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VALORISATION OF INDUSTRIAL WASTE: EXTRACTION OF BIOACTIVE COMPOUNDS FROM BREWER'S SPENT GRAIN

by

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DECLARATION

I, Sipiwe Shoko, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

Brewer's spent grain (BSG), a solid residue obtained from brewing beer, is gaining attention in the food, cosmetics and pharmaceutical industry due to its use as natural source of colorants, texturisers, functional ingredients and preservatives. It is therefore necessary to develop an economically viable method for the extraction, isolation or enrichment of these compounds. Although literature shows the technical feasibility of extraction of bioactive compounds from BSG at laboratory bench scale, none of the reviewed literature could provide adequate information necessary to determine the economic feasibility of the process at commercial scale. The aim of this study was to investigate the technical and economic viability of a commercial process for the recovery of antioxidant rich polyphenolic compounds from brewers spent grain using organic solvents and/or water. The objectives were to select the best solvent, perform the optimisation and kinetic study, as well as to model and simulate the extraction process with the aim of performing an economic analysis. In selecting the best solvent, maceration and soxhlet extraction were used for the recovery of polyphenolic compounds. Acetone and acetone: water mixtures, ethanol and ethanol: water mixtures as well as pure water were used as solvents. The evaluation of the best solvent was measured by the total phenolic content (TPC), flavonol content, the antioxidant activity using 2.2-diphenyl-1-picrylhydrazyl (DPPH) assay and ferric reducing antioxidant power (FRAP) assay. The study performed optimisation for various operational parameters (time, temperature, solvent to feed ratio and shaking speed) using response surface method. The effect of temperature on the extraction kinetics was also investigated with experiments being carried out at 20°C, 40°C and 80°C. Antioxidant activity was detected in all BSG extracts, but water showed the highest global yield and rates of extraction. The optimum conditions were found at 15 min reaction time, temperature of 40 °C, shaking speed of 185 rpm and solvent to solid ratio of 27.5: 1. So-Macdonald model was a suitable fit for the experimental data with a R^2-value range of ($0.85 < r^2 < 0.995$).

A processing scenario was proposed as a base case, upon which subsequent scenarios were generated to improve the operation or the economics. SuperPro Designer® (Intelligen, Inc) was used for modelling the proposed process, for simulation and for the economic evaluation. Four alternative schemes from the base case simulation were developed for optimisation of the process. The process was found to be economically feasible and attractive with a return of investment (ROI) of 48.45 % for alternative scheme 4. The results in this thesis highlight the likely economic feasibility of the extraction of polyphenolic compounds from BSG at commercial scale by the maceration method.

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DEDICATION

Dedicated to my loving parents, Rabson Shoko and Alice Shoko for their prayers and support in my life and through the course of my research work

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GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
ANOVA	Analysis of variance
ARP	Antiradical power
BSG	Brewers spent grain
C_d	The final solute concentration in solution due to the diffusion stage alone
C_w	The final solute concentration in solution due to the washing stage alone
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
EC_{50}	Effect of concentration for a 50% response
FA	Ferulic acid
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalents
HBAs	hydroxybenzoic acids
HCAs	hydroxycinnamic acids
IIR	Internal rate of return
k_d	The kinetic coefficient for the diffusion stage
k_w	The kinetic coefficient for the washing stage
LOD	Limit of detection
ME	Maceration extraction
NPV	Net present value
p-CA	p-coumaric acid
QE	Quercetin equivalents

ROI	Return on investments
SD	Standard deviation
t	Extraction time
SE	Soxhlet extraction
SFE	Supercritical fluid extraction
SUBCWE	Subcritical water extraction
TE	Trolox equivalents
TPC	Total phenolic content

TERMS AND CONCEPTS CITED

Antioxidant	An antioxidant is a molecule that when present in minute quantities has the capacity to inhibits the oxidation of other molecules
Brewer's spent grain	A fibrous by-product from beer production mostly composed of cellulose, hemicellulose and lignin
Microencapsulation	A method of masking the unpleasant odour of polyphenolic compounds by incorporating a wall or coating
Net present value	The net amount of cash that goes into a project at a given time
Modelling	A method of developing a set of differential and algebraic equations whose solution yields the static and dynamic behaviour of a process
Payback period	An indicator in capital investment which refers to the period of time required for the project to recover all the money invested in it
Polyphenols	Phytochemicals that act as antioxidants and are mainly found in the cellulose, hemicellulose of grains.
Return on Investment	The amount that is obtained as profit for the investment done

CHAPTER 1 BACKGROUND AND MOTIVATION

1.1 INTRODUCTION

Industrial waste in the form of biomass is a relatively new focus as a source of not only energy, but also of both bulk chemicals such as ethanol and fine chemicals such as polyphenols. In the recent years, value addition has been introduced to produce new products from industrial waste such as biofuels, functional foods and specialty chemicals (Ravishankar, 2016). In the food industry, there has been interest in the bioconversion of brewery industry wastes such as brewers spent grain (BSG), surplus yeast and spent hops into added-value products (Aliyu & Bala, 2013) because of their high content in fibre, carbohydrates, proteins, vitamins and biologically active compounds (polyphenols) (Ravishankar, 2016).

The bioprocessing of BSG has been reported to produce value added products such as functional foods and food additives (Halliwell, 2008). Polyphenolic compounds (see Section 2.6.2) are natural antioxidants found which are added in food to increase the shelf life of the product by retarding oxidation of lipids (Niki, 2014). This oxidation is a destructive process that results in food spoilage altering its chemical composition as well as nutritional value of food. Antioxidants can act as inhibitors to cellular damage mainly through their free radical scavenging property (Halliwell, 1995). They reduce oxidative damages in cells by donating electrons to free radicals and neutralizing them. The inhibition of cellular damage in humans assists in reducing diseases such as cancer, cardiovascular, diabetes and obesity (Kurutas, 2016).

Polyphenols occur in six categories and the largest type of these compounds is flavonoids, which are further divided into six groups. These are flavonols, flavanols, isoflavones, flavanones, anthocyanins and flavones (Goupy et al., 1999). The second class are phenolic acids, which are hydroxycinnamic and hydro benzoic acids. Other types of compounds include tannins, stilbenes and lignans. The most abundant polyphenols in BSG have been reported to be phenolic acids, namely ferulic acid and p-coumaric acid (Aoife L McCarthy et al. 2013). Thus, the recovery of these polyphenolic compounds is important.

Numerous methods such as maceration (Meneses et al., 2013a), soxhlet extraction (Moreira, 2012), microwave assisted extraction (Moreira et al., 2012), supercritical fluid extraction (Spinelli, Conte, Lecce, et al., 2016) and subcritical water extraction (Asl & Khajenoori, 2013) have been used in the extraction of bioactive compounds from BSG. The maceration and soxhlet methods are the conventional methods that have remained in use in the industry mainly because of their ability to use different solvents and aqueous phases. Other

advantages include rapid extraction rates for many separations, large volume throughput and the possibility of recycling the solvents.

1.2 CURRENT STATE OF KNOWLEDGE AND AVENUES FOR FURTHER RESEARCH

Although research has been conducted to determine the extraction of bioactive compounds from BSG at lab scale using conventional methods, none of the reviewed literature could provide adequate information necessary to determine the economic feasibility of the process at industrial scale. The potential for use of antioxidants from BSG in the food and cosmetics industry as valuable compounds has been shown in several studies (Mussatto et al., 2006; Aoife L. McCarthy et al., 2013; Parr & Bolwell, 2000; Kalia et al., 2008; Kitrytė et al., 2015). Extraction using organic solvents was done in the past and continues to play a big role in the recovery of antioxidants (Punín Crespo & Lage Yusty, 2005; Meneses et al., 2013a). Currently research has also been focusing on high pressure methods such as supercritical fluid extraction and subcritical water extraction (Mesomo et al., 2013; Coelho et al., 2014; Campos et al., 2005; Asl & Khajenoori, 2013). Therefore, this research focuses on generating adequate data from lab scale experiments to determine the technical and economic feasibility of the process.

1.3 PROBLEM STATEMENT

Brewers spent grain has been shown to be an important source of antioxidants, yet it is largely regarded as industrial waste. It has been used as a cheap animal feed, and more recently, in the production of bioethanol. In South Africa, the major brewing company has an annual brewing capacity of 3.1 billion litres of beer (SAB, 2017). According to Aoife L McCarthy et al. (2013) such a brewing capacity produces approximately 6,2 million tons of BSG. Recently, attention has been given to the research of the extraction of bioactive compounds from BSG. The efficient recovery of these bioactive compounds depend on many factors such as the extraction method, type of solvent, solvent to feed ratio, extraction temperature or extraction time. Although research (Tang et al., 2010; Luis F. Guido & Moreira, 2017; Spinelli, Conte & Del Nobile, 2016) has been conducted to determine the feasibility of extraction of bioactive compounds from BSG, none of the reviewed literature could provide adequate information necessary to determine the economic feasibility of the process. This study is aimed at optimising the operating conditions for the extraction of polyphenols from BSG and to use these to propose and develop a commercial scale process, and also to test the economic viability of the developed process.

1.4 AIM AND OBJECTIVES

In general terms, the aim of this study is to investigate the extractive capacity of some selected solvents in the extraction of bioactive compounds from BSG. The views are to determine the optimum operating parameters and the economic feasibility of the process.

More specifically, the objectives of this research proposal are:

1. To determine the extractive capacities of some selected solvents with a view to identify a suitable solvent for extraction of bioactive compounds from BSG.
2. To determine the extraction yield, composition and antioxidant activities of the extracts obtained using different solvents.
3. To determine the optimum operating parameters with respect to antioxidant activity and extraction yield.
4. To determine the extraction kinetics at the optimum conditions.
5. To develop a process model using a commercial simulation package and perform an economic analysis of the process with a view to determine its economic feasibility.

1.5 RESEARCH KEY QUESTIONS

The aforementioned objectives would provide answers to the following questions:

1. What bioactive compounds of value are extractable from BSG using organic solvents, and which solvent is most efficient at their extraction.
2. What is the rate of extraction, and which are the best operating conditions?
3. Can the extraction of bioactive compounds from BSG be economically viable at commercial scale?

1.6 HYPOTHESIS

Based on literature survey, extraction of bioactive compounds using conventional techniques is a technically and economically viable method for the extraction of bioactive compounds, particularly the polyphenolic compounds, from brewers' spent grain.

1.7 DELINEATION

In relation to the objectives, the following delineations were set as guidelines for this research project.

- This study is limited to the extraction of polyphenolic compounds using maceration with organic compounds and with water only.

- Experiments are carried out using standard analytical methods only
- With the exception of a specific compounds, the individual components found in BSG were not quantified in this work. Instead, the study was based on their measured antioxidant activity using two different methods.

1.8 SIGNIFICANCE OF RESEARCH

Although there are laboratory bench scale studies on extraction of bioactive compounds from BSG, no information could be found in the literature on the economic feasibility of such a process. This research would provide reference data and a baseline study on the extraction of polyphenolic compounds from BSG, and it has the potential of forming the basis of a patent application. The data obtained from this study, in addition to providing baseline information concerning the feasibility and economic viability of extraction of polyphenolic compounds using solvent extraction method, would be a useful reference in the scale up of the process.

1.9 THESIS OUTLINE

The experimental work consisted of three parts which were: the selection of the effective solvent from conventional extraction; optimisation of process parameters and extraction of kinetic studies. The thesis contains six chapters with chapter one and two providing the background and literature data of this work. The experimental work is discussed in chapter three and four providing the outcomes from the experiments. Chapter five highlights the economic analysis with the summary of the whole research illustrated in chapter six. Figure 1.1 shows the flow diagram of the overall thesis which was used as a guideline for this work.

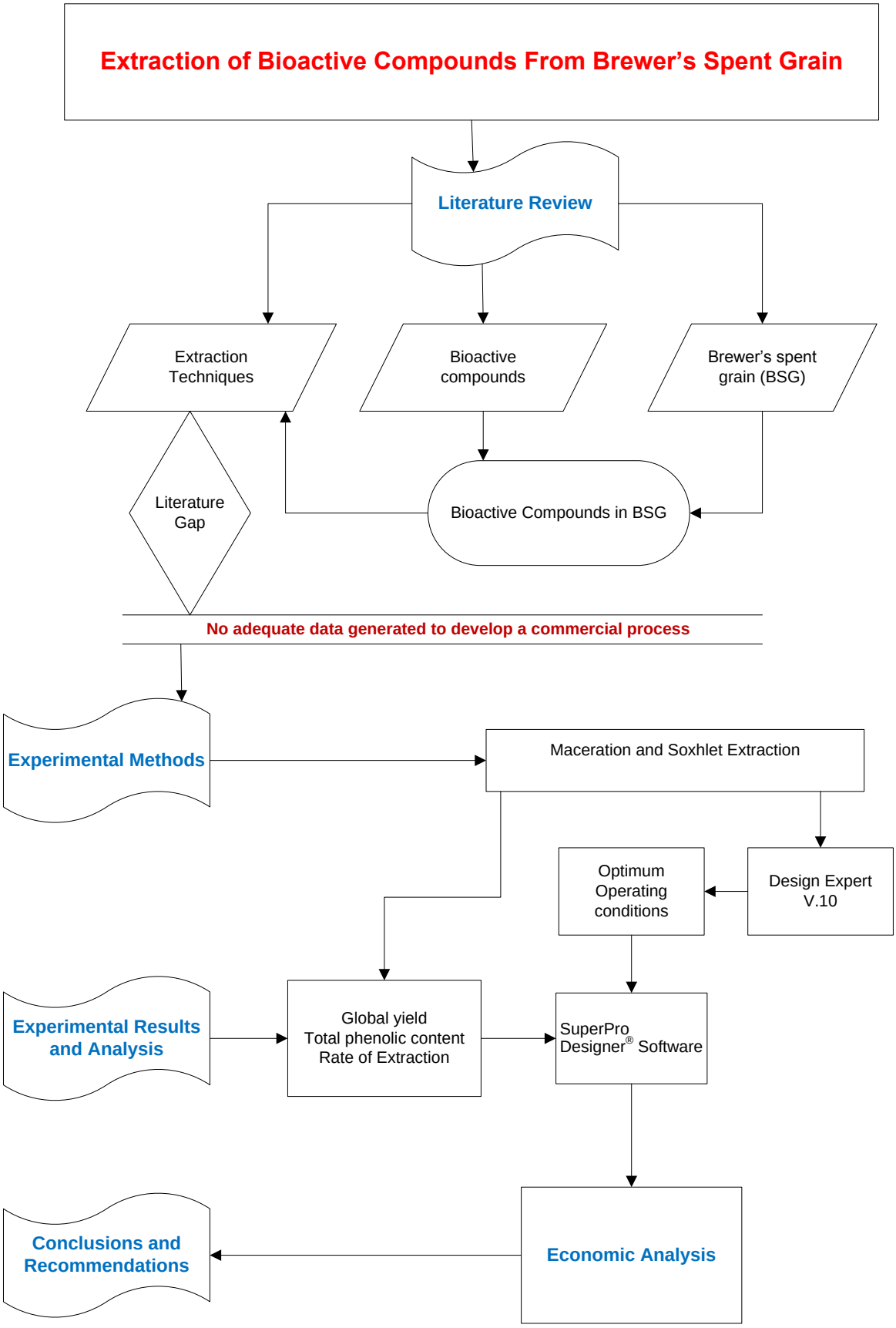


Figure 1.1: Flow diagram of overall thesis structure and mind map

1.9.1 Literature review and fundamental theory (Chapter 2)

The literature review is divided into three major sections which are polyphenolic compounds, BSG and extraction techniques. The polyphenolic compounds give a brief background on the polyphenols and explains them as a natural source of antioxidants. The BSG section describes the spent grain and how it is generated as waste from beer production. Lastly, the extraction techniques are then discussed. The conventional methods that has been used were explored in depth. Previous literature on the conventional and high pressure methods is discussed to give a detailed overview of the research and to show the gap to be filled by this work.

1.9.2 Experimental Methods (Chapter 3)

The methodology followed to carry out the experimental aspect of this research work is presented in Chapter 3 and the block flow diagram is shown in Figure 1.2. The extraction of polyphenols from BSG using conventional methods was carried out in the laboratory at CPUT.

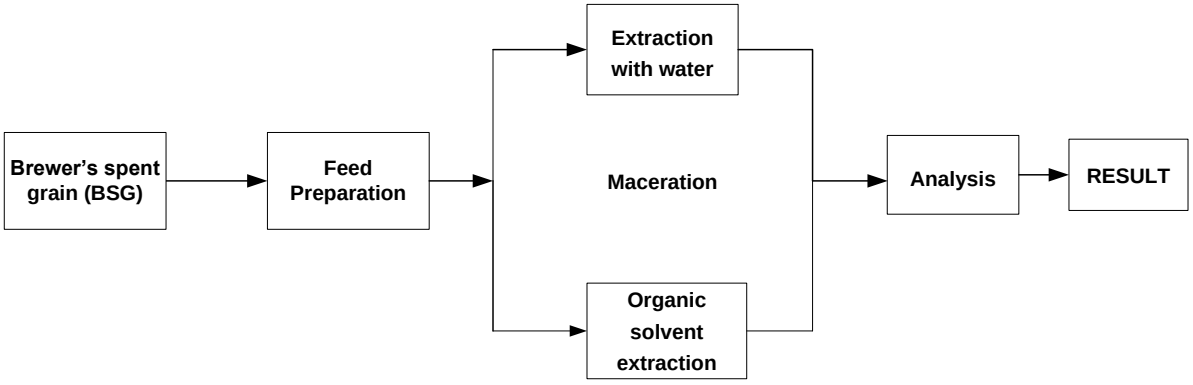


Figure 1.2: Block flow diagram representing experimental steps to be done

1.9.3 Results and discussion (Chapter 4)

The results and discussion for all the laboratory experiments done using the maceration extraction have been combined into one chapter. The first part discusses the selection of the best solvent based on the measurement of total phenolic compound, the antioxidant potential and the amount of individual components found in BSG. The second part evaluates the experiments done to optimize the process conditions of the selected solvent. Lastly, Kinetic extraction results are then discussed to conclude on the rate of extraction.

1.9.4 Economic evaluation of the process (Chapter 5)

Chapter 5 describes the results obtained from modelling and simulation using SuperPro designer® (Intelligen, Inc.). The base case simulation was constructed from the laboratory experiments with 4 other alternative schemes developed for optimisation. Three profitability indicators, return on investments (ROI), payback period and net present value (NPV) were used to select the most economically attractive scheme. A sensitivity analysis was also done to predict the variation the profitability indicators with the change in the annual production throughput.

1.9.5 Summary, Conclusions, Recommendations and contributions of this work (Chapter 6)

This section brings together the main points highlighted and described each chapter and summarizes the entire research work. This chapter also highlighted the key technical areas which still need to be worked on and improved in the existing maceration procedure. Recommendations for further research were also outlined.

CHAPTER 2 THEORY AND LITERATURE REVIEW

2.1 INTRODUCTION

The main aim of this chapter is to provide a brief and concise theoretical foundation on the development of commercial process for the extraction of polyphenolic compounds from brewers spent grain (BSG) using modelling and simulation. This first section discusses BSG, its structure and composition, and how BSG is generated from the brewing industry, as well as different preservation and pre-treatment methods. The second section describes antioxidants and polyphenolic compounds in details. The description includes the types of polyphenols, their nature and their function as a natural source of antioxidants. Following the polyphenols section, extraction techniques were briefly discussed with an overview on the future trends of the recovery of polyphenols. Lastly, the development of commercial processes of extraction of bioactive compounds from plants was highlighted. Previous and current works done on the extraction of bioactive compounds were discussed to narrow down the techniques to the most recent development in BSG valorisation.

2.2 BREWERS SPENT GRAIN (BSG)

Efforts to utilize BSG for phenolic recovery has been reported in several literature (Spinelli, Conte & Del Nobile, 2016; Fernandez-Pérez et al., 2008; Moreira, 2012; Meneses et al., 2013a). BSG is produced in high volume accounting for about 85% of the total by-products produced during the brewing. It is available at a cheap price and consists of valuable compounds such as fibre, cellulose, protein and polyphenols (Ravishankar, 2016). The chemical composition of the BSG depends on the type of grain used, the geographical place of farming and the conditions under which it was generated. This section will review the chemical composition and potential application of BSG, with specific focus on the bioactivities of individual polyphenols found in BSG. In the case of SAB Newlands Brewery, Cape Town, the spent grain is sold to the farmers for use as animal feed.

2.2.1 Structure of brewers spent grain

BSG generally is made up of the germ (embryo), the endosperm and the husk as shown in Figure 2.1. Mussatto et al. (2006a) showed that the endosperm consist of the aleurone and starch and the grain can contain some cereals such as maize, rice and wheat which would have been used during the mashing stage.

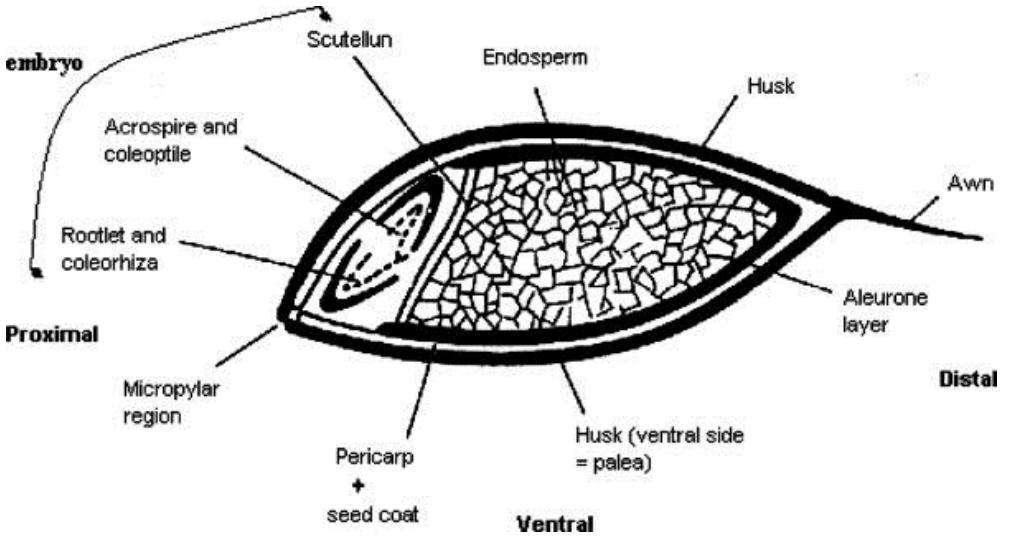


Figure 2.1: **Schematic representation of a barley kernel (Adapted from Mussatto et al., 2006b)**

The husk acts as a shell of protection which is made up of dead tissues. The multi-layered lignocellulosic wall has three sections namely the seed coat, the aleurone and the pericarp layer (Kunze & Kunze, 2010). Studies show that most of the phenolic acids (mainly Ferulic acid and p-coumaric acid) are found in the husk and aleurone layer (Nordkvist et al., 1984). The total phenolic acids are reported to be concentrated in the lignocellulosic wall with about 0.6–0.9 % in concentration whilst lower in the endosperm section with less than 0.1%.

2.2.2 Chemical characterization

As mentioned in section 2.2.1 BSG has a high protein content and contains a large concentration of cellulose and hemicellulose (Aliyu & Bala, 2013; Moreira, 2012). Table 2.1 shows the composition ranges found in literature for BSG.

Table 2.1: chemical composition of BSG as reported by several authors (adapted from Aliyu & Bala, (2013))

Component	*Composition range (% dry weight)*	*References*
Cellulose	16-25	(Adeniran et al., 2010; Khidzir, K, 2010; Mussatto et al., 2006)
Hemicellulose	28-35	(Dai & Mumper, 2010; Mussatto et al., 2006)
Lignin	11-27	(Carvalho et al., 2015; Meneses et al., 2013a)
Proteins	15-24	(Mussatto et al., 2006; Khidzir, K, 2010; Meneses et al., 2013a)
Ashes	2-7	(Khidzir, K, 2010; Mussatto et al., 2007; Thiago et al., 2014; Meneses et al., 2013a)
Carbohydrates	79.9	(Adeniran et al., 2010)

Cellulose and hemicellulose together have been reported to contain about 50% (w/w) of the spent grain composition (Hernanz et al., 2001; Mussatto et al., 2007; Aoife L. McCarthy et al., 2013). This accounts for the high content of sugars such as xylose, arabinoxylans and glucose to be found in the grain (Mussatto et al., 2006). Thiago et al. (2014) found 3.4 % of ashes of BSG and several authors reported a range of 2-7

2.2.3 Generation of Brewer's Spent Grain

BSG as shown in Figure 2.2 is generated from beer production. Beer, one of the most consumed drink in the world, is produced through two main processes which are malting and brewing (Aron & Shellhammer, 2010). The main ingredients used in beer production include barley (malt), hop and yeast. The beer in South Africa is domestically brewed with the main ingredients (barley malt and hope cones) being imported from neighbouring countries such as Zimbabwe, Mozambique and Tanzania (Wood, 2016; SAB, 2017). There are some traditionally brewed beer that were once South Africa's favourite, that is sorghum-based. However, the consumers nowadays prefer a variety of "clear beer" hence attention has shifted to the "western beers". The brewers spent grain is the solid residue that is produced during beer production and has about 85% of the by-products. The BSG used in this research is a by-product of beer made from barley malt alone (SAB Newlands, 2017).

Figure 2.2: **Brewers spent grain from SAB Miller Cape Town**

The production of beer is a diverse process and can allow different types and quality of beer to be produced through adjusting parameters (Carvalheiro, 2004; Moreira, 2012). There are two major steps in beer production: malting and brewing. Figure 2.3 is a schematic representation of the beer production with a brief discussion of the malting and brewing process in the next section.

2.2.4 Malting process

Malting is done to convert the insoluble starch and protein found in grain, into a soluble substrate which can be extracted by hot water to produce wort (Macleod, n.d.) as shown in Figure 2.3. The wort is the liquor containing fermentable carbohydrates and soluble proteins. There are essential changes that occur during the malting stage. During this stage, the grain is exposed to manipulated conditions which enhances its swelling and germination (Moreira, 2012).

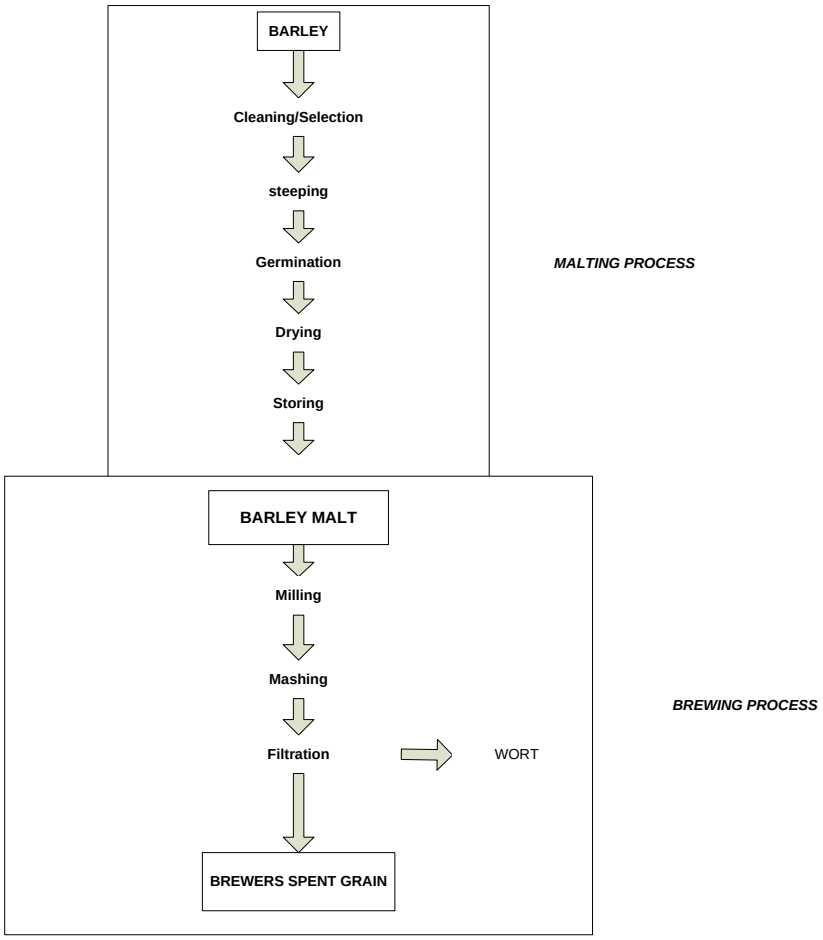


Figure 2.3: Schematic representation of the beer production process to generate BSG Adapted from (Mussatto et al., 2006)

Steeping is a stage used to increase the chances of germination for the grain (Moreira, 2012). The steeping stage consists of two alternating periods: under water and air rest periods. During the underwater period, the moisture content of the grain is increased by immersion in water for more than 15 hours. This will allow the grain to germinate. After the underwater period, air rest period follows, in which water is drained and air is sucked into the grain for 12-24 hours. The embryo is exposed to oxygen for respiration to occur. Due to the variation of the properties of barley over time and places, the steeping stage is also modified for the grain to reach the required moisture content (Thiago et al., 2014). The germination stage is to ensure that enzymes are developed so that they can hydrolyse the cell wall,

proteins and the whole grain. Brewing begins after kilning, the final heat treatment, which reduce the moisture content of green malt from germination conditions (45%) to 4 to 5%. The reduction of moisture makes transportation easier, and prevents further modification of the malt.

2.2.5 Brewing process

In brewing the barley, malt is mixed with water at an elevated temperature of 78˚C. The high temperatures assist in enzymatic hydrolysis which converts starch into both fermentable sugars such as maltose and non-fermentable sugars such as dextrins (Forssell et al., 2008). The dextrins are the ones responsible for the production of sweet liquor called wort (Aron & Shellhammer, 2010). The sweet wort is then filtered and separated from the insoluble residual fraction, BSG (Kunze & Kunze, 2010; Moreira, 2012; Mussatto, 2014). The sweet wort will be processed further whilst the BSG is sold to farmers or transported for disposal.

2.2.6 The effect of brewing and malting on the phenolic compounds present in BSG

Phenolic compounds exist as free, soluble and insoluble, and well as bound forms and, they have different levels of release from the plant material. In BSG generation, Cai et al., (2015) have investigated the effect of brewing and malting on the free phenolic compounds. During brewing, water acts as the extracting solvent resulting in the release of soluble phenolic compounds only in the wort. The wort is reported to have high antioxidant activity and total phenolic content than the original barley grain (Leitao et al., 2011). Although the process affects these compounds, however studies have shown that polyphenols influence the processes to a greater extent (Leitao et al., 2011; Cai et al., 2015). Phenolic compounds in beer are believed to be involved in flavour characteristics, foam maintenance, physical and chemical stability of the beer (Qing et al., 2012).

2.3 POTENTIAL USES OF BREWERS SPENT GRAIN

Although BSG is cheap and readily available, its market is greatly compromised and has major problems in the disposal of the high volume residue. Therefore, BSG has been underutilized in most of the countries like South Africa (Moreira et al., 2013). Nevertheless, researchers have reported new innovative ways of making use of BSG in biotechnological processes. Some of the potential uses are discussed in sections 2.3.1 to 2.3.4

2.3.1 BSG as a cattle feed: Animal nutrition

BSG has been mainly used as cattle feed due to the abundant fibre and protein content found in it. Several researches have been done to investigate the use of BSG as animal nutrition for chickens, pigs and fish (Mussatto et al., 2006a; Aoife L McCarthy et al., 2013; Buffington, 2014). The use of BSG supplementation in cattle feed increased the nutritional value and promoted milk production without affecting animal fertility (Aoife L McCarthy et al., 2013). BSG is usually used as a wet residue just after brewing for cattle feed. However, due to the moisture content, BSG cannot be stored for longer days (Wilhelmson et al., 2009). Therefore, other outputs for the by-product are necessary in areas where no demand for cattle feed exists.

2.3.2 BSG in human diet

There has been an increase in the interest of the addition of BSG in food to enhance the quality of food products (Mccarthy et al., 2014). Since BSG comes from edible food suitable for human consumption, it can be added to foods such as bread and snacks as a nutrient booster. The incorporation of BSG in food can also decrease the calorific content at the same time increasing the protein content, hence improving the nutritional quality of bread (Hassona, 1993). Prentice & D'Appolonia, (1977) and Yildiz-ozturk et al, (2014) investigated the addition of BSG in bread and snacks. 10 % BSG was added in traditional breads by Prentice & D'Appolonia, (1977) and this resulted in doubling crude fibre bread content. An addition of 15% spent grain in cookies gave 27 % increase in protein and a 3 fold increases in the dietary fibre contents (Yildiz-ozturk et al., 2014; Prentice & D'Appolonia, 1977). When incorporated in extruded snacks BSG improved the physical, nutritional value, dietary fibre and protein content.

2.3.3 Bioethanol production

BSG, is a lignocellulosic biomass that is a suitable raw material for the production of bioethanol (Wilkinson et al., 2017). The use of BSG in the bioethanol production results in low CO_2 emissions and reduces the greenhouse effect. However, a low yield of ethanol is produced due to inadequate technologies that are involved so far. Also, high production costs are involved with the process. The increasing demand of ethanol has led researchers to investigate more about this technology as well as the composition of BSG as described earlier in this section (Alam et al., 2007, 2009). The hemicellulose, lignin and the cellulosic material in the spent grain contributes to BSG being a good source of energy. Wilkinson et al., (2017) investigated the production of bioethanol from BSG using consolidated bioprocessing. They reported that 1 ton of BSG (dry weight) yielded 94 kg of ethanol using

36 hL of water in the process. The conversion of BSG to ethanol requires a large amounts of enzymes for fermentation and this makes the technology to be expensive (Xiros & Christakopoulos, 2009).

2.3.4 BSG as a carrier in Brewing

BSG can be reused as an antifoaming agent for yeast immobilization in brewing. Roberts, (1976) showed that the addition of BSG increased the performance of yeast but reduced the quality of beer. Kado et al. (1999) suggested that the BSG addition should be neutralised by acid to maintain the flavour and taste. Hence the addition of neutralised acid BSG extracts enhances the brewery economics.

2.4 BSG IN VALUE ADDITION PRODUCTS

BSG has been used to produce several other products such as polysaccharides and antioxidants derived products. These added-value products have got health promoting benefits and reduce the risk of chronic diseases and cancer, and can also be used as preservatives.

2.4.1 Polysaccharides production

The grain husk of BSG consist of polysaccharides such as arabinoxylan (22 − 28%), cellulose (17 − 25%) and lignin (12−28%) (Fărcaş et al., 2017). Hydrolytic methods such as hydrothermal, enzymatic or acidic have been used to break down these polysaccharides into useful products. Glucose is produced from cellulose polysaccharides that undergo hydrolysis. Moreira, (2012) reported on the hydrothermal hydrolysis treatment of BSG and produced dietary fibre rich arabino-oligoxylosides that can be used as functional foods. Recently Vieira et al., (2014) developed a commercial process that extracts polysaccharides with the aim of optimising the valorisation of BSG. Polysaccharides are also used as a carbon source in microbial fermentations.

2.4.2 BSG as a natural source of antioxidants

BSG has been regarded as a potential natural source of antioxidants (Moreira et al., 2013). Antioxidants, also known as bioactive compounds have been investigated to have potential use as a food preservative/antimicrobial agent, anti-inflammatory agent, chemo-protectant and have found interest in the food, pharmaceutical and cosmetic industry.

2.5 ANTIOXIDANTS

An antioxidant is defined as a substance that, when present minute quantities in a particular living cell, significantly delays or prevents oxidation of that cell (Halliwell, 1995). These cells are proteins, lipids, carbohydrates and DNA. In the food industry an antioxidant is a substance having the technical function of delaying the oxidation of nutrients, such as lipids, sugars and proteins, whose oxidation leads to an inevitable deterioration of the organoleptic qualities of a food. Detrimental effects include undesirable formation of chemical compounds like aldehydes, ketones and organic acids that yield off-flavours (Schillaci et al., 2014). Antioxidants inhibit oxidation by reacting with lipid free radicals, forming inactive products. Halliwell, (2008) reported that antioxidants have got properties such as scavenging of free radicals (mainly O_2^* and chelation of metal ions that inhibits cellular damage.

2.5.1 Natural antioxidants vs synthetic antioxidants

Natural antioxidants such as polyphenols are primarily derived from plants, while the synthetic antioxidants are chemically produced. Before World War 2, natural antioxidants were used as additives (Gupta & Sharma, 2014). However, synthetic antioxidants were soon introduced and preferred over natural antioxidants mainly because they were cheaper, of more consistent purity and possessed more uniform antioxidant properties. As years progressed the use of synthetic antioxidants was challenged by consumers due their toxicity nature (Oroian & Escriche, 2015). Natural antioxidants were preferred again over synthetic substances and were considered more acceptable for dietary intake (Porkony, 2012). Examples of synthetic antioxidants include BHA (butylated hydroxyl anisole), BHT (butylated hydroxyl toluene), TBHQ (tert butyl hydroquinone) and PG (propyl gallate).

2.5.2 Major classes of natural antioxidants

There are three major classes of compounds with antioxidant activity namely vitamins (vitamin C and vitamin E), carotenoids (carotenes and xanthophylls) and polyphenols (flavonoids, phenolic acids, lignans and stilbenes) (Schillaci et al., 2014). Table 2.2 is a brief discussion of vitamins and Carotenoids. Polyphenols will be discussed in detail in the next section.

Table 2.2: The sources of vitamins and carotenoids and their health benefits

Antioxidants	*Natural source*	*Uses and industry*	*Reference*
Vitamin C	Apple Banana Bayberry Broccoli Citrus peel	Can be used as supplements for diet. Vitamin C reduces chronic and degenerative diseases such as stroke	(Shahkar et al., 2015).
Vitamin E	Green tea Olives Olive oil Palm oil Pumpkin seeds	Vitamin E can be used as a vital ingredient in lotions for the cosmetic industry. It acts as a protectant from sun burning by preventing lipid pre-oxidation	(Iacopini et al., 2008; Porkony, 2012)
Carotenoids	Grains Orange, celery, basil, beetroot, dill Peppermint Spearmint	Carotenoids can act as colorants in the food industry. This antioxidant is in the form of pigments responsible for the red, orange and yellow colour in foods	(Jomova & Valko, 2013)

2.6 POLYPHENOLS

The polyphenols section is presented and targeted at highlighting the description of polyphenols and their classifications. Polyphenols, also referred to as biologically active compounds are found naturally in plants. Polyphenolic compounds have gained interest especially in the cosmetic industry for the production of body oils and lotions (Kris-Etherton et al., 2002; Dillard & German, 2000). The uses of polyphenolic compounds will be explained in detail in the section below.

2.6.1 Uses of polyphenols

2.6.1.1 Polyphenols as food preservatives

Polyphenols can act as oxidation Inhibitors in food preservation. They can be added to fats and oils to prevent lipid deterioration thereby increasing the shelf life of foods (Parbunath, 2013). Lipid formation occurs in almost all food raw materials as well as processed foods such as mayonnaise, margarine and frying oils. These lipids such as triglycerides and phospholipids are a potential source of the oxidative off-flavour and deterioration in foods (Porkony, 2012). Hence the addition of polyphenols to food is an important way of preservation.

2.6.1.2 Polyphenols as dietary supplements

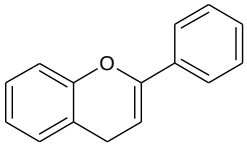
Polyphenols have been reported to be potential dietary supplements such as α-tocopherol, β-carotene and rutin (Figuereo et al., 2016). These compounds have been available on the market in a pure form or in defined solutions so that they can be added very easily in the amount desired. However, clinical trials performed by Stockfield, (2017) have not found polyphenols to provide substantial health benefits. They have suggested several reasons for this which includes that polyphenols may not be the only substance that has got effect on health in foods as well as the difference in chemical composition of antioxidants in foods and those already processed as supplements.

2.6.1.3 Polyphenols as cosmetics additives

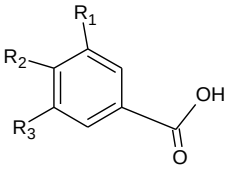
Polyphenols have been approved as cosmetics additives. This is because of their special properties in skin protection and prevention of aging (Juncan, 2011). Research have shown that extracts from green tea, avocado oil and grapeseed extract are precious ingredients in most cosmetic products (Whitney P & Silvina, 2016; Pyo et al., 2016). This is very attractive especially in the cosmetic industry as most of the body lotion and oils on the market do not contain natural products.

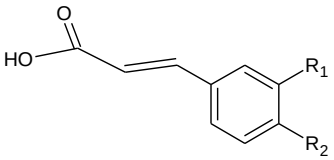
2.6.2 Classification of polyphenolic compounds

Polyphenols are categorized based on the structure and number of the phenol ring of the molecule (Manach et al., 2004). They can be divided in five major classes; Phenolic acids, flavonoids, stilbenes, lignans and tannins as shown in Figure 2.4.

