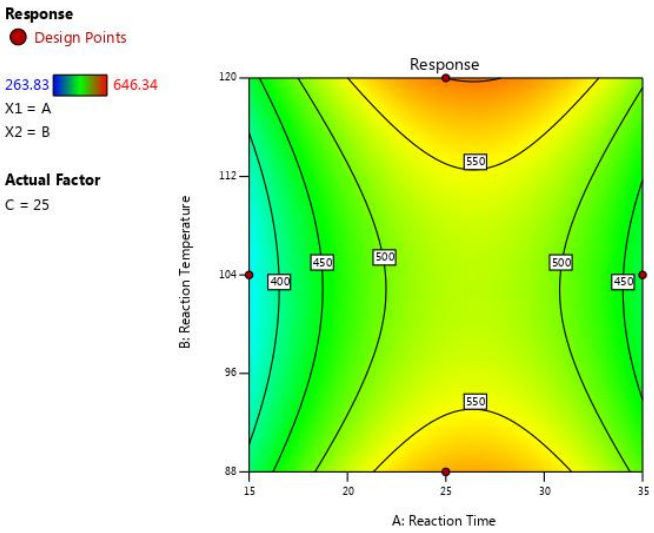


Figure 0.32: Responce plot of caffeine extraction with variation of input parameters.

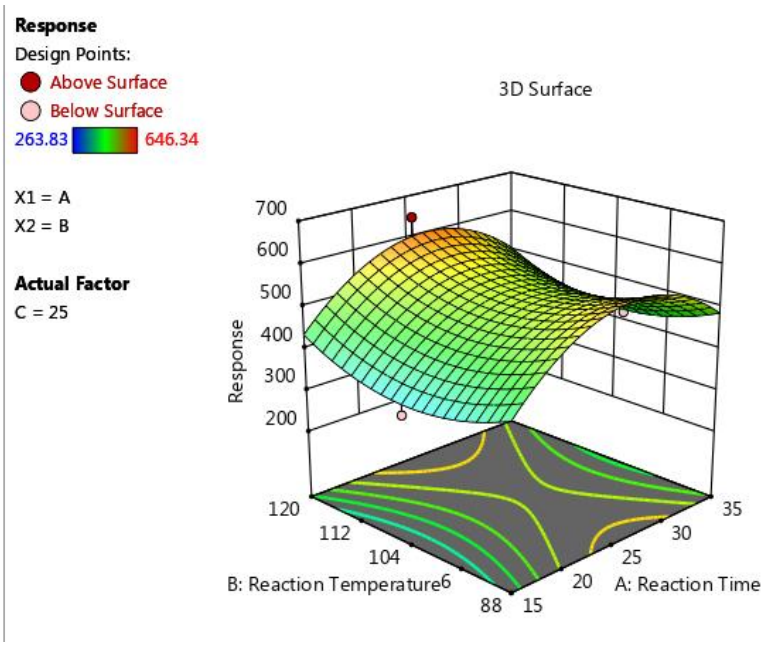


Figure 0.33: Contour plot for the caffeine concentration extracted

CHAPTER 5

COST ANALYSIS

5.1 Overview of cost analysis and feasibly

A study by Antunes, *et al.* (2018), found the typical market prices for product achieved from conventional sources. The retail market price of caffiene is dependant on the grade of caffiene sold, which is solely dependant on the purity. Prices of caffeine ranged from 10-175 £/kg, which is 204.40-3577 R/kg. This justifies the high value material, caffeine.

The overall cost and feasibility study of the extraction of caffeine from spent coffee grounds using three different extraction solvents were analysed in tables 5.1, 5.2 and 5.3. Quotations were obtained from international chemical companies and laboratories such as Merck, Shalom Laboratories, Sigma Aldrich (Merck, 2021) and Masiye Laboratories (Masiye, 2021). Sigma-Aldrich prices were used during the costing as they were the cheapest sources, hence displaying the cost and profit made and at a minimum. It is noted that for large scale production, materials would be purchased in bulk, obtaining it a lower price. Electrical cost factors were excluded during the costing; further studies are allowable to calculate the factors of electrical cost to add onto the production/extraction cost. Each scenario will decrease by the same electrical cost factor, hence justifying the ratio between each extraction scenario. For this study, prices were used according to that of small scale production. In the case where bulk purchasing is made, all costing per solvent will decrease per same cost factor; hence the accuracy and conclusions of the cost analysis is viable.

