PRODUCTION OF COOKING OIL FROM CORN GERM USING SUPERCRITICAL CO_2 AS SOLVENT

by

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ABSTRACT

This work was aimed at the production of edible, non-toxic oil from three different corn samples with little or no waste of raw materials. Moisture contents of the samples were determined to ensure that structural damage and rupture to the germ did not occur. Sohxlet extraction and supercritical fluid extraction methods were used to extract the oil and study the effect of pretreatment of the corn germ, pressure, temperature and flow rate on the process. Transmission Electron Microscope (TEM) was also used to monitor the corn sample surface morphology before and after extraction. Out of the three samples used UK sample 1 had the lowest oil content of 9.00 %, UK sample 2 has the highest oil content of 16.37% and Nigerian (NGN) sample had oil content of 12.10 %. Apparent solublity of corn oil in supercritical CO_2 measurement shows that the crossover pressure is 240 bars. Supercritical fluid extraction of corn oil occurred in two stages namely slow and fast extraction periods. The oil recovered in the slow extraction period was negligible as compared to that recovered in the fast extraction period. It was therefore not economical to continue the extraction in the slow extraction period. Based on the result it is therefore better to carry out the test at a flow rate of 4 l/min. For both UK sample 2 and the NGN samples, extraction at 300 bar had higher oil yield and faster recovery, even though Nigerian germ was processed using improvised method. The results of this study on supercritical extraction revealed that that UK sample 2 has high economic potentials followed by NGN sample.

DEDICATION

In the name of Allah, the most Gracious, the most Merciful. Peace and blessings of Allah be on his noble Prophet Muhammad (S.A.W.), the Opener and the Seal.

This work is dedicated to our beloved Prophet Muhammad *(S.A.W.),* his *Khalifa, Sheikh Tijjani (R.A.)* and their *Khalifa, Sheikh Ibrahim Inyaas (R.A.).*

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ABREVIATIONS

ASE Accelerated Solvent Extraction

BPR Back Pressure Regulator

CV Check Valve

ESE Enhanced Solvent Extraction

FMAE Focused MAE

GC Gas Chromatography

HPLC High Performance Liquid Chromatography

M:F Methanol:Fat

MAE Microwave-Assisted Extraction

ME Microwave Extraction

MV Micro-metering Valves

NGN Nigerian

N.P.T. National Pipe Thread

NPT Non Pretreated

PFE Pressurized Fluid Extraction

PHB Poly (R-Hydroxybutyrate)

PLE Pressurized Liquid Extraction

PMAE Pressurized MAE

PSE Pressurized Solvent Extraction

SC-CO_2 Supercritical Carbon dioxide

SDE Simultaneous Distillation-solvent Extraction

SEM Scanning Electron Microscopy

SF Supercritical Fluid

SFE Supercritical Fluid Extraction

TEM Transmission Electron Microscopy

UAE Ultrasound-Assisted Extraction

V:V Volume:Volume

W/W Weight per Weight

W_1 Weight of Thimble

W_2 Weight of Thimble + sample

W_3 Weight of empty evaporator flask

W_4 Weight of empty evaporator flask + extracted oil

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THESIS LAYOUT

This thesis comprises of five chapters. Chapter one is made up of the background information stating clearly the aim and objectives of the research. Chapter two is mainly a review of relevant literature on corn oil, its properties, potential benefits and the corn milling processes. The chapter also provides highlight on various extraction process. It also further provide a review on the concept of SFE, factors affecting it, application and research on SFE. Chapter three centred on materials and methods used to achieve the stated aim and objectives of the research. While chapter four discussed all the results of the experiment carried out. Finally chapter five helps in drawing a conclusion and providing appropriate recommendations for future work.

CHAPTER ONE

1 INTRODUCTION

1.1 Brief Overview

Three types of agricultural products make up the bulk of farm products that enter into chemical process industries. Animal fats, both edible and inedible are the largest in quantity and value. Starch runs a close second and vegetable oils are next. Fat and oils are found widely distributed in nature in both the plant and animal kingdoms. Waxes likewise are natural products, but differ slightly from fats and oils in basic composition. Waxes are mixed esters of higher polyhydric alcohols other than glycerol and fatty acids, whereas fats and oils are mixtures of the glycerides of various fatty acids (George, 1984).