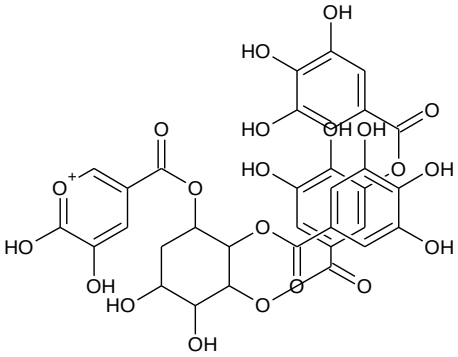


Flavanoids

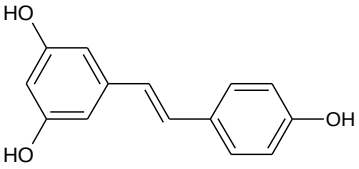




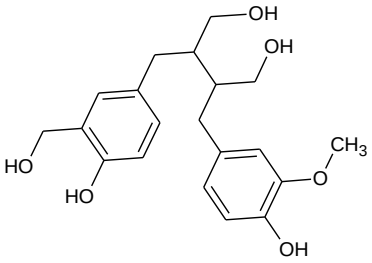
Phenolic acids (hydroxy-benzoic & cinnamic acids)



Tannins



stilbenes



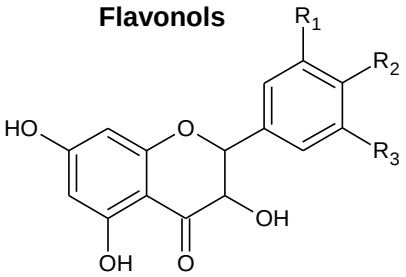
Lignans

Figure 2.4: **Generic classes of five major classes of plant phenols. R_1= COOH, R_2= OH, R_3=CH_3**

2.6.3 Flavonoids

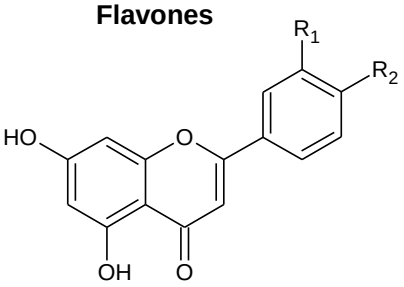
The largest group of the polyphenols found naturally are Flavonoids or bioflavonoids (Haminiuk et al., 2012; Manach et al., 2004). Anca et al., (2013) analysed the quantity of flavonoids found in BSG by comparing the types of grains used. They found out that the richest sample in flavonoids was the black malt sample, 17.55 mg QE/g, while the other two malt sample (caramel and base) had the lowest content of flavonoids. Flavonoids exist as pigment with a C_6-C_3-C_6 carbon framework (Marais et al., 2006). Flavonoids are known as glycosides when connected to one or more sugar molecules, and aglycones when no sugar molecules are involved (Skinner & Hunter, 1999). The antioxidant activity of these compounds are mainly determined by the degree of glycosylation (Skinner & Hunter, 1999) which also results in the classification of flavonoids as shown in Figure 2.5. This classification gives six categories of flavonoids; flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanals.

Flavonols



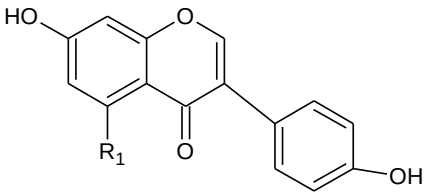
R_2=OH; R_1=R_3=H : Kaempferol
R_1=R_2=OH; R_3=H : Quercetin
R_1 =R_2=R_3=OH : Myricetin

Flavones



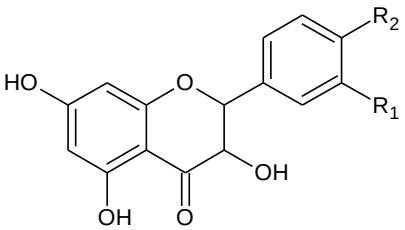
R_1=H; R_2=OH : Apigenin
R_1=R_2=OH : Luteolin

Isoflavones



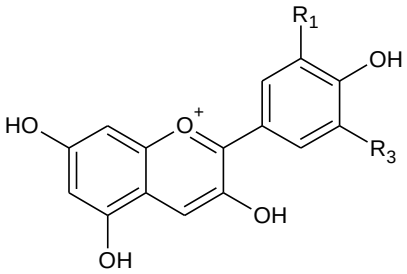
R_1=H : Daidzen
R_1=OH : Genistein

Flavanones



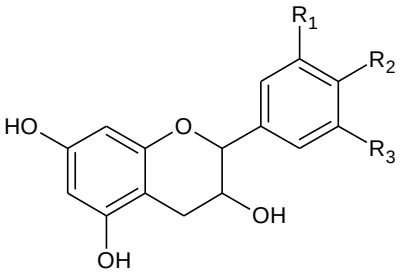
R_1=H; R_2 =OH : Naringenin
R_1= R_2 = OH : Eriodictyol
R_1=OH; R_2=OCH_3 : Hesperetin

Anthocyanins



R_1=R_2=H : Pelargonidin
R_1 = OH; R_2 =H : Cyanidin

Flavanols



R_1=R_2=OH; R_3=H : Catechins
R_1=R_2=R_3=OH : Gallocatechin

Figure 2.5: **Categories of flavonoids compounds**

2.6.3.1 Flavonols

Flavonols include kaempferol, quercetin and myricetin. These polyphenols occur mainly in vegetables and fruits and differ from other flavonoids by the position of the alcohol group on the C ring (Oroian & Escriche, 2015). They are also present in BSG especially kaempferol and rutin (Maestri et al., 2006). However, suitable and cost effective ways to recover these compounds are needed. Singh et al., (2014) reported that flavonols have well known antioxidant activity at low concentrations and can act by several pathways to inhibit oxidation

or break chain reactions. Therefore, flavonols are significantly used in pharmaceutical and food industries.

2.6.4 Phenolic acids

Phenolic acids make up a large content of the total phenols found in BSG. They are categorized into hydroxybenzoic and hydroxycinnamic acids. The four most abundant hydroxycinnamic acids are p-coumaric, caffeic, ferulic, and sinapic acids, while the corresponding hydroxybenzoic acids are p-hydroxybenzoic, protocatechuic, vanillic, and syringic acids. Several authors suggest that the antioxidant activities of phenolic acids dependent on the structure (Oroian & Escriche, 2015; Holteklen et al., 2006; Aoife L McCarthy et al., 2013). The acidic nature is mainly because of the COOH group present in the phenolics (Annie & Jean-Jacques, 2003). Furthermore, the CH=CH-COOH group is responsible for the higher antioxidant activities in the cinnamic acid derivate than the COOH group in benzoic acids (Nordkvist et al., 1984). This may explain the different antioxidant activities found in different fractions of the BSG because the different phenolic acids are unevenly distributed in the layers of the grain.

2.6.5 Other polyphenols

In addition to flavonoids and phenolic acids, there are other polyphenols including tannins, stilbenes and lignans that are found in plant cells. However, these polyphenols have not yet been investigated in BSG. There also has considerable amount of antioxidant activity. The tannins have been reported to be insoluble substance that develop in BSG samples when left for a period of time (Oroian & Escriche, 2015). Table 2.3 shows the polyphenolic compounds found in BSG and their biological effect.

Table 2.3a: Polyphenolic compounds found in BSG and their biological activities

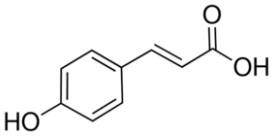
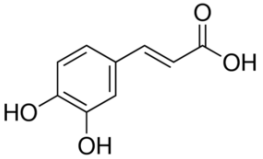
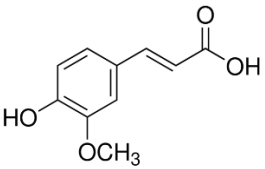
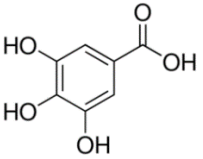
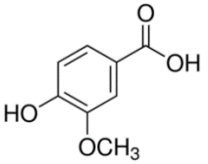
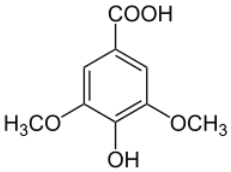
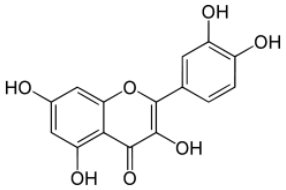
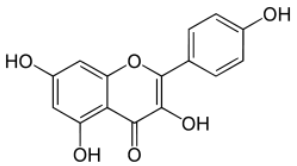
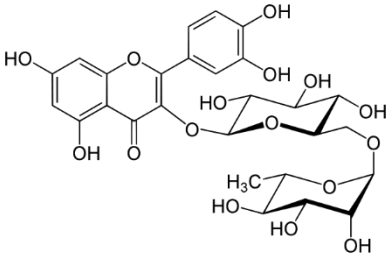
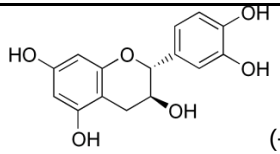
Main class	*Sub-class*	*Biological activities*	*Examples*	*Chemical structure*	*Reference*
Phenolic acids	Hydroxycinnamic acids	Reduces Diabetes, Obesity, Memory improvement	p-coumaric acid		(McCarthy et al., 2012)
			Caffeic acid		(Pereira et al., 2009)
			Ferulic acid		(Graf, 1992)
	Hydrobenzoic acids	Reduces obesity, improve visual function, Impact on Leucorrhea, dysentery, bronchitis, biliousness, urinary discharges, in haemorrhage from the uterus, lungs, or intestine	Gallic acid		(Kitrytė et al., 2015)
			Vanilic acid		(Balasundram et al., 2006)
			Syringic acid		(Moreira et al., 2013)

Table 2.4: Polyphenolic compounds found in BSG and their biological activities

Main class	*Sub-class*	*Biological activities*	*Examples*	*Chemical structure*	*Reference*
Flavonoids	Flavonols	Reduced risk of cancer, stroke, coronary heart diseases, Obesity, Diuretic, laxative, dropsical affections, virulent gonorrhoea, malaria, dysentery, hepatoprotective	Quercetin		(Anca et al., 2013)
			Kaempferol		(Vellosa et al., 2011)
			Rutin		(Parbunath, 2013)
	Flavanols	Impact on Cancer, Used in bronchitis, chest troubles, urinary complaints, aphrodisiac, piles, leprosy, tumours, jaundice, Memory improvement,	Catechin	 (-)	(Montuschi et al., 2004)

2.7 RECOVERY OF POLYPHENOLS

Extraction of the valuable compounds is the first activity done in the conceptual development stage of a commercial process. There are several factors that affect the efficient recovery of polyphenols from plant. These include the extraction method and the nature of solvent employed. Figure 2.6 shows different methods and stages that can be used to extract polyphenols in plants. The first step is the pretreatment stage in which drying and grinding are initiated. In this stage, the structure of the plant cell is reduced to enhance extraction. There are several methods that can be used for extraction as discussed in this section including soxhlet extraction and maceration. Some research has included more than one method of extraction to ensure maximum recovery e.g. using maceration after mechanical stirring (Stalikas, 2007; Routray & Orsat, 2012; Moreira, 2012).

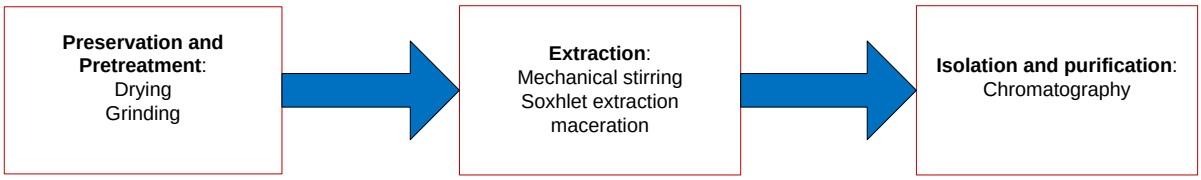


Figure 2.6: **Stages of extraction of phenolic compounds**
(Adapted from Routray & Orsat, (2012))

2.7.1 Preservation of BSG

Due to the high moisture (77- 81 %)) (Aliyu & Bala, 2013) and fermentable sugar contents, the shelf life of wet BSG is usually 7 to 9 days (Aboltins & Palabinskis, 2015). Various methods such as oven drying, freeze drying and lyophilisation is used for preserving BSG. Drying is necessary for BSG as it prolongs the shelf life. The shelf life of dried BSG is increased to 1 month after drying. Santos et al., (2003) studied the variability of BSG within a brewery in Mahou SA, Madrid, Spain, comparing results obtained using oven- and freeze-drying techniques for 18 h. They found that results in drying efficiency were similar for oven drying and freeze drying. Heredia-Olea et al., (2015) reported that it takes 24 h to oven dry BSG from a moisture content of 77 % w/w to 13 % w/w.

2.7.2 Pre-treatment methods

Grinding is one way of the pre-treatment step that can be used to improve extraction of phenolic compounds from BSG. This method decreases particle size and destructures the cell wall making the polyphenols more accessible (Hendriks & Zeeman, 2009). Another

possible pre-treatment method is milling. Niemi, (2016) demonstrated the effect of different milling pre-treatments on the microstructure of BSG. The use of a pin-disk mill showed the same unmilled structure of BSG, although the size of particles was clearly reduced. Grinding with a tuborotor was more effective than a pin disc mill to break down the aleurone cell walls. However, sieving the grinded BSG has been proven to be difficult by (Silva et al., 2012) because of the uneven polyphenols distribution in the grain. (Niemi, 2016).

2.7.3 Extraction of polyphenolic compounds

In BSG, the phenolic components are usually concentrated in the cell walls of the aleurone layer and the husk. The recovery of these compounds is greatly affected by the technique that is employed. Pinelo et al., (2005) reported that extraction methods are usually based on the sequential extraction process incorporating one or more solvent in combination with the washing steps. There are several techniques that have been suggested in literature for the extraction of polyphenols from brewers spent grain (Dhar et al., 2017). These includes solid-liquid extraction such as maceration (ME) and soxhlet (SE), assisted method such as microwave assisted extraction (MAE) and ultrasound assisted extraction (UAE), and high-pressure methods such as supercritical fluid extraction (SFE) and subcritical water extraction method (SubCWE). Due to the diverse nature of the polyphenols to be extracted, there is not one standard method that is ideal for all extractions (Che Sulaiman et al., 2017).

2.7.4 Solid-liquid extraction

This is a traditional extraction method, also known as solvent extraction that has been used to recover most polyphenols. This method uses stirring or shakers to open the cell structures and remove these bio actives based on the polarity of a solvent such as ethanol or acetone and its mixtures (Guido & Moreira, 2017; Moreira et al., 2013). Maceration and soxhlet are one of the commonly used for the extraction methods for phenolics.

Traditional methods have remained in use in the industry mainly due to their flexibility in the choices of solvents used (Cui et al., 2014). Other advantages of these conventional techniques include faster rate of extraction, large volume throughput, and the potential recycling of the solvent used (Marinsky & Marcus, 1997). Although the traditional methods have been efficient in the recovery of many phenolics, there have been some challenges with solid-liquid extraction. These include the utilization of large volumes of solvent, longer periods of extraction and degradation of compounds due to the high temperatures involved (Toda et al., 2016). Moreover, this type of extraction is not selective to non-phenolic compounds hence unwanted products such as chlorophyll, fats may also be extracted. This

will increase production cost in attempt to purify the polyphenolic extracts (Guido & Moreira, 2017)

2.7.4.1 Soxhlet extraction

Soxhlet method remains the standard method of conventional methods because of its efficiency (Luque de Castro & Garcia-Ayuso, 1998). According to the literature survey conducted in this research, there are no studies that have investigated the extraction of polyphenolic compounds from BSG. Nevertheless, studies have reported extractions from barley grain. Conde et al., (2008) evaluated the polyphenolic compounds extracted from barley husks using soxhlet. They reported a highest 0.459mg GAE/g extract of total phenolic content for ethyl acetate in a period of 9 hours. The most active individual extracts were that of 3.4-dihydroxybenzaldyde (3.07 mg/g) and p-coumaric acid (1.36mg/g). Soxhlet extraction utilizes the boiling point of the solvent and this has limited its use due to the degradation of polyphenolic compounds at elevated temperatures.

2.7.4.2 Maceration extraction

Maceration employs different solvents with stirring in a shaker. Different solvents can be used at a range of temperature depending on the type of shaker used. This method is effective in extracting phenolic compounds especially at elevated temperatures. However, the incomplete purification of the solvent from the extracts has restricted the application of this method in the food industry. This extraction technique was used by Meneses et al. (2013a) when they evaluated the efficiency of different solvent compositions (methanol, ethanol, acetone, hexane, ethyl acetate, water, methanol/water mixtures, ethanol/water mixtures, and acetone/water mixtures) from BSG. Their findings were that all the produced extracts showed antioxidant activity, but the highest total phenolic content (9.90 ± 0.41 mg Gallic acid equivalents (GAE)/g DW) was produced with 60% (v/v) acetone. The method has also been of interest for extractions of other antioxidants such as polysaccharides from BSG. Nevertheless, researchers are also giving attention to advanced techniques such as assisted extraction methods.

2.7.5 Assisted extraction methods

There are generally two assisted extraction methods that have been investigated in the recovery of polyphenolic compounds from BSG, microwave assisted extraction (MAE) and ultrasound assisted extraction (UAE). These will be discussed in the following section

2.7.5.1 Microwave assisted method

Microwave-assisted extraction (MAE) is an advanced technique (Moreira et al., 2013), which uses the solvent with an assistance of microwave energy. The microwave generate non-ionising electromagnetic (EM) waves and enhances the rate of extraction of polyphenols by rapid division of the plant matrix (Vollmer, 2003). This method uses solvent which is ten time smaller than that of traditional solvent extraction. The extraction time is also greatly reduced to range of 15 to 30 min. Different phenolic compounds have been separated from various plant matrices such as wheat bran, rice bran, grape seeds, rooibos (Stalikas, 2007). Several authors have also done a comparison between microwave assisted extraction methods and conventional methods for various medicinal plants such as *Salvia miltiorrhiza bunge* (Pan et al., 2002), *Dendrobium candidum* (Cui et al., 2014). MAE was found to be more effective than the conventional methods used. (Moreira et al., 2012) also proved that MAE of BSG gives higher yield of FA (1.31 %w/w), lower solvent consumption (NaOH 0.75%) and less extraction time (15min) as compared to conventional method. This method is thus effective and cost effective than tradition method. Although rapid mass transfer is facilitated for microwave assisted extraction, the use of elevated temperatures results in the degradation of useful polyphenolic compounds.

2.7.5.2 Ultrasound assisted method

Ultrasonic-assisted extraction (UAE) is a new technology that uses sound waves with frequencies greater than 25 kHz. The sound waves collide with the plant cell causing the loosening and release of the polyphenols. The method utilizes less time of extraction than traditional methods and also gives a higher efficiency than other assisted method. Studies have been made for the extraction of polyphenols from barley and wheat bran using UAE and have shown higher content of total phenols being released for barley (19 mg GAE/g Barley). However, no reports have shown the extraction of polyphenols from BSG using UAE. Although this method produces faster extraction rates than traditional extraction methods, a large amount of toxic and expensive solvents.

2.7.6 High pressure extraction methods

High pressure extraction (HPE) as a novel technique is used for extraction of active ingredients with pressure ranging from 100 to 800 MPa or even more up to 1000 MPa. HPE is considered as an alternative to both traditional and assisted extraction methods, which is proven to be fast and more effective (Prasad et al., 2009). High pressure extraction methods include supercritical fluid extraction and subcritical fluid extraction.

2.7.6.1 Supercritical fluid extraction (SFE)

Supercritical fluid extraction is a method which uses solvents whose temperature and pressure would have been elevated above the critical values (Rozzi & Singh, 2002). In this region, the solvent has abilities to act both as a liquid and a gas (Stalikas, 2007). This property enables the density of the fluid to change drastically with a slight variation of its pressure or temperature. The flexibility of the fluid enhances the mass transfer making supercritical fluid extraction an efficient method to recover compounds. The most known solvent for this technique is CO_2 which is generally recognized as safe (GRAS). CO_2 has a critical point of 31.06 °C and 7.386 MPa and at this low temperature, degradation of compound is minimized. However, CO_2 is expensive, and thus make the set up and production cost of the process high. Fernandez-Pérez et al., (2008) developed a process for the recovery of tocopherols from BSG using SFE. Raw and milled BSG was used at temperatures 313, 333 and 353K and at pressures from 10 to 35MPa. 2mgL^{-1} of tocopherols was obtained optimum conditions of particle size, temperature and pressure, 0.85mm, 313K and 35 MPa respectively. In another work, Spinelli et al., (2016) optimized the extraction conditions of extracting polyphenolic compounds and flavonoids from BSG. They found out that 0.35 ± 0.01 mg GAE/g BSG total phenolic content was obtained at a pressure of 35 MPa, 40 °C and when using the fluid CO_2 + 60% ethanol (v/v).

2.7.6.2 Subcritical water extraction (SubCWE)

In this advanced technology, water is the solvent whose property is adjusted by controlling the temperature and pressure to improve the extraction ability. In SubCWE, the temperature is increased up to 374°C and the pressure is kept just high enough to keep water in the liquid state as shown in Figure 2.7. The properties of subcritical water such as polarity, surface tension and viscosity are to a greater extent lower than those of water at normal conditions. This significantly adjusts the chemical properties of subcritical water to approximate those of water (Shi, 2007). Although several studies have presented the use subcritical water in the recovery of polyphenolic compounds, no literature data was found concerning the extraction from BSG.

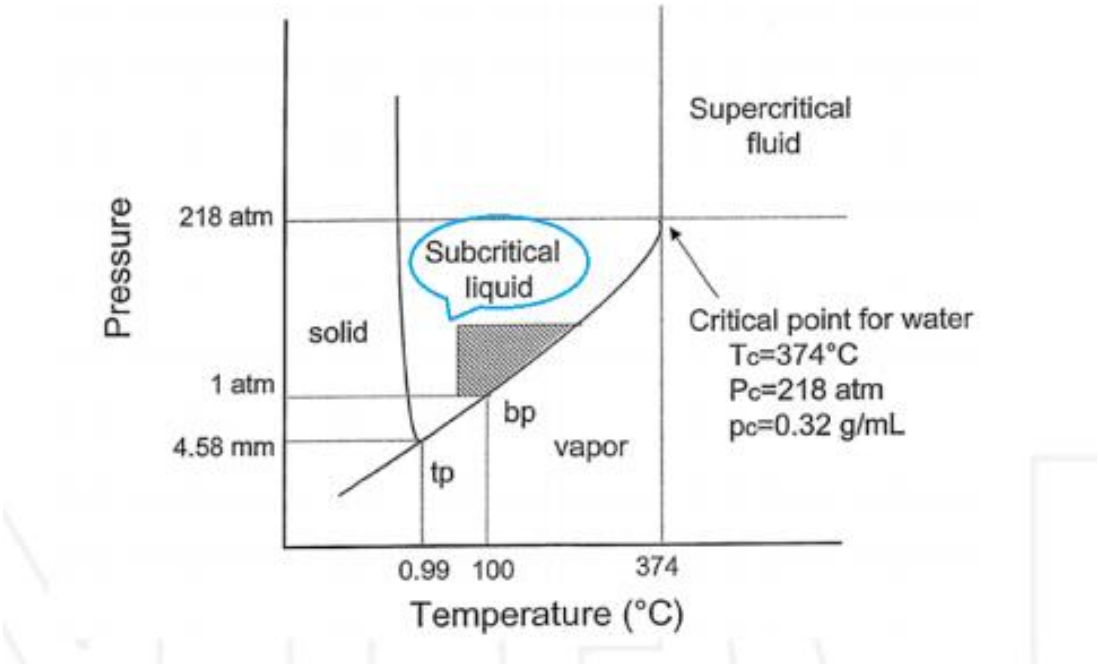


Figure 2.7: **Phase diagram of water as function of temperature for subcritical water extraction (Adapted from Asl & Khajenoori, (2013))**

2.8 FACTORS AFFECTING EXTRACTION OF POLYPHENOLS

The method used for recovery has got high effect on the efficiency of extraction. (Deng et al., 2016) has reported several other factors that influences the extraction of polyphenols such as solvent selection, solid to solvent ration, shaking speed, temperature and time. These parameters affect both the rate of extraction and the antioxidant activity of the polyphenols extracted.

2.8.1 Types of solvent used

Do et al., (2014) highlighted that the efficiency of extraction does not depend only the techniques but also on the nature of solvent used. The polarity of the solvent determines the solubility of the phenolic compounds in that particular solvent (Addai et al., 2013). The more polar the solvent, the higher the recovery of the phenolic compounds from the matrix. The mass transfer of polyphenols from the plant matrix depend on the presence of the hydroxyl groups in the solvent (Iloki-Assanga et al., 2015; Mussatto et al., 2006; Meneses et al., 2013a). The best solvent is usually the one that has high selectivity of the desired polyphenols. When extracting phenolic acids which are covalent molecules, polar solvents like water are more efficient to break the energy of interaction between the hydroxyl groups in water. This also explains why flavanols, non-covalent molecules are less extractable with water (Routray & Orsat, 2012).

Dent et al., (2013) have highlighted that a mixture of a solvent such as ethanol, acetone, methanol and ethyl acetate are more efficient in extracting covalent molecules. The solubility of the phenolic compounds does not necessarily have follow a trend with the variations of the composition of the mixture solvent. For example, Meneses et al., (2013b) investigated the effect of five different solvents (ethanol, methanol, acetone, ethyl acetate and hexane) and their mixtures in water (ethanol: water mixtures, acetone: water mixtures and methanol: water mixtures) on the extraction of polyphenols from BSG. Although all the extracts produced in their work showed antioxidant activity, the highest total phenolic content (9.90 mg GAE/g Dry BSG) was obtained for extracts produced using 60% v/v acetone: water mixture. Some solvents like methanol can be efficient in extracting polyphenols but can be toxic especially for the food industry.

Addai et al., (2013) investigated the influence of solvents on the extraction of polyphenolic compound from papaya cultivar. The solvents included pure acetone, ethanol, and methanol, as well as their respective aqueous solutions at 50 and 70% concentrations. Under the best maceration conditions (1g of papaya into 10ml solvent, 24000rpm, 1 min), 50% methanol obtained the highest TPC recovery (46.65mg/ 100g Papaya) with no significant difference ($p<0.05$). This survey showed that the type of solvent to be selected depend on the nature of polyphenols to be extracted as well as the non-toxicity of the solvent for industries which produce polyphenols that are consumed by humans (Abu Bakar et al., 2009; Meneses et al., 2013a).

2.8.2 Effect of solvent to feed ratio

The effect of the solvent to feed ratio has been investigated by several authors for different raw materials (Pinelo et al., 2005; Cacace & Mazza, 2003; Herodež et al., 2003). They concluded that the higher the solvent to feed ratio, the higher the recovery of polyphenols, despite the solvent used. This is due to the higher rate of mass transfer when the concentration gradient is greater (Pinelo et al., 2005). A higher solvent to feed ratio enhances the net transfer of molecules from a region of greater concentration (solid matrix) to a region of less concentration (bulk of the solvent) (Ho et al., 2011; Al-Farsi & Lee, 2008).

The improved diffusion rate enhances the release of phenolic compounds from the plant matrix. The trend was observed in a study of Dukung Anak (Phyllanthus niruri) conducted by Wong et al., (2013). They concluded that the total phenolic compounds yield and antioxidant activities (FRAP and DPPH assays) increased until an optimum level of ratio 1:15 (1 parts of solid to 15 parts of solvent). Deng et al., (2016) supported the findings by suggesting that there is an equilibrium that will be reached between the extracting solvent and the polyphenols above which no extraction takes place. Cacace & Mazza, (2003) showed different results, that total phenolic content level was negatively influenced by solvent to feed

ratio. Their results showed that the use of high solvent to feed ratio resulted in more dilute extracts which produced lower antioxidant activities.

2.8.3 Effect of temperature

The amount of phenolic content and antioxidant activity increase with increasing temperature. As you increase the temperature, the solubility of polyphenolic compounds increase according to Diptee, (1989) due to an increase in diffusion rates. Che Sulaiman et al., (2017) reported that extractions of phenols at temperatures above 60°C have higher efficiency than those obtained at room temperatures. Solvents have decreased surface tension and viscosity at higher temperatures which enhances the rate of diffusion. However, higher temperatures have got a limit above which most of the phenolic extractions become negatively affected. This is due to the decomposition of some phenolic compounds which would have been mobilized at lower temperatures (Liyana-Pathirana & Shahidi, 2005). Moreover, there are reactions that might occur at elevated temperatures which alters the desired individual compounds (Abad-Garcia et al., 2007). At these high temperatures, the global yield might remain the same but the total phenolic content and antioxidant activity might be greatly affected. For solvents such as acetone with a boiling point of 56.2°C, an increase in temperature may result in the evaporation of the solvent and hence greatly hinders extraction (Deng et al., 2016). Therefore, temperature is an important parameter to consider in this work.

2.8.4 Effect of particle size

Particle size has a great effect on the efficiency of extraction. The smaller the particle size, the higher the amount of polyphenols extracted (Baldosano et al., 2015). Smaller particles increase the surface area thereby enhancing the diffusion of polyphenols from the plant matrix. The reduction of particle size should not exceed fines as particles tend to agglomerate at smaller sizes. This agglomeration blocks the solvent from partitioning in the plant matrix and hence decreases the release of phenolic compounds. Pinelo et al., (2007) investigated the phenomena of particles agglomeration during extraction of grape skins leading to channelling and offside zones. They found out that particles with a range of (0.16-0.125mm) released more phenolics when the extraction was conducted with ethanol: water mixture (50% v/v) at 80°C and solvent to feed ration of 1:40. The extraction temperature influence differed with the particle size range, more polyphenols were extracted at high temperatures and small particle size (Pinelo et al., 2007). This shows that process parameters can have a combined effect on the efficiency of extraction. Hence their interaction needs to be studied and optimized.

2.9 OPTIMIZATION OF PROCESS PARAMETERS USING RESPONSE SURFACE METHOD (RSM)

Experimental design is being used for the optimization of different operating conditions of various processes in the field of quantitative research. Experimental design is an approach used to solve problem systematically and is applied in collection and analysis of data to obtain adequate information on the outcomes of experiments (Sharif et al., 2014). This approach also considers the process interactions of operating parameters and their combined effect on the responses. Although theoretically, a number of factors have a simultaneous effect on a process, application of experimental design is a way to screen and optimize response variables by systematic modification of the significant factors. The design also predetermines factors and their impact on the experimental outcome (Montgomery et al., 2016). A software, Design Expert (Stat Ease, Inc. (Minneapolis)) has been used since 1988 for the design and analysis of experiments. A number of statistical techniques used in this software includes response surface methodology (RSM) and factorial design. RSM is mainly used for optimization of process parameters while factorial design is to screen significant factors.

2.10 EXTRACTION KINETICS

To develop a cost effective process for commercial applications, a deep understanding of the mass transfer properties such as diffusion and hydrodynamic data and solubility is required (Ricardo et al., 2016). The behaviours of such properties under particular conditions such as type of solvent, type of raw material, temperature or pressures, determine the size and cost of extraction equipment together with the operational cost of the process. Therefore, kinetics studies help in generating data for the development of a commercial process.

In order to characterize the extraction kinetics, curves can be used that illustrates the concentration of the extract versus extraction times (Bart, Hans-Jörg, 2014). The extraction kinetics can be determined by taking samples during the extraction process at defined time intervals. The samples are then analysed with respect to the extract and this results in an exponential curve Figure 2.8. The behaviour shown by the curve depend on the mass transfer during extraction. According to several researchers, the extraction curve mainly consists of two steps which are washing and diffusion stage (Chan et al., 2013; Perez et al., 2011; So & Macdonald, 1986). In the washing stage, known as the fast extraction step, the rate of extraction is fast and increase gradually as the polyphenols which are already on the surface of the solid matrix are released into the solvent (Crossley & Aguilera, 2001; Rakotondramasy-Rabesiaka et al., 2009). For most extractions from BSG, this stage is considered the predominant stage as most polyphenolics would already be on the surface due to the brewing stage. In the diffusion stage, also known as the slow extraction step, the

solvent molecules penetrate through the plant matrices and causes a net transfer of the individual polyphenols from the inner cell to the bulk of the solvent. The extraction yield during this step is greatly dependent on the proportion of broken and intact cells after the washing stage or the pre-treatment stage, e.g. grinding (Crossley & Aguilera, 2001; So & Macdonald, 1986).

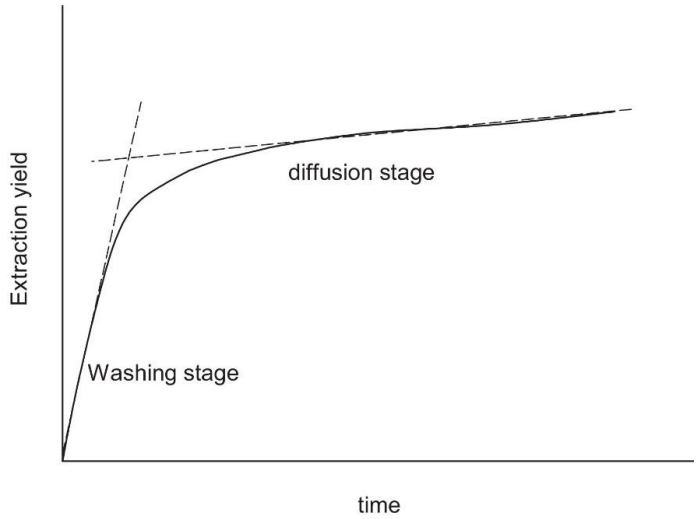


Figure 2.8: **Schematic representation of the extraction curve for extraction from plant matrices (Adapted from Chan et al., 2013)**

2.10.1 Modelling of extraction kinetics

There are many mathematical models such as Fick's law of diffusion, chemical kinetic equations and empirical equations that can be fitted into experimental data to determine the rate of extraction of a process. The models that have been mostly used in extraction includes Fick's law model, rate law, Peleg's and So and Macdonald (Chan et al., 2013).

2.10.1.1 Fick's law model

The Fick's law model uses the mass transfer principle and suggest that the rate of extraction is depended on the movement of solute from the plant matrix to the bulk of the solvent as well as the time of contact involved (Bird et al., 2006). The model is shown in

Equation 2.1.

$$\frac{C}{C_\infty} = 1 - (1-b)e^{-kt}$$ Equation 2.1

Where C is the concentration of solute after time t and C_∞ is the concentration remaining in the solute after infinite time of extraction. b and k are coefficients of extraction in the washing and diffusion step respectively.

2.10.1.2 Rate law model

The rate law model applies the second order kinetic rate in the dissolution of the bioactive compounds into the bulk solvent. From the rate model shown in

Equation 2.2, the second order extraction rate constant can be obtained using

Equation 2.3.

$$c = \frac{t}{(1/h) + (t/c_\infty)}$$ Equation 2.2

$$h = k_1 c_\infty{}^2$$ Equation 2.3

Where C is the concentration of solute after time t and C_∞ is the concentration remaining in the solute after infinite time of extraction.

2.10.1.3 So and Macdonald Model

So & Macdonald, (1986) have proposed a two-exponential kinetic model for solvent extraction method shown in Equation 2.4. This model was subsequently validated by several authors (Moubarik et al., 2011; Hadrich et al., 2017; Xi et al., 2015; Chan et al., 2013). It proposes that extraction occurs in both the washing and diffusion stages as explained in the section 2.10 (El-Belghiti et al., 2005). These two mechanisms were summed to obtain the following two-exponential Equation 2.4:

$$\mathrm{C} = C_w(1 - e^{-k_w t}) + C_d(1 - e^{-k_d t})$$ Equation 2.4

Where, C_w is the final extract solute concentration in washing stage alone, C_d is the final extract solute concentration in diffusion stage alone, k_w (s^{-1}) is the kinetic coefficient for the washing stage, k_d (s^{-1}) is the kinetic coefficient for the diffusion stage and t (s) is the time.

2.10.1.4 Peleg's model

Peleg, (1988) also developed an empirical model that can be used to describe the sorption curves in extraction using conventional method shown in

Equation 2.5. Fitting this model into experimental data will give the model rate constant K_1 and model capacity constant K_2.

$$C = C_0 + \frac{t}{K_1 + K_2 t} \qquad \text{Equation 2.5}$$

In all the above mentioned models, the rate of extraction is mainly affected by parameters such as the effect of temperature, solvent to feed ratio and particle size. Higher rates of extraction are observed for higher temperatures due to the enhanced diffusion especially for the diffusion stage (Santos et al., 2015). All models coefficients increase to indicate a faster rate and increased mass transfer.

The data that is generated from the laboratory experimental results, the optimisation and kinetic study is necessary to conduct an economic feasibility study. The economic evaluation determines if the process to recover BSG extracts using conventional method is profitable or not.

2.11 DESIGN OF LARGE SCALE PROCESS FOR BSG

The development of an economically feasible process for the extraction of polyphenolic compounds from BSG is of great importance. The BSG extracts provides many benefits for human health on the prevention of cardiovascular diseases, certain types of cancer and atherosclerosis (Moreira, 2012). Furthermore, the extracts can be used as natural sources of colorants, texturizers, functional ingredients or preservatives in the food, pharmaceutical and cosmetics industries (Moreira, 2012). The valorisation of BSG can also contribute to the sustainable development of the brewing industry, a sector with a great influence on the economy of a country. Thus, it is very important to develop a commercially viable process for the extraction of polyphenolic compounds from BSG.

Process design is the conceptual work which involves synthesis and analyses of a plant prior to the implementation of the actual building (Petrides et al., 2014). Petrides, (2013) defines synthesis as the selection and set up of unit operations capable of producing the desired product economically and with quality while process analysis is a comparison between various proposed schemes. Several authors have investigated computer aided simulation tools called process simulators that can be used to synthesize, purify, characterise, and formulate batch bioprocesses (Papavasileiou et al., 2007; Petrides et al., 2014; Petrides, 2013).

Models developed from simulation tools enables scale up from the development to the manufacturing stage. The models provide a comprehensive overview of the process to all types of recipients. Petrides et al., (2014) explained that the developed models can be adjusted so as to scale up from the development stage to the manufacturing stage. Moreover, determination of the size of equipment as well as the system of utilities is made available from model in the case of a new plant or retrofitting of an existing plant.

Process simulators are software programs that have been employed by engineering industries since the early 1960s. Established simulators for those industries include: Aspen Plus and HYSYS from Aspen Technology, Inc. (Cambridge, MA), ChemCAD from Chemstations, Inc. (Houston, TX), and PRO/II from SimSci-Esscor, Inc. (Lake Forest, CA). However, these programs have been developed for continuous processes and are not suitable to model batch or semi-continuous processes

2.11.1 Modelling of Batch and Semi-continuous processes

Batch and semi-continuous processes are best modelled with batch process simulators. This is because they account for sequential and time dependency unit operations. Petrides et al., (2014) gave a brief history of the developed batch simulators. The first simulator that was developed in mid-1980s was BATCHES by Batch Process Technologies (West Lafayette, USA). The program models processes using mathematical differential equations over time. Aspen Technology also introduced Aspen Batch Process Developer in the mid-1990s and the program is mainly for pharmaceutical processes. During the same time, Intelligen (Scotch Plains. NJ, USA) introduced SuperPro Designer® for bioprocesses. Later the scope of the program was extended to other types of batch or semi-continuous processes.

SuperPro Designer® is mainly used for the modelling of bioprocessing industries (Pharmaceutical, Biotech, Specialty Chemical, Food, Consumer Goods, Mineral Processing, Microelectronics, Water Purification, Wastewater Treatment, Air Pollution Control, etc.) (Intelligen, 2017). The window based simulation software is used for modelling biochemical, food, pharmaceutical, specialty chemical, as well as other continuous and batch

manufacturing processes (Athimulam et al., 2006). This software is sometimes preferred to Aspen Batch Process Developer as it estimates mass and energy balances, purchase costs as well as capital and manufacturing costs without being provided the thermodynamic properties of the raw materials and systems involved (Santos et al., 2010).

They are key features of SuperPro designer which are listed below.

- Perform material and energy balances to determine the production throughput
- Estimation of equipment sizing and costing
- Calculate demand for labour and utilities with respect to time
- Evaluate the cycle time reduction
- Perform thorough process economic
- Perform the environmental assessment
- Allows for Debottlenecking strategies and sensitivity analysis.

Athimulam et al., (2006) used SuperPro Designer® in the modelling and optimization of Eurycoma Longifolia water extract production. Their work selected one scheme from the four alternative schemes they had developed. The selected scheme produced a yield of 3% with an annual production of 1137.72 kg of Eurycoma Longifolia extract and a minimum cycle time of 8.32h. The process had an annual revenue of $6.32M, 86% gross margin and a return on investment (ROI) of 55% make the production economically viable. According to the literature survey done in this work, no reports have been found in this research that used SuperPro Designer® to model the extraction of bioactive compounds from brewers spent grain.

2.12 MICROENCAPSULATION OF BSG EXTRACTS

From laboratory studies, BSG extracts are mainly produced in powder form but have unpleasant flavours and aromas (Aliyu & Bala, 2013). Spinelli, Conte & Del Nobile, (2016) reported on the use of microencapsulation technology to minimize the problem. Microencapsulation is a method of masking the unpleasant odour by incorporating a wall or coating that protects the phenolic components from various environmental stresses, such as exposure to oxygen. Spinelli et al., (2016) performed microencapsulation by means of a spray dryer using maltodextrin capsul® as an encapsulating agent. Maltodextrin are used for encapsulation due to their low viscosity, good film-forming properties and thermo-protective effect during the exposure to high temperatures (Marchal, 1999). This technology enhances the quality of the product thus increasing the selling price and can make the commercial process attractive to investors.