From table 5.1, the extraction of caffeine using 1-ethyl-3-methylimidazolium as the extraction solvent with the best optimum conditions established in section 4.9 are presented. The costing is carried out using ratios corresponding to the initial 5g SCG as per the DOE, thereafter scaled up to 1000g (1kg). From scenario 1, the cost of the ionic liquid is extremely high. For 5g of SCG, R447 worth of solvent is required to perform the full extraction, obtaining 0,7g of caffeine which can be sold for R1004.50. Scaling up the reaction on 1000g

of spent coffee grounds, R89 400 extraction of solvent is required. R208425-00 of product can be obtained from this scenario, which is much higher than the cost value. Although the ionic liquid obtained high extraction yields of high-grade pure caffeine while following green principles, the cost can further be reduced by recycling and recovering the solvent.

Scenario 2 (Table 5.2) depicts an alternate green method for the extraction of caffeine from SCG using water as the solvent. Using water as the extraction solvent cuts the cost drastically, while still adhering to the green principles of engineering. Although the purity of caffeine decreased from 96% to 90%, the grade of caffeine can still be utilized for high end processes. From this extraction, for 5g of SCG, R9,95 was the total cost of the extraction process yielding 0.646 g of caffeine which can be sold for R110.20. Scaling it up to 1000g, the cost required was R1990,84 which in turn generated a total of R22052,77 of product. This is an excellent turnover that can be generated using waste coffee. Tonnes of SCG waste are generated therefore more caffeine will be extracted; hence the ratio of profit will multiply.

Table 5.3 illustrates the cost analysis of the extraction of caffeine using dichloromethane. Compared to that of the ionic liquid, dichloromethane is much cheaper. However, this solvent is not a green solvent and will not be a viable option to expand large scale production. Using this solvent also extracted a lower grade caffeine overall, which could not be used for high end pharmaceutical products. Besides the challenges faced about sustainability, Scenario 3 is capable of extracting caffeine and still making profits. According to table 5.3, for 1000g of spent coffee grounds, R8854, 12 product value can be generated with a R6081, 24 cost of materials.

Concluding from the above three scenarios, it is evident that all three extraction process differ with cost, purity caffeine obtained and the expected profits. When high purity caffeine of 96% is obtained, the process cost is higher, also generating a much higher profit. Decreasing the cost of the process by eliminating IL and using water, a good but lower grade of 90% pure caffeine is obtained. Attaining this from waste, is excellent as R20 061, 93 of caffeine can be extracted per every kilogram of waste coffee. Looking at the ionic liquid scenario, the process can be drastically improved even further by recovering and reusing the extraction solvent. However, both green methods illustrate exceptional cost and return predictions of the overall caffeine extraction from waste coffee grounds, hence justifying that the overall study is a feasible and profitable process, while benefitting the environment.

Table 5.1: Cost of extraction per 1kg SCG using 1-ethyl-3-methylimidazolium

Option 1 : 1-ethyl-3-methylimidazolium	**Quantity (g)**	**Prices(R)**	**Product number**	**Reference**	**% pure caffeine obtained = 96%**				
Material used					For a run of 5g coffee grounds (Optimum conditions)	5g SCG	1000g SCG (1kg)		
1-ethyl-3-methylimidazolium choride	25	1788	272841	(Merck, 2021)	6.25	447	89400		
water (mL)	1000000	28.41	Municipal water		25	0.00071025	0.14205		
Sodium carbonate	500	115	222321	(Merck, 2021)	1.500000002	0.069	13.8		
Sodium chloride	1000	1097	S9888	(Merck, 2021)	5	5.485	1097		
calcium hydroxide	500	963	239232	(Merck, 2021)	0.25	0.4815	96.3		
Anhydrous magnesium sulphate	500	141	M7506	(Merck, 2021)	5	1.41	282		
methyl chloride	25000	1650	MECHL001	(Masiye, 2021)	2	2	400		
Ethanol 99.9% (mL)	5000	350	E7023	(Masiye, 2021)	30	2.1	420		
					Total cost	458.5462102	91709.242		
					Yield obtained	mg/L	mg/L	g/L	**Price**
						726.22	145244	145.244	**208425.1**

Price of caffeine (R)		**Supplier**	**Product code**
1435	1g	Sigma-Aldrich (Merck)	SKU56396-100MG

Table 5.2: Cost of extraction per 1kg SCG using water

Option 2 : Water	Quantity (g)	Prices(R)	Product number	Reference	% pure caffeine obtained = 90 %				
Material used					For a run of 5g coffee grounds (Optimum conditions)	5g SCG	1000g SCG (1kg)		
Water as solvent (mL)	1000000	28.41	Municipal water		25	0.00071025	0.14205		
Sodium carbonate	500	115	222321	(Merck, 2021)	1.500000002	0.345	69		
Sodium chloride	1000	1097	S9888	(Merck, 2021)	5	5.485	1097		
calcium hydroxide	500	963	239232	(Merck, 2021)	0.25	0.4815	96.3		
Anhydrous magnesium sulphate	500	141	M7506	(Merck, 2021)	5	1.41	282		
methyl chloride	25000	1650	MECHL001	(Masiye, 2021)	2	0.132	26.4		
Ethanol 99.9% (mL)	5000	350	E7023	(Masiye, 2021)	30	2.1	420		
					Total cost	9.95421025	1990.842		
					Yield obtained	mg/L	mg/L	g/L	**Price**
						646.33	129266	129.266	**22052.7796**