Today, corn oil has become an important item in the mix of products manufactured from America's most important crop, and is no longer thought of as simply another co-product of starch manufacture. The growth of corn oil in the market place is based on its functionality, economy and acceptability in relation to other fats and oils. Among these factors, functionality is foremost. For health reasons, corn oil has replaced a significant amount of saturated fats and is also a top choice for trans-fat reduction in numerous food products. In 1950s, medical researchers found that corn oil was effective in reducing serum cholesterol in humans. This research gave rise to an increase in demand for corn oil that continues today. The current development in corn refining, characterized by advanced technology and variety of industries it serves demonstrate that corn kernel like crude petroleum has become an important source of chemical feedstock (Erickson, 2006a).

It is reported in Corn Refiners Association Annual Report, 2006, that if an average American is asked about corn's role in a sustainable environment, the response will likely focus on ethanol, with good reason. However, there are many more ways in which corn contributes to a sustainable environment. As a rich carbohydrate source, corn provides the backbone to a number of products that reduce our use of petrochemicals and non-renewable resources. Corn-based chemicals,

solvents and fuels not only have a positive impact by reducing our dependence on fossil fuels, but they are also better for the environment. The versatility of carbohydrate chemistry ensures that new ways of making corn to benefit the environment will be found and we will continue to ensure that new ways of beneficial usage of corn will be harnessed (Erickson, 2006b). For instance, it has been discovered, from the literature (Sigh and Cheryan, 1998), that edible oil can be extracted from corn.

Oil is extracted from plant material (oil seeds) using a solvent, usually hexane. Published research indicates that about 50-70% of the hexane can be recovered and recycled using nano filtration membranes instead of the evaporators used today, thus reducing energy consumption substantially. The extracted crude oil is mostly triglycerides, but it also contains small amounts of free fatty acids, phosphatides (lecithin/gums) and waxes, among other impurities (King, 2000).

Organic solvents lack the desired solute specificity and often require downstream refining to produce quality oil and meal. Supercritical CO_2 is proven to be an efficient solvent with better transport properties than most organic solvents (Del Valle ***et al.,*** 2008). Supercritical fluid extraction is the most technologically advanced extraction system (Shobben ***et al.,*** 2011). Supercritical carbon dioxide (SC-CO_2) extraction of oilseeds is a viable method to expeller and/or hexane extraction methods. There has been a great deal of interest in supercritical fluid technology in the past ten years as evidenced by literature. One of the most active areas involves the extraction of oil with SC-CO_2 because CO_2 is used in food products such as carbonated beverages. It is commonly regarded as a safe solvent for the extraction of variety of agricultural products, including oilseed. An additional advantage of SC-CO_2 is its low cost, non-flammability and ease of separation from extracted products by phase separation (List ***et al.***, 1989).

The vegetable oil industry must advance to meet up with universal and competitive economy through minimizing production costs and producing high quality oil whose properties must comply with international standard for edible oil (Santos, 2000). Incorporating supercritical fluid chromatography (SFC) for the separation and detection of food-related solutes eliminates not only most of the traditional solvent needs associated with high performance liquid

chromatography (HPLC), but also any solvent utilized in the extraction or sample work-up steps prior to analysis. In this regard, SFC is an excellent tool for monitoring the end-result of an extraction or reaction of a food component using supercritical fluid media. Also, by using SFC, food related analytes that are thermally liable to degradation via oxidation are not exposed to the harsh conditions that often accompany their analysis by gas chromatography (GC) or HPLC. This advantage can be attributed to the protective action of CO_2 which excludes oxygen and the low temperatures used when separating components via SFC (King, 2000).