2.13 OUTCOMES OF THIS CHAPTER

This chapter reviewed the literature that has been done before on the extraction of polyphenols, from plant materials particularly BSG. Information was provided that enables the reader to identify the stages involves in developing a process of extraction of BSG extracts. The methodology involved in the conceptual development stage will be explained in detail in the next chapter. The study of the literature data identified gaps that indicated that the research of extraction of polyphenolic compounds from BSG has not been fully exploited.

The key findings of this chapter are

- To the author's knowledge, no work has been reported on development of a commercial process for the extraction of polyphenols from BSG
- To the best of the author's knowledge, no work has investigated the extraction kinetics of polyphenols from BSG using both conventional methods (maceration and soxhlet) and high pressure methods (supercritical fluid and subcritical water extraction)
- It was also found that no work has been done to investigate the global yield of the extraction of BSG extracts using maceration and soxhlet extraction.
- Several authors have investigated the use of organic solvents in maceration and soxhlet extraction. BSG extracts obtained using acetone 70% v/v was found to contain the highest antioxidant activity.

This literature gap has caused the potential use of BSG as a source of polyphenols in the industry to be neglected. Although contributions have been made for academic purposes, the extraction of polyphenols from BSG has not been implemented on the commercial scale. This work might open up new avenues of commercialising this underutilized by-product of beer production and bring sustainable development for the beer industry. The next chapter explains the experimental methods that were used to generate the data missing in literature.

2.14 NOMENCLATURE

BSG	Brewers spent grain
C	Concentration of solute after time
C_d	final extract solute concentration in diffusion stage alone
C_w	final extract solute concentration in washing stage alone
C_0	Initial solute concentration before extraction
C_∞	Concentration remaining in the solute after infinite time
DPPH	1,1-diphenyl-2-picrylhydrazyl
DW	Dry weight
FRAP	Ferric reducing antioxidant power
HBAs	hydroxybenzoic acids
HCAs	hydroxycinnamic acids
k_d	kinetic coefficient for the diffusion stage
k_w	kinetic coefficient for the washing stage
p-CA	p-coumaric acid
SUBCWE	Subcritical water extraction
SFE	Supercritical fluid extraction
t	Time of extraction
TPC	Total phenolic content
ROI	Return on investment

CHAPTER 3 EXPERIMENTAL METHODS, OPTIMISATION AND KINETIC STUDIES

3.1 INTRODUCTION

This work aims at discussing the materials and method used in this research work to develop an economically attractive process of extractions of polyphenols from BSG. The provision of BSG samples and chemicals were highlighted in the following section. A section of the method used to dry BSG and select the best solvent from maceration and soxhlet extraction was discussed. Following this section was how the optimisation of operating conditions in this study was performed using Response surface method (RSM). The methodology of the kinetic study was then discussed. Lastly, a section on how the modelling and simulation was done using SuperPro Designer® Software was also highlighted.

3.2 MATERIALS

3.2.1 Brewer's spent grain (BSG)

BSG investigated was a residue from the brewing process which was kindly supplied by SAB Newlands Brewery, Cape Town. The starting material was dried in a tray dryer for 10 hrs, afterwards it was stored in a sealed box and used in experiments within a period of 1 month.

3.2.2 Chemicals

The bulk of the chemicals used in this work was for experimental stage of best solvent selection. Three solvents (water, acetone and ethanol) were chosen from literature data for the extraction process. Water was used as deionized from the purification system available in the Cape Peninsula University of Technology (CPUT), chemical engineering laboratory. Acetone and ethanol was purchased as HPLC grade, >99.5 % (Kimix, chemical and laboratory suppliers). The analysis of the extracts obtained using water, acetone and ethanol solvent was done at the oxidative stress unit (CPUT). For analysis of sample, measurement of Total phenolic content (TPC), antioxidant activity (DPPH radical scavenging activity assay and ferric reducing antioxidant power (FRAP) assay), individual components using HPLC-DAD analysis was done. The chemicals involved in the TPC measurements were Folin-Ciocalteu's reagent (Saarchem) and sodium carbonate (Sigma Aldrich). Gallic acid was used for the calibration curve (Sigma Aldrich).

For the DPPH radical scavenging determination, 2,2-diphenyl-1-picrylhydrazyl (Sigma Aldrich) and methanol (>99.5 %, Sigma Aldrich) was used. The FRAP assay was carried out

using acetate buffer (Saarchem), HCl (Saarchem), 2,4,6-tri[2-pryridyl]-s-triazine (TPTZ) (Sigma Aldrich), iron (III) chloride hexahydrate (Saarchem) and L-ascorbic acid (Sigma Aldrich).

For the HPLC system, methanol, deionized water and formic acid (HPLC grade, Sigma Aldrich) were used as solvents. A nylon filter of 0.45µm pore size was used to filter the eluents. Calibration curves were constructed for gallic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid and cinnamic acids, rutin, kaempferol and (+) – catechin (Sigma Aldrich).

3.3 EXPERIMENTAL METHODS

3.3.1 Pre-treatment of BSG

Wet BSG was received from SAB and immediately dried using a tray dryer as shown in Figure 3.1. The drying time was determined experimentally and the drying kinetics was obtained. The tray was filled uniformly flat at the top to maintain constant drying. The BSG was stored in a sealed container until further use and was grinded without sieving before being used in extraction. The storage of BSG was not allowed to exceed 1 month to avoid reactions and decomposition of compounds that occur over time. The grinding was done for 5 min using a food blender.

Figure 3.1: **Drying of BSG using a tray dryer**

3.3.2 Solvent selection

To select the best solvent. Soxhlet extraction and liquid-solid extraction was employed. Experiments carried out using soxhlet method were used as standards to determine the yield of extraction. The measurements of total phenolic content TPC, total antioxidant activity and the composition of individual components (p-coumaric acid) were used in the selection of best solvent.

3.3.2.1 Soxhlet extraction

Soxhlet extraction method performed was modified from the procedure developed by (Kalia et al., 2008). 5 g of BSG was put into a soxhlet thimble. The apparatus was fitted with a 500ml round bottom flask containing 150ml of water, acetone, and ethanol as extracting solvents. The extraction temperature was set with tuning to a temperature that is sufficient enough for boiling. Extraction was performed for 4h and the solvent was refluxed. After the extraction process, the extract water was filtered using a filter paper and a funnel. The Liquor obtained was taken for chromatographic analysis at the oxidative stress unit (CPUT).

3.3.2.2 Maceration extraction

The extraction procedure of polyphenolic compounds from BSG was performed according to Pandey & Tripathi, (2014) with minor modification. 5g of BSG was mixed with a solvent in a 500ml sealed bottle to prevent any leakages of the solvent. The solvents used were water (deionized), acetone and acetone: water mixtures, ethanol and ethanol: water mixtures in the same proportions done for soxhlet extraction. For mixtures, deionized water was mixed with the correct solvent using a measuring cylinder and a syringe in the correct proportions. The mixtures were based on the % composition by volume e.g. for every 100 ml, 70% v/v acetone: water mixture will have 70 ml acetone and 30 ml of water. The mixture was shaken in an oven shaker for 60min. The speed of the oven shaker was set at 200rpm and the temperature was kept at 60°C. The extract was filtered from the solvent mixture, dried and weighed to measure the global yield. After determining the global yield, 5 ml of solvent was added to the sample in vials, and were taken for analysis. The experiments were repeated once.

3.3.3 Methods of analysis for solvent selection

The global yield was determined by weighing the masses as shown in section 3.3.3.1. The total phenolic content and antioxidant activity of the extracts was analysed at the oxidative

stress unit (CPUT). Figure 3.2 shows some of the dried extract after adding 5 ml of solvent which will be taken for analysis.

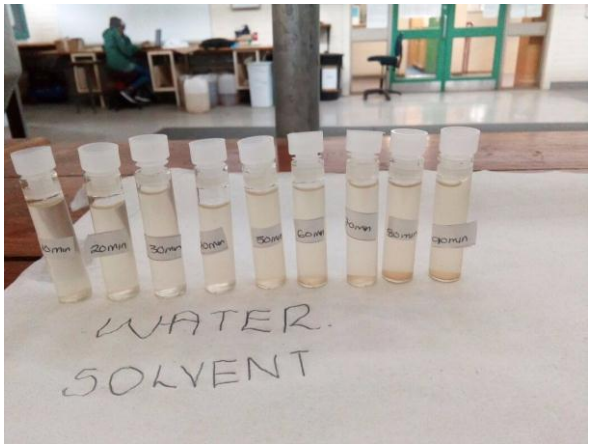


Figure 3.2: **Samples ready for analysis**

3.3.3.1 Determination of global yield

The global yield was calculated using the following formula:

$$\text{Global yield} = \frac{\text{Mass of BSG extract}}{\text{Initial mass of BSG}} \qquad \text{Equation 3.1}$$

The initial mass of BSG was weighed in a weighing boat using the weighing balance. The mass of the BSG extract was determined from the difference between the mass of the round bottom flask used to evaporate the solution after extraction and filtration and the mass of the empty round bottom flask. After weighing, a fresh solvent was added to the dried extract and the sample was taken for analysis of the total phenolic content and the antioxidant activity.

3.3.3.2 Determination of total phenolic content

The TPC of the extracts was determined by the Folin-Ciocalteu method as modified from Moreira, 2012. The Folin's reagent was diluted with water at 1:10 ratio. The samples were prepared in a plate that has 26 wells. Each well was filled with 25µL sample, 125µL Folin's reagent and 100µL of $NaCO_3$ solution (7.5% w/v) in the relative order using a pipette. The pipette tips were changed after a single use to avoid contamination of wells. One sample was filled in three consecutives well so as to take the mean reading. The first well was filled with a blank followed by 7 standards of gallic acid (GA). After 2h of incubation at room temperature in the dark, the absorbance was measured at 740nm using a multiskan® spectrum (Germany). The TPC measures the oxidative strength of the extracts relative to the

gallic acid. The total phenolic concentration was calculated from the calibration curve, using GA as standard (5-250mgL^{-1}). Data for the TPC were reported as mean ± SD for duplicates.

3.3.3.3 Measurement of antioxidant activity

The antioxidant activity is influenced by several factors to be considered when selecting the best antioxidant for a specific application. These factors include structural features of the antioxidants, concentration, temperature, reaction kinetics and location of the system as well as presence of pro-oxidants and synergists (Shahidi & Zhong, 2015). Moreira, (2012) reported several methods used to determine the total antioxidant activity of food and beverage such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, Oxygen radical absorbance capacity (ORAC) assay, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and ferric reducing ability of plasma (FRAP) assay. These assays differ in the chemical reactions that take place with the targeted compounds and hence produce different results from each other. Depending on the chemical reactions involved, these assays fall into two categories of hydrogen atom transfer (HAT) reaction-based assays and single electron transfer (ET) reaction-based assays (Shahidi & Zhong, 2015).

There is no standard or best assay that can be considered since each assay react different with antioxidant. Therefore at least two assays should be used for evaluation to authenticate the research. In this work two methods, DPPH and FRAP assay were used to determine the antioxidant activity of the BSG extracts.

3.3.3.4 DPPH radical scavenging activity assay

DPPH is a stable free radical and with a deep purple colour. The method is based on the theory that antioxidants are hydrogen donors. The DPPH measures the electron transfer ability of antioxidants to neutralize the DPPH radical and therefore is an ET-based method (Prior et al., 2005). The scavenging of the DPPH molecules is accompanied with a colour change of DPPH from purple to yellow and a decrease of UV absorption at 517nm. The extent of the decolourisation indicates the efficacy of the antioxidant. In this work, the radical scavenging activity of each extract was measured according to the procedure of Brand-Williams et al., (1995).

The DPPH solution in methanol (6.6×10^{-5} M) was prepared and 275 µL of this solution was mixed with 25 µL of the sample extracts using a pipette. Trolox also known as 6-hydro-2,5,7,8-tetramethylchroan-2-2carboxylic acid (Aldrich) was used as standard. The filling of the wells was similar to that of the measurement of TPC. The absorbance decrease of DPPH was measured using the spectrophotometer at 517nm after 10min. The disappearance of the purple colour indicates higher free radical scavenging activity. The antiradical power (ARP,

%) which is defined as ($1/EC_{50}$ x 100) was used to determine the activity of extracts. EC_{50} is defined as the amount of BSG extract that can halve the initial concentration of the DPPH solution.

3.3.3.5 Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power was measured according to the method described by (Benzie & Strain, 1996) with some modifications. The FRAP assay is a typical ET-based method that measures the ability of the BSG extract to reduce the ferric ion (Fe^{3+})–ligand complex to the deep blue ferrous (Fe^{2+}) complex in acidic media (Shahidi & Zhong, 2015). However, the assay does not directly measure the antioxidant capacity of a potential antioxidant. Its antioxidant activity is determined as increase of absorbance at 593 nm, and results are expressed as micromolar Fe^{2+} equivalents or relative to an antioxidant standard, trolox (5-250mg L^{-1}) (Antolovich M, Prenzler PD, Patsalides E, McDonald S, 2002). The FRAP reagent was prepared from 300mM acetate buffer at pH 3.4, 10mM 2,4,6-Tripyndyl-s-triazine (TPTZ) in 40mM HCl and 20mM $FeCl_3$ solution in proportions of 10:1:1 (v/v), respectively. 300µL of this solution was added to 10µL of sample or blank or standards using a pipette for 30min. The filling of the wells was similar to that of the measurement of TPC. Readings of the Prussian blue, ferrous tripyridyltriazine complex were then taken at 593nm. The measurements were carried out for 2 sets of experiments and results expressed as mg TE/g DW of sample.

3.3.3.6 HPLC-DAD analysis for individual components

The phenolic composition for the extracts with high TPC and antioxidant activity was analysed using an HPLC system (Agilent technologies, 1200 series) modified from the method described by (Rubilar et al., 2007). The HPLC system consisted of a low-pressure quaternary gradient unit with an inline degasser and a photodiode array detector.

Separation of polyphenols was achieved on a C_{18} column (150mm x 4.6mm x 4µm) with temperature being kept at 30 °C. The chromatography had a flow rate of 0.3mLmin^{-1} and the analytical standard and injection volume was 20µL. Water was used as a mobile phase A and methanol used as mobile phase B. The following gradient system was used in this analysis: 100% B in 0 min, from 100% to 0% B in 110 min, followed by 100% A for 20 min and back to 100% B in 10 min and a period of 10 min was set in between injections. The photodiode array detection was performed at 320 nm for phenolic acids (p-CA) and at 370 nm for quercetin, kaempferol and rutin. The chromatography data system obtained identified the analytes by comparing their peak integration area. Peak purity was also monitored to minimize interference of other peaks. Results in samples were expressed in mg/g BSG.

3.3.4 Statistical analysis

All data points are the mean and standard deviation values of two independent experiments. Excel was used for analysis of variance (ANOVA). The p-value less than 0.05 ($p < 0.05$) was considered as statistically significant.

3.4 OPTIMISATION STUDY

After analysing the results obtained using the methods mentioned in the sections 3.3.1 to 3.3.4 and selecting the best solvent, optimisation for operating conditions was investigated. Response surface method (RSM) was used to optimise the operating conditions. The strategy was carried out according to Montgomery, (1991). Montgomery, (1991) described RSM as a collection of statistical methods useful for the modelling and analysis of problems in which a response of interest is influenced by several factors and their relationship with each other. The objective is to optimise this response as well as quantifying the relationship between one or more responses and the vital input factors.

3.4.1 Response surface design

Design Expert software v.10 was used to develop the experimental plan for RSM according to Noordin et al., (2004). The experimental domain was defined taking into account the results obtained for water, which was selected as the best solvent in the extraction of polyphenols from BSG, and the limitations provided by the instruments used. As mentioned in chapter 2.8, the following significant parameters were chosen for independent process variables for this work: Extraction time (X_1; in minutes), Extraction temperature (X_2; in degrees Celsius), solid to feed ratio (X_3) and shaking speed (X_4; in rpm). TPC, FRAP and p-coumaric acid were selected as the responses of the independent variables. All the experiments were performed in duplicate, and the average values of the responses were reported.

A central composite design (CCD) with the axial points set at 1.681 coded units from the centre was used to determine the best combination of extraction variables. Table 3.1 shows the range and centre point values of four dependent variables used in the CCD.

Table 3.1: Levels of independent variables

Factor	***Name***	***Low Level***	***Level***	***High Level***
A	Time	20.00	40.00	60.00
B	solvent to feed ratio	15.00	27.50	40.00
C	Temperature	20.00	40.00	60.00

D	Shaking speed	150.00	185.00	220.00

3.4.2 Design of Experiments

This design included 30 experiments to estimate the model coefficients and a centre point with 4 replicates as shown in Table 3.2. The 4 replicates at centre point allowed estimating experimental error and checking fit. The experiments are taken from Central composite design arrangement in terms of the independent variables (X_1, X_2, X_3 and X_4) and their observed responses, including TPC, FRAP and p-coumaric acid. The TPC is measured in mg GAE/ g BSG, FRAP is measured in mM Fe (II)/g BSG and p-coumaric acid is measured in mg/g BSG.

Table 3.2: Central composite design arrangement for experiments 1 to 15

Run	*X_1:Time*	*X_2:solvent to feed ratio*	*X_3:Temperature*	*X_4:Shaking speed*	*TPC*	*FLAVONOL*	*FRAP*	*P-COUMARIC ACID*
1	40.00	27.50	40.00	185.00				
2	20.00	40.00	20.00	150.00				
3	60.00	40.00	60.00	150.00				
4	20.00	40.00	20.00	220.00				
5	20.00	15.00	20.00	150.00				
6	60.00	40.00	20.00	220.00				
7	40.00	27.50	80.00	185.00				
8	20.00	15.00	60.00	220.00				
9	40.00	27.50	40.00	255.00				
10	40.00	27.50	20.00	185.00				
11	40.00	52.50	40.00	185.00				
12	60.00	15.00	20.00	220.00				
13	20.00	15.00	60.00	150.00				
14	40.00	2.50	40.00	185.00				
15	80.00	27.50	40.00	185.00				

Table 3.3: Central composite design arrangement for experiments 15 to 30

Run	*X_1:Time*	*X_2:solvent to feed ratio*	*X_3:Temperature*	*X_4:Shaking speed*	*TPC*	*FLAVONOL*	*FRAP*	*P-COUMARIC ACID*
16	20.00	15.00	20.00	220.00				
17	40.00	27.50	40.00	185.00				
18	40.00	27.50	40.00	185.00				
19	60.00	40.00	60.00	220.00				
20	10.00	27.50	40.00	185.00				
21	40.00	27.50	40.00	185.00				
22	60.00	15.00	60.00	150.00				
23	20.00	40.00	60.00	220.00				
24	60.00	15.00	20.00	150.00				
25	20.00	40.00	60.00	150.00				
26	40.00	27.50	40.00	115.00				
27	60.00	40.00	20.00	150.00				
28	40.00	27.50	40.00	185.00				
29	40.00	27.50	40.00	185.00				
30	60.00	15.00	60.00	220.00				

3.4.3 Analysis of variance (ANOVA)

The experiments shown in Table 3.2 were performed in the laboratory using maceration extraction and soxhlet extraction methods, as discussed in section 3.3.2.1 and section 3.3.2.2 respectively. The extracts obtained from the best solvent was taken for analysis following the same procedure in section 3.3.3. The results obtained from the lab were fit into Table 3.2 and analysed using response surface regression. The default response transformation was selected to select the appropriate model. By clicking "fit summary" tab, Design Expert software fits the linear, 2FI, quadratic and cubic polynomials to the responses. The polynomial is in the form as given in Equation 3.2.

$$\mathrm{Y} = \beta_{\mathrm{i}} + \sum \beta_i X_i + \sum \beta_{ii} X^2{}_i + \sum \beta_{ij} X_i X_J \qquad \text{Equation 3.2}$$

Where Y is the predicted response, β_0 is an intercept, $\beta_i, \beta_{ii}, \beta_{ij}$ are the coefficients of the linear, quadratic, and interaction terms, respectively. X_i and X_j are coded independent variables. The best model is selected based on the probability ("Prob>F") to see if it falls below the selected significance level. A significance level of 0.05 was selected for this work. Design Expert also suggest the best model and discard the model that would be aliased even if it was significant.

In order to validate a model, the lack of fit, coefficient of determination (R^2), and the Fischer test value (F-value) was generated from analysis of variance (ANOVA) by the Design Expert software. The fitted models are considered significant if the p value is less than 0.05 for a 95% confidence level and their lack of fit is not significant.

3.4.4 Verification of the model parameters

The optimum extraction conditions for bioactive compounds from BSG were obtained using RSM. To verify the models developed, experiments of the extraction of polyphenols from BSG was performed under the optimal conditions developed by Design Expert. The extracts were then analysed at the oxidative stress unit. These experimental results were compared to the predicted values obtained from optimisation for verification of the developed models.

3.5 EXTRACTION KINETIC STUDY

The extraction kinetics were used on the optimum conditions to determine the time that is technically feasible and the rate of extraction. The optimum conditions obtained using RSM were used for kinetic experiment. Water was used as the solvent at the solid to feed ratio of (27.5:1), temperature of 40°C and shaking speed of 185 rpm. Experiments with 6 same beakers with the same solid to feed ratio was carried out at the same time with the same operating conditions. The assumption made was that all the beakers are operating at the same conditions and volumes of measurements. The experiments were done in replicates. This method was compared with the one where only one beaker was put and samples were drawn after every 15 min interval. The assumption made for the second method was that the sample withdrawn at each interval is small resulting in an insignificant change of volume. The experiments were repeated twice.

3.5.1 Kinetic models

Equation 3.3 Equation 3.4

Equation 3.5Equation 3.6 were used in this study to model the extraction and these are based on mass transfer within solid particles, diffusion of the solute to the solvent, the movement of solute based on the variation of temperature as discussed in section 2.10.1. These models were fitted into the experimental data.

So and Macdonald Model

$$C^* = C^*_w(1-e^{-k_w t}) + C^*_d(1-e^{-k_d t}) \qquad \text{Equation 3.3}$$

Modified fick's law

$$\frac{C}{C_0} = (1-b^{'})e^{-k^{'}t} \qquad \text{Equation 3.4}$$

Rate law

$$c = \frac{t}{(1/h)+(t/c_\infty)} \qquad \text{Equation 3.5}$$

Peleg's model

$$c = c_0 + \frac{t}{K_1 + K_2 t}$$ Equation 3.6

Non-linear regression and solver in excel was used to fit the models into the experimental data. The best fit was selected based on the R^2 values which was calculated using data analysis in excel. The best model was further used to investigate the effect of temperature on the extraction kinetics in the recovery of BSG extracts.

3.6 MODELLING AND SIMULATION USING SUPERPRO DESIGNER®

The development of a process which is economically feasible was done using modelling and simulation in SuperPro Designer®. SuperPro Designer® is a window based simulation software for modelling biochemical, food, pharmaceutical, specialty chemical, as well as other continuous and batch manufacturing processes (Athimulam et al., 2006). This software estimates the mass and energy balances as well as evaluate the profitability of the process developed (Santos et al., 2010).

The base case simulation was constructed from the laboratory experiments using the batch operation mode. A comparison was done between the base case simulation and four other alternative schemes to select the best process. The selection of the best process was based on the profitability indicators, payback time, return on investment (ROI) and net present value (NPV). The best process was concluded to be the technically and economically viable for the extraction of bioactive compounds from BSG. A sensitivity analysis was also done to evaluate the change in the profitability indicators when the annual throughput was either increased or decreased. The sensitivity analysis was done using the SuperPro Designer® and Excel.

3.6.1 Modelling of the base case scheme

The base case was modelled based on the data generated in the laboratory. These data include the drying time for BSG, the best solvent selected, the optimum conditions of extraction, the global yield and the rate of extraction. Batch operation mode was selected with an annual operating time of 7920 h as shown in Figure 3.3.

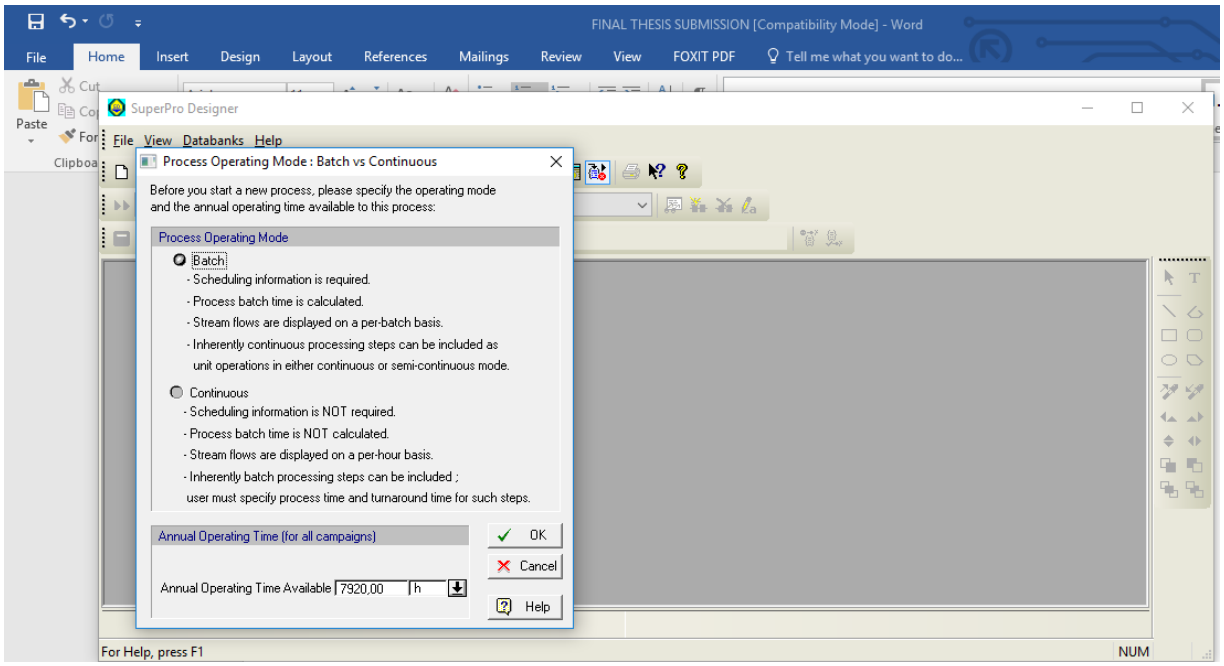


Figure 3.3: Selection of operation mode and time

3.6.1.1 Registration of components

Water solvent and air for drying of BSG were registered from the databank of SuperPro Designer®. Since BSG is a biomass and its properties are unknown with the software, it was user defined as shown in Figure 3.4. The density and mass composition of BSG was taken as similar to that of water. The price of BSG was registered as adopted from (Fernandez-Pérez et al., 2008; Buffington, 2014; Lynch et al., 2016).

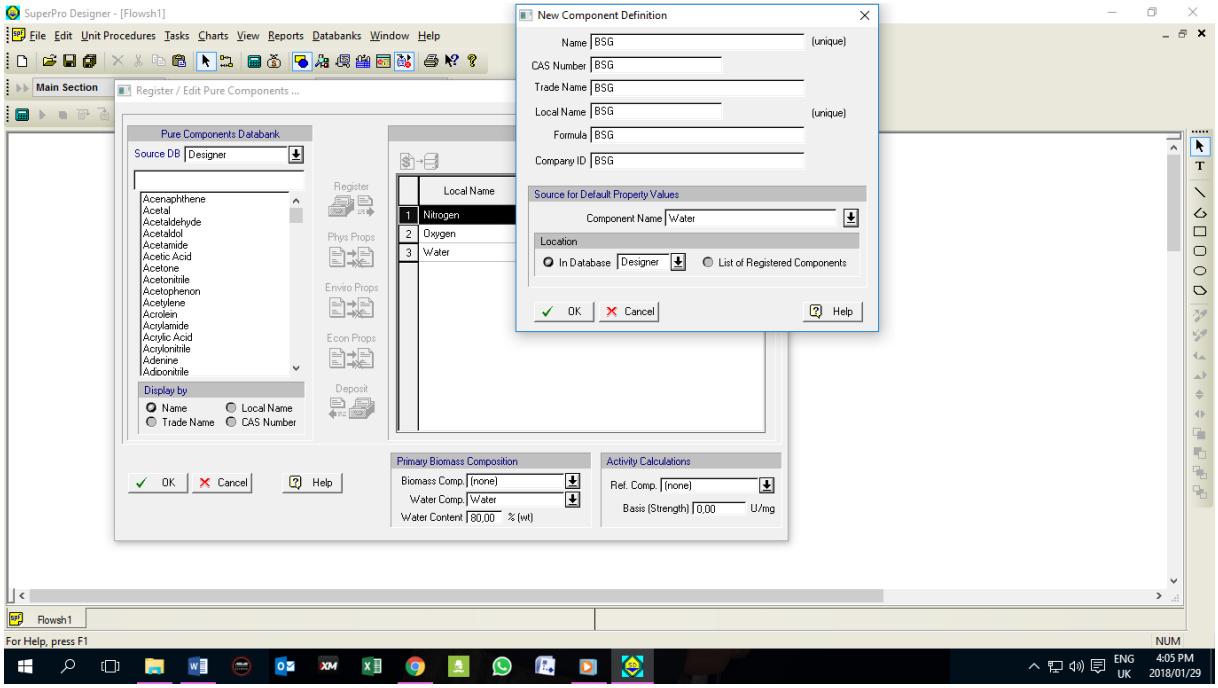


Figure 3.4: Registration of BSG

3.6.1.2 Estimation of Prices

The purchasing cost of BSG was obtained from literature sources as mentioned in section 3.6.1.1. The selling price of the BSG residue after the extraction process was obtained from Fernandez-Pérez et al., (2008). This BSG residue is considered for combustion purposes is sold at 0.38 $/KWh. The selling price of the main product BSG extract was calculated based on the Sigma Aldrich. Although the price indicated by Sigma-Aldrich may not necessarily reflect market prices, they are a good indicator of the relative price of the compounds.

According to Sigma Aldrich, (2017) 50 mg of p-coumaric acid (98 %) is sold for $ US 408.5 making 1 kg of p-coumaric acid (98 %) to cost $ 8 170. This report used the pricing of 1 % of final cost of the commercial product of p-coumaric acid as adapted from the study done by Fernández-Ronco et al., (2013). They modelled the production of BSG extract using supercritical fluid extraction with ASPEN plus. From the tocopherols found in BSG, 1 % of its commercial price was used as the selling price for their BSG extracts at 0.30 Euros/kg. Modifying the same method, this study took 1 % of the commercial product of p-coumaric acid as the price of BSG extract and sold it at $ 8,170/kg. This enabled direct comparison of literature results with those obtained in this work.

3.6.2 Initialization of unit procedures and scheduling

Five unit procedures (Drying, storage, extraction, filtration and evaporation) were selected following the experiments done in the laboratory. Each unit procedure was sequenced with appropriate operations such as charge or transfer quantities and the scheduling relationships between different consecutive procedures. The scheduling details were documented for each step. Operation started either at the beginning of the batch or at the end of the previous operation. The extraction operation in this research work was taken as a reaction which has a 95% conversion as shown in Figure 3.5. The rest of the procedures were taken as done in the laboratory.

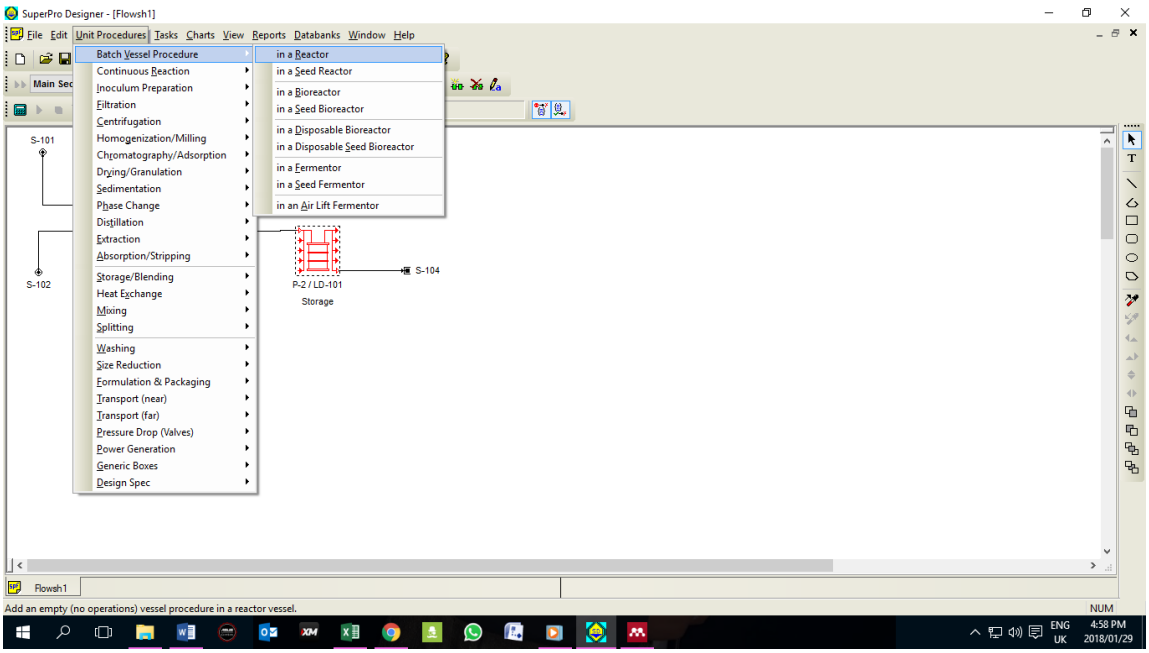


Figure 3.5: Selection of extraction procedure

Table 3.4 illustrates the sequence of the extraction process. The reaction was taken as shown in Equation 3.7. The water solvent was considered as a carrier of the reaction.

$$\text{BSG} \rightarrow \text{BSG Extract} + \text{BSG Residue} \qquad \text{Equation 3.7}$$

Table 3.4: Unit operations for the extraction stage

Equipment Tag	***Unit operation***
R-101	Transfer BSG from storage drum
	Charge fresh water
	Heat
	Batch Stoic. Reaction
	Transfer out extraction mixture

3.6.3 Process simulation and economic evaluation

The process was simulated by clicking the "mass and energy" and "economic analysis" buttons. After simulation, the process was analysed through the "charts" and "reports" tabs shown on the main ribbon of the flowsheet page as shown in Figure 3.6.

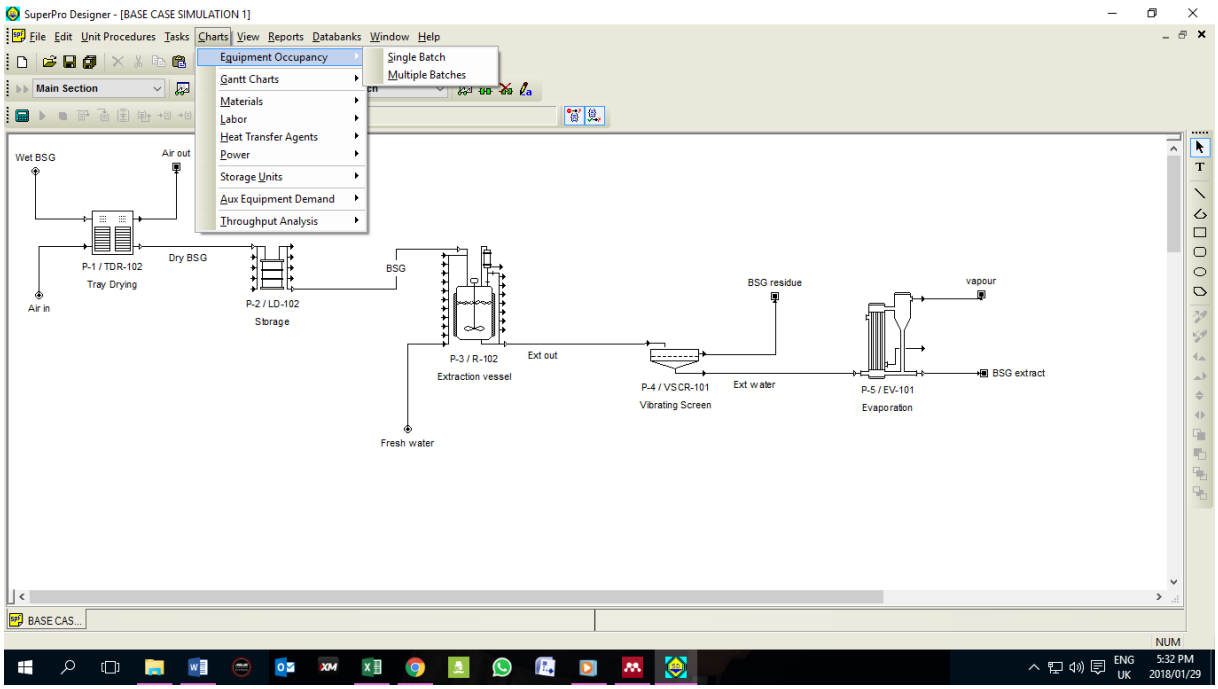


Figure 3.6: SuperPro Designer® interface that gives charts and reports

The charts provided information of the number of batches involved in the process. The reports evaluated the economic feasibility of the base case using the profitability indicators payback period, net present value (NPV) and return on investment (ROI). Debottlenecking strategies were employed to increase the attractiveness of the process to potential investors and alternative schemes were developed.

The economics of this process were calculated using the following equations. The total capital investment was calculated using Equations 3.8, 3.9 and 3.10.

$$\text{Total Capital Investment (TCI)} = \text{Fixed Capital Investment (FCI)} + \text{Working Capital}$$

Equation 3.8

$$\text{Fixed Capital Investment (FCI)} = \text{Direct Costs} + \text{Indirect Costs}$$

Equation 3.9

$$\text{Working Capital} = \text{Fixed Capital Investment (FCI)} \times 0.15$$

Equation 3.10

To calculate FCI in Equation 3.9, direct costs were added to indirect costs of the developed process. Direct cost is the addition of equipment cost, piping and installations, instrumentation, electrical, building and auxiliary facilities. The engineering and construction costs sum up the indirect costs. The annual revenue and gross profit were calculated using Equation 3.11 and 3.12.

$$\text{Annual Revenue} = \text{BGS Selling Price} \times \text{Production Rate} \qquad \text{Equation 3.11}$$

$$\text{Gross Profit} = \text{Annual Revenue Sales - Annual Operating Costs} \qquad \text{Equation 3.12}$$

The TCI, annual revenue, gross profit, annual operating costs, tax depreciation will give the cash net flow per period. The payback period and the net present value (NPV) is then calculated from the cash net flow per period.

$$\text{Payback Period} = \frac{\text{Total Capital Investment (TCI)}}{\text{Cash Netflow Per Period}} \qquad \text{Equation 3.13}$$

$$\text{Return on Investment (ROI)} = \frac{\text{Cummulative Cash Net Flow at the end of the Investment}}{\text{Life Of Project} \times \text{Total Capital Investment (TCI)}} \times 100\% \qquad \text{Equation 3.14}$$

3.6.4 Sensitivity analysis

Sensitivity analysis done for the best scheme, the annual outputs was adjusted to determine the influence of the production scale on the product unit costs. The tasks “Adjust Process Throughput” option was selected as shown in Figure 3.7.

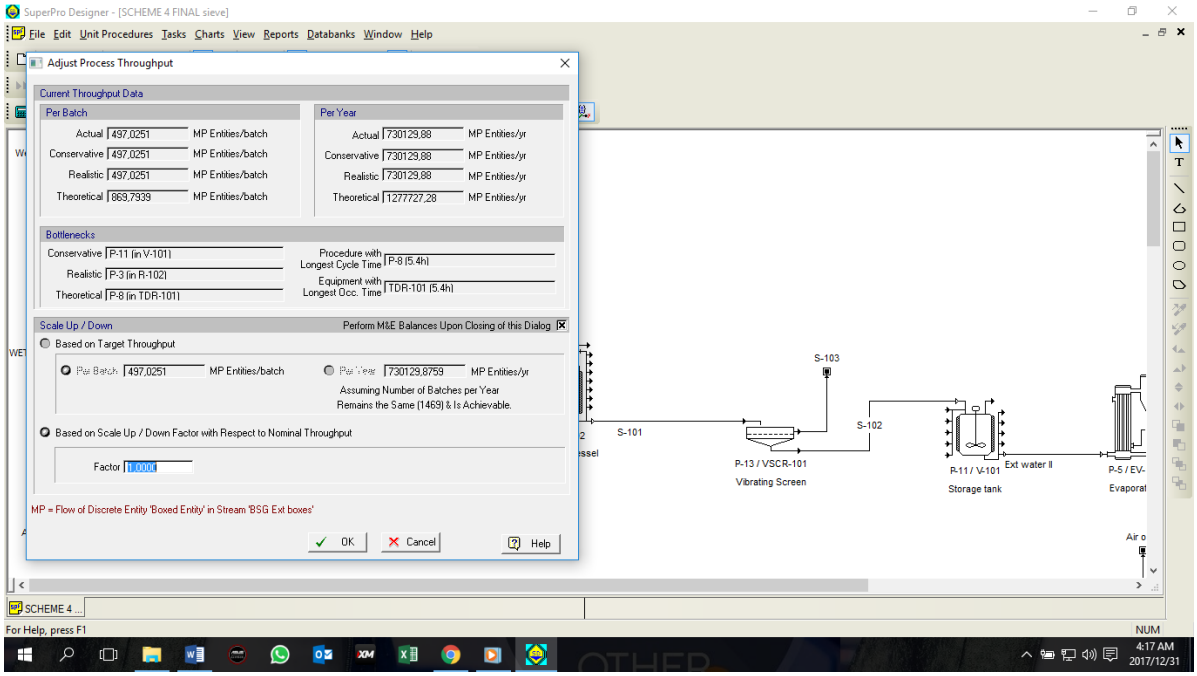


Figure 3.7: Example dialogue box for the sensitivity analysis

By adjusting the scale up or scale down factor in Figure 3.7, SuperPro Designer® simulated the new process by solving new mass and energy balances as well as perform the new economic analysis.