Price of caffeine (R)		Supplier	Product code
853	5g	Sigma-Aldrich (Merck)	W222402-sample-k
170.6	1g	Sigma-Aldrich (Merck)	W222402-sample-k

Table 5.3: Cost of extraction per 1kg SCG using dichloromethane

Option 3 : Dichlormethane	Quantity (g)	Prices(R)	Product number	Reference	% pure caffeine obtained = <85 %				
Material used					For a run of 5g coffee grounds (Optimum conditions)	5g SCG	1000g SCG (1kg)		
Dichloromethane (mL)	2000	1712	270997	(Merck, 2021)	6.25	5.35	1070		
water	1000000	28.41	Municipal water		25	0.00071025	0.14205		
Sodium carbonate	2500	1769	222321	(Merck, 2021)	7.500000008	5.307000001	1061.4		
Sodium chloride	1000	1097	S9888	(Merck, 2021)	5	5.485	1097		
calcium hydroxide	500	963	239232	(Merck, 2021)	0.25	0.4815	96.3		
Anhydrous magnesium sulphate	500	1155	M7506	(Merck, 2021)	5	11.55	2310		
methyl chloride	25000	1650	MECHL001	(Masiye, 2021)	2	0.132	26.4		
Ethanol 99.9% (mL)	5000	350	E7023	(Masiye, 2021)	30	2.1	420		
					Total cost	25.05621025	6081.2421		
					Yield obtained	mg/L	mg/L	g/L	**Price**
						566.12	113224	113.224	**8854.1168**

Price of caffeine (R)		Supplier	Product code
391	5g	Sigma-Aldrich (Merck)	SKU-CO750-5g
78.2	1g	Sigma-Aldrich (Merck)	SKU-CO750-5g

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

This study considered using three different extraction solvents namely (i) 1-ethyl-3-methylimidazolium, (ii) dichloromethane and (iii) water for the extraction of caffeine from spent coffee grounds at different temperature (88-120 °C); time durations (15-35 minutes) and varying solid-solvent loading ratio (20g/10 mL-25 mL). The characteristics of coffee indicate its suitability for the extraction process and its potential to supply the necessary bioactive compounds. Results had been used to distinguish the best extraction conditions which was further used to analyse the effect of each parameter on the overall process. Lastly, the responses obtained were inserted into Design- experiment which fitted design models.

The following conclusions can be established from this study:

- Extraction of caffeine by the routine home or machine brewing and percolation removed caffeine but a majority of the amount present in coffee beans still remained behind. All extraction solvents, 1-ethyl-3-methylimidazolium chloride, dichloromethane and water have great extraction potential, however according to the established optimum conditions; the ionic liquid 1-ethyl-3-methylimidazolium extracted a higher concentration of caffeine to that of water and dichloromethane. At 120 °C for 25 minutes using 25 mL volume of extracting solvent, the caffeine extracted from 1-ethyl-3-methylimidazolium chloride, water and dichloromethane was 726.22 mg/L, 646.33 mg/L and 566.12 mg/L respectively.

- 1 ethyl-3-methylimidazolium chloride extracted 0, 00363 g caffeine / 1 g SCG, while water extracted 0, 00323 g caffeine / 1 g SCG and lastly dichloromethane extracted 0, 00283 g caffeine / 1 g SCG.

- The use of the ionic liquid will continue the sustainability and green principles of engineering. Large profits were generated with a large startup cost, generating an almost pure grade of caffeine. However, as stated in section 2.25.1, mostly all ionic liquids are recoverable meaning they can be recycled, therefore should considered for future study. Recycling the solvent will drastically reduce the extraction cost while still maintaining the large profits generated.

- Solvent recycling and recovery will further justify the use of the 1-ethyl-3-methylimidazolium as the highest yielding caffeine extraction solvent with the highest profit achieved. Using water as the extraction solvent also achieved economic feasibility and cost effectiveness. Changing the solvent use drastically reduced the cost and almost achieved the same extraction/concentration yield of caffeine; however the purity of caffeine obtained decreased.

- With the analysis and results, caffeine is a valuable and much needed product in the health, food and medicinal field that can be obtained from waste coffee. Therefore, the need for caffeine is endless. Considering this process on large scale will generate revenue, create employment, reduce waste build ups and most importantly produce high quality caffeine for the food and medicinal industry. With the yields of caffeine being extracted, large profits can be made from the overall process.