Abdulkadir and Isah (2010) used a by-product obtained from flour production to produce corn oil. Other work to produce crude corn oil locally includes that of Adeoti, 2006 using solvent extraction but virtually no effort has been expended on trying to design an equipment to extract the oil. Corn varieties vary in the number of days needed from planting to maturity. Long season varieties take up to 180 days to mature while short season varieties mature in around 120 days. Some short season varieties mature within 90 days after emergence (Bishop *et al.*, 1983). Considering the ease of cultivation of maize and its availability in Nigeria, coupled with the economic and medicinal importance of corn and its oil as stressed earlier, this work is going to lay foundation for design of an indigenous plant for the process.

1.2 Aim

The aim is to produce edible, low cholesterol, non-toxic oil from corn with little or no waste of raw materials using supercritical fluid extraction with CO_2 as solvent.

1.3 Objectives

The aim of this research was achieved through the realization of the following objectives:

1. Determination of moisture content of corn germ powder for the extraction
2. Determination of oil content of corn germ powder using solvent extraction
3. Evaluation of the process parameters for supercritical fluid extraction (SFE)
4. Generation of design data

1.4 Scope

This work will focus on the use of solvent extraction and supercritical fluid extraction to produce oil from corn and also study the effect of process variables on SFE of corn oil. This will serve as the basis for later work in lipids and resins from plants and herbs.

1.5 Justification

According to Koh and Mohammed (2011), edible oils are limited in supply in many countries of the world. In India for instance, considerable amount of edible oil are imported to cater for short fall (Jain and Sharma, 2011). Van Kasteren and Nisworo (2007) made it clear that China imports more than 400 million tonnes of edible oil annually to satisfy it consumption need. Also in Nigeria research has shown that local production cannot satisfy the edible oil requirement (Oghenejoboh and Umukoro, 2011). In fact, Sam *et al.* (2008) declared that there is acute shortage of edible oil in Africa with the shortfall being met by import of oil from developed countries of the world.

Corn oil despite its nutrition and medicinal value is under explored in Nigeria. This could be due to the obvious reason that corn is a staple food in the country. This work will use only the germ for the production thereby boosting the corn flour production and improving the quality of the by-product for animal feed. More so, considering the ease of cultivation of maize and its availability in Nigeria, coupled with the economic and medical importance of corn and its oil as stressed earlier, this work is going to serve as a spring board for the design of an indigenous plant for the process for the commercialization exploitation of corn oil.

In the last few decades solvent extraction using commercial grade hexane has been perceived as the most efficient means of recovery of oil from its seeds. This technique has time and again

resulted in the production of undesirable residues and the resulting oil usually undergoes oxidative transformation during solvent removal; this transformation has the capacity to cause deterioration in the oil quality (Nimet *et al.,* 2011). The unique properties of supercritical fluids bring certain advantages to chemical separation processes over solvent extraction. This has made this novel approach a very attractive alternative to the conventional solvent extraction method as supercritical extraction using pressurized fluid enable efficient removal of the oil, aid the complete solvent recovery due to fluid volatility. These are important factors which make the supercritical fluid extraction process more economically attractive.

The versatility of corn coupled with the wide application of SFE will no doubt bring a lot of benefits to the industrialization process of Nigeria and West Africa in terms of modern day processing of its agricultural, mineral and water resources.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Maize Kernel

This crop is one of the world's most important grain crops and geographically the most widely planted and distributed food plant, surpassed in acreage planted only by wheat. It is grown from latitudes up to 58^0N in Central Europe, Canada, Russia and throughout the tropics, to latitudes of about 40^0S in Southern America and New Zealand. Corn as a crop matures every month of the year somewhere around the world. Maize as shown in Figure 2.1 is also called corn or Indian corn in English, mais in French, milho in Portuguese, maiz in Spanish and botanically as *Zea mays*, is a cereal plant of the tribe Mayadeae of the grass family gramineae (Anonymous, 1973).