3.7 OUTCOMES OF THIS CHAPTER

This chapter was the experimental part of this work. The materials and methodology used in this work were explained in detail. The results of these experiments were analysed and are discussed in chapter four.

3.8 NOMENCLATURE

Symbol	Description
ANOVA	Analysis of variance
ARP	Antiradical power
CCD	Central composite design
DW	Dry weight
EC_{50}	Effect of concentration for a 50% response
FCI	Fixed capital investment
FRAP	Ferric reducing antioxidant power
GA	Gallic acid
GAE	Gallic acid equivalents
HPLC	High performance liquid chromatography
NPV	Net present value
mM	Milli moles
QE	Quercetin equivalents
SD	Standard deviation
TCI	Total capital investment
TE	Trolox equivalents
rpm	Revolutions per min
µL	Micro Litre
R^2	Coefficient of determination

CHAPTER 4 EXPERIMENTAL RESULTS AND ANALYSIS

4.1 INTRODUCTION

This chapter discusses the experimental results for selecting the best solvent, optimisation study and kinetic study. The drying kinetics that was also performed in chapter 3 as a pre-treatment method for BSG is analysed.

4.2 DRYING OF BSG

Figure 4.1 shows the drying of wet BSG prior to the extraction procedure. The results show that the free moisture was completely removed after 12 hours of drying. The drying time is lesser than that reported by literature data. Heredia-Olea et al., (2015) and Santos et al., (2003) dried BSG using oven drying for 24h and 18h respectively. The difference can be attributed to the difference in the type of grains which may result from different processing conditions, as well as climates and their ability to store water. The drying was done to preserve the BSG samples to be used for the selecting of the solvent and the optimisation experiments.

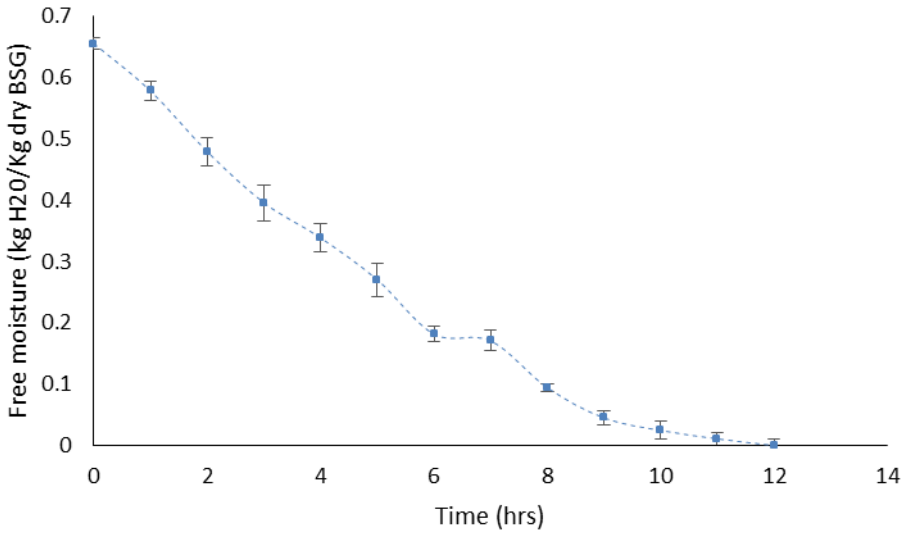


Figure 4.1: **The curve of drying a 1,2 kg BSG sample in a Tray dryer at 60 °C**

4.3 SOLVENT SELECTION

The selection of solvents was determined from experiments performed using soxhlet extraction and maceration extraction. For soxhlet extraction, water, acetone and ethanol were used as solvents. The solvents that were used for maceration extraction includes acetone, ethanol, water, acetone: water mixtures and ethanol: water mixtures. The best solvent was evaluated using the measurements of the global yield, TPC, flavonol content and antioxidant activities (FRAP and DPPH). The results were used to select the best solvent to be used for the extraction of polyphenolic compounds from BSG.

4.3.1 Global yield

Figure 4.2 shows the global % yield obtained for extraction of polyphenolic compounds from BSG using soxhlet extraction. The global % yield was calculated using the mass of the extract and the original BSG mass sample.

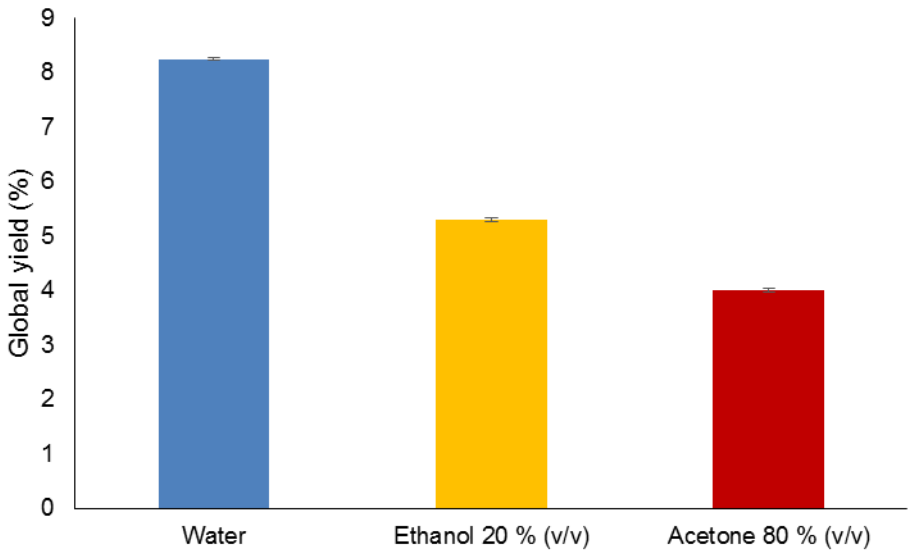


Figure 4.2: Global yield % for soxhlet extractions using water, acetone 80 % (v/v), ethanol 20 % (v/v)

Figure 4.2 shows the global % yield produced by soxhlet extraction method from different solvents using optimum solvent compositions observed from maceration extraction of Figure 4.3. The global yield produced is generally low compared to that reported in literature data for the extraction of other plant materials other than BSG. According to the research done in this work, there is no literature on the global yield for the extraction of polyphenols from BSG using soxhlet extraction. The results also highlighted that the global yield was highest for

extraction done using water solvent. However, there was an insignificant difference between the global % yield of acetone and that of ethanol. There was no literature found in this research to compare with the results in Figure 4.2. Figure 4.3 shows the global % yield of the extraction of BSG using maceration method.

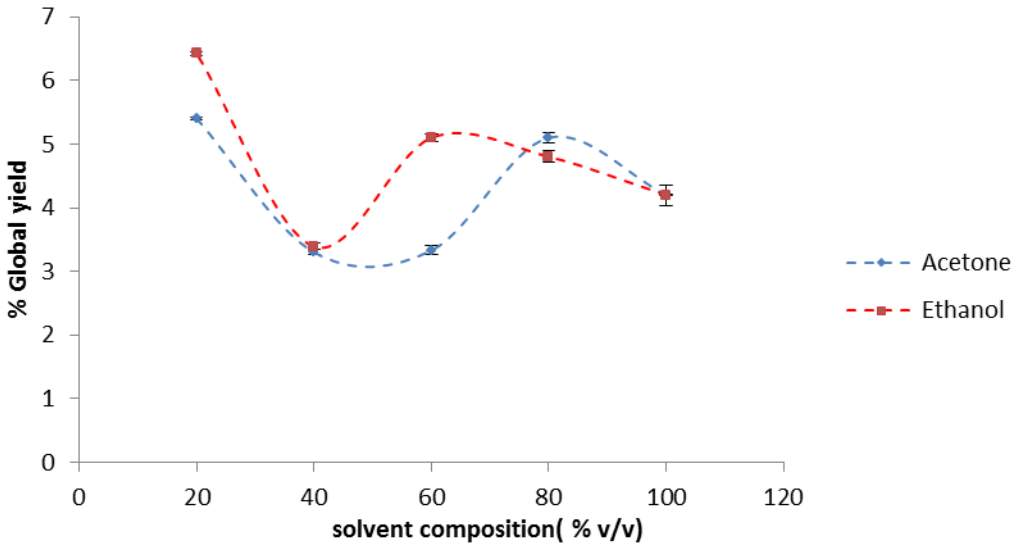


Figure 4.3: Global yield % for maceration extractions using acetone, ethanol and their water mixtures

Figure 4.3 illustrates the global % yield for maceration extractions done using different solvents and different solvent compositions. The global % yield was highest for extractions done using water (9.31 ± 0.002 %). The optimum solvent compositions were found to be 80% acetone (5.1 ± 0.08 %) and 20 % acetone (5.4 ± 0.02 %), and 20 % ethanol (6.42 ± 0.03 %). The standard deviation for all data points was high. The variations in the data maybe possibly due to the degradation and deterioration of the BSG when stored for many days. Although no literature was found with the maceration extraction of polyphenolic compounds from BSG, comparison with studies for the same extraction from plants was considered. It was observed that the global % yields obtained in this work for BSG is lowest compared with other yields from other studies. Most of the extraction yields ranges from 4 % to 35% (Dhanani et al., 2017; Dent et al., 2013; Do et al., 2014). This can be attributed to the fact that some polyphenols would have been extracted already during brewing. At the beginning of brewing, malted barley is mixed with hot water in a mash mixer for several periods of time The mash mixer is usually operated in acidic conditions to enhance the conversion of starch molecules into fermentable sugars (American Society of Brewing Chemists. & Ono, 1996; Thiago et al., 2014; Kunze & Kunze, 2010). The polyphenols are soluble in water when the pH is less than 5.5 and therefore separated from BSG during brewing.

4.3.2 Total Phenolic Content (TPC)

Figure 4.4 shows the effect of different solvents on the total phenolic content extracted from BSG using soxhlet extraction.

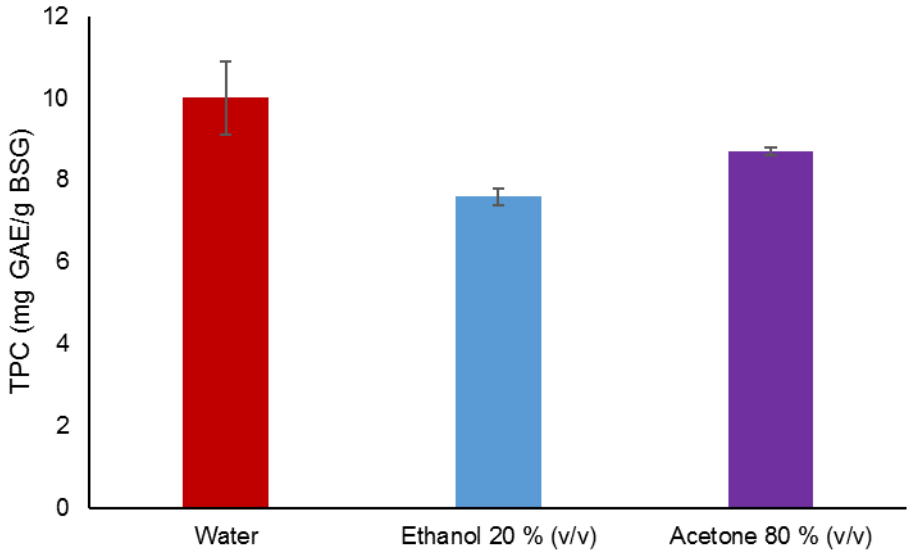


Figure 4.4: TPC values for soxhlet extractions for water, acetone 80 % (v/v), ethanol 20 % (v/v)

The results shown in Figure 4.4 illustrates the TPC values for soxhlet extraction technique. Soxhlet extraction method has been reported by Dhanani et al. (2017) to measure the maximum extractable polyphenols from natural herbs. Only free phenolic compounds are extractable by conventional solid-liquid extraction. However, due to higher extraction temperatures used in soxhlet extraction, both free and bound phenolics are recovered (Bonoli et al., 2004; Dvorakova et al., 2008). Since soxhlet extractions occurs at boiling point of the solvents, authors have found that the use of higher temperatures causes degradation of phenolic compounds thereby affecting antioxidant activity (Dorta et al., 2012). Extracts from water showed the highest TPC and this was in agreement with the results obtained for the global yield shown in Figure 4.2. The results also showed that ethanol 20 % (v/v) had a higher global yield than acetone 80 % (v/v) but the amount of TPC was less for ethanol 20 % (v/v). This indicates that soxhlet extraction might have released some undesired compounds which are not polyphenols when ethanol was used as a solvent resulting in a high global yield. Table 4.1 compares the results obtained in this research with the work done by Moreira, (2012) for soxhlet extractions. However, their work indicates that extracts obtained using acetone 70 % (v/v) solvent gives higher TPC than extracts obtained using water.

Table 4.1: TPC for BSG extracts obtained from soxhlet extraction method and literature data. Values are expressed as mean ± standard deviation.

Solvent composition	*TPC (mg GAE/ g BSG)*	
	Research work	**(Moreira, 2012)**
Water	10,01 ± 0,9	4,3 ± 0,3
Acetone 80 % w/w	8,7 ± 0,1	11,6 ± 0,3[1]
Ethanol 20 % w/w	7,6 ± 0,2	-

Figure 4.5 shows the effect of different solvents on the total phenolic content of extracts from BSG using maceration. The results show the influence of different solvent and solvent composition on the extraction. Moreira, (2012) reported that the nature of the extracting solvent used has got the greatest significance on the extract yields, the total phenolic content and the resulting antioxidant activities of the sample. In this work, the total phenolic content for all extracts obtained from maceration was highest for water, a more polar solvent.

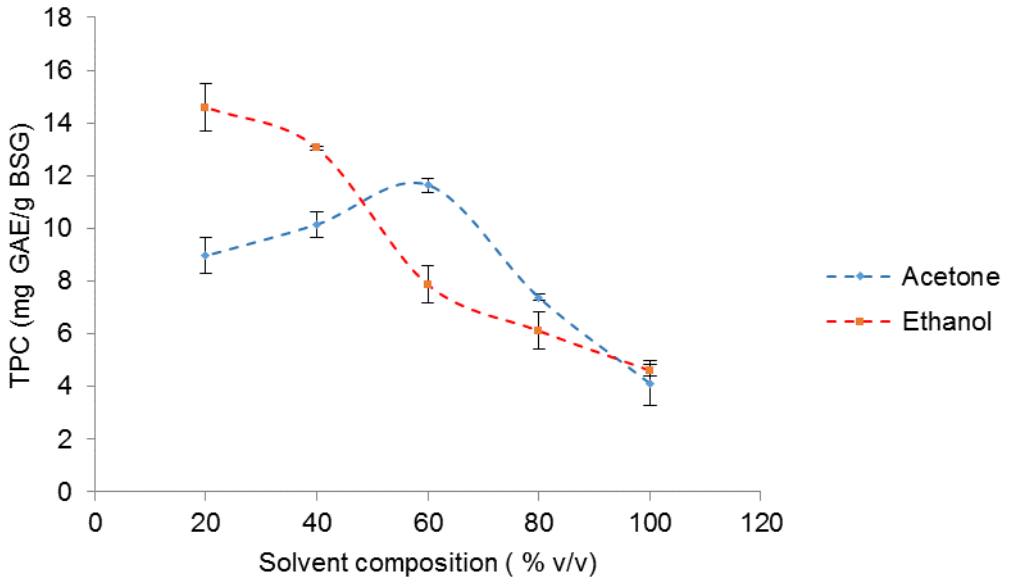


Figure 4.5: TPC values for maceration extractions done using ethanol, acetone and their water mixtures

Acetone solvent had a different behaviour from ethanol solvent in the efficiency of extraction. For acetone solvent, 60% v/v had the highest phenolic content whilst for ethanol solvent, the lesser the solvent in the mixture the greater the total phenolic content. This means that the highest amount of total phenols was obtained when there was no ethanol at all and it was only pure water. The results agree with those of Meneses et al., (2013a) as shown in Table

[1] The value was a result of an extract done by Moreira, (2012) for Acetone 70 % w/w

4.2. They studied the influence of solvents on the extraction of polyphenols from BSG. Meneses et al., (2013a) evaluated methanol, ethanol, acetone, hexane, ethyl acetate in different compositions of 100%, 80%, 60%, 40% and 20%), and water. They reported that TPC was higher for 60% acetone (v/v) than all other cases.

Table 4.2: TPC for BSG extracts obtained from Maceration extraction method. Values are expressed as mean ± SD

Solvent compositions (%)	***TPC (mg GAE/ g BSG)***	
	Research work	(Meneses et al., 2013a)
Water	14,86 ± 0,77	3,59 ± 0,46
Acetone		
100	4,13 ± 0,85	5,66 ± 1,00
80	7,38 ± 0,11	5,37 ± 0,11
60	11,63 ± 0,28	9,90 ± 0,41
40	10,13 ± 1,49	6,26 ± 0,61
20	8,95 ± 0,68	5,94 ± 0,22
Ethanol		
100	4,61 ± 0,23	4,60 ± 0,35
80	6,11 ± 0,70	5,54 ± 0,31
60	7,86 ± 0,69	7,13 ± 0,24
40	13,04 ± 0.084	6,18 ± 0,57
20	14,58 ± 0,14	4,26 ± 0,51

Meneses et al., (2013b) also reported that the more elevated total phenolic content was obtained when using 70% (v/v) acetone as compared to using water and ethanol. Their findings show that a less polar solvent (acetone) is able to recover higher amounts of phenolic compounds as compared to higher polar solvents such as water. The main reason for the verified differences may be due to the sample and type of origin or the differences in extraction procedures. The method of brewing and malting in the generation of the BSG may also be a contributor to the contrast findings in our results. Moreover, the application of aqueous acetone as solvent may lead to an unacceptable level of acetone residue in the extracts On the other hand our results are in agreement with several authors who reported the influence of the polarity of the extracting solvent as shown (Babbar et al., 2014; Roby et al., 2013; Zhao et al., 2006).

Roby et al., (2013) evaluated the influence of extracting solvents on the extraction of polyphenolic compounds from three aromatic plants (thyme, sage and marjoram) extracts. They concluded that methanol and ethanol were better solvents in the extraction of polyphenols from thyme than ethyl acetate and hexane with a range of 8.1 mg GAE/g DW to 3.9 mg GAE/g DW. Although Roby et al. (2013) did not evaluate water as a solvent, their results show that higher polar solvents were more efficient in extracting the three plant

materials than the less polar solvent. Dent et al. (2013) reported similar results to ours when they evaluated the effect of extraction solvent on the composition of polyphenols in Damlatian wild sage. They concluded that water was a greener and better solvent in extracting sage as compared to ethanol and methanol volume fractions in water.

Although our results do not agree with other authors in terms of the solvent with higher TPC, they have similarities in the trends exhibited by each solvent. The TPC values obtained in our results is lower than that observed by several authors which may again be attributed to the extraction procedure used. Our results showed that the amount of total phenolic content extracted from BSG is greatly influenced by the polarity of the solvent as shown by Dent et al., (2013) and Roby et al., (2013). However, this result was not in agreement with previous reports suggesting that binary solvent systems such as ethanol/water or acetone/water are more efficient than a mono-solvent system like water in the extraction of polyphenolic compounds in regards to their relative polarity (Moreira, 2012; Meneses et al., 2013a).

4.3.3 Antioxidant activity

This work evaluated the antioxidant activity based on two assays (FRAP and DPPH). Due to the complexity of phenolic compounds in BSG, there is need to perform more than one type of testing radical system.

4.3.3.1 Reducing power using FRAP assay

Figure 4.6 shows the reducing capacity of BSG extracts using the ferric reducing antioxidant power (FRAP) method. The results are expressed as millimoles of ferrous equivalent per dry BSG sample (mM Fe (II)/ g BSG). The assay uses the reduction of ferric ions to ferrous as an indication to the reducing capacity of a phenolic compound.

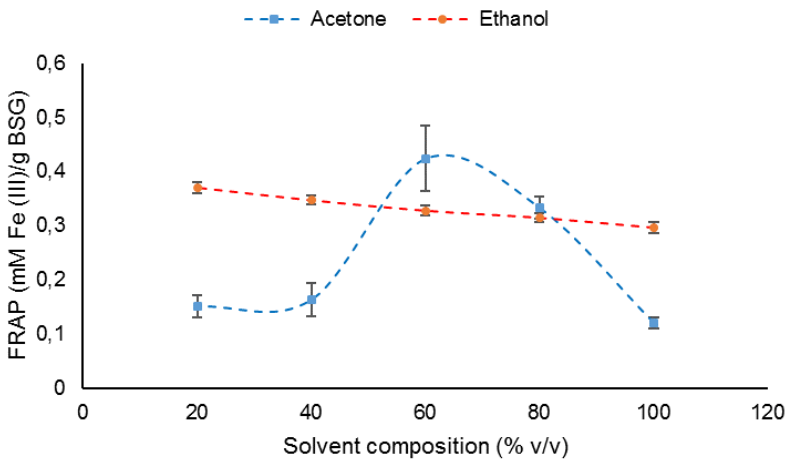


Figure 4.6: FRAP assay for the maceration extractions for acetone and ethanol solvents

Payne et al., (2013) reported that the FRAP assay is the only assay that directly measures antioxidants (or reductants) in a sample compared to other assays measuring inhibition of free radicals. The reduction capacity of a polyphenolic compound is directly related to the electron transfer ability of that compound and can therefore be used as a measure of its antioxidant activity. The presence of an antioxidant compound result in the reduction of yellow ferric tripyridyltriazine complex (Fe (III)-TPTZ) to a blue ferrous (Fe (II)-TPTZ) by the action of electron donating antioxidants as illustrated by

Equation 4.1

$$Fe^{3+} + e^{-} \rightarrow Fe^{2+} \qquad \text{Equation 4.1}$$

Table 4.3: Radical scavenging activity of BSG extracts assessed by FRAP. Values are expressed as mean ± SD

Solvent compositions (% w/w)	FRAP (mM Fe (III)/g BSG)	
	Research work	(Meneses et al., 2013a)
Water	0,615 ± 0,046	0,88 ± 0,15
Acetone		
100	0,121 ± 0,01	0
80	0,333 ± 0,02	2,75 ± 0,1
60	0,424 ± 0,06	4,15 ± 0,24
40	0,164 ± 0,03	2,53 ± 0,19
20	0,151 ± 0,02	1,66 ± 0,11
Ethanol		
100	0,297 ± 0,01	1,29 ± 0,12
80	0,315 ± 0,008	2,88 ± 0,40
60	0,328 ± 0,009	2,87 ± 0,18
40	0,347 ± 0,008	1,30 ± 0,23

20	0,370 ± 0,01	0,94 ± 0,22

The calculation of FRAP values is shown in Appendix C. The higher the FRAP value, the higher the reducing power and consequently the higher the antioxidant potential. All the extracts from BSG shown in Table 4.3 showed a low standard deviation from the average in their reducing power. The results show that extracts from water (0.615 ± 0.067 mM Fe(III)/ g BSG) has a significantly higher reducing power as compared to the extracts from acetone and ethanol solvent. These results indicated that extraction solvent had greater influence on the reducing power of BSG.

Our results do not agree with those of Meneses et al., (2013b) who also evaluated the antioxidant activity of BSG extracts using the FRAP method and obtained the highest reducing power value for extracts produced with 60% and 70% acetone. However, these results are in accordance with the TPC values obtained previously. This is supported the reports that the reducing power of extract might be due to the presence of phenolic acids such as ferulic acid, p-coumaric acid and caffeic acid, which contributes to the antioxidant activity by various mechanisms (Dent et al., 2013; Moreira, 2012; Babbar et al., 2014)

4.3.3.2 Radical scavenging activity towards DPPH free-radical

In this study, the extracts from all the solvents (water, ethanol and acetone) were also evaluated using the 2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) method and the results were expressed as antiradical power (ARP, %) as shown in Figure 4.7. DPPH possesses a proton free radical which decreases significantly on exposure to proton radical scavengers and is unaffected by certain side reaction of polyphenols such as metal ion chelation and enzyme inhibition (Babbar et al., 2014).

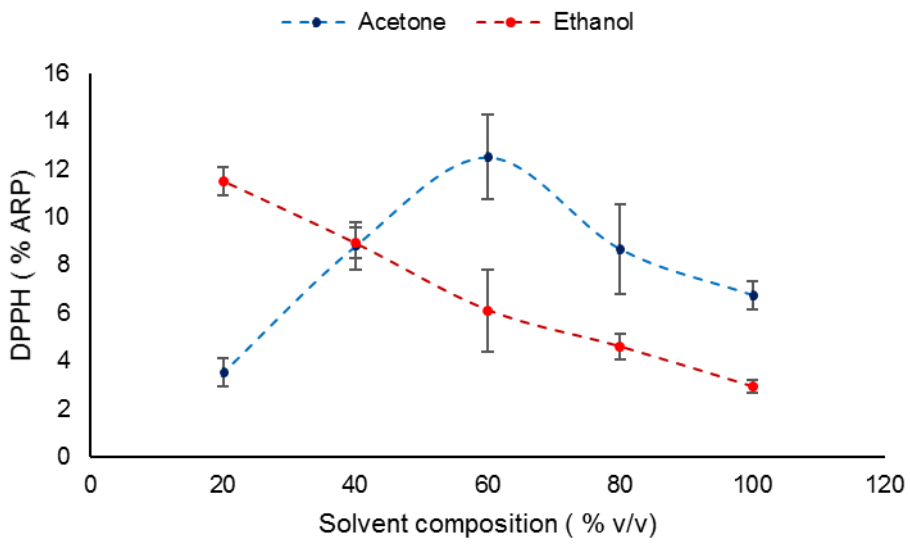


Figure 4.7: DPPH assay for maceration extraction using water, ethanol and acetone solvents

The results show that the best results in the DPPH assay were achieved when water (14.1 ± 0.5 % ARP) was used as a solvent and has the same trends as those obtained in the FRAP assay. The highest antioxidant activity was found to be for extracts obtained using water solvent. The optimum composition for acetone and ethanol was acetone 60 % (v/v) and ethanol 20 % (v/v) respectively. However, the standard deviation indicated by the error bars is big for most of the data points. This may have been caused by nature of the thick extracts obtained which were difficult to detect in the DPPH analysis.

Table 4.4: The reducing power of BSG extracts assessed by DPPH method. Values are expressed as mean ± SD

Solvent compositions (% w/w)	*DPPH (% ARP)*	
	Research work	(Meneses et al., 2013a)
Water	14.1 ± 0,50	3,33 ± 1,79
Acetone		
100	6,75 ± 0,58	13,5 ± 1,62
80	8,66 ± 1,85	20,6 ± 1,53
60	12,5 ± 1,77	18,5 ± 0,95
40	8,81 ± 0,98	13,0 ± 2,06
20	3,53 ± 0,59	7,46 ± 1,29
Ethanol		
100	2,93 ± 0,26	5,03 ± 0,56
80	4,6 ± 0,55	12,1 ± 0,07
60	6,12 ± 1,71	16,9 ± 0,78
40	8,91 ± 0,64	1,64 ± 0,41
20	11,5 ± 0,60	0

Table 4.4 shows the comparison of literature and this research work on the scavenging activities of the BSG extracts towards the radicals of DPPH. Extraction using water solvent had a higher DPPH scavenging activity, which is in accordance with TPC results. However, Meneses et al. (2013a) and Moreira (2012) reported that the highest DPPH inhibition were obtained with 70% v/v acetone (20.55 ± 1.53 % for BSG) and 60% v/v acetone (8.2 ± 0.3% for BSG) respectively. These authors also reported that the 70% v/v and 60% v/v acetone extracts were found to contain the highest levels of (+)- catechin and ferulic, caffeic, vanillic and p-coumaric acids and the water extracts had the highest values of protochateic and gallic acids. The values obtained in this work are generally less than those reported by Moreira, (2012) and Meneses et al., (2013a). These authors evaluated the antiradical activity of free, soluble and insoluble bound phenolics fraction showing the efficacy of their extraction procedure in extracting the polyphenols.

Authors have reported on the relationship between the chemical structures of the phenolic compounds and the DPPH scavenging activities. Brand-Williams et al., (1995) reported that polyphenols such as caffeic acid have a higher antiradical activity compared to monophenols such as p-coumaric acids. Moreover, they reported that the position of the hydroxyl group on the antioxidant determines the efficiency of the scavenging activities. Therefore, the diverse chemical structures of the polyphenols might contribute to the differences in the DPPH scavenging activities given for the three solvents. The results found in this work indicate that water might be a better antioxidant extraction solvent from BSG against DPPH antiradical power evaluation.

It can be concluded that water is the best solvent for this research work based on the results discussed above. Moreover, water is a green, non-toxic solvent which is beneficial to the environment and can increase the application of maceration extractions from BSG especially in the food industry.

4.4 OPTIMISATION STRATEGY OF BSG EXTRACTION

For a process to be developed for the extraction of BSG using water solvent, optimization of extraction conditions needs to be conducted to select the best operating conditions. The optimization of the extraction yield was carried out using RSM, according to (Montgomery et al., 2016).

4.4.1 Preliminary tests

Preliminary experiments were carried out to choose relevant variables. These included the study of the effect of grinding where the yield of unground BSG was compared to that of ground BSG without sieving. The effect of shaking speed was studied and the kinetic studies will be discussed later. Baldosano et al., (2015), Herodež et al., (2003), Pinelo et al., (2007) and Cisse et al., (2012) reported that particle size has a significant effect on the extraction of polyphenols. Figure 4.8 shows the study of the effect of grinding for the extraction of BSG using water.

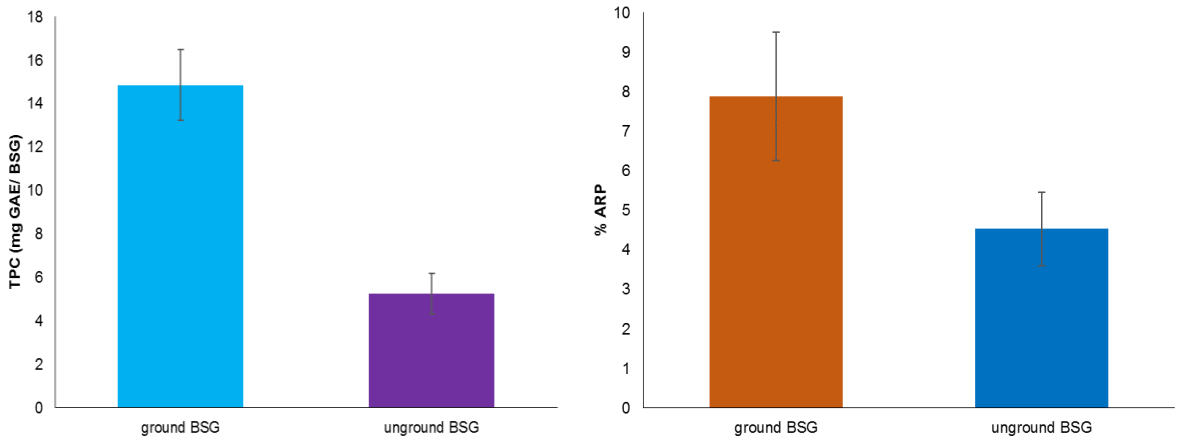


Figure 4.8: TPC and DPPH assay for ground extracts using water solvent. The bars were expressed as mean with error bars showing standard deviation

The results show that the highest TPC is obtained when using ground BSG (14.86 ± 0.772 mg GAE/g BSG). The grinding of BSG had a significant influence on the yield of BSG extracts. The sieving of BSG after grinding is not suitable for spent grains due to the uneven distribution of the polyphenols in the grain. Hence, it was preferred to grind and not sieve.

Sieving after grinding was attempted but the results obtained were not significant enough ($p > 0.05$) as shown in Appendix A. Therefore, the results could not be verified and reproduced. A noticeable difference was observed between the ground and unground BSG for the radical scavenging of the DPPH radical. Therefore, it can be concluded that grinding has a significant effect on the extraction of polyphenols from BSG.

4.4.2 Response Surface Method (RSM)

Response surface method (RSM) was used for process optimization. The experimental domain was based on literature and the solvent used on the results obtained in the selection of best solvent. The following parameters were selected (extraction time, solvent to feed ratio, temperature and shaking speed) based on literature data. The names of the process factors and levels are shown in Table 4.5. The process was studied using central composite design (CCD), a rotatable design with the axial star points set at 1.68179 coded units from the centre. Five responses: TPC, flavonol, FRAP, DPPH and p-coumaric acid were evaluated.

Table 4.5: Factors for response surface study

Factor	*Name*	*Level*	*Low Level*	*High Level*
A	Time	40.00	20.00	60.00
B	solvent to feed ratio	27.50	15.00	40.00
C	Temperature	40.00	20.00	60.00
D	Shaking speed	185.00	150.00	220.00

4.4.3 Actual Responses

The responses from the experiments carried out in the lab were entered into Design-Expert in the order shown in Table 4.6. The TPC is measured in mgGAE/g BSG, FRAP is measured in mM Fe (II)/g BSG and p-coumaric acid is measured in mg/g BSG

Table 4.6: Central composite design arrangement with responses for run 1 to 15

Run	*X_1:Time*	*X_2:solvent to feed ratio*	*X_3:Temperature*	*X_4:Shaking speed*	*TPC*	*FLAVONOL*	*FRAP*	*P-COUMARIC ACID*
1	40.00	27.50	40.00	185.00	2,51	1,63	7,51	0,13
2	20.00	40.00	20.00	150.00	4,33	0,68	5,43	0,08
3	60.00	40.00	60.00	150.00	2,34	1,23	6,54	0,13
4	20.00	40.00	20.00	220.00	1,17	0,56	3,50	0,12
5	20.00	15.00	20.00	150.00	11,48	3,55	17,82	0,30
6	60.00	40.00	20.00	220.00	159,32	86,07	171,78	11,14
7	40.00	27.50	80.00	185.00	0,95	0,58	34,46	0,07
8	20.00	15.00	60.00	220.00	11,34	5,34	8,13	0,64
9	40.00	27.50	40.00	255.00	7,90	4,19	18,84	0,33
10	40.00	27.50	20.00	185.00	0,54	0,29	2,87	0,02
11	40.00	52.50	40.00	185.00	1,30	0,66	4,63	0,03
12	60.00	15.00	20.00	220.00	4,47	1,07	6,89	0,13
13	20.00	15.00	60.00	150.00	0,78	0,29	2,06	0,04
14	40.00	2.50	40.00	185.00	2,35	0,89	6,45	0,13
15	80.00	27.50	40.00	185.00	7,20	6,56	21,38	0,37

Table 4.7: Central composite design arrangement with responses for run 16 to 30

Run	*X_1:Time*	*X_2:solvent to feed ratio*	*X_3:Temperature*	*X_4:Shaking speed*	*TPC*	*FLAVONOL*	*FRAP*	*P-COUMARIC ACID*
16	20.00	15.00	20.00	220.00	0,57	0,24	2,12	0,04
17	40.00	27.50	40.00	185.00	0,91	0,36	2,86	0,05
18	40.00	27.50	40.00	185.00	2,36	1,22	6,97	0,12
19	60.00	40.00	60.00	220.00	1,59	1,56	5,27	0,01
20	10.00	27.50	40.00	185.00	1,44	0,77	6,07	0,09
21	40.00	27.50	40.00	185.00	2,35	1,21	6,93	0,13
22	60.00	15.00	60.00	150.00	0,62	0,25	2,45	0,04
23	20.00	40.00	60.00	220.00	2,35	1,21	7,40	0,12
24	60.00	15.00	20.00	150.00	0,90	0,47	3,23	0,06
25	20.00	40.00	60.00	150.00	2,33	1,85	9,76	0,09
26	40.00	27.50	40.00	115.00	5,91	18,89	24,46	0,22
27	60.00	40.00	20.00	150.00	8,53	6,61	27,75	0,61
28	40.00	27.50	40.00	185.00	9,28	1,12	14,55	0,04
29	40.00	27.50	40.00	185.00	7,73	5,45	26,18	0,55
30	60.00	15.00	60.00	220.00	2,29	1,22	7,78	0,11

4.4.4 Selection of Model

Design-Expert provides a full array of response transformation and fits polynomial into the actual responses. The fit summary for TPC response shown in Table 4.8 and models for all responses were selected likewise.

Table 4.8: A summary of the statistics model of TPC response from Fit Summary on Design Expert

Source	*Standard deviation*	*R-Squared*	*Adjusted R-Squared*	*Predicted R-Squared*	*PRESS*	
Linear	28	0.173	0.040	-0.289	30600	
2FI	**23**	**0.561**	**0.329**	**-1.150**	**50900**	**Suggested**
Quadratic	26	0.570	0.168	-1.470	58600	
Cubic	15	0.930	0.709	-8.750	231000	Aliased

The suitable model was selected based on the adjusted R-squared and predicted R-squared values. The model comes out best when it exhibits low standard deviation, high R-squared values and a low “PRESS”. Although all models had low values of adjusted R-squared and predicted R-squared, the best suggested response surface model was 2 factor interactions (2FI) as shown in Table 4.8. The cubic model had the highest adjusted R-squared, however the model was aliased and could not be selected. The 2F1 model was suggested for all responses.

4.4.5 Analysis of variance (ANOVA) for the 2FI model

The analysis of variance ANOVA was performed to confirm the adequacy of the 2FI model (the model Prob>F is less than 0.05). the probability values for each individual term in the model was analysed.

Table 4.9: Responds surface equations and ANOVA results of the central composite optimisation of TPC, flavonol content, FRAP and P-coumaric acid by varying extraction time solvent to feed ratio, temperature and shaking speed

Response	*Model Equation*
TPC	$47.6 - 2.47X_1 - 3.41X_2 + 3.93X_3 - 0.48X_4 + 0.044X_1X_2 - 0.02X_1X_3 + 0.014X_1X_4 - 0.041X_2X_3 + 0.02X_2X_4 - 0.012X_3X_4$
Flavonol content	$24.69 - 1.21X_1 - 1.79X_2 + 2.19X_3 - 0.27X_4 + 0.024X_1X_2 - 0.015X_1X_3 + 7.17 \times 10^{-3}X_1X_4 - 0.022X_2X_3 + 0.011X_2X_4 - 6.33 \times 10^{-3}X_3X_4$
FRAP	$54.6 - 2.48X_1 - 3.31X_2 + 4.08X_3 - 0.55X_4 + 0.04X_1X_2 - 0.02X_1X_3 + 0.015X_1X_4 - 0.042X_2X_3 + 0.02X_2X_4 - 0.011X_3X_4$
p-coumaric acid	$1.75 - 2.48X_1 - 3.31X_2 + 4.08X_3 - 0.55X_4 + 0.04X_1X_2 - 0.02X_1X_3 + 0.015X_1X_4 - 0.042X_2X_3 + 0.02X_2X_4 - 0.011X_3X_4$

X_1 – Time (min), X_2 –solvent to feed ratio, X_3 - Temperature (^oC), X_4 –shaking speed (rpm)

The fitness of the 2FI model was evaluated based on 1) non-significant lack of fit test, 2) reasonable and significant R^2 values (Table 4.10) and the F-value.

Table 4.10: Anova for response surface 2FI model

SOURCE	*TPC*		*FLAVANOL*		*FRAP*		*P-COUMARIC ACID*	
	F Value	**p-Value (probe>F)**	**F Value**	**p-Value (probe>F)**	**F Value**	**p-Value (probe>F)**	**F Value**	**p-Value (probe>F)**
MODEL	2,420	0,046	2,210	0,065	2,200	0,067	2,460	0,044
X_1 (time)	1,880	0,187	2,220	0,152	2,650	0,120	2,030	0,171
X_2 (S/F ratio)	1,650	0,214	1,810	0,195	2,100	0,164	1,740	0,202
X_3 (Temp)	2,100	0,164	1,760	0,200	0,989	0,332	1,890	0,185
X_4 (shaking speed)	1,840	0,191	0,672	0,423	1,000	0,330	1,890	0,185
Lack of fit	63,000	0,0001	70,800	< 0,0001	12,400	0,006	100,000	0.0001
R-Squared	0,561		0,561		0,537		0,564	
Adj R-Squared	0,329		0,329		0,293		0,334	
Pred R-Squared	-1,140		-1,150		-1,090		-1,130	
Adeq Precision	7,430		7,430		7,350		7,420	

The results show that the model was significant for TPC (Prob>F = 0.046) and p-coumaric acid (Prob>F = 0.044) responses. Although there are small chances (< 5 % for all responses) that Model F-values could occur due to noise, all the responses have Model F-values less than 2 and this indicates that the model is significant. The “Adeq Precision" which measures the signal to noise ratio had a ratio greater than 4 for all models . The ratios for all responses are above 7 meaning the model used can be used to navigate the design space. Moreover all response models had negative “Pred. R-squared” values implying that the overall mean is a predictor of all my responses than the 2F1 model.