- The effect of reaction time, reaction temperature and solid-solvent loading ratio all show that they do not function independently. Interactional factors had a greater influence on the yield of caffeine.

- The best experimental run with the highest caffeine extraction was run 2. This makes the optimum extraction conditions of 120°C, 25 minutes and 25mL solvent.

- In conclusion, caffeine extraction from spent coffee grounds shows promising potential as caffeine yields of 566.12 – 726.22 mg/L was obtained.

- At these optimum conditions $0.0028306 g/g^{-1}$ SCG was extracted using DCM, $0.00363112 g/g^{-1}$ SCG using IL and $0.00323170 g/g^{-1}$ SCG using water.

6.2 Recommendations for future work

The following are the recommendations observed in this study for further research:

- The effect of moisture content on the extraction of caffeine from spent coffee grounds should be investigated as this is the most varied factor when obtaining combinations coffee waste from landfill sites or shops. For this study, the extraction methodology was carried out as the raw material was obtained without alterations, as this is the actual state of the coffee which will be obtained making the results a true reflection of SCG contents. Further study can investigate whether altering the moisture content, alters the yield of caffeine attainable.

- Overall cost analysis should be carried out in detail taking into consideration electrical costs as well, for a better understanding of the cost of the extraction process, energy efficiency and the estimation of the return on investment.

- The overall costing of the extraction displayed is purely based on raw materials and product generated. However, it should be noted that taking into consideration the equipment costs, electrical costs and other operating factors, the generated figures will differ.

- The recovery of the ionic liquids/solvents uses should be investigated and a recovery process established. This will then be evaluated to observe the efficiency of the recycled solvent on the extraction process; under the same optimum conditions.

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APPENDICES

Appendix 1

Table 7.1: Box Behnken design matrix generated for experimental runs

		Factor 1	Factor 2	Factor 3
Std	Run	A:Reaction Time	B:Reaction Temperature	C:S/L Ratio
9	1	25	88	10
12	2	25	120	25
15	3	25	104	17.5
6	4	35	104	10
11	5	25	88	25
4	6	35	120	17.5
16	7	25	104	17.5
2	8	35	88	17.5
17	9	25	104	17.5
13	10	25	104	17.5
8	11	35	104	25
7	12	15	104	25
14	13	25	104	17.5
5	14	15	104	10
10	15	25	120	10
1	16	15	88	17.5
3	17	15	120	17.5

Table 7.2: Raw data and calculation set-up for IL-caffeine extraction

				Sample mL	25	5	5000	1000
Sample	**Concentration mg/1000mL (Diluted)**	**Concentration mg/1000mL**	**mg/25mL**	**mg/mL**	**Converted to m/M per 100g sample**	**g per 1g SCG sample**	**g per 5g SCG sample**	**g per 1kg SCG sample**
Run 1	6.098007968	609.8007968	15.24501992	0.6098008	0.3049004	0.00304900	0.01524502	3.049003984
Run 2	7.262241941	726.2241941	18.15560485	0.7262242	0.3631121	0.00363112	0.01815560	3.631120971
Run 3	2.964378848	296.4378848	7.410947121	0.2964379	0.14821894	0.00148219	0.00741095	1.482189424
Run 4	4.474339008	447.4339008	11.18584752	0.4474339	0.22371695	0.00223717	0.01118585	2.237169504
Run 5	6.363074973	636.3074973	15.90768743	0.6363075	0.31815375	0.00318154	0.01590769	3.181537486
Run 6	3.202372329	320.2372329	8.005930822	0.3202372	0.16011862	0.00160119	0.00800593	1.601186164
Run 7	4.737631293	473.7631293	11.84407823	0.4737631	0.23688156	0.00236882	0.01184408	2.368815647
Run 8	3.910449113	391.0449113	9.776122782	0.3910449	0.19552246	0.00195522	0.00977612	1.955224556
Run 9	4.551629844	455.1629844	11.37907461	0.455163	0.22758149	0.00227581	0.01137907	2.275814922
Run10	4.358728721	435.8728721	10.8968218	0.4358729	0.21793644	0.00217936	0.01089682	2.179364361
Run 11	4.746432452	474.6432452	11.86608113	0.4746432	0.23732162	0.00237322	0.01186608	2.373216226
Run 12	3.744549076	374.4549076	9.361372691	0.3744549	0.18722745	0.00187227	0.00936137	1.872274538
Run 13	4.33536762	433.536762	10.83841905	0.4335368	0.21676838	0.00216768	0.01083842	2.167683810
Run 14	4.411734879	441.1734879	11.0293372	0.4411735	0.22058674	0.00220587	0.01102934	2.205867439
Run 15	6.478576603	647.8576603	16.19644151	0.6478577	0.32392883	0.00323929	0.01619644	3.239288301
Run 16	3.794585295	379.4585295	9.486463238	0.3794585	0.18972926	0.00189729	0.00948646	1.897292648
Run 17	3.065175661	306.5175661	7.662939152	0.3065176	0.15325878	0.00153259	0.00766294	1.532587830