Bishop *et al.*, 1983 outlined that there are both yellow and white varieties of corn where dent corn and flint corn are types of field corn. Dent is identified by the depression in the crown of the seed, relatively soft endosperm, while the flint corn has a hard endosperm. In the United States dent corn is the principal field corn grown while flint is mostly grown in South America and Europe. The yellow field corn is used primarily as a feed for livestock, but it is also used for corn-meal and snack foods such as corn chips. On the other hand, white field corn is used primarily for food products such as corn chips, snack foods, hominy and grits. Other types of corn that exist include popcorn, sweet corn, waxy maize, flour corn, pod corn, and decorative corn. The dent corn containing more oil is widely grown for industrial purposes.

2.1.1 Composition of maize kernel

The composition of corn germ obtained by dry milling can vary widely. According to Djerdj *et al.,* 1992 corn germ contains from 10 to 24% oil. Previous investigations have shown that the quality of corn germ may vary even within the same de-germination plant. Lofland *et al.*, 1954 in their work on distribution of fatty acids in corn oil made it clear that it has been known for many years that oil content of corn grain is variable and percentage of oil varies among strains.

Christianson and Friedrich (1985), in their work, instead of extracting oil from corn germ, rather produced high-protein, food-grade product by de-fatting the corn. The authors discovered that when a dry-milled corn germ fraction is subjected to extraction by carbon dioxide under supercritical conditions the residual lipid content reduced to less than half that obtainable by hexane extraction. The peroxidase activity is also reduced by sevenfold. Therefore, the flour produced from SC-CO_2 extracted corn germ residue has an acceptable flavour and extended shelf life.

This justifies the fact that when supercritical extraction is used to extract corn oil there is no waste of raw material because even the de-fatted corn germ could be a useful as a source of a high-quality, food-grade flour for both humans and animal without toasting.

Figure 2.1: Picture of the corn kernel

2.1.2 Corn milling process

Two basic corn milling processes (dry and wet) are available which are used depending on the interest of the manufacturer. The combination of these two processes is known as the combined dry-wet milling process. Wet milling is mostly used to recover corn germ. Wet milling is made up of starch and oil recovery processes are mostly affected by method of harvesting and drying. It was reported by Freeman (1973) that the introduction of field shelling initiated a decline in the oil content of corn received by wet milling plants. In his work, it was explained that kernel impacts due to field shelling of corn containing 20-25% (wet basis) moisture caused structural damage and rupture of the germ which allows oil to flow into the endosperm. Since only the germ is processed for oil recovery, oil that is lost from the germ is not recoverable after wet milling.

2.2 Fat and Oil

Oils are used to describe all substances that are greasy or oil fluids at room temperature, while fats are solids at room temperatures. The most abundant group of lipids is the neutral fat and oil, called triglycerides. Lard, tallow, butterfat (animal fat) and olive oil, cottonseed oil, corn oil, peanut oil, linseed oil, coconut oil, and soybean oil (vegetable oils) are included in this group. Their molecules consist of esters between glycerol and long chain fatty acids. The three acid units in typical triglycerides are not identical and are usually from three different fatty acids (Holum, 1979).

A careful review of the literature revealed that some work had been carried out on extraction and oil content of oil using samples from different locations in the world. Yermanos ***et al.,*** 1972 studied oil content and composition of the seed in the World collection of sesame where they used 721 samples from 20 countries. The study showed that there is considerable quantitative variability of traits in the collection. They concluded that the samples analysed were in many cases quite heterogeneous, representing local unselected populations, segregating populations, or mechanical mixtures.

Emil ***et al.***, 2010 extracted oil from Jatropha seed collected from different origins i.e. Malaysia, Indonesia and Thailand. The physicochemical properties of the extracted jatropha seed oil were evaluated. The fatty acid composition was also evaluated using gas chromatography (GC). The research revealed that jatropha oil from Indonesian seed has the highest amount of polyunsaturated fatty acids and can find application in surface coating industries. While Thailand jatropha oil containing a high amount of monounsaturated fatty acid can find application as

biodiesel feed stock. Generally, the high yield of jatropha oil compared to those of other vegetable oils is an advantage for selecting this oil to produce cost competitive products.