Based on literature data, it was expected that these factors would have a significant impact on the TPC and the antioxidant activity of the extracts obtained. However, the lack of fit F-values of all responses (TPC=62.999, Flavonol =70.782, FRAP =12.441, p-coumaric =100.074) were significant with a < 0.01 % chance that these lack of fit F-values of this large could occur due to noise. This maybe possibly because the selected variables (time, temperature, solvent to feed ratio and shaking speed) have no impact on the responses. The lack of fit might also mean the investigated variables affects the responses, however the effect is inadequate due to random error or noise. The small sample size used for experiments might have been too small to predict the impact of independent variables on the responses. Moreover, the R-squared value was calculated to be just above 0.500 for all models Table 4.10. This value is not close to 1 and implies that the variation of 50 % for the

responses is attributed to the independent variables, and another half of the total variation cannot be explained by the model.

4.4.6 Normal probability plot

Figure 4.9 shows the normal distribution of residuals for the TPC response. The normal probability plot is not linear for all response models indicating that the error terms are not normally distributed. There are too many extreme positive and negative residuals making the distribution to be “heavy tailed”. The relationship between the sample and theoretical percentiles is not linear for all response models.

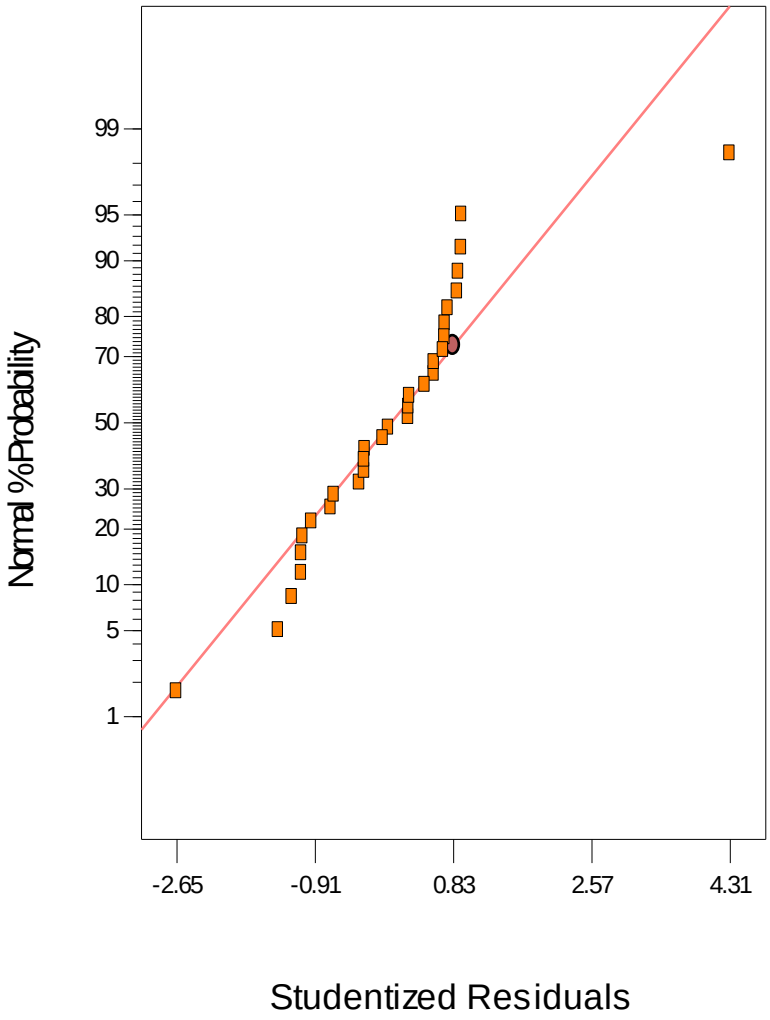


Figure 4.9: Normal probability plot for TPC response

Therefore, the optimization of this research work provided data that could not be fitted into any model. It can be concluded that the model does not provide a good explanation of the relationship between the independent variables and the responses (TPC, flavonol, FRAP and

p-coumaric acid). The model just happens to fit the data and does not signify the impacts that the independent variables have on the response but is insignificant.

1. 3D Surface Plots

The three-dimensional displays of response surface 3D plots shown in Figure 4.10 graphically illustrate how response varies as a function of two factors. The following plots shows how solvent to feed ratio and extraction time affects the responses (TPC, FRAP, p-coumaric acid and flavonol) at shaking speed of 185 rpm and temperature of 40°C.

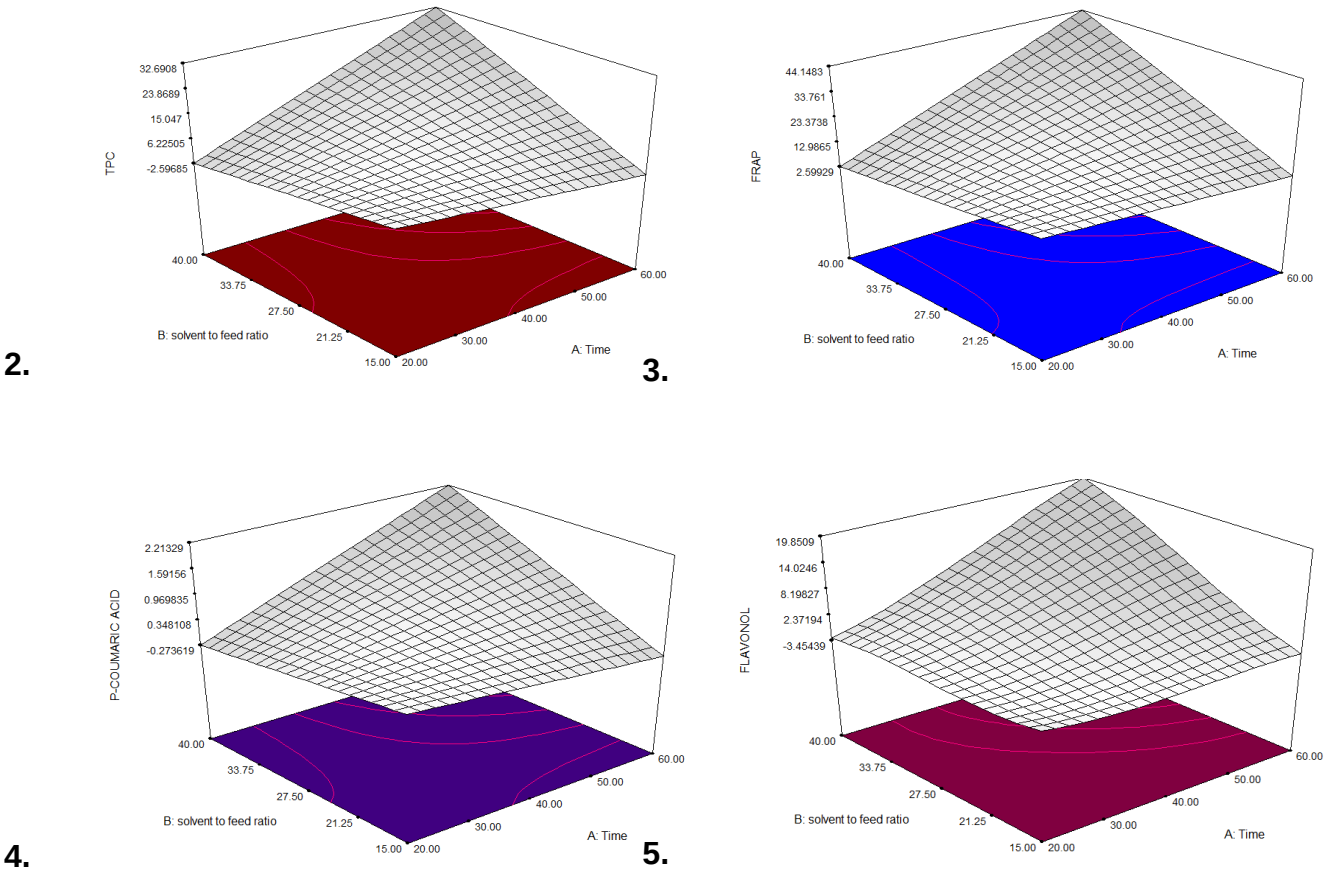


Figure 4.10: 3D Surface plots for TPC, FRAP, p-coumaric acid and Flavonol responses

The plots show that at high solvent to feed ratio and high time, more TPC, p-coumaric acid and flavonol are obtained. This can be explained to be due to the more contact time that will be created between the solvent and the solute during extraction which results in the recovery of more polyphenolic compounds. The more the polyhenolic compounds extracted, the greater the antioxidant activity of the BSG extracts. This is also shown by the higher reduction potential of the Fe (III) to Fe (II) complex compounds indicated by high FRAP values.

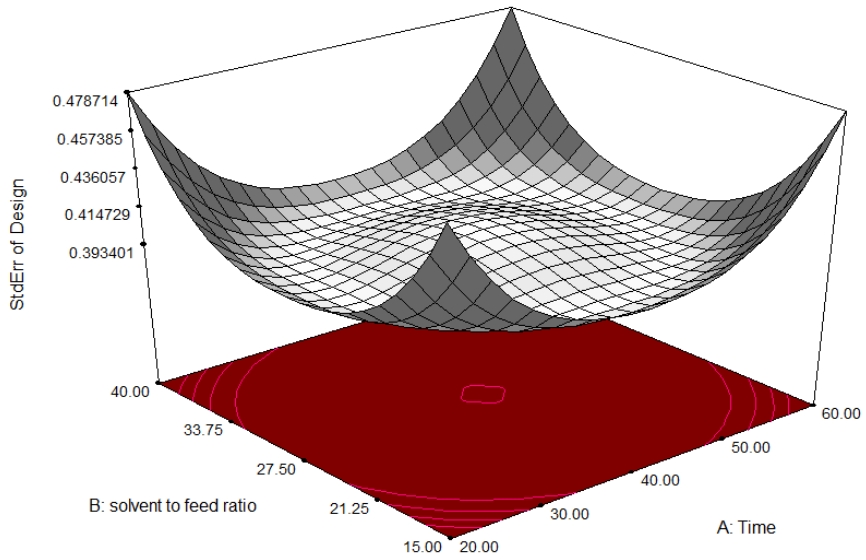


Figure 4.11: 3D plot of standard error

Figure 4.11 shows the 3D plot of the standard error of design. The response surface has a small standard error of design of 0.393 further indicating that the overall mean is a more accurate reflection and predictor of the response as a function of solvent to feed ratio and time than the model. The plot produces a bumpy bottom showing an inaccurate precision for predictions throughout the experimental region.

4.4.7 Verification of predicted data

Although the lack of fit was significant for all responses when using the 2FI model, the predicted values from the optimisation was very close to the experimental values obtained for the same responses at the same operating conditions. The predicted responses from the optimisation using RSM shown in Table 4.11, were compared with the results obtained in the laboratory at the following optimum conditions: time (40mins), S/F ratio (27.5:1), temperature (40℃) and shaking speed of 185rpm. Therefore, the optimisation conditions can be used for the kinetic studies.

Table 4.11: ANOVA prediction for the extraction of brewers spent grain at the optimum condition.

Response	*RSM prediction value*	*Actual experimental value*
TPC	8,904	7.01
FLAVONOL	1,831	0.245
FRAP	15,736	14.3
P-COUMARIC ACID	0,527	0.32

4.5 KINETIC STUDY

Figure 4.12 shows how the So and Macdonald model, Rate law, Peleg's and Fick's law models fit into the experimental data results obtained from optimum operating conditions. The fick's law model did have a good fit while other models fitted well. Non-linear regression was used to fit the model as shown in Appendix E and the best model was selected based on the highest R^2 value shown in Table 4.12.

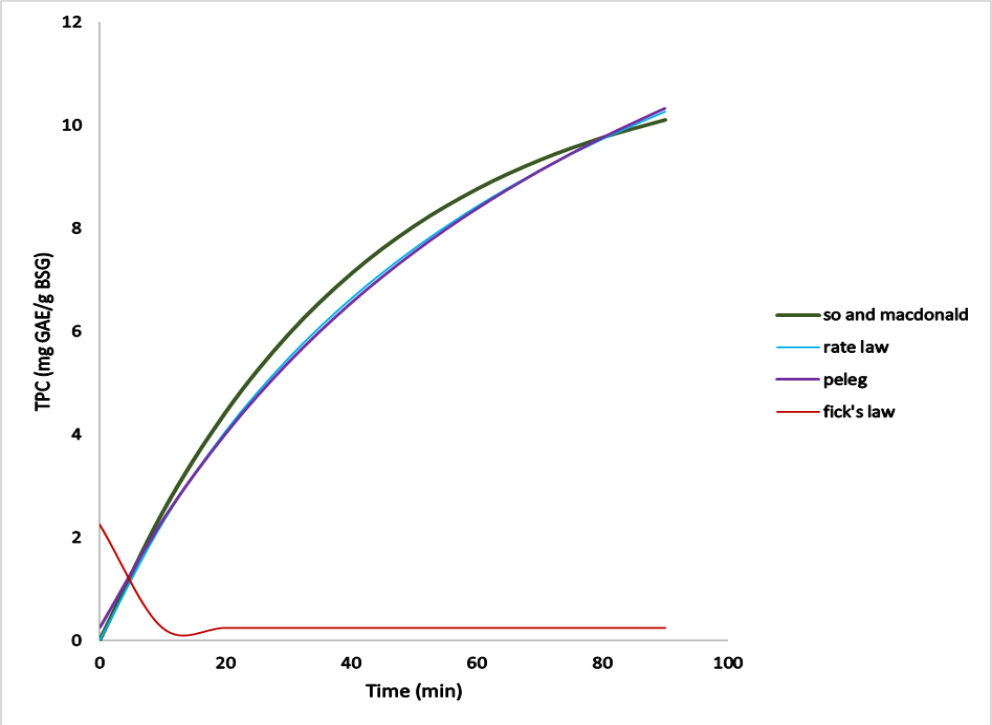


Figure 4.12: Fitting the models into experimental data

Table 4.12: R^2 values for the model fitted into experimental data

Model	*Equation*	*R^2 values*	*p-value*
So and Macdonald	$C^* = C^*_w(1-e^{-k_w t}) + C^*_d(1-e^{-k_d t})$	0.958	0.019
Rate law	$c = \frac{t}{(1/h)+(t/c_\infty)}$	0.95	0.017
Peleg's	$c = c_0 + \frac{t}{K_1 + K_2 t}$	0.915	0.007
Fick's law	$\frac{C}{C_0} = (1-b')e^{-k't}$	0.272	0.022

The So and Macdonald model with an R^2 value of 0.958 was selected as the best model for the extraction of polyphenols from BSG using water as a solvent. The model had been used

by Moubarik et al., (2011) for the extraction of polyphenolic compounds using conventional methods and was used in this kinetic study.

Figure 4.13 shows the influence of temperature on BSG extraction at a shaking speed of 185rpm, solvent to feed ratio of 27.5: 1. Water was used as the solvent and the experiments were carried out at 20℃, 40℃ and 80℃. The kinetic data obtained showed that the rate of BSG extraction increases with temperature. The highest rates of extraction were obtained for 80℃ after 15min. The results in Figure 4.13 showed that water extracts obtained at the extraction temperature of 80 °C contained the highest TPC.

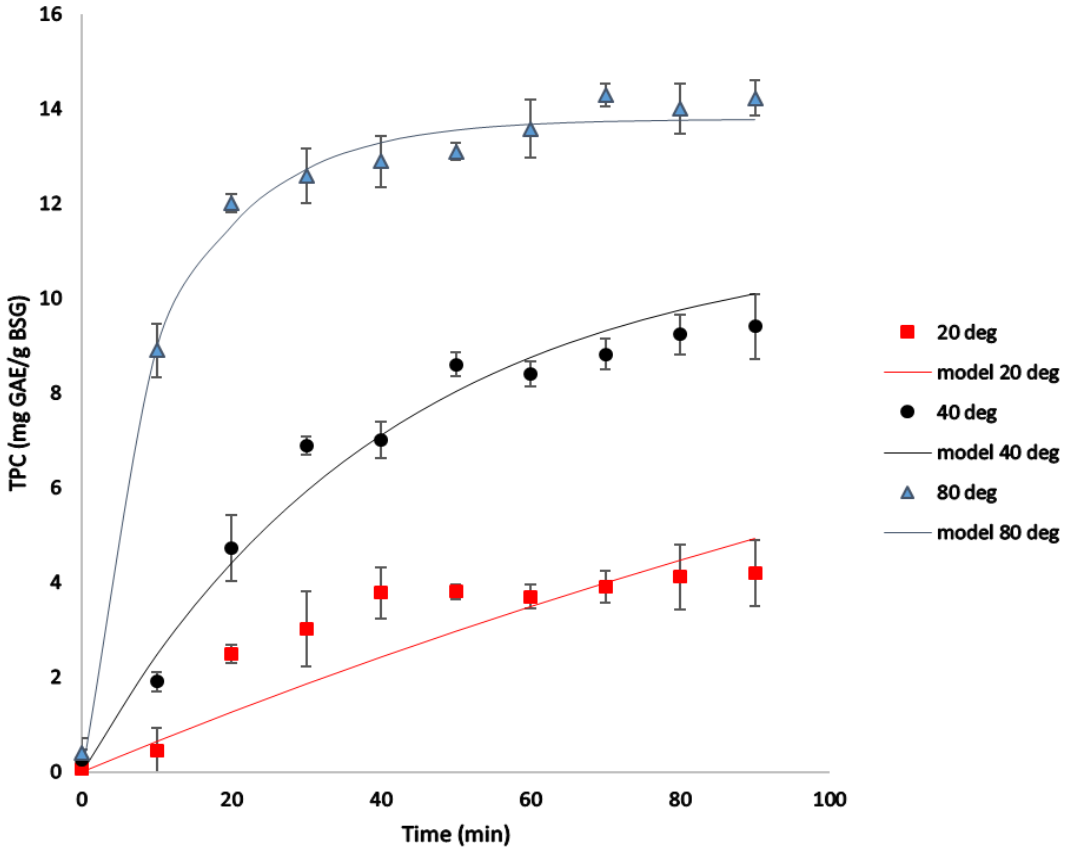


Figure 4.13: Influence of temperature on the extraction kinetics. The experiments were carried out at 20℃, 40℃ and 80℃

The results also showed a significant increase in TPC when the temperature rises. Several authors have found that for most plant materials, extraction at higher temperatures improves the extraction efficiency due to the permeabilisation of cell walls, increased solubility and diffusion coefficients of polyphenols, reduced viscosity of the solvent, reduced surface tension as well as enhanced hydrolysis and break-down reactions. Harbourne et al., (2009) and Vergara-Salinas et al., (2013) have shown that this rise in temperature increases the movement of solutes in the cells and reduces the strength of the hydrogen bonds, thereby

decreasing the energy required to break the matrix-solute interactions. Moreover, Ricardo et al., (2016) concluded that increasing the temperature above 65℃ also modifies the cell membranes by breaking part of the cell structure thereby increasing the mass transfer process. At high temperatures, the diffusion coefficient increases and the surface tension between the solvent and the solid matrix decreases thereby reducing the contact time. In addition, Budrat & Shotipruk, (2009) attributed the improve in extraction efficiency when temperature is increased to the decrease in the dielectric constant of the solvent which increases the solubility and extraction of low-polarity polyphenols.

Table 4.13 shows clearly that in the beginning of extraction the process is dominated by the washing mechanism ($k_w > k_d$). In this step, the particles on the surface of the grain are readily wetted by water and the release of total phenols and the antioxidant capacity increases over time as illustrated by Figure 4.8. In the first 15 min, TPC of up to 8mg GAE/g BSG is observed for 80℃ and this accounts for 56 % of the final TPC (14.4mg GAE/g BSG). Similar behaviour is observed for kinetics at 40℃ and 20℃, the washing rate appears to proceed for about 30 min irrespective of temperature as shown in appendix B. After the washing stage, the extraction rate decreases substantially as diffusion becomes the predominant process.

These results are in accordance with previous studies performed by (Meziane & Kadi, 2008) who concluded that the calculated mass transfer coefficients were for the washing stage were higher than the coefficients of the diffusion process ($k_w > k_d$). Table 4.13 present the mass transfer coefficients and concentrations at the equilibrium conditions calculated for the extraction of BSG at 20℃, 40℃ and 80℃ using the model proposed by So & Macdonald, (1986). Values expressed as mean ± standard deviation

Table 4.13: Mass transfer coefficient and concentration at equilibrium condition calculated by So and Macdonald's model (1986)

T (°C)	*Mass transfer coefficient*		*Concentration at equilibrium condition (mass %)*		*R^2*
	K_W	K_d	C_w	C_d	
20	0,930 ± 0,0262	0,0047 ± 0,0006	0,041 ± 0,013	11,10 ± 0,26	0,850
40	0,920 ± 0,0059	0,0308 ± 0,008	0,242 ± 0,003	15,02 ± 0,60	0,997
80	1,080 ± 0,0894	0,0915 ± 0,001	3,480 ± 0,012	10,41 ± 0,27	0,993

For the extraction of polyphenols, the predominance of the washing stage compared to the diffusion stage was observed for all temperature ranges. On average, the washing coefficients are 20 times higher than the diffusion coefficients $(k_w > k_d)$. There was a significant temperature effect among the concentrations at equilibrium conditions. The higher

the temperature, the greater amount of BSG extracts thus the equilibrium constants c_d and c_w increase with increasing temperature. These results were verified by (Toda et al., 2016) from a study of kinetics of soybean oil extraction using ethanol as a solvent and a temperature from 40℃ to 60℃. They observed that higher concentrations were found at higher levels of temperatures

In addition, the parameters of So & Macdonald model were fitted to the experimental data. The So & Macdonald model showed a very good fit into the experimental data for all temperatures 20℃, 40℃ and 80℃ with a coefficients of determination of r^2=0.85, r^2=0.997 and r^2=0.993, respectively as shown in Table 4.13. In adjusting the parameters to the experimental data it was considered that the washing step occurs in the first 15min for 80℃, 40min for 40℃ with a yield of extract representing 69% for 80℃ and 74% for 40℃ of the total phenolic content (TPC).

Figure 4.14 shows the effect of increasing the temperatures beyond 80℃. The rate was high at the beginning for 100℃, however it becomes constant after 20 min of extraction. This might have been as a result of degradation of compounds that were initially recovered. Several authors have reported on the negative effect which may result from increasing temperatures (Hanim et al., 2012; Pinelo et al., 2005). They concluded that higher temperatures cause degradation of polyphenols thereby decreasing the activity of extracts. Moreover, reaction of polyphenols with other compounds may occur and therefore inhibiting their extraction.

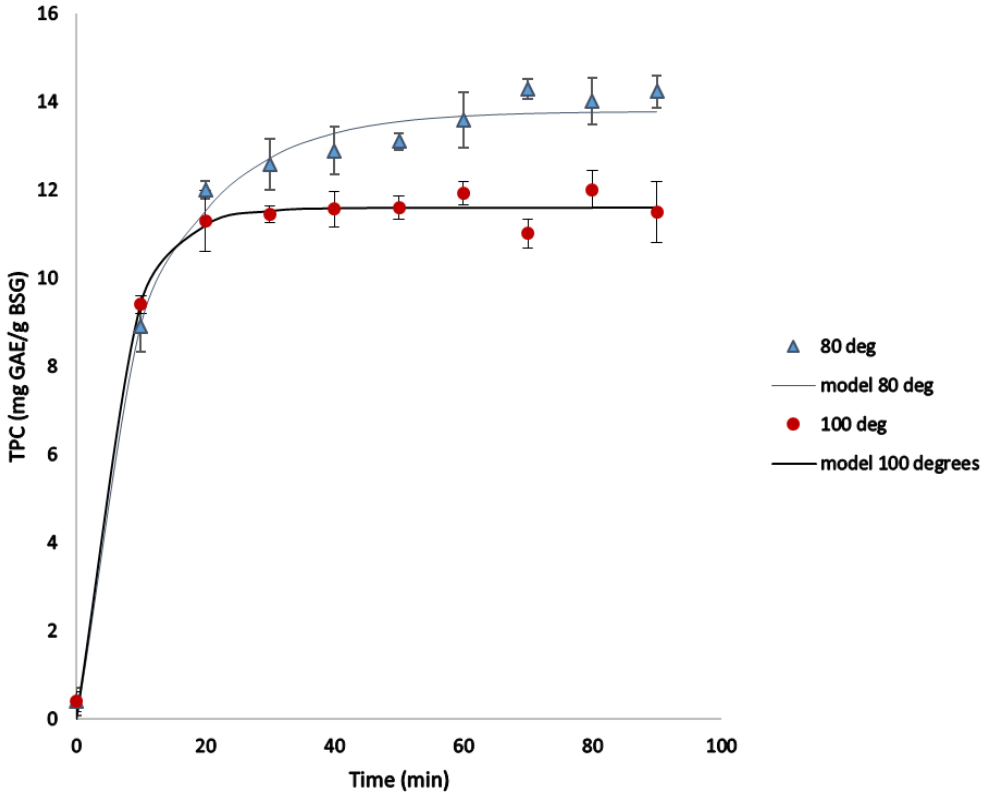


Figure 4.14: Influence of temperature at 80℃ and 100℃ on the extraction kinetics

4.6 OUTCOMES OF THIS CHAPTER

The aim of this chapter is to provide the necessary information for modelling and simulation of a process on the extraction of polyphenols from BSG, thereby achieving objective 1 and objective 2. In this chapter it was concluded that it takes 12 hours to remove all the free moisture from BSG. Based on the measurement of global yield, total phenolic content and antioxidant activity, water was found to be the best solvent. Moreover, the optimum conditions were evaluated to be at a temperature and time of 40 and 40 min respectively, with a solvent to feed ratio of 1: 27.5 and a shaking speed of 185 rpm. This analysis generated adequate data to commercialise this process. The key findings of this chapter are:

- The global yield was found highest in extracts obtained using water and a small difference was observed between acetone and ethanol solvents
- Experimental results showed significant increase in the global yield with temperature variations
- There were factors such as the pH that were not taken into consideration by the author that might have had an influence on the variations in the antioxidant activity of each BSG extracts from different batches. This made the reproducibility of data to be difficult because of different pH during extraction experiments and perhaps different types of BSG from the SAB

- The highest yield, TPC, reducing power using FRAP assay and the radical scavenging activity towards DPPH free radical was for extracts obtained using water solvent. This is in contradiction with literature data which indicates that solvent mixtures solubilizes polyphenols better than water
- In most of the analysis, a small difference was observed for extracts between ethanol and acetone solvents as well as their mixtures of the same composition
- Before planning experiments in experimental design, optimization was done on single factors; temperature, solvent to feed ratio and particle size. It was found out that each of these factors affect the amount of total phenolic content (TPC) extracted from BSG. An increase in temperature as well as solvent to feed ratio, increases TPC. The results for different particle size were found to be inconsistent
- The parameters such as particle size, extraction temperature and shaking speed suggested by literature data as significant did not have impact on the outcomes in this research work as indicated from the results of optimization experiments using response surface method
- The 2FI model selected for the optimization of the experiments using response surface method was found to be significant and that is good, however the lack of fit was also significant which is bad for the model. Hence the results from the optimization showed that the selected independent variables from literature data did not have impact on the responses for this work
- The rate of extraction was found to be 15 min, irrespective of the increase in temperature and the washing step was calculated to be more predominant than the diffusion stage
- There were different individual components that were produced from different samples of BSG. For example, in the first set of samples, the individual components that were identified were p-coumaric acid, rutin and kaempferol. However, in the second and third set of samples for exactly the same experiments, only p-coumaric acid was identified
- The use of high temperature during extraction resulted in the degradation of compounds hence the instability of results
- The BSG extracts obtained using water and acetone were difficult to analyse mainly because of the formation of a precipitate in the samples. This was especially during the analysis using DPPH
- An insignificant colour change was observed for all samples at different times. The liquor only got thicker but with minor colour changes
- Sieving the BSG after grinding resulted in results that were not consistent hence the effect of grinding had to be studied instead. This might be because of the uneven distribution of the polyphenols in the grain

4.7 NOMENCLATURE

Symbol	Description
ANOVA	Analysis of variance
ARP	Antiradical power
CCD	Central composite design
C	the solute concentration at time (t)
C_d	The final solute concentration in solution due to the diffusion stage alone
C_w	The final solute concentration in solution due to the washing stage alone
FCI	Fixed capital investment
FRAP	Ferric reducing antioxidant power
GA	Gallic acid
GAE	Gallic acid equivalents
HPLC	High performance liquid chromatography
k_d	The kinetic coefficient for the diffusion stage
k_w	The kinetic coefficient for the washing stage
mM	Milli moles
QE	Quercetin equivalents
SD	Standard deviation
t	Time
TCI	Total capital investment
TE	Trolox equivalents
rpm	Revolutions per min
µL	Micro Litre
R^2	Coefficient of determination

CHAPTER 5 MODELLING, SIMULATION AND ECONOMIC ANALYSIS

5.1 INTRODUCTION

This chapter presents modelling, simulation and economic analysis of the production of BSG extracts using a simulation tool, SuperPro Designer® v9.0 (Intelligen, 2017). A base case simulation was constructed from the laboratory extraction process. The analysis of four other alternative schemes enabled the optimization of the economics. The selection of the best profitable scheme was based on three indicators, payback period, return on investment (ROI) and net present value (NPV). To the best of our knowledge, this is the first report to evaluate the commercial viability of the maceration extraction of polyphenols from BSG using SuperPro Designer®.

5.2 MODELLING AND SIMULATION

The base case simulation model is a scenario constructed from the laboratory experiments carried out at the Chemical Engineering Department Laboratory, Cape Peninsula University of Technology (CPUT). The model was developed based on the following:

- The global yield, drying time, extraction time were adopted from the laboratory experiments done in this work
- The flow rates of all unit procedures was assumed to be 600 kg/h
- The throughput size of unit procedures was adapted from Couper, (2005) and the rules of thumb
- The set up time (SUT) for all unit procedures was assumed to be 5 min.

5.2.1 Process description

Figure 5.1 shows the process flow diagram of a lab scale production of BSG extract. The production consists of three main operations, drying of the BSG feed, solid-liquid extraction (in an oven shaker) and evaporation.

Wet BSG from SAB, Newlands Brewery, Cape Town, is put in a tray dryer for 12 h (determined from the lab). The moisture content has been reported in literature to be about (77 % to 85 %) (Aliyu & Bala, 2013) for wet BSG which is reduced to a range between (10 % - 4 %) (Ström, n.d.; Aboltins & Palabinskis, 2015).

The solvent to solid ratio determined from the optimisation of the lab experiments using response surface method was 27.5 : 1. However this ratio is not economically feasible hence this study considered a solvent to solid ratio of 10 : 1. In the first stage of extraction, the samples are heated in an oven shaker at 40 ℃ and a speed of 185 rpm. These extraction

operation continues for a period of 30 min. The time was determined from the extraction kinetics done in this research. The results show that maximum rate of extraction is at 30 min. Upon completion of extraction, the samples are then filtered and the extract water is taken for analysis. To determine the production yield and the time of extraction, experiments were done at lab scale as illustrated with the process flow diagram in Figure 5.1. BSG was put in an extraction vessel with the solvent-to-feed ratio maintained at 10: 1. The extract water filtered was dried in a spray dryer and the powder extract was weighed to get the mass per batch and the overall process yield.

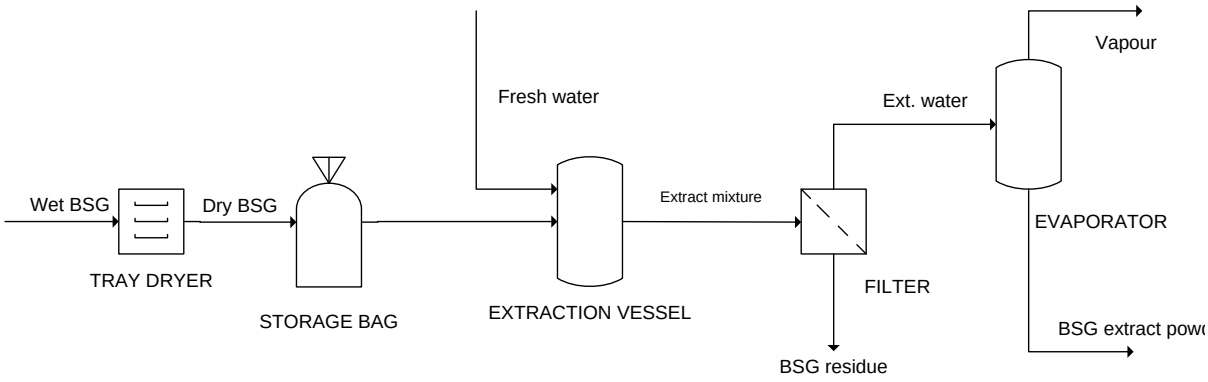


Figure 5.1: **PFD for the laboratory experiments**

5.2.2 Scaling up

The scale up procedure assumed that the pilot and industrial scale units have the same performance as the laboratory scale when the solvent-to-solid ratio (S/F) between the mass of solid and solvent are kept constant. In addition, the true density of the substrate and operational conditions are kept constant (Veggi et al., 2011). In this work, using the solvent to solid ratio of 10:1, 56kg of dry BSG was extracted using 560 L of fresh water.

5.3 DEVELOPMENT OF BASE CASE SCHEME

Figure 5.2 shows the development of the base case simulation model. BSG is a biomass and the component does not exist in the simulator database. Therefore, user defined components were approximated for BSG, BSG extract and BSG residue. Batch operating conditions were used in the simulation model to give a clear presentation of the nature of the process. This involved a few operations that take place in a sequence within a single unit procedure (intelligen, 2017). Individual operations such as charging of material, material heating and extraction processes take place in a single vessel.

5.3.1 Operations modes

Based on the laboratory experiments, the extraction of bioactive compounds from BSG is in the batch mode instead of continuous with a default annual operating time of 7920 h.

5.3.2 User-defined components

In this thesis, BSG was the user-defined component. Since the grain is a mixture of many materials with different composition. The author kept the density and mass composition as that of biomass as well as registered the actual price for BSG to be used in the economic analysis.

5.3.3 Estimated prices for simulation

Table 5.1 illustrates the prices for materials that were used in this process and the sources from which they were taken from. The price of BSG extract was based on the price of p-coumaric acid sold on the world market, as indicated in Sigma Aldrich sales catalogue, as explained in chapter 3.6.1.2.

Table 5.1: Purchase or Selling Price of materials used in the process

Materials	*Purchase/Selling Price ($)*	*Source*
Wet BSG/Kg	0,53	Fernandez-Pérez et al., 2008; Buffington, 2014; Lynch et al., 2016
BSG Extract/Kg	8,17	Sigma Aldrich
BSG Residue/KWh	0,38	Fernandez-Pérez et al., 2008
Water solvent/kL	0.006	City of Cape Town

5.3.4 Unit operations construction

Based on the BSG extractions carried out in the lab. The unit operations were simulated as described below. The base case simulation is shown in Figure 5.2.

5.3.4.1 Tray dryer

The first operation P-1 involves charging of 100 kg raw materials of wet BSG in a tray dryer. Literature has reported that the drying takes 18 h to dry wet BSG from a moisture content of 80 wt.% to 4 wt. % (Aboltins & Palabinskis, 2015; Ström, n.d.; Aliyu & Bala, 2013). Laboratory experiments were conducted to obtain a drying curve shown in Figure 4.1, and from the curve, the drying time was determined to be 12 h. The internal circulating rate was

adapted as 5 % wt. gas/wt. evaporated and the specific power used was 6.5 kW/m^2 (Couper, 2005: 248). The drying time was set based of rate of evaporation of 99.3 kg H_2O /h (Couper, 2005: 248).

5.3.4.2 Storage drum

The dried BSG is transferred into the next operation which is storage. A hermetically sealable storage vessel was used for storage until the material was transferred to the next subsequent procedure, extraction P-2.

5.3.4.3 Extraction vessel

Charging of 560 L extraction solvent (fresh water) is done at the end of transferring dried BSG. After charging of water, heating of the water-BSG mixture is done to 60 ℃. The transfer rate was estimated to be 60 kg/hr. The extraction process took place when the materials was transferred out of the storage drum. The extraction vessel was maintained at 60 ℃ for 30 min. A product yield of 4.4 wt % was considered based on the overall global yield obtained for extractions using water solvent from the laboratory experiments done in this work.

5.3.4.4 Vibrating screen

The extract water and solid mixture was transferred to a vibrating screen P-3 in the model representing the filter in the laboratory. This operation completely removed the BSG residue and 2 % of the extract-water was lost. The separation was almost complete with the overflow only containing liquid as adapted from Couper, (2005). The remaining extract water was transferred to an evaporator P-4 to dry the extract into a powder.

5.3.4.5 Evaporator

The standard power of the evaporator was estimated to be 10 kW (kg/h) (Branan, 2002). From the rules of thumb evaporation product losses are 1 % of the circulation for every 42 ℃ (Branan, 2002). The evaporation process was analysed from laboratory experiments done in this work. It was observed that 95% of the water was removed using the rotary evaporator after 4 hours. The weighing was done using a mass balance. After adding necessary operations to each unit procedure, scheduling is done to quantify the number of batches. The following section describes how scheduling was done in this thesis.

5.3.5 Scheduling time

As the manufacturing process is carried out in batch operation, effort have been made to document the scheduling details of each processing steps. This includes the setup time (SUT), process time (PT), and start time (ST) of each individual process. Where the time periods for certain operations were unknown and could not be reasonable estimated, default times were adopted. The scheduling summary sheet is shown in Table 5.2.

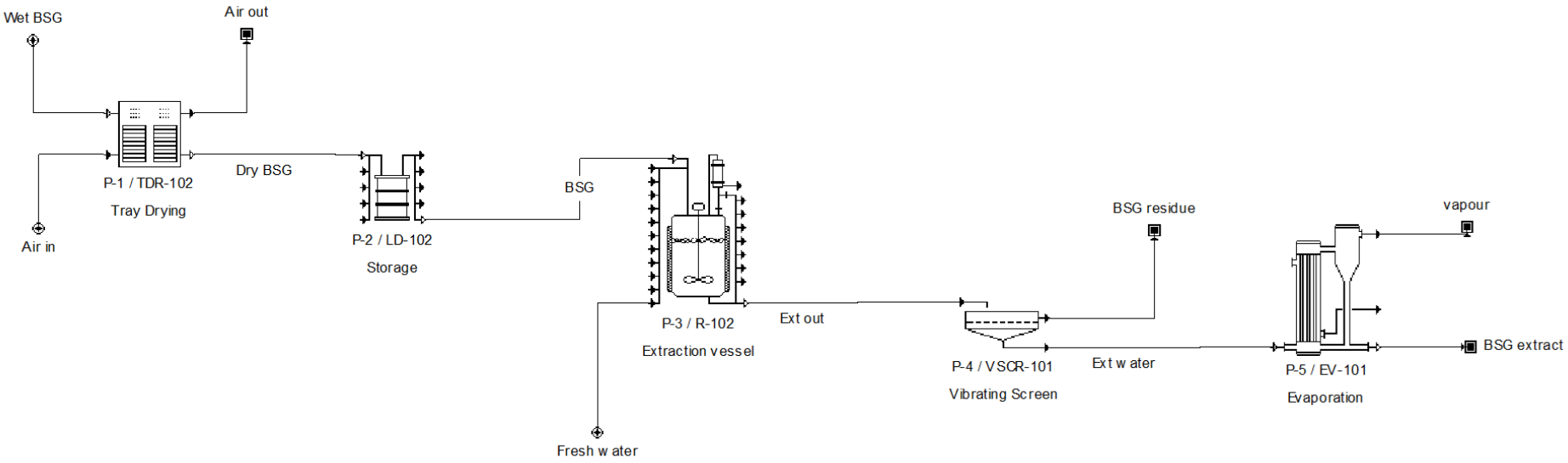


Figure 5.2: Base case simulation flowsheet

Table 5.2: Scheduling summary of the base case model

Procedure	***Operation***	***Process scheduling***
P-1/TDR	Charge BSG	SUT 5min
		PT = 5min
		ST= Beginning of the batch
	Dry 1	SUT = 5min
		PT = 99.3Kg H_2O/hr
		ST= after charge BSG ends
	Transfer out I	SUT 5min
		PT = 600kg/h
		ST= after dry ends
P-2/Storage	Transfer in I	SUT 5min
		PT = 5min
		ST= start with transfer out
	Store 1	SUT= 5min
		PT = 2 hrs
		ST= after transfer in
	Transfer out II	SUT 5min
		PT = 600kg/h
		ST= after dry ends
P-3/R-101	Transfer BSG	SUT 5min
		PT = Based on mass flowrate of 60kg/hr
		ST= Beginning of the batch
	Charge fresh water	SUT = 5min
		PT = Based on volumetric flow rate of 60kg/hr
		ST=After charge BSG ends
	Heat 1	SUT=5min
		PT =30mins
		ST = After charge fresh water ends
	Extract (batch stoic. react)	PT =1 h
		starts with heat 1
	Transfer out extraction mixture	SUT =5min
		PT= master slave relationship with P-2
		ST= after extract 1 ends
P-4/ VSCR-101	Solid-liquid separation (filtration)	SUT =5min
		PT = 1h
		ST = Starts with trans-out-1 in P-1

Table 5.3: Scheduling summary of the base case model (cont.)