Table 7. 3: Raw data and calculation set-up for dichloromethane-caffeine extraction

				Sample mL	25	5	5000	1000
Sample	**Concentration (Diluted) mg/L**	**Concentration mg/L**	**mg/25mL**	**mg/mL**	**Converted to m/M per 100g sample**	**g per 1g SCG sample**	**g per 5g SCG sample**	**g per 1kg SCG sample**
Run 1	5.136180286	513.6180286	12.84045071	0.513618029	0.25680901	0.0025681	0.012840451	2.5680901
Run 2	5.661225263	566.1225263	14.15306316	0.566122526	0.28306126	0.0028306	0.014153063	2.8306126
Run 3	6.028501089	602.8501089	15.07125272	0.602850109	0.30142505	0.0030143	0.015071253	3.0142505
Run 4	6.360609791	636.0609791	15.90152448	0.636060979	0.31803049	0.0031803	0.015901524	3.1803049
Run 5	6.730461131	673.0461131	16.82615283	0.673046113	0.33652306	0.0033652	0.016826153	3.3652306
Run 6	5.652949531	565.2949531	14.13237383	0.565294953	0.28264748	0.0028265	0.014132374	2.8264748
Run 7	6.343130385	634.3130385	15.85782596	0.634313039	0.31715652	0.0031716	0.015857826	3.1715652
Run 8	4.966859199	496.6859199	12.417148	0.49668592	0.24834296	0.0024834	0.012417148	2.4834296
Run 9	6.066698229	606.6698229	15.16674557	0.606669823	0.30333491	0.0030333	0.015166746	3.0333491
Run10	6.090275542	609.0275542	15.22568886	0.609027554	0.30451378	0.0030451	0.015225689	3.0451378
Run 11	7.0329893	703.29893	17.58247325	0.70329893	0.35164947	0.0035165	0.017582473	3.5164947
Run 12	4.1646435	416.46435	10.41160875	0.41646435	0.20823217	0.0020823	0.010411609	2.0823217
Run 13	6.048915822	604.8915822	15.12228956	0.604891582	0.30244579	0.0030245	0.01512229	3.0244579
Run 14	3.168468895	316.8468895	7.921172237	0.316846889	0.15842344	0.0015842	0.007921172	1.5842344
Run 15	5.301278288	530.1278288	13.25319572	0.530127829	0.26506391	0.0026506	0.013253196	2.6506391
Run 16	4.397045734	439.7045734	10.99261434	0.439704573	0.21985229	0.0021985	0.010992614	2.1985229
Run 17	4.404582899	440.4582899	11.01145725	0.44045829	0.22022914	0.0022023	0.011011457	2.2022914

Table 7. 4: Raw data and calculation set-up for water-caffeine extraction

				Sample mL	25	5	5000	1000
Sample	**Concentration mg/1000mL (Diluted)**	**Concentration mg/1000mL**	**mg/25mL**	**mg/mL**	**Converted to m/M per 100g sample**	**g per 1g SCG sample**	**g per 5g SCG sample**	**g per 1kg SCG sample**
Run 1	5.427227092	542.7227092	13.56806773	0.5427227	0.27136135	0.00271361	0.01356807	2.713613546
Run 2	6.463395328	646.3395328	16.15848832	0.6463395	0.32316977	0.00323170	0.01615849	3.231697664
Run 3	2.638297175	263.8297175	6.595742937	0.2638297	0.13191486	0.00131915	0.00659574	1.319148587
Run 4	3.982161717	398.2161717	9.955404292	0.3982162	0.19910809	0.00199108	0.00995540	1.991080858
Run 5	5.663136726	566.3136726	14.15784181	0.5663137	0.28315684	0.00283157	0.01415784	2.831568363
Run 6	2.850111373	285.0111373	7.125278432	0.2850111	0.14250557	0.00142506	0.00712528	1.425055686
Run 7	4.216491851	421.6491851	10.54122963	0.4216492	0.21082459	0.00210825	0.01054123	2.108245925
Run 8	3.48029971	348.029971	8.700749276	0.34803	0.17401499	0.00174015	0.00870075	1.740149855
Run 9	4.050950561	405.0950561	10.1273764	0.4050951	0.20254753	0.00202548	0.01012738	2.025475281
Run10	3.879268562	387.9268562	9.698171405	0.3879269	0.19396343	0.00193963	0.00969817	1.939634281
Run 11	4.224324882	422.4324882	10.56081221	0.4224325	0.21121624	0.00211216	0.01056081	2.112162441
Run 12	3.332648678	333.2648678	8.331621695	0.3332649	0.16663243	0.00166632	0.00833162	1.666324339
Run 13	3.858477182	385.8477182	9.646192955	0.3858477	0.19292386	0.00192924	0.00964619	1.929238591
Run 14	3.926444042	392.6444042	9.816110105	0.3926444	0.1963222	0.00196322	0.00981611	1.963222021
Run 15	5.765933176	576.5933176	14.41483294	0.5765933	0.28829666	0.00288297	0.01441483	2.882966588
Run 16	3.377180913	337.7180913	8.442952282	0.3377181	0.16885905	0.00168859	0.00844295	1.688590456
Run 17	2.728006338	272.8006338	6.820015846	0.2728006	0.13640032	0.00136400	0.00682002	1.364003169

7.1 Sample calculations

7.1.1 Preparation of sample for each run as per DOE

Preparation of experimental samples for the Parr reactor: According to the DOE matrix that was generated, the volume of the solvent exceeded the maximum capacity of the Teflon cup. Hence, the volume was divided by 4.

DOE generated values of X mL solvent → (X mL solvent ÷ 4) = amount of volume inserted into the Teflon cup.

20g SCG → 5g of SCG

6g of sodium carbonate → 1,5g of sodium carbonate

7.1.2 HPLC standard calculations

Preparation of 200ppm standard: weight of caffeine weighted = 0.0206 g of pure caffeine

\+ 100 mL of water

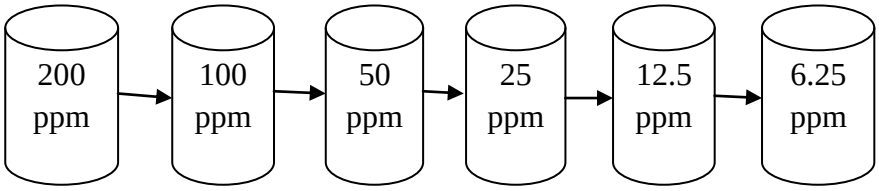
Preparation of 100ppm standard: Add 5 mL of 200 ppm standard solution to 10 mL of deionised water.

Preparation of 50 ppm standard: Add 5 mL of 100 ppm standard solution to a 10 mL volumetric flask and top up volume with deionised water.

Preparation of 25 ppm standard: Add 5 mL of 50 ppm standard solution to a 10 mL volumetric flask and top up volume with deionised water.

Preparation of 12.5 ppm standard: Add 5 mL of 25 ppm standard solution to a 10 mL volumetric flask and top up volume with deionised water.

Preparation of 6.25 ppm standard: Add 5 mL of 12.5 ppm standard solution to a 10 mL volumetric flask and top up volume with deionised water.



During HPLC analysis, samples that exceeded the 200ppm standard injection were diluted 100 times (1 mL extracted sample in 100 mL deionised water). The diluted runs concentrations mg/mL was multiplied by 100 to obtain the sample concentrations.

7.1.3 Moisture content sample calculation:

$$\%\ \boldsymbol{Moisture\ content} = \frac{\boldsymbol{W_2 - W_1}}{\boldsymbol{W_2 - W_T}} \times \mathbf{100}$$

$$= \frac{24.74-24.31}{24.75-23.75} \times \mathbf{100}$$

$$= \mathbf{61,97\%}$$

7.1.4 Ash content sample calculation:

$$\%\ \boldsymbol{Ash} = \frac{\boldsymbol{A}}{\boldsymbol{B}} \times \mathbf{100}$$

$$\%\ \boldsymbol{Ash} = \frac{\mathbf{0.0470}}{\mathbf{1}} \times \mathbf{100}$$

$$\%\ Ash = 4.7\%$$

7.1.5 Particle size analysis sample calculation:

$$\%\ \boldsymbol{P} = \left(\frac{\boldsymbol{M_2 - M_1}}{\boldsymbol{M_{Total}}}\right) \times \mathbf{100}$$

$$\%\ \boldsymbol{P} = \left(\frac{\mathbf{477 - 310}}{\mathbf{350}}\right) \times \mathbf{100}$$

$$\%\ \boldsymbol{P} = \mathbf{47.7}\ \%$$

7.1.6 Average particle size diameter

$$\boldsymbol{D = \frac{\sum_{i=1}^{n}\left[\frac{S \times P}{100}\right]}{n}}$$

$$\boldsymbol{D = \frac{\sum_{1}^{6}\left[\frac{25 \times 47.7}{100}\right]}{6}}$$

$$\boldsymbol{D = 202.73\ \mu m}$$

7.1.7 Extraction yield sample calculations

Sample injected into HPLC ⟶ (Value) in mg/L

If sample was diluted ⟶ (Value x 100) mg/L

Sample size used was constant size 25 mL (size of Teflon cup); therefore mg/L is converted to mg / 25mL

mg / 25mL = $\frac{\frac{mg}{L} \times 25}{1000}$ = (Value) mg/25mL

(Value) ÷25 = mg/mL

---

mg/mL converted to m/M per 100g sample.