Procedure	*Operation*	*Process scheduling*
P-5/ V-102	Transfer in Ext. Water	SUT =5min
		PT= master slave relationship with P-2
		ST=Starts with filtration in P-2
	Temporary storage	PT=2h
		ST= starts with TRANS-IN-1
	Transfer out storage product	SUT =5min
		PT =based on mass flow rate of 600kg/hr
		ST= after store-1 ends
P-5/EV-101	Evaporation (Evaporate-1)	SUT = 5min
		PT =4h
		ST = Starts with trans-out-2 in P-3
	Chamber cleaning (CIP-1)	PT= 15min
		ST = after DRY-1 ends

After the process is developed, scheduling was done to ensure that all the activities were correctly sequenced and sufficient labour was allocated to each procedure (Pedrites et al., 2011). The following assumptions were made:

1. The set up time (SUT) was assumed to 5 min for all operations
2. The process time (PT) based on the mass flow rate was estimated to be 600 kg/h for all units.
3. The volume for all unit operation was adapted from the book chemical process equipment- selection and design (Couper, 2005)

5.4 SIMULATION PROCESS

After the process specifications were completed, the process model simulated by selecting the “Tasks: Solve M & E balances” and “Tasks: Perform Economic analysis” option from the main menu, the full results were viewed and analysed for the material balances and the economics of the base case simulation. The same simulation procedure was done for all the developed schemes.

5.5 SIMULATION RESULTS

After simulating the process, the results are made available in the form of charts, reports and executive summary. These outputs are found on the main menu and the reports can be generated in excel spreadsheets by the software.

5.5.1 Material balances

The main process parameters of the base case simulation are summarized in Table 5.4. The annual operating time is 7913 h with an annual throughput of 22947 kg of BSG extract per year. 770 batches can be executed per year and the amount of BSG extract that can be produced per batch is 29.80 kg. The tray dyer is the bottleneck equipment resulting in a process cycle time of 12.42 h. The material balances of each individual equipment are shown in the next section.

Table 5.4: Main process parameters of the base case simulation

Process parameter	***Quantity***	***Units***
Annual Operating Time	7914	h
Annual Throughput	22900	kg MP/yr
Batch Throughput	29,80	kg MP
Batch Time	12,42	h
Number of Batches per Year	770	

Table 5.5, Table 5.6, Table 5.7 and Table 5.8 show the material balance generated around the dryer, extractor, centrifuge and the evaporator respectively. These results were calculated by SuperPro Designer®, based upon the input parameters specified for relevant operations such as material charges. In addition to calculating the overall raw material requirements, process simulators calculate the amounts and compositions of each individual stream (inputs, intermediates and outputs). This provides useful information for verifying results related to material transformation and separations, liquid and solid waste management, emissions, and equipment capacity requirements.

Table 5.5: Material balance for a tray dryer P-1

Stream Name	Wet BSG	Air in	Air out	Dry BSG
Temperature (°C)	25,00	170,00	100,00	70,00
Component Flowrates (kg/batch)				
BSG	56,00	0,00	0,00	56,00
Nitrogen	0,00	139,04	139,04	0,00
Oxygen	0,00	36,96	36,96	0,00
Water	44,00	0,00	35,20	8,80
TOTAL (kg/batch)	100,00	176,00	211,20	64,80

From the material balances around a tray dryer, the moisture content was reduced from 66 % to 13.7 %. This is in agreement with (Ström, n.d.)**,** who reported that the moisture content of different test of BSG ranged from 65.7 % to 10.6 %. the drying time was determined experimentally as mentioned earlier in chapter 4. In optimizing the plant design for this simulation, considerations maybe given to other drying technologies such as superheated steam drying. Stroem et al., (2009) evaluated the superheated steam drying of BSG in a rotary dryer as a way of saving energy and minimizing the sticking of BSG to the drum. The value of BSG in their study was improved by increasing the energy efficiency to a range between 60 % to 76 %. Therefore, more drying options can be explored in this study.

Table 5.6: Material balance for extractor P-2

Stream Name	BSG	Fresh water	Ext out
Temperature (°C)	70,00	25,00	25,00
Component Flowrates (kg/batch)			
BSG	56,00	0,00	5,60
Ext out	0,00	0,00	1,51
Water	8,80	557	566
TOTAL (kg/batch)	64,80	557	622

BSG and water solvent were fed into the extraction vessel with a ratio of 1:10. SuperPro Designer® modelled a global yield of extraction of 27 % (1.51kgof Extract out of 5.6kg of BSG fed). This global yield is higher than the one obtained in the laboratory experiments for the extraction of BSG using water as a solvent (9.31 % ± 0.002). These results may mean that the simulation did not take into account the polyphenols that would have been lost during brewing.

Table 5.7: Material balance around a Vibrating screen P-3

Stream Name	Ext out	BSG residue	Ext water
Temperature (°C)	25,00	25,00	25,00
Component Flowrates (kg/batch)			
BSG	5,60	5,60	0,00
BSG-EXTRACT	1,51	0,00	1,51
BSG-RESIDUE	48,89	48,89	0,00
Water	566	11,32	555
TOTAL (kg/batch)	622	65,80	556

The extract mixture from the extraction vessel is very watery with a few solids. A vibrating screen was selected to separate the extract water from the BSG residue. The vibrating screen separates all solids from the water.

Table 5.8: Material balance around the evaporator

Stream Name	Ext water	vapour	BSG extract
Temperature (°C)	25,00	40,00	40,00
Component Flowrates (kg/batch)			
BSG	0,00	0,00	0,00
BSG-EXTRACT	1,51	0,00	1,51
Water	554	527	27,73
TOTAL (kg/batch)	556	527	29,24

Based on the annual operating time of 7914 h and minimum cycle time of 12.42 h, the annual batch production for extraction of BSG extracts is calculated as 770 batches. This corresponds to 22500 kg of BSG extracts per year. This production rate is not sufficient enough to meet the local and international demand. Table 5.12 shows the equipment data with 2017 prices from the market.

Figure 5.3 shows the Gantt chart which illustrate the execution of operations to get a batch of 12.42 h. The longest procedure is the tray dryer with 10.27 h followed by the evaporator with 4 h. This mean that this process can only accommodate 2 batches per day. Normally a day has three working shifts with 8 h in each shift. This implies that one worker will work on both batches and there is need to reduce the cycle time and add the third shift.

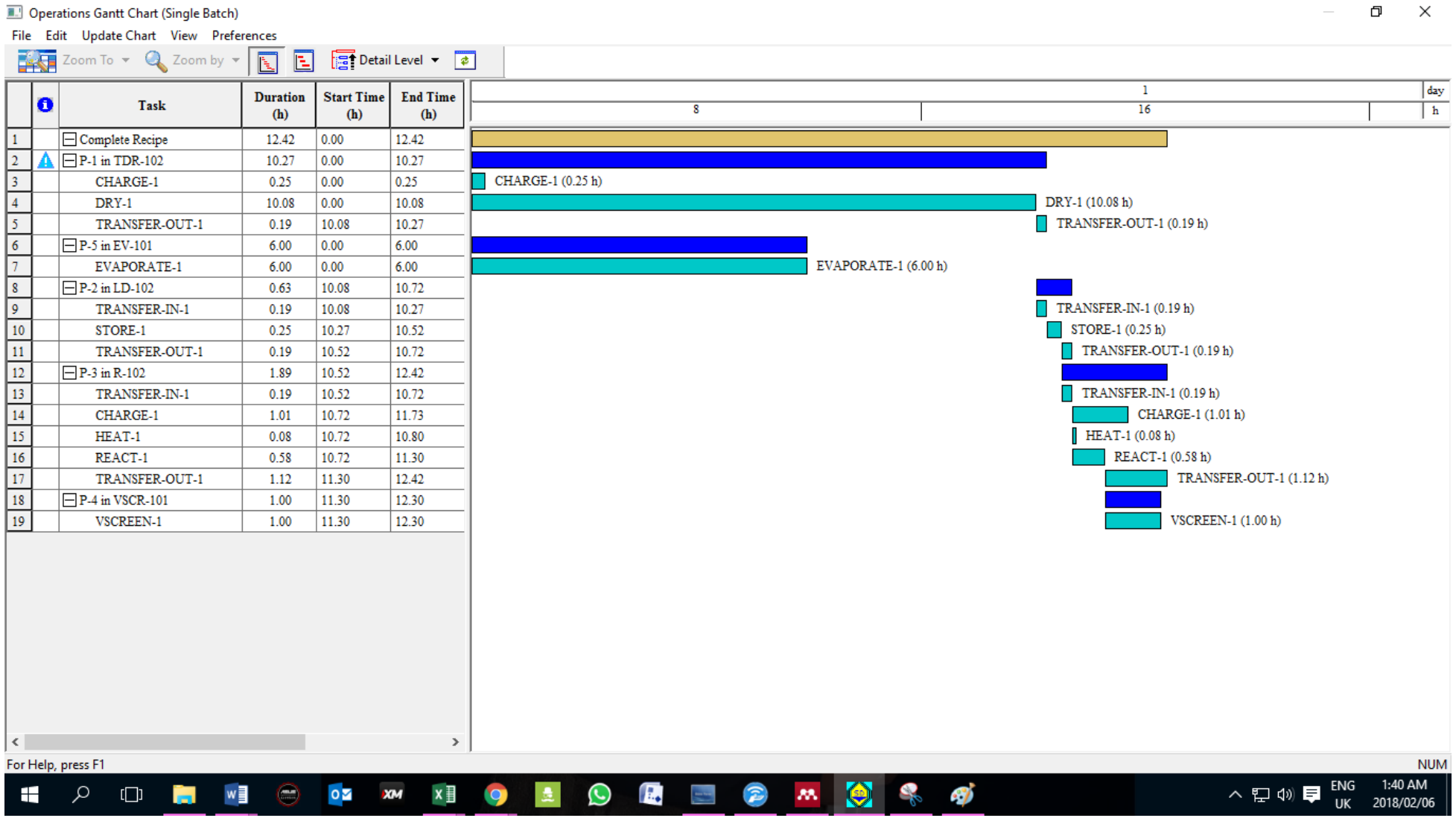


Figure 5.3: Gantt chart for the base case scheme

5.6 ECONOMIC ANALYSIS FOR THE BASE CASE SIMULATION

Economic analysis was conducted on the base model. Table 5.9 shows the economic analysis for the extraction of BSG extracts for the base case. The base model is economically infeasible due to the lower annual production and consequently lower revenue produced. The return on investment (ROI) is as low as -51.10 %. The process makes a loss because the payback time is infinity meaning the production will not return the investments made. Hence to increase the process throughput, efforts were made to identify the process bottleneck that limits the current production. Athimulam et al. (2006) describes bottlenecks as process limitations that are related to equipment or resource such as demand for various utilities. In batch manufacturing, size bottleneck and scheduling bottleneck can be identified.

Table 5.9: Economic analysis for the base case scheme

Process parameter	***Quantity***	***Units***
Throughput		
Batch Throughput	29,80	kg MP/batch
Annual Throughput	22947,00	kg MP/yr
number of batches per year	770	batches
plant batch time	12,42	h
Annual operating time	7914	h
Economics		
Total Investment	5682901,00	$
Total Revenues	0,00	$/yr
Operating Cost	3400146,00	$/yr
Unit Production Ref. Rate	22948,00	kg MP/yr
Gross Margin	-1,00	%
Return On Investment	-51,10	%
Pay-back time	N/A	yr
IRR After Taxes	N/A	%
NPV at (7.00 % interest)	0,00	$

The base case is economically unattractive and efforts were made to improve it. The process of improving it is known as debottlenecking. This strategy helps to identify the bottlenecks that limits the current production. The bottlenecks are either related to equipment or resources and are known as size bottlenecks or scheduling bottlenecks. SuperPro Designer® provides charts that helps to identify the bottlenecks.

5.6.1 Debottlenecking strategy to improve production and profitability

Figure 5.4 shows the throughput analysis chart for the base case simulation that helps identifies bottlenecks of the process. Throughput Analysis Chart that displays capacity utilization, equipment uptime and combined utilization.

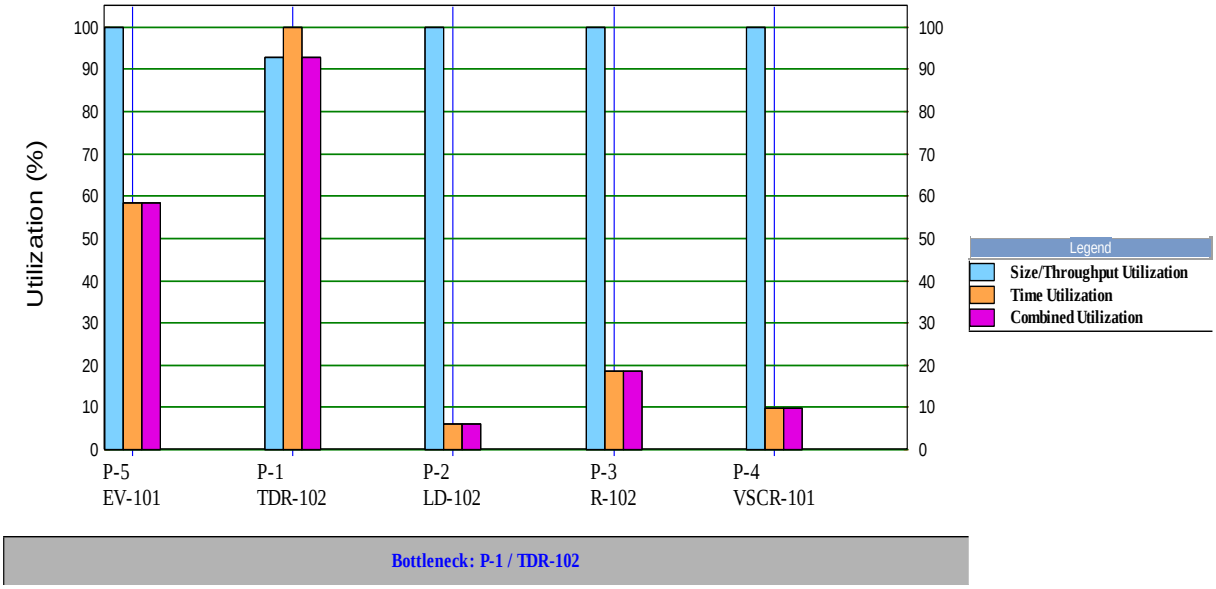


Figure 5.4: Throughput Analysis Chart for base case simulation.

The annual throughput of a batch process is the product of the batch size and the number of batches produced per year (Pedrites et al., 2011). Therefore, to increase the annual throughput, either the batch size or the number of batches has to be increased. In this base case simulation, most equipment has maximum size utilization showing operation is at maximum for the process. Addition and alteration of extra equipment was considered to increase the number of batches. This debottlenecking strategy was used to reduce the cycle time on the evaporator EV-101 and tray dryer TDR-102.

The equipment occupancy chart in Figure 5.5 further suggests that there is need to reduce the time occupied by the tray dryer and the storage as they flow of production and the number of batches. The unit procedures of each batch are shown with a different colour. The tray dryer and the evaporator have the longest cycle time and therefore constitute the scheduling bottlenecks for the base case. Although the evaporator is not a bottleneck, the produced BSG extract is not in the desired form. The evaporator was not able to remove all water to give a dry powder. Hence, additional equipment such as a spray dryer is needed to increase the quality of BSG extract powder.

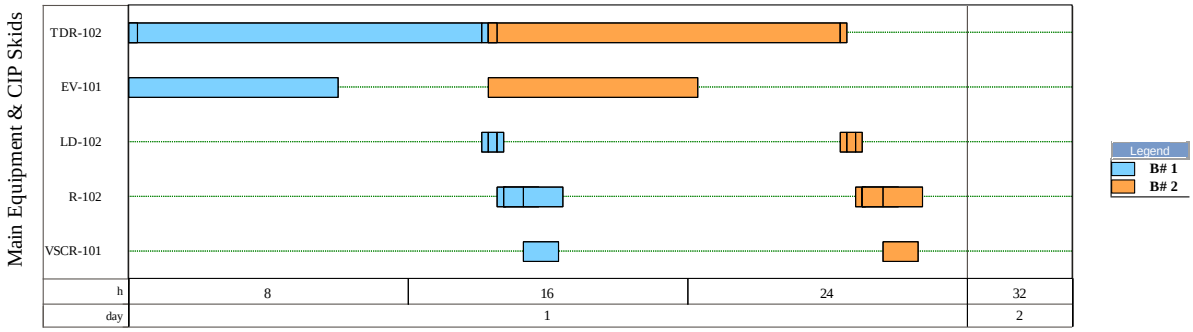


Figure 5.5: Equipment occupancy chart/ 2batch recipes

5.7 ALTERNATIVE SCHEMES DEVELOPMENT

Four debottleneck schemes as shown in Table 5.10 were developed based on the base case simulation to improve the annual production by increasing the number of batches. Their throughput and economics were evaluated to identify the most feasible scheme.

5.7.1 Alternative Scheme 1

An alternative scheme was considered, in which the product is not a liquid concentrate, but a powder, which comprised of the soluble solids contained in the concentrate. The powdered form of plant extracts appeared to be more common on the market than the aqueous concentrates, most likely due to the longevity of the dried form, as well as the convenience of storage and transportation of dry powders. The dried powders are also much easier to repackage into consumer attractive formats, such as tablets. Figure 5.6 shows scheme 1. In this scheme, a spray dryer was added to the process just after the evaporator. This enabled the reduction of the drying time from 10 h using an evaporator alone to 5 h of drying using both an evaporator and a spray dryer. A grinder was added to the process as a pre-treatment method of extraction. The size reduction of particles will increase the mass transfer and consequently increase the rate of extraction. This significantly increase the yield to about 5 wt % as determined experimental from the experiments carried in this study.

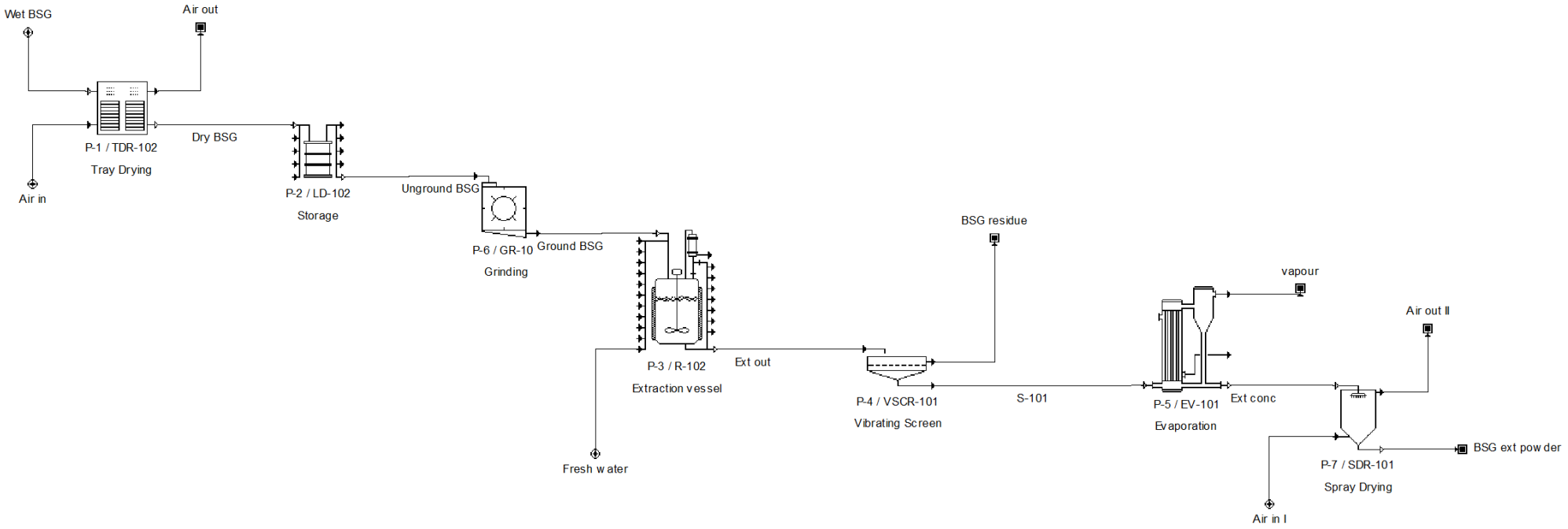


Figure 5.6: Simulation flowsheet of debottlenecking scheme 1

Table 5.10 includes the throughput analysis and economics summary of scheme 1. The number of batches per year remained 770 batches as well as the batch time 12.42 h. These results indicate that although the evaporator is a limiting equipment in this process, it is not the main bottleneck. Therefore, to increase the number of batches, the tray dryer needs to be changed. Moreover, the addition of a grinder which increased the product yield from 3 wt % to 5 wt % did not have significant impact on the number of batches. The increase in product yield might be the contributor to the slight increase in the return on investment (ROI). However, the process remained economically infeasible because of the negative return on investment of -37.76 %. Hence other alternatives were considered.

Figure 5.7 shows scheme 2. In this scheme, cycle time reduction study was done on the drying process at the beginning of the batch. To eliminate the scheduling bottleneck made by P-1/ TDR-102, two tray dryers in parallel were introduced.

Table 5.10: Executive summary for the Economic analysis for the base model and debottlenecking schemes

Process parameter	*Units*	*Scheme 1*	*Scheme 2*	*Scheme 3*	*Scheme 4*
Throughput					
Batch Throughput	kg MP/batch	2,52	2,52	3,81	497
Annual Throughput	kg MP/yr	1940	8770	5020	656000
number of batches per year	batches	770	1319	1319	1319
plant batch time	h	12,42	12,53	8,33	8,51
Annual operating time	h	7913	7915	7916	7916
Economics					
Total Investment	$	7240000	7530000	12800000	8340000
Total Revenues	$/yr	15800	27100	502000	10600000
Operating Cost	$/yr	3750000	51000000	5800000	51200000
Unit Production Ref. Rate	kg MP/yr	1930	8770	5020	656000
Gross Margin	%	-23600	-18700	-10500	51,90
Return On Investment	%	-37.76	-59.19	-32,16	48,45
Pay-back time	yr	N/A	N/A	N/A	1.76
IRR After Taxes	%	N/A	N/A	N/A	56.91
NPV at (7.00 % interest)	$	-29900000	-40000000	-43000000	24900000

5.7.2 Alternative scheme 2

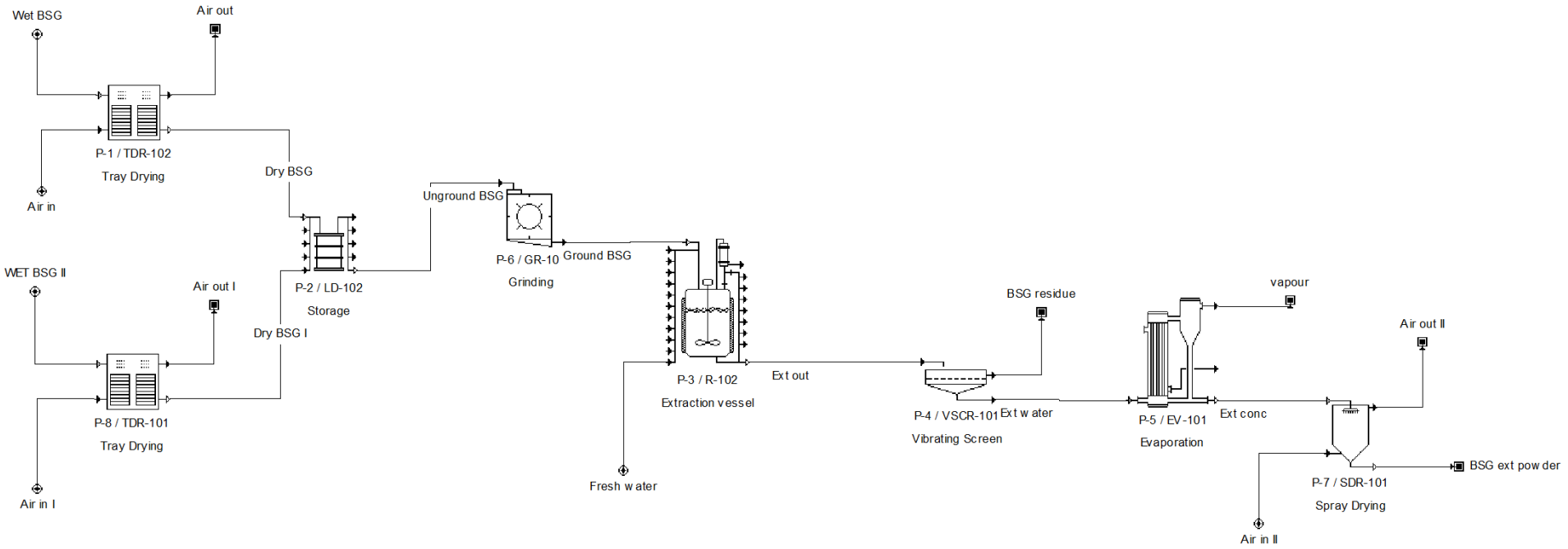


Figure 5.7: Simulation flowsheet of debottlenecking scheme 2

Scheme 2 increased the number of batches to 1319. However, the return on investment (ROI) decreased to -59.19 % and the process remained economically unattractive. More efforts are still needed to make the process economically viable. Despite the reduction of the cycle time, the process remained a loss. The increase of the number batches seems to be ineffective as the return of investment remains negative. This implies that, in this work, the increase in the number of batches has got an insignificant impact on the profitability of the process. Consequently, it was assumed that the bottleneck was product specific. This led to the development of scheme 3 in Figure 5.8 in which a two stage counter current solid liquid extraction was introduced.

5.7.3 Alternative scheme 3

Figure 5.8 shows further effort to make the process economically viable by employing not one, but two extraction vessels, arranged in a counter–current configuration to enhance mass transfer by increasing the contact time. The residence time, S/F, temperature and rate of agitation in both stages were similar. The fresh make –up solvent is added to the second vessel. The number of batches remained 1319. However, the return on investment (ROI) remained unprofitable at -32.16 % making scheme 3 economically infeasible.

The introduction of another extractor increased the product yield but was not effective enough to make the process profitable. This shows that an increase in the yield has got and insignificant effect on the profit of the process. The introduction of a second stage extraction is also not technically feasible as the raw material BSG is a product of the brewing process. Some of the polyphenols would have been lost before the maceration process and this makes the second stage extraction ineffective with a few polyphenols left for extraction.

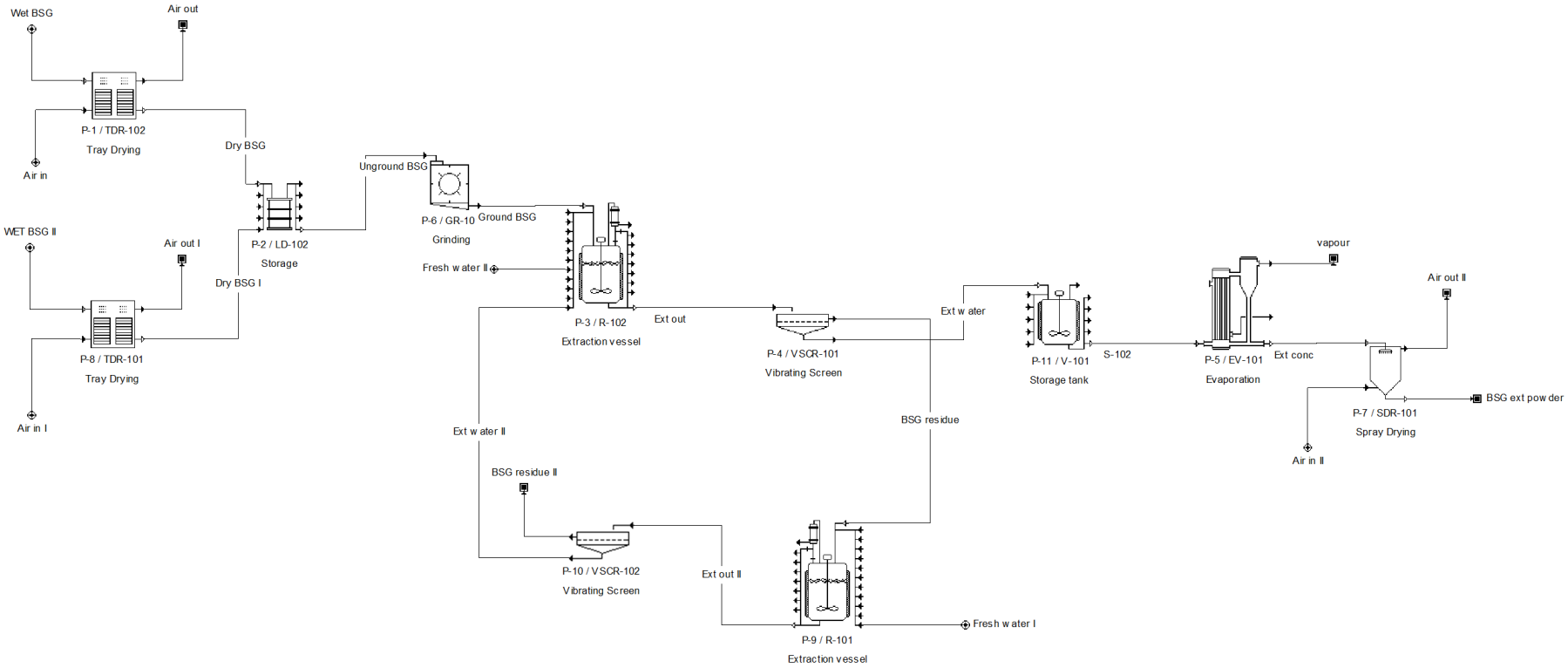


Figure 5.8: Simulation flowsheet of debottlenecking scheme 3

Although the annual throughput has been increased, the economic analysis of all of the three proposed schemes shows the ROI values (Table 5.10) are still well below the desired 30 %. Due to the addition of new equipment, the total capital and operating costs are higher than the total revenues in all three scenarios. Consequently, the values of ROI are low and the payback period is unachievable (Athimulam et al., 2006). The main constraint to all the developed schemes is the low revenue of the final product.

Efforts were then made to increase the value of the end product whilst reducing the operating cost. In scheme 4, the process economics were improved by producing encapsulated BSG extract 500mg bottles. To produce the encapsulated BSG extract, new equipment was added before the spray dryer and in the packaging section.

5.7.4 Alternative scheme 4

Figure 5.9 shows the proposed scheme 4 where the value added product was a consumer product rather than for industrial processing, i.e. in the form of capsules instead of bulk concentrate or powder. As mentioned above, the pricing was set at 1% of that specified on the online marketing source (Yuantai Biological Technology Co., Ltd, 2018) for p-coumaric acid, a polyphenol present in BSG. This was found to be the standard pricing method according to (Fernandez-Pérez et al., 2008). In the schemes developed above, BSG extract was sold at $ 8.17/kg. Microencapsulation using maltodextrin, user defined carbohydrate made from natural starch was introduced to improve the product quality and stability. The type of natural starch was selected from Spinelli et al, (2016) with the price of the maltodextrin ($1.50/ kg) adapted from (Athimulam et al., 2006). The BSG extract was filled in 250mg plastic containers (purchase price of $ 0.110/ container). After, 12 containers were packed in boxes (purchase price of $ 0.13/box). The price of the container and the boxes was set based on an online source (Yuehui Packing Material Factory Co., Ltd, 2018). The selling price of BSG extract was doubled to $ 16.24 per box of 12 according to the pricing method set by (Athimulam et al., 2006). They proposed that the price of the products can be doubled when the packaging is reduced as it is sold directly to end users.

It can be seen in Table 5.11 that the packaging equipment increased the capital cost to $ 8 350 000, and yet the change in the flowsheet also increased the increased the revenue to $ 11 900 000. Consequently, a return on Investment (ROI) of 56.91 % was recorded for scheme 4 with a payback period of 1.76 years. The final column of Table 5.10 shows that the annual throughput increased due to the increase in the batch throughput. This indicates that introducing the packaging line did not cause any time bottlenecks making the process more attractive. The total capital and operating costs also increased as compared to the base case and schemes 1 and 2 due to the installation of the packaging line. However, the increase in these costs was compensated by the drastic increase in revenues. Consequently, the gross

margin and ROI improved significantly and a reasonable payback period was attained. The calculations of this scheme show that the capital investment can be recovered in 1.76 years since the operating cost exceed the total revenues. Hence scheme 4 can be implemented for the production of BSG extracts.

Table 5.11: Executive summary of scheme 4

Entity	***Amount***	***US $***
Direct Fixed Capital	7650000	$
Working Capital	316000	$
Startup Cost	382000	$
Total Investment	8350000	$
Total Revenues	11900000	$/yr
Annual Operating Cost (AOC)	5150000	$/yr
Net Unit Production Cost	7,06	$/MP Entity
Gross Profit	16,24	$/MP Entity
Taxes (40%)	6700000	$/yr
Net Profit	2680000	$/yr
Gross Margin	4750000	$/yr
Return On Investment	48.45	%
Payback Time	1,76	years

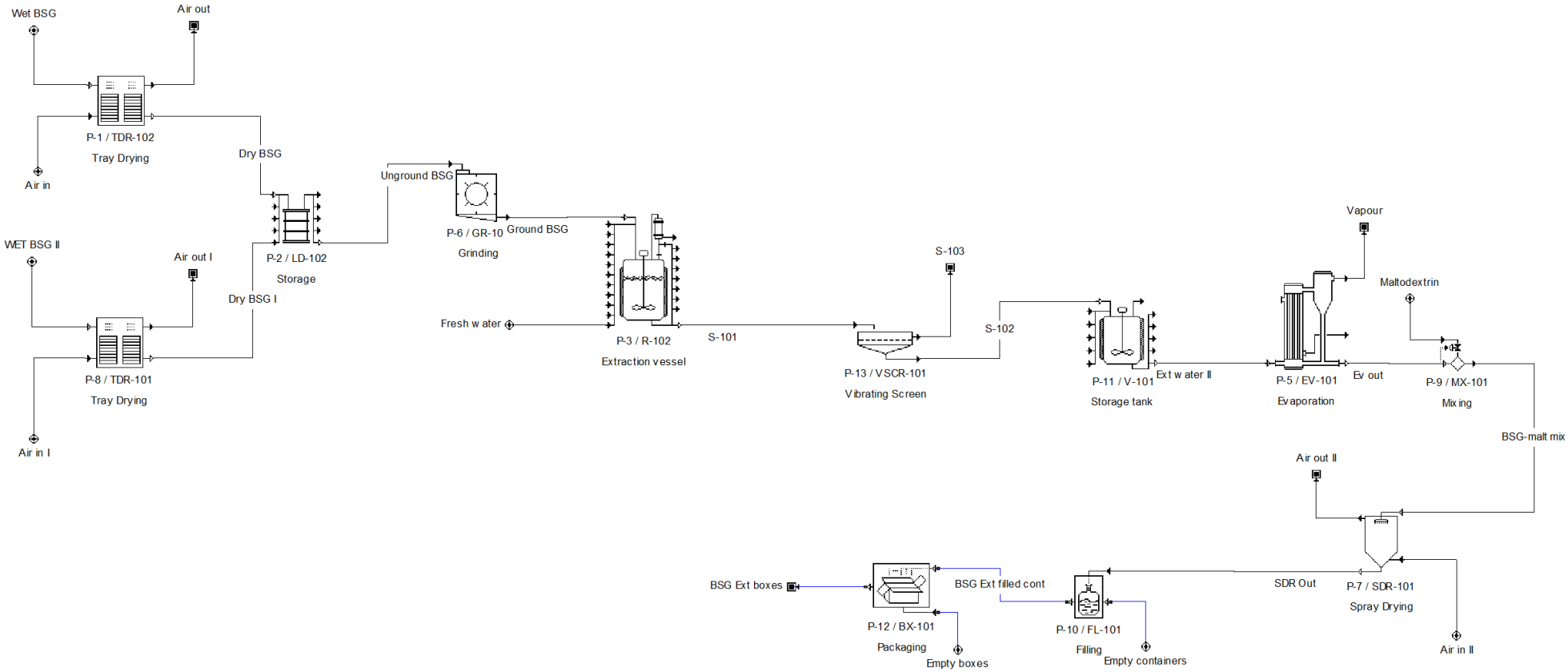


Figure 5.9: Simulation flowsheet of debottlenecking scheme 4

The equipment pricing and the profitability analysis is discussed further for potential investors whose attention would have been drawn by the attractive scheme 4 for the production of BSG extracts. The details of the economic data are presented below.

5.7.5 Prices for equipment in selected scheme 4

Table 5.12 shows the prices of equipment for proposed scheme 4. All the prices for the equipment was taken as 50 % of the prices obtained from an online source (Zhengzhou Pasen Machinery Co., Ltd, 2018). The capacities of the equipment were adapted from the book Chemical process equipment: selection and design (Couper, 2005).

Table 5.12: Equipment pricing

Name	*Type*	*Size (Capacity)*	*Units*	*Material of Construction*	*Purchase Cost ($/Unit)*
R-101	Stirred Reactor	70,88	L	SS316	460000,00
EV-101	Evaporator	63,79	L/h	SS316	1180000,00
V-101	Blending Tank	52,79	L	SS316	160000,00
R-102	Stirred Reactor	29,72	L	SS316	460000,00
VSCR-101	Vibrating Screen	13,37	L/h	SS316	2000,00
GR-101	Grinder	8,73	kg/h	CS	70000,00
SDR-101	Spray Dryer	76,30	L	SS316	126000,00
BX-101	Packer	2902	entities/h	SS316	20000,00
FL-101	Filler	3482	entities/h	SS316	30000,00
SDR-102	Spray Dryer	2,09	L	SS316	126000,00
MX-101	Mixer	46,13	kg/h	CS	40000,00
SDR-103	Spray Dryer	76,30	L	SS316	126000,00
FSP-101	Flow Splitter	46,13	kg/h	CS	10000,00
MX-102	Mixer	0,35	kg/h	CS	10000,00

Table 5.12 provides a list of the major equipment items in the selected scheme 4, along with their purchase costs. The total equipment cost for a plant of this capacity is approximately $ 3 million. The reactors and the spray dryers are the most expensive equipment accounting for almost half the equipment cost. The materials of construction suitable for this process is stainless steel grade 316 and carbon steel. Dailey & Vuong, (2015) used SuperPro Designer® to optimize aqueous conditions for recovery of phenolic content and antioxidant

properties from Macadamia. Their evaluated a total equipment cost for a plant of approximately $ 36 million dollars.

5.7.6 Unit cost analysis for selected scheme 4

A unit cost is the total expenditure incurred by a company to produce, store and sell one unit of a particular product or service and is of particular interest to the economists and engineers (Richard Turton, Richard C. Bailie, Wallace B. Whiting, 2013). Direct fixed capital (DFC) and operating costs must be calculated in order to estimate unit costs. Table 5.13 shows the various items included in the direct fixed capital (DFC) investment. Based on the specification of major equipment costs shown in Table 5.12, direct fixed capital cost includes the total plant direct cost (TPDC), Total plant indirect cost (TPIC) and Contractor's fee and contingency (CFC).

Table 5.13: Fixed Capital Estimate Summary (Prices in US $)

Type of Cost	*Amount US$*
Total Plant Direct Cost (TPDC)	
1. Equipment Purchase Cost	1270000,00
2. Installation	501000,00
3. Process Piping	444000,00
4. Instrumentation	508000,00
5. Insulation	38000,00
6. Electrical	127000,00
7. Buildings	571000,00
8. Yard Improvement	190000,00
9. Auxiliary Facilities	508000,00
TPDC	4150000,00
3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	1030000,00
11. Construction	1450000,00
TPIC	2490000,00
3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	6650000,00
3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	332000,00
13. Contingency	665000,00
CFC = 12+13	997000,00
3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	7650000,00

The factor method within the SuperPro Designer® software was used in the estimation of physical costs and economic parameters are the default numbers in the software as shown in Figure 5.10.