During experimental runs, 5g of SCG was used in a 25mL total solution volume.

$\%\ caffeine\ in\ solution = \frac{x\ mg/25mL}{5000\ mg} \times 100 = (caffeine\ value\ \%\frac{m}{M})$ per 100 g SCG sample

↓

$(Caffeine\ value\ \%\frac{m}{M})$ per 100 g SCG sample ÷ 100 $= (caffeine\ value)$ per 1 g SCG sample

↓

$(caffeine\ value)$ per 1 g SCG sample x 5 $= (\boldsymbol{caffeine\ value})$ **per 5 g SCG sample used**

Using the above, the amount of caffeine attainable from 1kg of SCG was calculated.

7.1.8 SEM sample calculations

Using the scales from the SEM images, the size of particles from the samples was calculated.

If scale states: 2 cm on the ruler = 50 µm

Then 1, 2 cm sample on the ruler = $\frac{50\ \mu m\ x\ 1{,}2\ cm}{2cm}$

Cross-multiplying to calculate the sample size = 30 µm

2cm	50 µm
1,2 cm	X µm

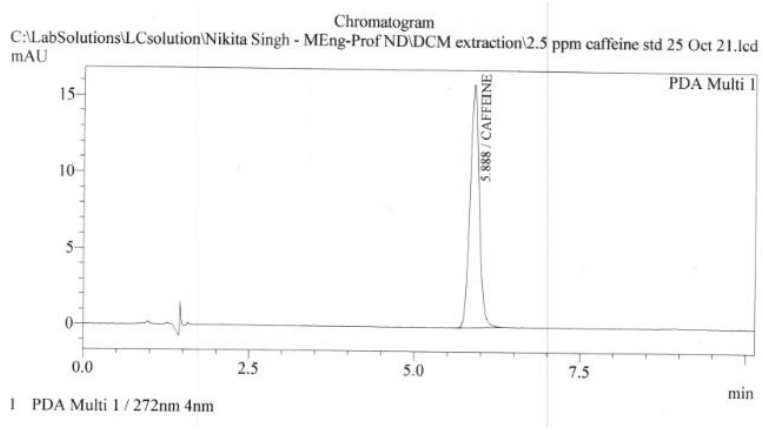