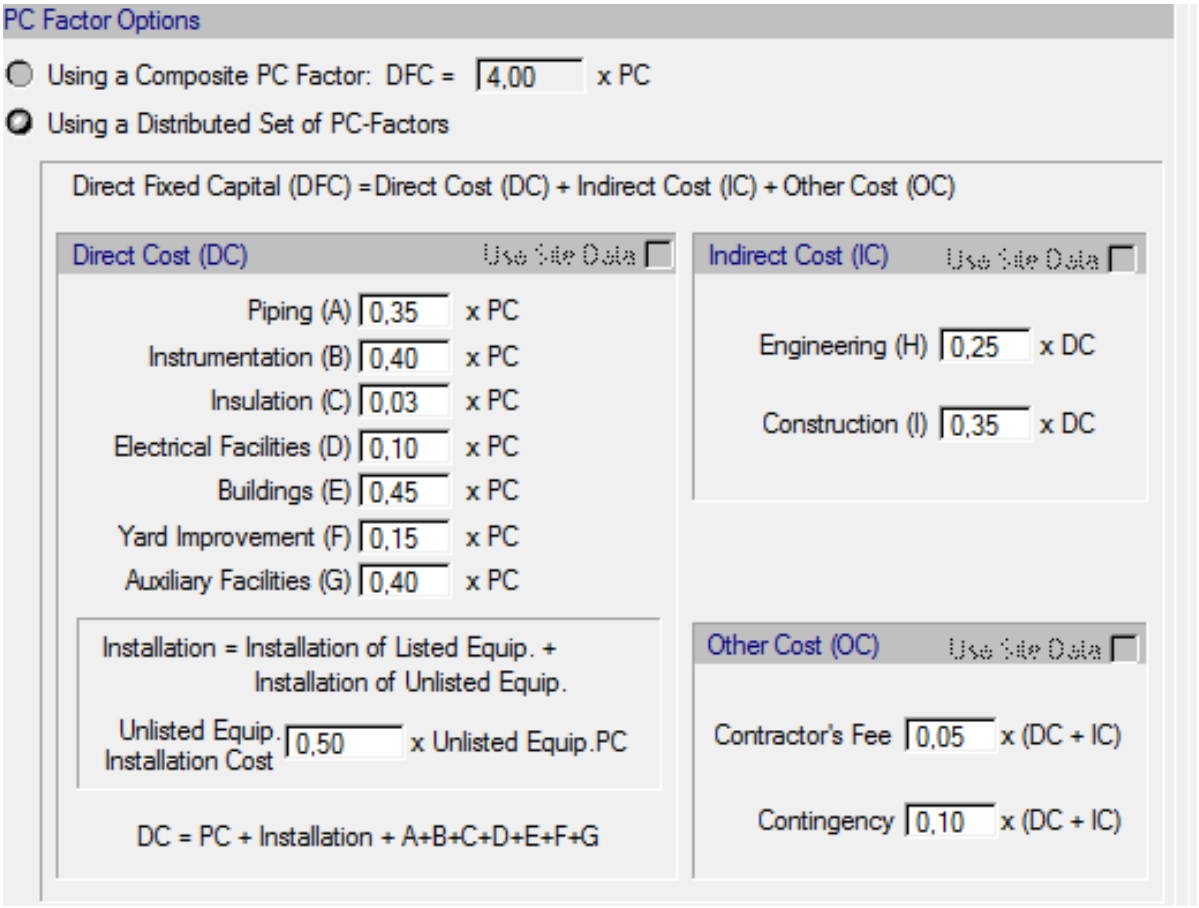


Figure 5.10: The factors used to calculate the direct, indirect and contingency costs

The direct fixed capital (DFC) cost $ 76 million, which is 6 times the equipment cost. The direct fixed capital costs take into account expenses that depends directly on production rate (Gustavo Barbosa-Cánovas et al., 2017). Raw materials, utilities and operations are the major contributors of the direct fixed capital cost. The total capital cost including the cost of start-up and validation is around $ 83 million. Indirect cost are the expenses which does not directly depend on the production rate such as taxes, insurance and depreciation and should be considered even if there is no production

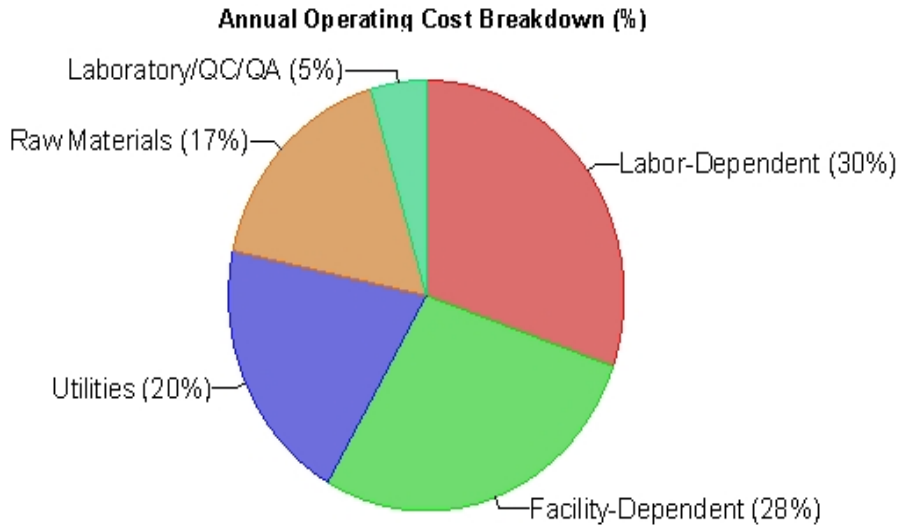


Figure 5.11: Annual operating costs breakdown for proposed scheme

Figure 5.11 shows the annual operating costs breakdown for scheme 4. The total annual operating cost in scheme 4 is $ 51 million. The is the greatest contributor to the annual cost is the labor-dependent with a 30 % contribution. The facility-dependent cost is calculated based on estimates of depreciation, maintenance, and miscellaneous factory expenses. The depreciation item is calculated using a straight-line depreciation method considering a salvage value fraction which is assumed by default to be 5 % in this analysis. Facility-dependent cost is the second largest, contributing 30 % to the annual operating cost. Utilities and laboratory/QC/QA contributes 20 % and 5 % respectively. Moreover, the annual operating cost also depends on the raw materials (15 %).

An increase in the equipment capacity has got a directly proportional relationship to the increase in the operational cost as more raw materials will be required. However, an increase in the equipment cost does not increase the operating costs.

5.7.7 Profitability analysis

This section analyzes the profitability of the production of BSG extract in the selected scheme and gives insights to potential investors. Profitability is measured by payback period, return on investment (ROI) and net present value (NPV). According to Fernandez-Pérez et al., (2008), the selling price of BSG extracts was identified to be 8.17 $/entity. The price level is higher that the unit production cost of 7.06 $/Entity. Table 5.14 shows the profitability summary of the selected scheme 4.

Table 5.14: Profitability analysis for the proposed scheme 4

Entity	***Amount***	***US $***

Direct Fixed Capital	7650000	$
Working Capital	316000	$
Startup Cost	382000	$
Total Investment	8350000	$
Total Revenues	11900000	$/yr
Annual Operating Cost (AOC)	5150000	$/yr
Net Unit Production Cost	7,06	$/MP Entity
Gross Profit	16,24	$/MP Entity
Taxes (40%)	6700000	$/yr
Net Profit	2680000	$/yr
Gross Margin	4750000	$/yr
Return On Investment	48.45	%
Payback Time	1,76	years

Scheme 4 is selected as the best-developed process for the extraction of BSG based on three profitability indicators: payback period, NPV and ROI. The three indicators were determined from the cash flow analysis shown in Table 5.15. If this scheme is implemented the capital investment can be recovered in less than 2 years.

The net present value was calculated to be $ 25 854 358 at the discount rate of 7 % (Table 5.10). This indicates the expected impact of the project on the value of the simulated BSG extract production plant. The higher the NPV, the more profitable the project is. The ROI was calculated to be 48.45 %, which is even higher than the acceptable range of 15-30 %. All the profitability indicators make scheme 4 more economically attractive with an annual revenue of $ 10 M.

Table 5.15: Cash flow analysis for scheme 4 (thousand $)

Year	Capital Investment	Sales Revenues	Operating Cost	Gross Profit	Depreciation	Taxes	Net Profit	Net Cash Flow
1	- 2,29	0	0	0	0	0	0	0
2	- 3,06	0	0	0	0	0	0	0
3	- 2,61	1970	3360	1	727	0	0	658
4	0	11800	5150	6700	727	6700	2680	4750
5	0	11800	5150	6700	727	6700	2680	4750
6	0	11800	5150	6700	727	6700	2680	4750
7	0	11800	5150	6700	727	6700	2680	4750
8	0	11800	5150	6700	727	6700	2680	4750
9	0	11800	5150	6700	727	6700	2680	4750
10	0	11800	5150	6700	727	6700	2680	4750
11	0	11800	5150	6700	727	6700	2680	4750
12	0	11800	5150	6700	727	6700	2680	4750
13	0	11800	4420	7430	0	7430	2970	4460
14	0	11800	4420	7430	0	7430	2970	4460
15	699	11800	4420	7430	0	7430	2970	4460

The cash flow analysis in Table 5.15 shows a payback period between 3 years which is different from the one simulation by SuperPro Designer® shown in Table 5.10 as 1.76 years. In Table 5.15, an assumption was made to take into account a 2 year construction period during which no operation was taking place hence no revenue was generated. The simulation done by software assumes operation begins at 0 years. The projected cash flow was made up to 15 years predicting a good plant life. After 15 years, the plant equipment would have to be replaced with new ones. The plant life can be increased by improving schedules for maintenance and cleaning.

5.8 SENSITIVITY ANALYSIS

In the economic analysis done for the base case simulation and all the alternative scheme, the production scale is set at 560 kilograms. This number may vary so as to analyse the influence of ±80 % change of the production rate (-80 %, -60 %, -40 %, -20 %, +20 %, +40 %, +60 %, +80%) on the unit costs to produce BSG extracts and on the profitability indicators for the simulated process. The sensitivity analysis was conducted using SuperPro Designer® software. Table 5.16 shows the results obtained by adjusting the process throughput in SuperPro Designer®.

Table 5.16: Sensitivity analyses for the influence of production scale on the unit costs and profitability

Sensitivity variables		*Unit cost of production ($/kg)*	*Payback time (year)*	*Net present value (NPV)*	*Return on investment (ROI)*
Production scale: (kg/batch MP)	(-80%): 112	29,56	N/A	-15 500 000	-11,06
	(-60%): 224	16,22	11,3	-5 720 000	8,85
	(-40%): 336	11,79	4,83	3 100 000	20,36
	(-20%): 448	9,48	3,08	10 200 000	32,46
	Base: 560	8,25	2,27	17 400 000	36.91
	(+20%): 672	7,36	1,8	24 600 000	55,77
	(+40%): 784	6,73	1,4	31 700 000	67,19
	(+60%): 896	6,27	1,27	38 900 000	78,58
	(+80%): 1008	5,89	1,11	46 100 000	89,98

Figure 5.12 shows the influence of the variation of the production scale on the unit costs of BSG extract process. The sensitivity analysis was done according to the procedure used by Zhuang, (2004).

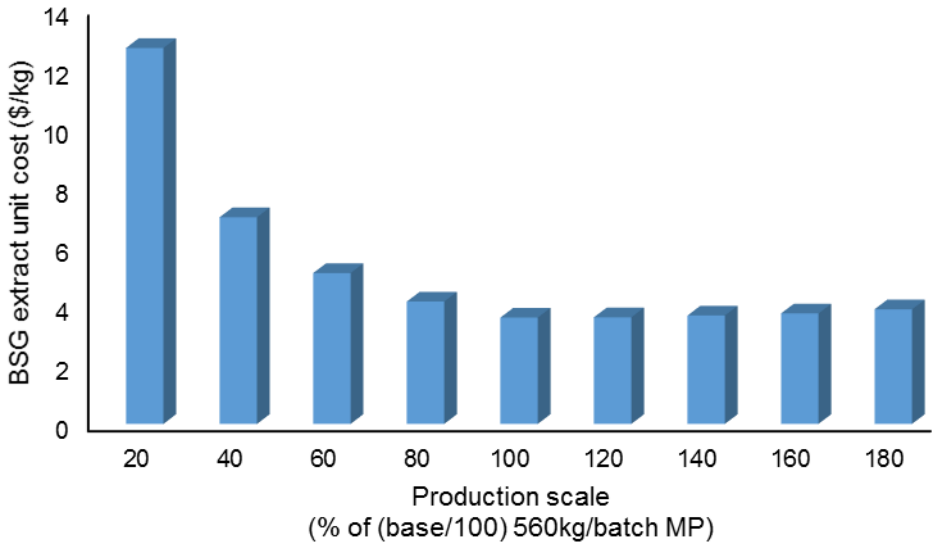


Figure 5.12: Influence of BSG production scale on the unit costs

The sensitivity analysis was carried out by adjusting systematically the process throughput. From Figure 5.12 and Figure 5.13, the base case is shown to be the best case in all the variations. The base case also has the lowest unit production cost. The return on investment has got a drastic increase when the production throughput is increased from below the base

case to the base case. However, the return on investment decreases slightly when the production throughput is increased above the base case.

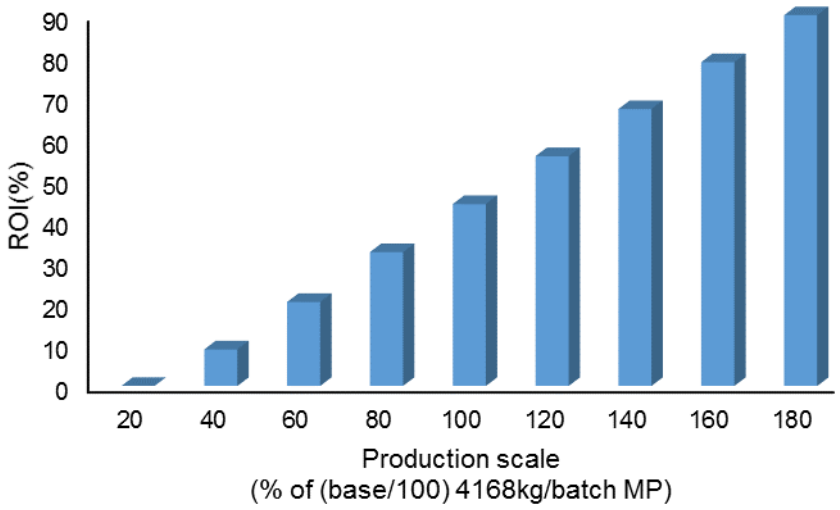


Figure 5.13: The variation of return on investments (ROI) with the production scale

5.9 OUTCOMES OF THIS CHAPTER

The aim of this chapter is to establish an accurate process model and to evaluate its economic feasibility. This achieves objective 3 of this research work. The feed raw materials were estimated to be 100 kg of wet BSG. Three profitability indicators were used on the base case simulation as well as the alternative schemes developed.

The key observations made in this chapter include:

- The material balances obtained from the software were reasonable. However, they might not have been accurate since the composition and the physical properties of BSG were not considered in this work. Only the price of BSG was used as a measure and identification of the biomass. The registration of BSG in the simulation was taken as biomass and could be identified mainly with the cost
- The base case simulation was economically infeasible and two equipment were identified as scheduling bottlenecks: tray dryer and evaporator
- The process remains economically unattractive for all alternative schemes in which efforts are being made to reduce the cycle time of the process and consequently increase the number of batches. However, the process became economically feasible when the product packaging and size was changed

- The solvent to feed ratio that was used in this work produced a very watery mixture which would only be filtered by a sieve or screen. Filters were found not suitable to separate
- SuperPro Designer® could not take into account the thermodynamic properties hence the prediction of the mixture behaviour and interactions might be limited
- The payback period simulated from the software was lower (1.76 years) than that calculated (3 years). This was because SuperPro Designer® did not consider the years of construction of the plant
- The time and schedule bottlenecks of the process had less impact on the economic hence all debottlenecking strategies were unsuccessful until value addition to the product was introduced

5.10 SIGNIFICANT CONTRIBUTIONS

The main contribution of this chapter was the development of a process model that is able to produce BSG extracts with profit. Results generated using SuperPro Designer® was combined with the experimental data to prove the feasibility of the process obtained for extraction of polyphenols from BSG using water as a solvent

5.11 NOMENCLATURE

SYMBOLS/ACRONYMS	DESCRIPTION
ANOVA	Analysis of variance
EV-101	Evaporator
IIR	Internal rate of return
MP	Main Product
NPV	net present value
PT	Process time
R-101	Extraction vessel
ROI	Return on investments
ST	Starting time
SUT	Set up time
wt.	weight
V-102	Storage tank

CHAPTER 6 SUMMARY, CONCLUSION, RECOMMENDATIONS AND CONTRIBUTIONS

6.1 SUMMARY

This research contributed to the ongoing development of economically feasible process of extracting bioactive compounds, specifically antioxidants comprising mainly of polyphenols, from brewers spent grains (BSG), and a brief summary of the main literature in the area is reviewed here to highlight the main gap addressed by this work. Publications from as early as 1977. Prentice & D'Appolonia, (1977) have reported the potential use of polyphenols from BSG, such as ferulic acid, as preservatives and colorants for the food industry. Most of the past work has showed the influence of different factors such as extraction method, type of solvent, temperature and solvent to fluid ratio on the efficiency of extracting these polyphenols from BSG (Meneses et al., 2013a; Luis F Guido & Moreira, 2017; Spinelli, Conte, Lecce, et al., 2016).

Guido & Moreira, (2017) reviewed the extraction techniques that have been employed over the years for the extraction of BSG. They reported that solid liquid extraction methods such as extraction with organic solvents, enzymatic and alkaline reactions are the most used for the recovery of BSG extracts. Among these methods, Meneses et al., (2013) proved that organic solvent extraction using 60% (v/v) acetone: water mixture for 30 min at 60 °C was highly efficient to extract antioxidant phenolic compounds from BSG (9.90 mg GAE/g dry BSG). However using solid liquid extraction methods has disadvantages of large quantity of solvents being used and are time consuming (Fernandez-Pérez et al., 2008). Hence, the research has intensified in the use of advanced techniques such as microwave assisted extraction, ultrasound assisted extraction and supercritical fluid extraction. Moreira, (2012) applied the microwave extraction process particularly to obtain the ferulic acid % yield. The results increased five times more than those obtained with solid liquid extraction methods, showing that at the optimal conditions (15 min extraction time, 100 °C extraction temperature, 20 mL of solvent, and maximum stirring speed), the yield of FA was 1.31 ± 0.04% (w/w) (Luis F Guido & Moreira, 2017; Moreira, 2012).

From laboratory experiments, BSG extracts are obtained in powder form but have unpleasant flavours and aromas. A method to mask the unpleasant odour and bitter taste by incorporating a coating on the extract powder has been investigated. This method, microencapsulation was investigated by Spinelli et al., (2016) on fish-burger formulations. Spinelli et al., (2016) performed microencapsulation using different ratios of mass of BSG to mass of the coating material (1:2, 1:4, 1:6, 1: 8) and spray drying the formulations at

temperatures of (90, 120, 150 °C) to obtain a dry and light-brown coloured BSG extract powder.

Despite the development that has been made in the research of the BSG extracts. The economic feasibility of the extraction of polyphenols from the beer production waste has not been explored. The current research work presented in this thesis covered the modelling and simulation as well as the economic evaluation for the process of extracting BSG extracts using SuperPro Designer® Software.

The batch process was selected as the mode of operation for the modelling and simulation of the process. The base case simulation was constructed from the experiments that had been done in the laboratory previously. The information used for unit procedures and equipment was mainly derived from Couper, (2005). Assumptions such as Set up time (SUT) of 5 min and Start time (ST) of 5 min were considered for all unit operations in this work. The process was simulated to obtain the material balances that could be generated by the software. The prices of BSG was adopted from Fernandez-Pérez et al., (2008) and the price of BSG extract powder was taken as 1% of p-coumaric acid price found on the world market (Alibaba, 2017).

6.2 CONCLUSIONS

The main outcome of this work was the development of commercial process for the extraction of polyphenols from BSG. The development of the process included the conceptual stage where experiments were done in the lab and the modelling and simulation done using the SuperPro Designer® Software.

In the conceptual stage, maceration extraction method was used to recover the BSG extracts. Based on the experiments done, water was found to be the suitable solvent for extraction. These results were however not in agreement with those of Meneses et al., (2013b) and Moreira, (2012) who reported acetone 70% v/v as the solvent that gave the best extracts. All the solvents used in this work showed antioxidant activity with extracts obtained using water giving the highest.

In selecting other factors response surface method was used to optimize the process. Temperature, solvent to fluid ratio and shaking speed were taken from literature data as independent variables. Central composite design was used with the 2FI model to investigate the impact of the variables on the responses. The model was found to be significant for all responses. However, the lack of fit was insignificant which might have implied that the independent variables have no impact on the responses. In order to investigate the time

required for maceration and the speed at which extraction occurs, a kinetic study was done. The samples were being withdrawn at an interval of 10 min. The study showed that the 15 mins is enough to extract most of the polyphenols. Temperature showed to have insignificant impact on the rate of extraction in this work. The data obtained from conceptual stage was used for the modelling and simulation using SuperPro Designer® software to evaluate the economic feasibility of this process

Three profitability indicators were used in this work namely Return on Investment (ROI), payback period and Net present value. The ROI of the base case simulation was found to be -51.90 %. This made the process to be economically unattractive having a ROI less than 30 %. This led to the development of alternative schemes with debottlenecking strategies to eliminate major limitations. The tray dryer and evaporator were the scheduling bottlenecks and eliminating these would increase the number of batches and consequently the ROI. However, scheme 1 to scheme 3, were not good enough to make the process economic feasible. Therefore, a value addition on the end product was introduced by adding a packaging line. The packaging line would produce encapsulated 500mg bottles of BSG extract instead of the bulk powder. This value addition made the process to be economically attractive with a ROI of 48.45 % and a payback period of 2.06 %.

6.3 RECOMMENDATIONS FOR FUTURE RESEARCH

Several technical difficulties were noted during the course of the experiments. These are described below for each of the test conducted.

- Exogenous factors

Although the BSG was obtained from the same brewing recipe throughout, there may have been factors outside of the control of this project that may have affected either the extraction or the model produced therefrom. Such factors could have been the natural variation in the particular batches of brew or their feed material, detail of which the researcher had no access to. However, these variations can be assumed to be limited, and of such a nature as to be expected in a commercial scale process, and therefore are deemed to be incorporated within the process model proposed.

- shaking of samples during experiments

The method of shaking, using a bench-top shaker, is one that is not commonly used in industry and may therefore be difficult to reproduce at commercial level. It is therefore recommended that the experiments be carried out at pilot scale in order to select the equivalent mixing regime using a different method of agitation, such as with a mixing paddle.

- Filtering of sample when using water as a solvent

The filtering of fine BSG extraction mixtures was difficult using a filter paper because of the thick mixture that would have been formed. An investigation of the use of centrifuge at commercial scale could be considered.

6.4 CONTRIBUTIONS

By completing this research work, the contributions made are as follows:

- Additional data has been generated regarding the extraction of bioactive compounds from BSG, including the kinetics of extraction.
- Additional data has also been generated regarding the antioxidant activity of the extracts from BSG.
- The developed process will assist in commercialising the extraction of BSG extracts with an attractive margin. Results showed that giving a good packaging to the products can add value to the process.

CHAPTER 7 REFERENCES

Aboltins, A. & Palabinskis, J. 2015. Research in brewer's spent grain drying process. : 230–235.

Abu Bakar, M.F., Mohamed, M., Rahmat, A. & Fry, J. 2009. Phytochemicals and antioxidant activity of different parts of bambangan (Mangifera pajang) and tarap (Artocarpus odoratissimus). *Food Chemistry*.

Addai, Z.R., Abdullah, A. & Abd Mutalib, S. 2013. Effect of extraction solvents on the phenolic content and antioxidant properties of two papaya cultivars. *JOurnal of Medicinal Plants Research*, 7(47): 3354–3359. http://www.academicjournals.org/JMPR.

Adeniran, H.A., Abiose, S.H. & Ogunsua, A.O. 2010. Production of fungal ??-amylase and amyloglucosidase on some nigerian agricultural residues. *Food and Bioprocess Technology*, 3(5): 693–698.

Al-Farsi, M.A. & Lee, C.Y. 2008. Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chemistry*, 108(3): 977–985. http://linkinghub.elsevier.com/retrieve/pii/S0308814607012654 22 June 2017.

Alibaba. 2017. natural suppliments.

Aliyu, S. & Bala, M. 2013. Brewer's spent grain: A review of its potentials and applications. *African Journal of Biotechnology*, 10(3): 324–331. http://www.ajol.info/index.php/ajb/article/view/92219.

American Society of Brewing Chemists., M. (Suntory L.. O.J.. & Ono, M. 1996. *Journal of the American Society of Brewing Chemists.* American Society of Brewing Chemists. http://agris.fao.org/agris-search/search.do?recordID=US1997055094#.WW4mY5DF080.mendeley 18 July 2017.

Anca, F., Maria, T., Sonia, S., Stancuta, S., Liana, S. & Carmen, P. 2013. Analysis of flavonoids in malt and brewers' spent grain by uv-vis spectrophotometry using different extraction technologies. , XII: 205–212.

Annie, F. & Jean-Jacques, M. 2003. *Phenolic acids in fruits and vegetables. Flavonoids in Health and Disease*. 2nd edn. New York, USA: CRC Press: Taylor and francis group.

Antolovich M, Prenzler PD, Patsalides E, McDonald S, R.K. 2002. Methods for testing antioxidant activity. *The Analyst*, 127(1): 183–198. http://xlink.rsc.org/?DOI=b009171p.

Aron, P. & Shellhammer, T. 2010. A discussion of polyphenols in beer physical and flavour stability. *Journal of the Institute of Brewing*, 116(4): 369–380. http://onlinelibrary.wiley.com/doi/10.1002/j.2050-0416.2010.tb00788.x/abstract.

Asl, A.H. & Khajenoori, M. 2013. Subcritical Water Extraction. *Mass Transfer - Advances in Sustainable Energy and Environment Oriented Numerical Modeling*: 459–87.

Athimulam, A., Kumaresan, S., Foo, D.C.Y., Sarmidi, M.R. & Aziz, R.A. 2006. Modelling and Optimization of Eurycoma longifolia Water Extract Production. *Food and Bioproducts Processing*, 84(2): 139–149.

http://www.sciencedirect.com/science/article/pii/S0960308506705284.

Babbar, N., Oberoi, H.S., Sandhu, S.K. & Bhargav, V.K. 2014. Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. *Journal of Food Science and Technology*, 51(10): 2568–2575.

Balasundram, N., Sundram, K. & Samman, S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, 99(1): 191–203. http://www.sciencedirect.com/science/article/pii/S0308814605006242 18 July 2014.

Baldosano, H.Y., Beatriz, M., Castillo, M.G., Elloran, C.D.H. & Bacani, F.T. 2015. Effect of Particle Size , Solvent and Extraction Time on Tannin Extract from Spondias purpurea Bark Through Soxhlet Extraction. *Proceedings of the DLSU Research Congress*, 3: 4–9.

Bart, Hans-Jörg, S.P. 2014. *Industrial Scale Natural Products Extraction*. 1st ed. New York, USA: wiley.

Benzie, I.F.F. & Strain, J.J. 1996. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of ‘“ Antioxidant Power ”’: The FRAP Assay. , 76: 70–76.

Bird, R.., Stewart, W.. & Lightfoot, E.. 2006. *Tranport phenomena*. 1 st. New York, USA: wiley.

Bonoli, M., Marconi, E. & Caboni, M.F. 2004. Free and bound phenolic compounds in barley (Hordeum vulgare L.) flours: Evaluation of the extraction capability of different solvent mixtures and pressurized liquid methods by micellar electrokinetic chromatography and spectrophotometry. *Journal of Chromatography A*, 1057(1–2): 1–12.

Branan, C. 2002. *Rules of Thumb for Chemical Engineers*. http://books.google.com/books?hl=en&lr=&id=lpA83iVcaGYC&oi=fnd&pg=PP2&dq=Rules+of+Thumb+for+Chemical+Engineers&ots=fUgjuLIjba&sig=g-o9GM01QY-QCYRBFPWfEKuFBxQ.

Brand-Williams, W., Cuvelier, M.E. & Berset, C. 1995. Use of a Free Radical Method to Evaluate Antioxidant Activity. , 30: 25–30.

Budrat, P. & Shotipruk, A. 2009. Enhanced recovery of phenolic compounds from bitter melon (Momordica charantia) by subcritical water extraction. *Separation and Purification Technology*, 66(1): 125–129. http://linkinghub.elsevier.com/retrieve/pii/S1383586608004577 11 September 2017.

Buffington, J. 2014. The Economic Potential of Brewer ’ s Spent Grain (BSG) as a Biomass Feedstock. *Advances in Chemical Engineering and Science*, (July): 308–318.

Cacace, J.E. & Mazza, G. 2003. Optimization of Extraction of Anthocyanins from Black Currants with Aqueous Ethanol. *Journal of Food Science*, 68: 240–248.

Cai, S., Han, Z., Huang, Y., Chen, Z.H., Zhang, G. & Dai, F. 2015. Genetic Diversity of Individual Phenolic Acids in Barley and Their Correlation with Barley Malt Quality.

Journal of Agricultural and Food Chemistry, 63(31): 7051–7057.

Campos, L.M.A.S., Michielin, E.M.Z., Danielski, L. & Ferreira, S.R.S. 2005. Experimental data and modeling the supercritical fluid extraction of marigold (Calendula officinalis) oleoresin. *The Journal of Supercritical Fluids*, 34(2): 163–170. http://www.sciencedirect.com/science/article/pii/S0896844604002864 16 April 2016.

Carvalheiro, F. 2004. Production of oligosaccharides by autohydrolysis of brewery's spent grain. *Bioresource Technology*, 91(1): 93–100. http://www.sciencedirect.com/science/article/pii/S0960852403001482 4 May 2016.

Carvalho, P.I.N., Osorio-Tobón, J.F., Rostagno, M.A., Petenate, A.J. & Meireles, M.A.A. 2015. Techno-economic evaluation of the extraction of turmeric (Curcuma longa L.) oil and ar-turmerone using supercritical carbon dioxide. *The Journal of Supercritical Fluids*, 105: 44–54. http://www.sciencedirect.com/science/article/pii/S0896844615001333 19 May 2016.

Chan, C., Yusoff, R. & Ngoh, G. 2013. Modeling and kinetics study of conventional and assisted batch solvent extraction. *Chemical Engineering Research and Design*, 92(6): 1169–1186. http://dx.doi.org/10.1016/j.cherd.2013.10.001.

Che Sulaiman, I.S., Basri, M., Fard Masoumi, H.R., Chee, W.J., Ashari, S.E. & Ismail, M. 2017. Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of Clinacanthus nutans Lindau leaves by response surface methodology. *Chemistry Central Journal*, 11(1): 1–11.

Cisse, M., Bohuon, P., Sambe, F., Kane, C., Sakho, M. & Dornier, M. 2012. Aqueous extraction of anthocyanins from Hibiscus sabdariffa: Experimental kinetics and modeling. *Journal of Food Engineering*, 109(1): 16–21.

Coelho, E., Rocha, M.A.M., Saraiva, J.A. & Coimbra, M.A. 2014. Microwave superheated water and dilute alkali extraction of brewers' spent grain arabinoxylans and arabinoxylo-oligosaccharides. *Carbohydrate Polymers*, 99: 415–422.

Couper, J.R. 2005. Chemical process equipment: selection and design. *Work*: 774. http://books.google.com/books?hl=en&lr=&id=IMkb4VBxqo4C&oi=fnd&pg=PA329&dq=Chemical+Process+Equipment,+Selection+and+Design&ots=49O_c4f-B-&sig=S58y5X8Fo7Ar4vnBI2Zpqyh8OVA.

CROSSLEY, J.I. & AGUILERA, J.M. 2001. MODELING THE EFFECT OF MICROSTRUCTURE ON FOOD EXTRACTION. *Journal of Food Process Engineering*, 24(3): 161–177. http://doi.wiley.com/10.1111/j.1745-4530.2001.tb00538.x 22 July 2017.

Cui, H.-Y., Niranjana Murthy, H., Moh, S.H., Cui, Y.Y., Lee, E.-J. & Paek, K.-Y. 2014. Comparison of conventional and ultrasound-assisted methods for extraction of nutraceutical compounds from *Dendrobium candidum*. *CyTA - Journal of Food*, 12(4): 355–359. http://www.tandfonline.com/doi/abs/10.1080/19476337.2014.888482 21 July 2017.

Dai, J. & Mumper, R.J. 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10): 7313–7352.

Dailey, A. & Vuong, Q. 2015. Optimization of Aqueous Extraction Conditions for Recovery of Phenolic Content and Antioxidant Properties from Macadamia (Macadamia tetraphylla) Skin Waste. *Antioxidants*, 4(4): 699–718. http://www.mdpi.com/2076-3921/4/4/699/ 5 August 2017.

Deng, J., Liu, Q., Zhang, C., Cao, W., FAN, D. & Yang, H. 2016. Extraction Optimization of Polyphenols from Waste Kiwi Fruit Seeds (Actinidia chinensis Planch .) and. *Molecules*, 21(832): 8–13.

Dent, M., Dragovi, V., Peni, M. & Brn, M. 2013. The Effect of Extraction Solvents , Temperature and Time on the Composition and Mass Fraction of Polyphenols in Dalmatian Wild Sage (Salvia officinalis L .) Extracts. , 9862(1): 84–91.

Dhanani, T., Shah, S., Gajbhiye, N.A. & Kumar, S. 2017. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. *Arabian Journal of Chemistry*, 10: S1193–S1199. http://dx.doi.org/10.1016/j.arabjc.2013.02.015.

Dhar, G., Akther, S., Sultana, A., May, U., Islam, M.M., Dhali, M. & Sikdar, D. 2017. Effect of extraction solvents on phenolic contents and antioxidant capacities of Artocarpus chaplasha and Carissa carandas fruits from Bangladesh. *Journal of Applied Biology & Biotechnology*, 5(3): 39–44. http://jabonline.in/abstract.php?article_id=210.

Dillard, C.J. & German, J.B. 2000. Review Phytochemicals : nutraceuticals and human health. , 1756(May).

Diptee, R. 1989. Process Optimization and Properties of Protein Concentrates From Brewers' Spent Grain. : 146.

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. & Ju, Y.H. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis*, 22(3): 296–302. http://dx.doi.org/10.1016/j.jfda.2013.11.001.

Dorta, E., Lobo, M.G. & Gonzalez, M. 2012. Reutilization of mango byproducts: Study of the effect of extraction solvent and temperature on their antioxidant properties. *Journal of Food Science*, 77(1): 80–88.

Dvorakova, M., Guido, L.F., Dostálek, P., Skulilová, Z., Moreira, M.M. & Barros, A. a. 2008. Antioxidant Properties of Free, Soluble Ester and Insoluble-Bound Phenolic Compounds in Different Barley Varieties and Corresponding Malts. *The Institute of Brewing and Distilling*, 114(1): 27–33.

El-Belghiti, K., Rabhi, Z. & Vorobiev, E. 2005. Kinetic model of sugar diffusion from sugar beet tissue treated by pulsed electric field. *Journal of the Science of Food and Agriculture*, 85(2): 213–218.

Fărcaş, A.C., Socaci, S.A., Mudura, E., Dulf, F.V., Vodnar, D.C., Tofană, M. & Salanță, L.C.

2017. World â€TM s largest Science , Technology & Medicine Open Access book publisher Exploitation of Brewing Industry Wastes to Produce Functional Ingredients.

Fernandez-Pérez, M., Rodiguez, J.. F., Garcia, M.T., de Lucas, A. & Gracia, I. 2008. Application of Supercritical Fluid Extraction to Brewer ' s Spent Grain Management. *Ind. Eng. Chem. Res.*, 47(5): 1614–1619.

Fernández-Ronco, M.P., De Lucas, A., Rodríguez, J.F., García, M.T. & Gracia, I. 2013. New considerations in the economic evaluation of supercritical processes: Separation of bioactive compounds from multicomponent mixtures. *Journal of Supercritical Fluids*, 79(2013): 345–355. http://dx.doi.org/10.1016/j.supflu.2013.01.018.

Figuereo, A., Roche, M., Trost, C., Albertini, L. & Fernandez-torres, L.C. 2016. Antioxidant capacity of common dietary supplements. , 2016(2): 2016–2017.

Forssell, P., Kontkanen, H. & Schols, H. 2008. Hydrolysis of brewers' spent grain by carbohydrate degrading enzymes. *Institute of Brewing*. http://onlinelibrary.wiley.com/doi/10.1002/j.2050-0416.2008.tb00774.x/full.

Goupy, P., Hugues, M., Boivin, P. & Amiot, M.J. 1999. Antioxidant composition and activity of barley (Hordeum vulgare) and malt extracts and of isolated phenolic compounds. *Journal of the Science of Food and Agriculture*, 79(12): 1625–1634.

Graf, E. 1992. Antioxidant potential of ferulic acid. *Free Radical Biology and Medicine*, 13(4): 435–448. http://linkinghub.elsevier.com/retrieve/pii/089158499290184I 11 May 2017.

Guido, L.F. & Moreira, M.M. 2017. Techniques for Extraction of Brewer's Spent Grain Polyphenols: a Review. *Food and Bioprocess Technology*, 10(7): 1192–1209.

Guido, L.F. & Moreira, M.M. 2017. Techniques for Extraction of Brewer's Spent Grain Polyphenols: a Review.

Gupta, V.K. & Sharma, S.K. 2014. Plants as natural antioxidants. *Indian Journal of Natural Products and Resources*, 5(4): 326–334.

Gustavo Barbosa-Cánovas, Pastore, G.M., Candoğan, K., Meza, I.G.M., Lannes, S.C. da S., Buckle, K., Yada, R.Y. & Rosenthal, A. 2017. *Global food securities and wellness*. New York.

Hadrich, B., Dimitrov, K. & Kriaa, K. 2017. Modelling Investigation and Parameters Study of Polyphenols Extraction from Carob (Ceratonia siliqua L.) Using Experimental Factorial Design. *Journal of Food Processing and Preservation*, 41(2): 1–10.

Halliwell, B. 2008. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? *Arch Biochem Biophys*, 476. http://dx.doi.org/10.1016/j.abb.2008.01.028.

Halliwell, B. 1995. How to characterize an antioxidant: an update. *Biochemical Society Symposium*, 61: 73 LP-101. http://symposia.biochemistry.org/content/61/73.abstract.

Haminiuk, C.W.I., Maciel, G.M., Plata-Oviedo, M.S. V & Peralta, R.M. 2012. Phenolic compounds in fruits - an overview. *International Journal of Food Science and*

Technology, 47(10): 2023–2044.

Hanim, S.S., Norsyabilah, R., Suhaila, M.H.N., Noraishah, A. & Kartina, A.K.S. 2012. Effects of Temperature, Time and Pressure on the Hemicelluloses Yield Extracted Using Subcritical Water Extraction. *Procedia Engineering*, 42: 562–565. http://www.sciencedirect.com/science/article/pii/S1877705812028482 1 April 2016.

Harbourne, N., Jacquier, J.C. & O'Riordan, D. 2009. Optimisation of the extraction and processing conditions of chamomile (Matricaria chamomilla L.) for incorporation into a beverage. *Food Chemistry*, 115(1): 15–19. http://dx.doi.org/10.1016/j.foodchem.2008.11.044.

Hendriks, A.T.W.M. & Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource technology*, 100(1): 10–8. http://www.sciencedirect.com/science/article/pii/S0960852408004574 12 January 2016.

Heredia-Olea, E., Perez-Carrillo, E. & Serna-Saldivar, S.O. 2015. Effect of extrusion conditions and hydrolysis with fiber-degrading enzymes on the production of C5 and C6 sugars from brewers' spent grain for bioethanol production. *Biofuel Research Journal-Brj*, 2(1): 203–208.

Hernanz, D., Sancho, A.I., Faulds, C.B. & Williamson, G. 2001. Hydroxycinnamic Acids and Ferulic Acid Dehydrodimers in Barley and Processed Barley. : 4884–4888.

Herodež, Š.S., Hadolin, M., Škerget, M. & Knez, Ž. 2003. Solvent extraction study of antioxidants from Balm (Melissa officinalis L.) leaves. *Food Chemistry*, 80(2): 275–282.

Ho, S.K., Tan, C.P. & Ho, C.W. 2011. Optimization of Binary Solvent Extraction of Antioxidant Compounds and Capacities from Dukung Anak , Phyllanthus niruri using Response Surface Methodology. , 1: 99.

Holteklen, A.K., Kinitz, C. & Knutsen, S.H. 2006. Flavanol and bound phenolic acid contents in different barley varieties. *Journal of Agricultural and Food Chemistry*, 54(6): 2253–2260.

Iacopini, P., Baldi, M., Storchi, P. & Sebastiani, L. 2008. Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, in vitro antioxidant activity and interactions. *Journal of Food Composition and Analysis*, 21(8): 589–598. http://www.sciencedirect.com/science/article/pii/S0889157508000653 1 May 2017.

Iloki-Assanga, S.B., Lewis-Luján, L.M., Lara-Espinoza, C.L., Gil-Salido, A.A., Fernandez-Angulo, D., Rubio-Pino, J.L. & Haines, D.D. 2015. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of Bucida buceras L. and Phoradendron californicum. *BMC Research Notes*, 8(1): 396. http://www.biomedcentral.com/1756-0500/8/396.

Jomova, K. & Valko, M. 2013. Health protective effects of carotenoids and their interactions with other biological antioxidants. *European Journal of Medicinal Chemistry*, 70: 102–110. http://dx.doi.org/10.1016/j.ejmech.2013.09.054.

Juncan, A.-M. 2011. Analysis of some antioxidants use in cosmetics by chromatographic methods. *Faculty of Chemistry and Chemical Engineering*.

Kalia, K., Sharma, K., Singh, H.P. & Singh, B. 2008. Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of potentilla atrosanguinea lodd. and quantification of its phenolic constituents by RP-HPLC. *Journal of Agricultural and Food Chemistry*, 56(21): 10129–10134.

Khidzir, K, M. 2010. *Brewery Spent Grain: Chemical Characteristics and Utilization as an Enzyme Substrate*.

Kitrytė, V., Šaduikis, A. & Venskutonis, P.R. 2015. Assessment of antioxidant capacity of brewer's spent grain and its supercritical carbon dioxide extract as sources of valuable dietary ingredients. *Journal of Food Engineering*, 167: 18–24. http://www.sciencedirect.com/science/article/pii/S0260877414005251 11 January 2016.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E. & Etherton, T.D. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine*, 113(9): 71–88.

Kunze, W. & Kunze, W. 2010. TECHNOLOGY Brewing & Malting.

Kurutas, E.B. 2016. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition Journal*, 15(1): 71. http://dx.doi.org/10.1186/s12937-016-0186-5.

Leitao, C., Marchioni, E., Bergaentzlé, M., Zhao, M., Didierjean, L., Taidi, B. & Ennahar, S. 2011. Effects of Processing Steps on the Phenolic Content and Antioxidant Activity of Beer. *Journal of Agricultural and Food Chemistry*, 59(4): 1249–1255. http://www.researchgate.net/publication/49780110_Effects_of_Processing_Steps_on_the_Phenolic_Content_and_Antioxidant_Activity_of_Beer.

Liyana-Pathirana, C. & Shahidi, F. 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry*, 93(1): 47–56.

Lynch, K.M., Steffen, E.J. & Arendt, E.K. 2016. Brewers' spent grain: a review with an emphasis on food and health. *Journal of the Institute of Brewing*, 122(4): 553–568.

Macleod, B.A.M. [J. Inst. Brew. THE PHYSIOLOGY OF MALTING—A REVIEW.

Maestri, D.M., Nepote, V., Lamarque, A.L. & Zygadlo, J.A. 2006. *Natural products as antioxidants*. https://www.trnres.com/ebook/uploads/imperato/T_1231133728Imperato-5.pdf.

Manach, C., Scalbert, A., Morand, C., Rémésy, C. & Jiménez, L. 2004. Polyphenols: food sources and bioavailability. *The American journal of clinical nutrition*, 79(5): 727–47. http://www.ncbi.nlm.nih.gov/pubmed/15113710 4 May 2017.

Marchal, L. 1999. Towards a rational design of commercial maltodextrins. *Trends in Food Science & Technology*, 10(11): 345–355.

http://linkinghub.elsevier.com/retrieve/pii/S0924224400000182 4 August 2017.

Mccarthy, A.L., O'Callaghan, Y.C., Connolly, A., Piggott, C.O., Fitzgerald, R.J. & O'Brien, N.M. 2014. Phenolic-enriched fractions from brewers' spent grain possess cellular antioxidant and immunomodulatory effects in cell culture model systems. *Journal of the Science of Food and Agriculture*, 94(7): 1373–1379.

McCarthy, A.L., O'Callaghan, Y.C., Connolly, A., Piggott, C.O., Fitzgerald, R.J. & O'Brien, N.M. 2012. Phenolic extracts of brewers' spent grain (BSG) as functional ingredients - Assessment of their DNA protective effect against oxidant-induced DNA single strand breaks in U937 cells. *Food Chemistry*, 134(2): 641–646. http://dx.doi.org/10.1016/j.foodchem.2012.02.133.

McCarthy, A.L., O'Callaghan, Y.C., Neugart, S., Piggott, C.O., Connolly, A., Jansen, M.A.K., Krumbein, A., Schreiner, M., FitzGerald, R.J. & O'Brien, N.M. 2013. The hydroxycinnamic acid content of barley and brewers' spent grain (BSG) and the potential to incorporate phenolic extracts of BSG as antioxidants into fruit beverages. *Food Chemistry*, 141(3): 2567–2574.

McCarthy, A.L., O'Callaghan, Y.C., Piggott, C.O., FitzGerald, R.J. & O'Brien, N.M. 2013. Brewers' spent grain; bioactivity of phenolic component, its role in animal nutrition and potential for incorporation in functional foods: a review. *The Proceedings of the Nutrition Society*, 72(1): 117–25. http://www.ncbi.nlm.nih.gov/pubmed/23137812.

Meneses, N.G.T., Martins, S., Teixeira, J.A. & Mussatto, S.I. 2013a. Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. *Separation and Purification Technology*, 108: 152–158. http://dx.doi.org/10.1016/j.seppur.2013.02.015.

Meneses, N.G.T., Martins, S., Teixeira, J.A. & Mussatto, S.I. 2013b. Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. *Separation and Purification Technology*, 108(April): 152–158.

Mesomo, M.C., Corazza, M.L., Ndiaye, P.M., Dalla Santa, O.R., Cardozo, L. & Scheer, A. de P. 2013. Supercritical CO2 extracts and essential oil of ginger (Zingiber officinale R.): Chemical composition and antibacterial activity. *The Journal of Supercritical Fluids*, 80: 44–49. http://www.sciencedirect.com/science/article/pii/S0896844613001290 8 April 2016.

Meziane, S. & Kadi, H. 2008. Kinetics and thermodynamics of oil extraction from olive cake. *JAOCS, Journal of the American Oil Chemists' Society*, 85(4): 391–396.

Montgomery, D.C. 1991. *Design and Analysis of Experiments*. 1st ed. New York: wiley.

Montgomery, D.C., Myers, R.H. & Anderson-Cook, C.M. 2016. *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*. 4th ed. New York: wiley.

Montuschi, P., Barnes, P.J. & Roberts, L.J. 2004. Isoprostanes: markers and mediators of

oxidative stress. *FASEB J*, 18. http://dx.doi.org/10.1096/fj.04-2330rev.

Moreira, M. 2012. *Extraction and Characterization of Natural Antioxidants from Brewing Industry By - products*. http://repositorio-aberto.up.pt/bitstream/10216/69892/2/9132.pdf.

Moreira, M.M., Morais, S., Barros, A.A., Delerue-Matos, C. & Guido, L.F. 2012. A novel application of microwave-assisted extraction of polyphenols from brewer's spent grain with HPLC-DAD-MS analysis. *Analytical and Bioanalytical Chemistry*, 403(4): 1019–1029.

Moreira, M.M., Morais, S., Carvalho, D.O., Barros, A.A., Delerue-Matos, C. & Guido, L.F. 2013. Brewer's spent grain from different types of malt: Evaluation of the antioxidant activity and identification of the major phenolic compounds. *Food Research International*, 54(1): 382–388. http://dx.doi.org/10.1016/j.foodres.2013.07.023.

Moubarik, A., El-Belghiti, K. & Vorobiev, E. 2011. Kinetic model of solute aqueous extraction from Fennel (Foeniculum vulgare) treated by pulsed electric field, electrical discharges and ultrasonic irradiations. *Food and Bioproducts Processing*, 89(4): 356–361. http://dx.doi.org/10.1016/j.fbp.2010.09.002.

Mussatto, S.I. 2014. Brewer's spent grain: A valuable feedstock for industrial applications. *Journal of the Science of Food and Agriculture*, 94(7): 1264–1275.

Mussatto, S.I., Dragone, G. & Roberto, I.C. 2006. Brewers' spent grain: Generation, characteristics and potential applications. *Journal of Cereal Science*, 43(1): 1–14.

Mussatto, S.I., Dragone, G. & Roberto, I.C. 2007. Ferulic and p-coumaric acids extraction by alkaline hydrolysis of brewer's spent grain. *Industrial Crops and Products*, 25(2): 231–237.

Niemi, P. 2016. Enz ymat ic frac t io nat io n o f bre w e r â€™ s spe nt grain and bio c o nve rsio n o f l h frac t io ns in a c o l o n mo de l in vit ro. *department of biotechnology*.

Niki, E. 2014. Antioxidants: Basic Principles, Emerging Concepts, and Problems. *Biomed J*, 37.

Noordin, M.Y., Venkatesh, V.C., Sharif, S., Elting, S. & Abdullah, A. 2004. Application of response surface methodology in describing the performance of coated carbide tools when turning AISI 1045 steel. *Journal of Materials Processing Technology*, 145(1): 46–58.

Nordkvist, E., Salomonsson, A.-C. & Åman, P. 1984. Distribution of insoluble bound phenolic acids in barley grain. *Journal of the Science of Food and Agriculture*, 35(6): 657–661. http://doi.wiley.com/10.1002/jsfa.2740350611 19 July 2017.

Oroian, M. & Escriche, I. 2015. Antioxidants: Characterization, natural sources, extraction and analysis. *Food Research International*, 74: 10–36. http://dx.doi.org/10.1016/j.foodres.2015.04.018.

Pan, X., Niu, G. & Liu, H. 2002. Comparison of microwave-assisted extraction and conventional extraction techniques for the extraction of tanshinones from Salvia

miltiorrhiza bunge. *Biochemical Engineering Journal*, 12(1): 71–77. http://linkinghub.elsevier.com/retrieve/pii/S1369703X02000396 21 July 2017.

Pandey, A. & Tripathi, S. 2014. Concept of standardization , extraction and pre phytochemical screening strategies for herbal drug. , 2(5): 115–119.

Papavasileiou, V., Koulouris, A., Siletti, C. & Petrides, D. 2007. OPTIMIZE MANUFACTURING OF PHARMACEUTICAL PRODUCTS WITH PROCESS SIMULATION AND PRODUCTION SCHEDULING TOOLS. , (July).

Parbunath, O.L. 2013. VALIDATION OF TWO BIO-ANALYTICAL ASSAYS FOR THE MEASUREMENT OF HYDROPHILIC ANTIOXIDANT CAPACITY IN SEVERAL FOOD AND BEVERAGE COMMODITIES IN ACCORDANCE WITH ISO 17025 REGULATORY GUIDELINES By OLIVIA LESHIA PARBHUNATH Thesis submitted in fulfilment of the r. , (March).

Parr, A.J. & Bolwell, G.P. 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture*, 80(7): 985–1012. http://doi.wiley.com/10.1002/%28SICI%291097-0010%2820000515%2980%3A7%3C985%3A%3AAID-JSFA572%3E3.0.CO%3B2-7 5 May 2016.

Payne, A.C., Mazzer, A., Clarkson, G.J.J. & Taylor, G. 2013. Antioxidant assays - consistent findings from FRAP and ORAC reveal a negative impact of organic cultivation on antioxidant potential in spinach but not watercress or rocket leaves. *Food science & nutrition*, 1(6): 439–44.

Pedrites, D., Siletti, C., Jimenez, J., Psathas, P. & Mannion, Y. 2011. Optimizing and the design of operation of fill-finish facilities using process simulation and scheduling tools. *Pharm. Eng*, 31(2): 1–10. http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Optimizing+the+Design+and+Operation+of+Fill-Finish+Facilities+using+Process+Simulation+and+Scheduling+Tools#0.

Peleg, M. 1988. An Empirical Model for the Description of Moisture Sorption Curves. *Journal of Food Science*, 53(1365): 1216–1217.

Pereira, D.M., Valentão, P., Pereira, J.A. & Andrade, P.B. 2009. Phenolics: From chemistry to biology. *Molecules*, 14(6): 2202–2211.

Perez, E.E., Carelli, A.A. & Crapiste, G.H. 2011. Temperature-dependent diffusion coefficient of oil from different sunflower seeds during extraction with hexane. *Journal of Food Engineering*, 105(1): 180–185. http://linkinghub.elsevier.com/retrieve/pii/S0260877411000896 22 July 2017.

Petrides, D. 2013. *Bioprocess Design and Economics*.

Petrides, D., Carmichael, D., Siletti, C. & Koulouris, A. 2014. Biopharmaceutical Process

Optimization with Simulation and Scheduling Tools. *Bioengineering*, 1: 154–187.

Piggott, C. & Fitzgerald, P.D. Brewers ' spent grain : a potential source of health enhancing ingredients Generation of Brewer ' s Spent Grain (BSG).

Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J. & N????ez, M.J. 2005. Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *Journal of Agricultural and Food Chemistry*, 53(6): 2111–2117.

Pinelo, M., Tress, A.G., Pedersen, M., Arnous, A. & Meyer, A.S. 2007. Effect of cellulases, solvent type and particle size distribution on the extraction of chlorogenic acid and other phenols from spent coffee grounds. *American Journal of Food Technology*, 2(7): 641–651.

Porkony, J. 2012. *Introduction of Antioxidant*.

Prasad, K.N., Yang, E., Yi, C., Zhao, M. & Jiang, Y. 2009. Effects of high pressure extraction on the extraction yield, total phenolic content and antioxidant activity of longan fruit pericarp. *Innovative Food Science and Emerging Technologies*, 10(2): 155–159. http://dx.doi.org/10.1016/j.ifset.2008.11.007.

Prentice, N. & D'Appolonia, B.L. 1977. High-fiber bread containing brewers spent grain. *Cereal Chemestry*, 54(5): 1084–1095.

Prior, R.L., Wu, X. & Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10): 4290–4302.

Punín Crespo, M.O. & Lage Yusty, M.A. 2005. Comparison of supercritical fluid extraction and Soxhlet extraction for the determination of PCBs in seaweed samples. *Chemosphere*, 59(10): 1407–1413.

Pyo, S., Meinke, M., Keck, C. & Müller, R. 2016. Rutin—Increased Antioxidant Activity and Skin Penetration by Nanocrystal Technology (smartCrystals). *Cosmetics*, 3(1): 9. http://www.mdpi.com/2079-9284/3/1/9.

Qing, H., Kai, Z., Zhang, C. & Chen, M. 2012. Packaging Design Research and Analysis Based on Graphic Visual. *IACSIT Coimbatore Conferences*, 28: 148–153.

Rakotondramasy-Rabesiaka, L., Havet, J.-L., Porte, C. & Fauduet, H. 2009. Solid–liquid extraction of protopine from Fumaria officinalis L.—Kinetic modelling of influential parameters. *Industrial Crops and Products*, 29(2–3): 516–523. http://linkinghub.elsevier.com/retrieve/pii/S0926669008001994 22 July 2017.

Ravishankar, R. V. 2016. *Advances in Food Biotechnology*. 1st ed. oxford: John Wiley & Sons.

Ricardo, P.-C., Vergara-Salinas, J.R. & Cuevas-valenzuela, J. eds. 2016. *Advances in Technologies for Producing Food-relevant Polyphenols*. 1st ed. New York: CRC Press: Taylor and francis group.

Richard Turton, Richard C. Bailie, Wallace B. Whiting, J.A.S. 2013. *Analysis, Synthesis and Design of Chemical Processes Third Edition*.

Roberts, R.T. 1976. Use of an Extract of Spent Grains As an Antifoaming Agent in Fermentors. *Journal of the Institute of Brewing*, 82(2): 96–96.

Roby, M.H.H., Sarhan, M.A., Selim, K.A.H. & Khalel, K.I. 2013. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. *Industrial Crops and Products*, 43(1): 827–831. http://dx.doi.org/10.1016/j.indcrop.2012.08.029.

Routray, W. & Orsat, V. 2012. Microwave-Assisted Extraction of Flavonoids: A Review. *Food and Bioprocess Technology*, 5(2): 409–424.

Rozzi, N.L.L. & Singh, R.K.K. 2002. Supercritical fluids and the food industry. *Comprehensive Reviews in Food Science and Food Safety*, 1(1): 33–44. http://doi.wiley.com/10.1111/j.1541-4337.2002.tb00005.x.

Rubilar, M., Pinelo, M., Shene, C., Sineiro, J. & Nu??ez, M.J. 2007. Separation and HPLC-MS identification of phenolic antioxidants from agricultural residues: Almond hulls and grape pomace. *Journal of Agricultural and Food Chemistry*, 55(25): 10101–10109.

SAB. 2017. No Title. http://country.southafrica.net/country/gh/en/articles/entry/sab-world-of-beer-enus1.

Santos, D.T., Veggi, P.C. & Meireles, M.A.A. 2010. Extraction of antioxidant compounds from Jabuticaba (Myrciaria cauliflora) skins: Yield, composition and economical evaluation. *Journal of Food Engineering*, 101(1): 23–31. http://linkinghub.elsevier.com/retrieve/pii/S0260877410002852 22 July 2017.

Santos, M., Jiménez, J.J., Bartolomé, B., Gómez-Cordovés, C. & Del Nozal, M.J. 2003. Variability of brewer's spent grain within a brewery. *Food Chemistry*, 80(1): 17–21.

Santos, S.B. Dos, Martins, M.A., Caneschi, A.L., Aguilar, P.R.M. & Coimbra, J.S.D.R. 2015. Kinetics and Thermodynamics of Oil Extraction from Jatropha curcas L. Using Ethanol as a Solvent. *International Journal of Chemical Engineering*, 2015.

Schillaci, C., Nepravishta, R. & Bellomaria, A. 2014. Antioxidants in food and pharmaceutical research. *Albanian Journal of Pharmaceutical Sciences*, 1(15): 15–25.

Shahidi, F. & Zhong, Y. 2015. Measurement of antioxidant activity. *Journal of Functional Foods*, 18: 757–781. http://dx.doi.org/10.1016/j.jff.2015.01.047.

Shahkar, E., Yun, H., Kim, D.-J., Kim, S.-K., Lee, B.I. & Bai, S.C. 2015. Effects of dietary vitamin C levels on tissue ascorbic acid concentration, hematology, non-specific immune response and gonad histology in broodstock Japanese eel, Anguilla japonica. *Aquaculture*, 438: 115–121. http://www.sciencedirect.com/science/article/pii/S0044848615000046.

Sharif, K.M., Rahman, M.M., Azmir, J., Mohamed, A., Jahurul, M.H.A., Sahena, F. & Zaidul, I.S.M. 2014. Experimental design of supercritical fluid extraction – A review. *Journal of*

Food Engineering, 124: 105–116. http://www.sciencedirect.com/science/article/pii/S0260877413005128 29 March 2016.

Shi, J. 2007. *Functional Food Ingredients and Nutraceuticals: Processing Technologies*. 1st ed. Boca Raton: CRC Press: Taylor and francis group.

Silva, G.G.D., Couturier, M., Berrin, J.G., Buléon, A. & Rouau, X. 2012. Effects of grinding processes on enzymatic degradation of wheat straw. *Bioresource Technology*, 103(1): 192–200. http://dx.doi.org/10.1016/j.biortech.2011.09.073.

Singh, M., Kaur, M. & Silakari, O. 2014. Flavones: An important scaffold for medicinal chemistry. *European Journal of Medicinal Chemistry*, 84: 206–239. http://dx.doi.org/10.1016/j.ejmech.2014.07.013.

Skinner, M. & Hunter, D. 1999. *Bioactives in Fruit: Health Benefits and Functional Foods*. 2nd ed. New York: wiley blackwell.

So, G.C. & Macdonald, D.G. 1986. Kinetics of oil extraction from canola (rapeseed). *The Canadian Journal of Chemical Engineering*, 64(1): 80–86. http://doi.wiley.com/10.1002/cjce.5450640112 22 July 2017.

Spinelli, S., Conte, A., Lecce, L., Padalino, L. & Del Nobile, M.A. 2016. Supercritical carbon dioxide extraction of brewer's spent grain. *Journal of Supercritical Fluids*, 107: 69–74. http://dx.doi.org/10.1016/j.supflu.2015.08.017.

Spinelli, S., Conte, A. & Del Nobile, M.A. 2016. Microencapsulation of extracted bioactive compounds from brewer's spent grain to enrich fish-burgers. *Food and Bioproducts Processing*, 100: 450–456. http://dx.doi.org/10.1016/j.fbp.2016.09.005.

Stalikas, C.D. 2007. Extraction, separation, and detection methods for phenolic acids and flavonoids. *Journal of Separation Science*, 30(18): 3268–3295.

Stockfield, B. 2017. Antioxidants: In Depth. *national center of complementary and integrative health*. https://nccih.nih.gov/health/antioxidants/introduction.htm.

Stroem, L.K., Desai, D.K. & Hoadley, A.F.A. 2009. Superheated steam drying of Brewer's spent grain in a rotary drum. *Advanced Powder Technology*, 20(3): 240–244. https://www.sciencedirect.com/science/article/pii/S0921883109000752 13 December 2017.

Ström, L.-L. Evaluation of Pilot Scale Drying of Brewer's Spent Grain in a Keith Rotary Superheated Steam Dryer -a Preliminary Investigation.

Tang, D.S., Tian, Y.J., He, Y.Z., Li, L., Hu, S.Q. & Li, B. 2010. Optimisation of ultrasonic-assisted protein extraction from brewer's spent grain. *Czech Journal of Food Sciences*, 28(1): 9–17.

Thiago, R.D.S.M., Pedro, P.M.D.M. & Eliana, F.C.S. 2014. Solid wastes in brewing process: A review. *Journal of Brewing and Distilling*, 5(1): 1–9. http://academicjournals.org/journal/JBD/article-abstract/0E6313746212.

Toda, T.A., Sawada, M.M. & Rodrigues, C.E.C. 2016. Kinetics of soybean oil extraction using

ethanol as solvent: Experimental data and modeling. *Food and Bioproducts Processing*, 98: 1–10. http://dx.doi.org/10.1016/j.fbp.2015.12.003.

Veggi, P.C., Prado, I.M., Vaz, N., Prado, J.M. & Meireles, M.A. a. 2011. Manufacturing Cost of Extracts from Jackfruit (Artocarpus heterophyllus) Leaves Obtained Via Supercritical Technology and Solvent Extraction. , 55(19).

Vellosa, J.C.R., Regasini, L.O., Khalil, N.M., Da Silva Bolzani, V., Khalil, O. a K., Manente, F.A., Netto, H.P. & De Faria Oliveira, O.M.M. 2011. Antioxidant and cytotoxic studies for kaempferol, quercetin and isoquercitrin. *Ecletica Quimica*, 36(2): 7–20.

Vergara-Salinas, J.R., Bulnes, P., Zúñiga, M.C., Pérez-Jiménez, J., Torres, J.L., Mateos-Martín, M.L., Agosin, E. & Pérez-Correa, J.R. 2013. Effect of pressurized hot water extraction on antioxidants from grape pomace before and after enological fermentation. *Journal of Agricultural and Food Chemistry*, 61(28): 6929–6936.

Vieira, E., Rocha, M.A.M., Coelho, E., Pinho, O., Saraiva, J.A., Ferreira, I.M.P.L.V.O. & Coimbra, M.A. 2014. Valuation of brewer's spent grain using a fully recyclable integrated process for extraction of proteins and arabinoxylans. *Industrial Crops and Products*, 52: 136–143. http://dx.doi.org/10.1016/j.indcrop.2013.10.012.

Vollmer, M. 2003. Physics of the microwave oven. *Physics Education*, 39(1): 74–81.

Whitney P, B. & Silvina, P. 2016. cosmetic benefits of natural ingredients. *Dermatology*, 11(10): 707–712. http://www.mdpi.com/2079-9284/3/1/9.

Wilhelmson, A., Pekka, L., Niklas von, W., Merja, I., Juhani, S., Raija-Liisa, H., Pirkko, F. & Buchert, J. 2009. Future applications for brewers' spent grain. https://www.newfoodmagazine.com/article/269/future-applications-for-brewers-spent-grain/.

Wilkinson, S., Smart, K.A., James, S. & Cook, D.J. 2017. Bioethanol Production from Brewers Spent Grains Using a Fungal Consolidated Bioprocessing (CBP) Approach. *Bioenergy Research*, 10(1): 146–157.

Wong, B.Y., Tan, C.P. & Ho, C.W. 2013. Effect of solid-to-solvent ratio on phenolic content and antioxidant capacities of 'Dukung Anak' (Phyllanthus niruri). *International Food Research Journal*, 20(1): 325–330.

Wood, L. 2016. South African Beer Report Presents an Analysis of the Fastest Growing Beer Industry in the World and Then Moves on to Examine Competition within the Industry.

Xi, J., He, L. & Yan, L. 2015. Kinetic modeling of pressure-assisted solvent extraction of polyphenols from green tea in comparison with the conventional extraction. *Food Chemistry*, 166: 287–291. http://dx.doi.org/10.1016/j.foodchem.2014.06.026.

Xiros, C. & Christakopoulos, P. 2009. Enhanced ethanol production from brewer's spent grain by a Fusarium oxysporum consolidated system. *Biotechnology for Biofuels*, 2(1): 4. http://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/1754-6834-2-4.

Yildiz-ozturk, E., Tag, O. & Yesil-celiktas, O. 2014. The Journal of Supercritical Fluids

Subcritical water extraction of steviol glycosides from Stevia rebaudiana leaves and characterization of the raffinate phase. , 95: 422–430.

Zhao, H., Dong, J., Lu, J., Chen, J., Li, Y., Shan, L., Lin, Y., Fan, W. & Gu, G. 2006. Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (Hordeum vulgare L.). *Journal of Agricultural and Food Chemistry*, 54(19): 7277–7286.

Zhuang, J. 2004. Economic analysis of cellulase production by Clostridium thermocellum in solid state and submerged fermentation. *University of Kentucky UKnowledge Graduate*: 102. http://uknowledge.uky.edu/gradschool_theses/170/.

APPENDICES

APPENDIX A: GLOBAL YIELD VALUES FOR MACERATION AND SOXHLET EXTRACTION.

For the soxhlet method, extractions were carried out at the boiling point of each solvent for 4 hours. Values are expressed as mean ± standard deviation of duplicate analysis. There is no literature data that shows the global yield of extraction of polyphenols from BSG.

Soxhlet extraction

Solvent used	***Global yield*** *%*	***Global yield % (references)***
Water	1,65 ± 0,02	-
Acetone 80 % w/w	0,8 ± 0,03	-
Ethanol 20 % w/w	1,06 ± 0,03	-

Maceration extraction

Solvent compositions *% v/v*	***Global yield*** *%*	***Global yield (literature data)*** *%*
Water	0,31 ± 0,002	-
Acetone		
100	0,14 ± 0,002	-
80	0,17 ± 0,08	-
60	0,111 ± 0,06	-
40	0,110 ± 0,04	-
20	0,18 ± 0,02	-
Ethanol		
100	0,14 ± 0,02	-
80	0,16 ± 0,08	-
60	0,17 ± 0,07	-
40	0,113 ± 0,05	-
20	0,214 ± 0,03	-

APPENDIX B: TABLE FOR P-VALUE OF GROUND AND SIEVED BSG

Error! Reference source not found. shows the regression statistics of the ground and sieved BSG. The standard error is high, the p-value> 0.05 and the R^2 value is 0.292, therefore it can be concluded that the experiments of the extraction of ground and sieved BSG could not be reproduced.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0,541095
R Square	0,292784
Adjusted R Square	-0,41443
Standard Error	4,258749
Observations	3

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	7,508591	7,508591	0,413994	0,63602
Residual	1	18,13695	18,13695		
Total	2	25,64554			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	9,970671	4,736359	2,105134	0,282323	-50,2105	70,15182
X Variable 1	-0,26868	0,417578	-0,64342	**0,63602**	-5,57451	5,037154

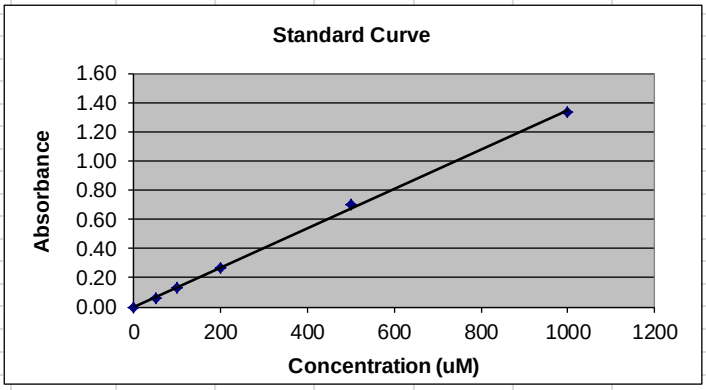
APPENDIX C: CALCULATION OF FRAP VALUES

To determine FRAP values, the absorbance of the standard is measured by the spectrometer. The absorbance standard values was plotted against concentration to get the standard curve. The absorbance from the spectrometer Δ_{593nm} of the samples was then inserted into the straight line equation of the standard curve as X to get Y the FRAP values (µmol/g) as shown by Equation 7.1. To convert the units to millimoles (mM Fe (II)/g BSG), the FRAP values are divided by 1000

$$\mathrm{FRAP}\,(\mu\mathrm{mol}\,(\mathrm{Fe}\,(\mathrm{II})/\mathrm{g}\,\mathrm{BSG}) = \frac{(\Delta_{593nm} - \mathrm{intercept})}{\mathrm{slope}} \qquad \text{Equation 7.1}$$

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.073	0.072	0.072	0.134	0.134	0.128	0.216	0.207	0.195	0.325	0.341	0.341
B	0.775	0.775	0.774	1.455	1.330	1.430	0.155	0.159	0.161	0.697	0.708	0.738
C	0.147	0.120	0.176	0.450	0.461	0.468	0.441	0.438	0.469	0.432	0.437	0.439
D	0.091	0.091	0.080	0.315	0.337	0.328	0.500	0.531	0.536	3.164	3.255	3.294
E	0.360	0.294	0.283	3.001	3.107	3.143	1.503	1.511	1.415	1.350	1.395	1.455
F	1.559	1.558	1.609	0.940	0.932	0.920	1.911	2.042	2.085	0.189	0.235	0.190
G	0.449	0.435	0.442	0.268	0.247	0.240	0.716	0.647	0.689	0.076	0.071	0.071
H	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	1.193	1.153	1.139

	Average	Ave - Blank	Conc	COV%	Predicted
Blank	0.07	0.00	0.00	0.80%	
Std 1	0.13	0.06	50.00	2.62%	97.70
Std 2	0.21	0.13	100.00	5.11%	152.73
Std 3	0.34	0.26	200.00	2.75%	249.14
Std 4	0.77	0.70	500.00	0.07%	575.55
Std 5	1.41	1.33	1000.00	4.71%	1044.24
Slope, m	0.0013				
y-intecept, b	0.0006				
R-squared, r2	0.999				

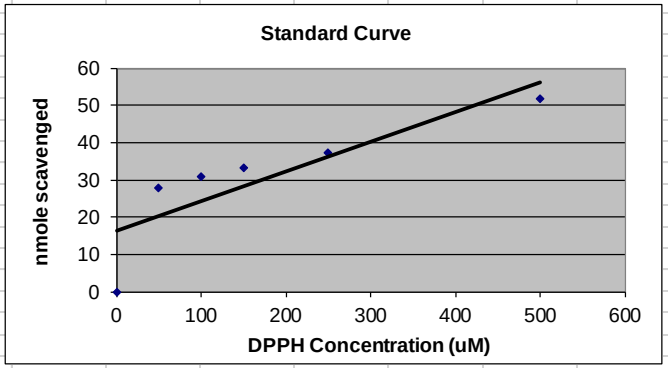


	Conc (umole/g	COV%
Sample 1	63.50	1.93%
Sample 2	476.91	2.97%
Sample 3	55.57	18.97%
Sample 4	511.51	1.97%
Sample 5	121.99	3.81%
Sample 6	1105.95	0.83%
Sample 7	95.97	7.27%
Sample 8	1118.98	3.39%
Sample 9	141.97	3.73%
Sample 10	0.44	2.06%
Sample 11	236.89	13.33%
Sample 12	871.00	2.39%
Sample 13	98.94	3.61%
Sample 14	150.99	3.76%
Sample 15	422.38	1.85%
Sample 16	794.65	1.08%
Sample 17	111.00	4.50%
Sample 18	952.23	12.84%
Sample 19	870.16	1.58%
Sample 20	125.34	5.79%
Sample 21	358.24	5.08%
Sample 22	502.36	3.97%
Sample 23	50.61	0.00%
Sample 24	781.35	0.00%
Sample 25	901.25	0.00%
Sample 26	10.25	2.41%

APPENDIX D: DPPH CALCULATIONS

	1	2	3	4	5	6	7	8	9	10	11	12
A	2,3	2,3	2,3	1,884			1,812	1,852	1,841	1,813	1,784	1,811
B	1,731	1,715	1,783			1,522	2,245	2,116	2,172	2,164	2,146	2,23
C	2,163	2,148	2,189	2,209	2,185	2,136	2,23	2,179	2,167	2,027	1,988	1,944
D	1,949	1,953	1,962	2,029	2,033	2,031	1,984	2,024	1,99	1,987	1,952	1,895
E	1,963	1,87	1,955	1,923	1,944	1,806	1,973	1,837	1,941	1,907	1,898	1,76
F	1,84	1,622	1,779	1,816	1,848	1,809	1,825	1,742	1,794	1,84	1,845	1,848
G	1,906	1,798	1,834	1,914	1,894	1,914	1,897	1,94	1,923	1,907	1,976	1,885
H	1,852	1,799	1,828	1,833	1,849	1,906	1,823	1,795	1,789	2,03	2,093	1,973

	Average	Conc	STDEV %	% DPPH use	Remaining	[DPPH]	nmole scavenged
Blank	2,30	0,00	0,00%	0,00	100,00	153,33	0,00
Std 1	1,88	50,00	0,05%	18,09	81,91	125,60	27,73
Std 2	1,84	100,00	1,13%	20,22	79,78	122,33	31,00
Std 3	1,80	150,00	0,90%	21,62	78,38	120,18	33,16
Std 4	1,74	250,00	2,04%	24,22	75,78	116,20	37,13
Std 5	1,52	500,00	0,07%	33,83	66,17	101,47	51,87
Slope, m	0,0795						
y-intecept, b	0,0000						
R-squared, r2	0,717						



	nmol				umole TE/g			Average	STDEV %
Control 1	8,53	3,67	12,27	8,53	46,1	154,3	107,3	102,56	2,97%
Control 2	8,00	9,07	10,27	4,67	100,6	114,0	129,1	114,58	2,03%
Sample 1	8,89	9,13	10,13	7,40	111,8	114,9	127,4	118,03	0,96%
Sample 2	8,22	6,07	7,67	10,93	103,4	76,3	96,4	92,04	1,71%
Sample 3	7,20	4,67	8,07	8,87	90,5	58,7	101,4	83,56	1,53%
Sample 4	20,91	18,20	20,80	23,73	263,0	228,9	261,6	251,14	2,09%
Sample 5	23,02	23,40	23,13	22,53	289,5	294,3	290,9	291,57	0,34%
Sample 6	17,93	18,07	17,80	17,93	225,5	227,2	223,8	225,52	0,10%
Sample 7	20,04	21,07	18,40	20,67	252,1	264,9	231,4	249,47	1,08%
Sample 8	23,69	20,87	23,20	27,00	297,9	262,4	291,8	284,03	2,39%
Sample 9	24,71	22,47	28,67	23,00	310,8	282,5	360,5	317,93	2,67%
Sample 10	27,27	25,13	23,73	32,93	342,9	316,1	298,5	319,14	3,93%
Sample 11	25,53	21,80	30,87	23,93	321,1	274,2	388,2	327,81	3,71%
Sample 12	29,67	26,20	26,80	36,00	373,1	329,5	337,0	346,53	4,44%
Sample 13	36,87	30,67	45,20	34,73	463,6	385,7	568,4	472,57	6,44%
Sample 14	31,71	32,27	30,13	32,73	398,8	405,8	378,9	394,51	1,14%
Sample 15	34,20	31,67	37,20	33,73	430,1	398,2	467,8	432,05	2,35%
Sample 16	30,38	30,67	30,33	30,13	382,0	385,7	381,5	383,05	0,22%
Sample 17	30,27	26,27	33,47	31,07	380,6	330,3	420,9	377,27	2,98%
Sample 18	26,18	25,73	27,07	25,73	329,2	323,6	340,4	331,07	0,61%
Sample 19	25,33	26,87	24,00	25,13	318,6	337,9	301,8	319,42	1,13%
Sample 20	25,16	26,20	21,60	27,67	316,3	329,5	271,6	305,82	2,47%
Sample 21	31,58	29,87	33,40	31,47	397,1	375,6	420,0	397,58	1,45%
Sample 22	29,16	31,13	30,07	26,27	366,7	391,5	378,1	378,76	2,06%
Sample 23	33,18	31,80	33,67	34,07	417,2	399,9	423,4	413,51	1,01%
Sample 24	17,87	18,00	13,80	21,80	224,7	226,4	173,5	208,20	2,95%

The antiradical power is calculated by the equation

$$\%\,\mathrm{ARP} = \frac{A_f}{A_0} \times 100$$

Equation 7.2

Where A_f and A_0 correspond to absorbance at 515nm of DPPH• .

APPENDIX E: FITTING THE MODELS INTO EXPERIMENTAL DATA BY NON-LINEAR REGRESSION

The following worksheet was used to model the rate law into the experimental data. Solver in data analysis was used in the non-linear regression.

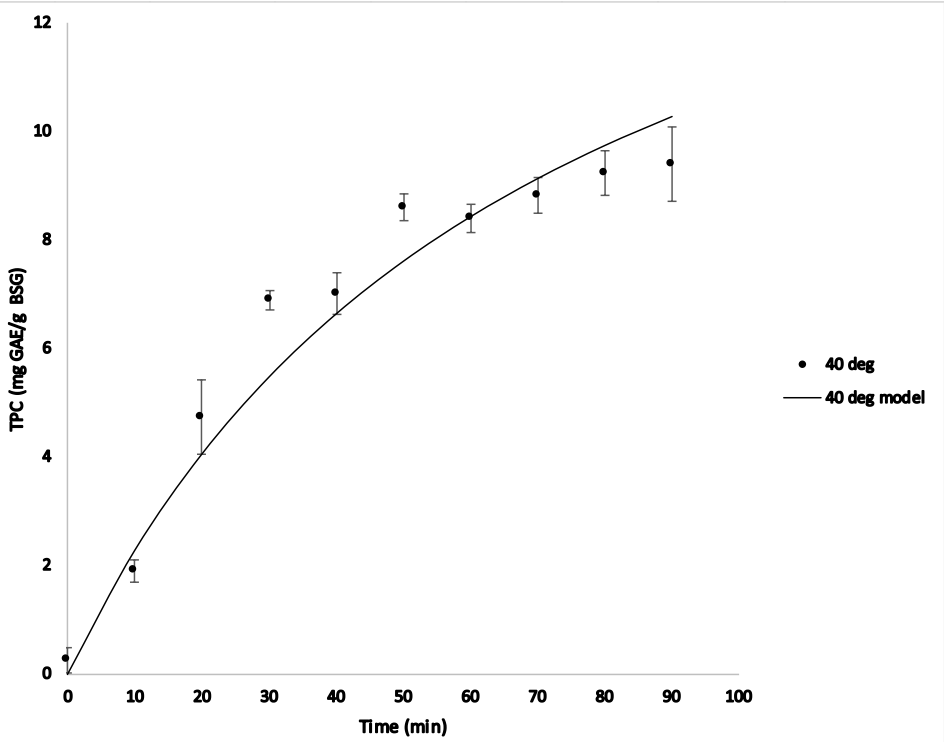
RATE LAW

$$c = \frac{t}{(1/h) + (t/c_\infty)}$$

Parameter	Amount
h	0.260558827
c_∞	18.27791521
1/h	32
k	1

40 degrees

Time (x) min	average TPC (y)	standard error	y (estimated)	SSE^2
0	0.255	0.22568461	0	1
10	1.9	0.20858513	2.280494818	0.04010424
20	4.73	0.691819704	4.055050061	0.020362019
30	6.89	0.191479186	5.475221933	0.042163649
40	7.01	0.394067073	6.637529793	0.002823235
50	8.6	0.255288236	7.606359751	0.013349391
60	8.4	0.260695155	8.426309472	9.80993E-06
70	8.82	0.328392765	9.129247968	0.001229353
80	9.24	0.418542529	9.738554278	0.00291126
90	9.4	0.6956465	10.27176811	0.008600947
			SSE^2	1.131553904



APPENDIX F: CALCULATION OF R^2 VALUES FROM EXCEL FOR THE SO AND MACDONALD'S MODEL

Excel was used to calculate the coefficient of determination for all models as shown below

SUMMARY OUTPUT									
Regression Statistics									
Multiple R	0.978866928								
R Square	0.958180463								
Adjusted R Square	0.952953021								
Standard Error	0.724135205								
Observations	10								
ANOVA									
	df	*SS*	*MS*	*F*	*Significance F*				
Regression	1	96.1163796	96.11638	183.2981	8.50692E-07				
Residual	8	4.194974366	0.524372						
Total	9	100.311354							
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*	
Intercept	1.509422298	0.425613229	3.546465	0.007548	0.527956433	2.490888163	0.527956433	2.490888163	
X Variable 1	0.107937344	0.007972466	13.53876	8.51E-07	0.089552804	0.126321884	0.089552804	0.126321884	

APPENDIX G: KINETIC STUDIES FOR MACERATION EXTRACTION USING WATER

The time of extraction rate was determined as shown.