Figure 7.4:Chromatogram obtained for 2.5 ppm caffeine standard injection on HPLC

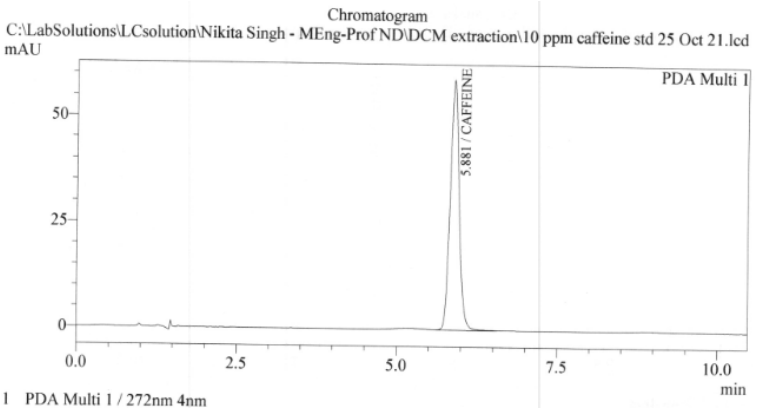


Figure 7.5: Chromatogram obtained for 10 ppm caffeine standard injection on HPLC

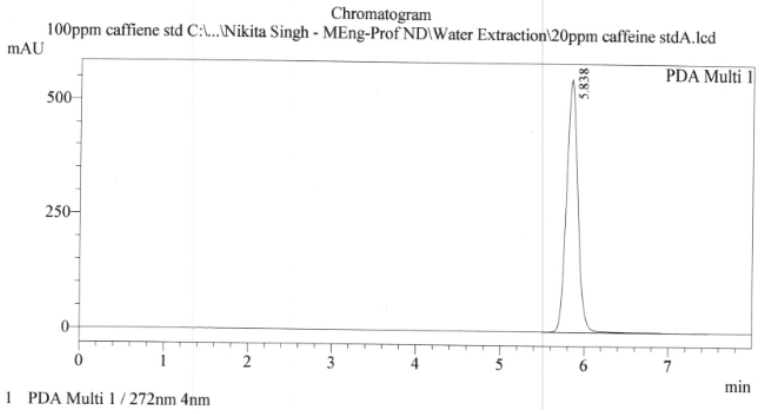


Figure 7.6: Chromatogram obtained for 20 ppm caffeine standard injection on HPLC

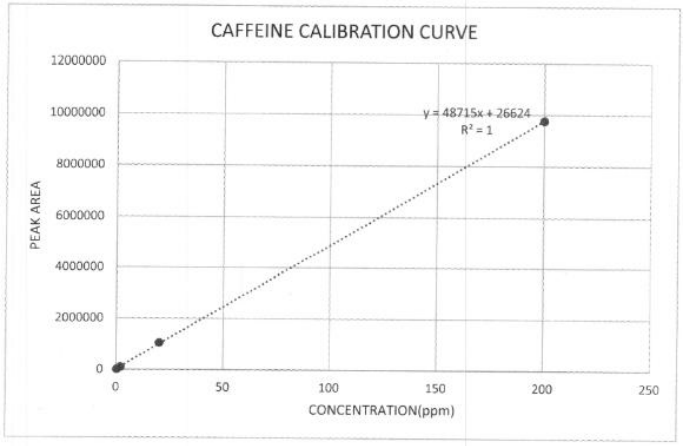
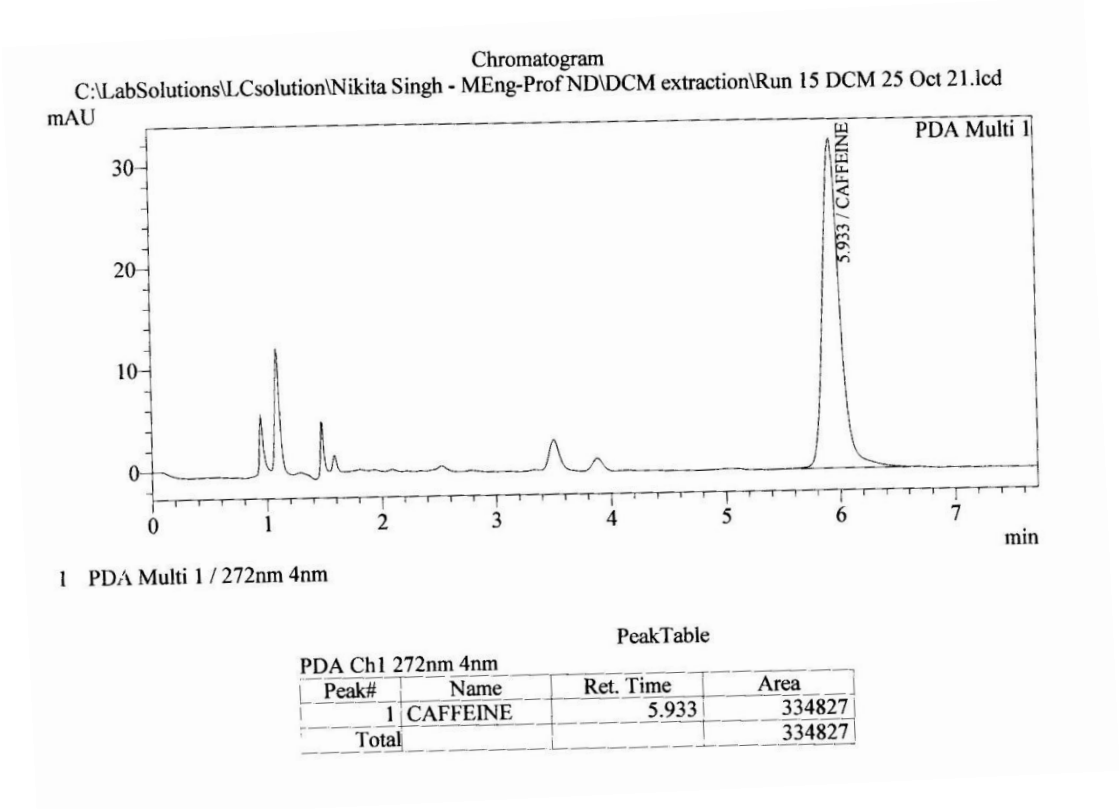


Figure 7.7: Calibration curve obtained using Microsoft Excel for distinguishing caffeine concentration per injected sample.



PeakTable

PDA Ch1 272nm 4nm

Peak#	Name	Ret. Time	Area
1	CAFFEINE	5.933	334827
Total			334827

Figure 7.8: Example of chromatogram, retention time data and peak area data obtained for each sample inserted into HPLC equipment.

Appendices 2

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