2.3 Corn Oil

Corn germ contains about 85 percent of the total oil of the kernel. The rest is dispersed in endosperm and hull fractions. Oil is usually extracted from the germ by a combination of expelling in continuous screw presses and solvent extraction of the press cake. The initial expeller can recover a little more than half of the oil and subsequent solvent extraction (with hexane or iso-hexane) will bring the total yield to about 95 percent. The solvent is mostly removed by evaporation for recovery and re-used. Corn oil as a concentrated source of energy (calories), is highly digestible and provides essential fatty acids and Vitamin E to the body. It is also a rich source of poly-unsaturated fatty acids which help regulate blood cholesterol levels and lower elevated blood pressure (Erickson, 2006a).

2.3.1 Health benefit of corn oil

The Corn Refiners Association, in its an article on benefit of corn oil, defined it as an effective component in lowering blood cholesterol and pressure levels because it offers high levels of polyunsaturated instead of saturated fats. Monounsaturated fats neither lower nor raise blood cholesterol levels while polyunsaturated fats lower blood cholesterol levels. On the other hand, saturated fats are approximately twice as powerful in raising cholesterol levels as polyunsaturated fats are in lowering them. Corn oil contains about 25 to 30 percent monounsaturated, 10 to 15 percent saturated fats and 60 percent polyunsaturated. In fact, the

U.S. Food and Drug Administration have acknowledged the unsaturated fat benefits of corn oil in reducing the risk of heart disease.

Corn oil research has shown that phytosterols play an important role in reducing blood cholesterol by inhibiting its absorption from the intestines. The U.S. Department of Agriculture reported that corn oil contains 968 milligrams of phytosterols per 100 grams of oil. The oil has one of the highest phytosterol levels of the refined vegetable oils. Rice-bran oil is the only oil that has higher phytosterol content at 1,190 mg/100 grams. However, corn oil is the only product that contains a natural mixture of free phytosterol, phytosterol esters, and phytostanol esters.

Linoleic acid is essential because it cannot be synthesized by the body and must be supplied in the diet. Corn oil is a rich source of linoleic acid, which is one of two essential acids necessary for growth, good skin and hair quality. Other benefit of corn oil is that it is recognized as an excellent source of tocopherols functioning as antioxidants which retard development of rancidity and provide a good source of Vitamin E.

2.4 Extraction Processes

Apart from Expeller-Pressed, Cold-Pressed and Chemical or Solvent Extraction methods of extraction which are the traditional methods of extraction, there are other recent methods being used in the literature for extraction of substance from solid matrix. Camel (2001) critically reviewed three extraction techniques which are: SFE, pressurized fluid extraction (PFE) and microwave-assisted extraction (MAE). In another development, Xu *et al.* (2011) in their work on recent advances on supercritical fluid extraction of essential oils which covers the period between 2005 and 2011 discussed ultrasound-assisted extraction (UAE) and microwave extraction (ME). ME is basically different from MAE as a result of addition of solvent in the

latter. Xu *et al.* (2011) also added simultaneous distillation-solvent extraction (SDE) as part of their review.

According to Camel (2001) pressurized fluid extraction (PFE), accelerated solvent extraction (ASE™, which is a Dionex trade mark), pressurized liquid extraction (PLE), pressurized solvent extraction (PSE) or enhanced solvent extraction (ESE) is an extraction technique which appeared few years ago. The pressurized fluid extraction is derived partly from supercritical fluid extraction based on the basic principle of its operation but in the PFE, the extractant is maintained in its liquid state as against that of SFE which is mostly in its supercritical state.

Pressurized fluid extraction is similar to Soxhlet extraction but the solvents used are near their supercritical region where they possess high extraction properties. In that region, the high temperature increases solubilization and diffusion rate of lipid solutes in the solvent (Camel, 2001). On the other hand, the high pressure keeps the solvent below its boiling point, enabling a high penetration of the solvent in the sample. PFE permits high extraction efficiency with little volume of solvent and short extraction time (Richter *et al.*, 1996).

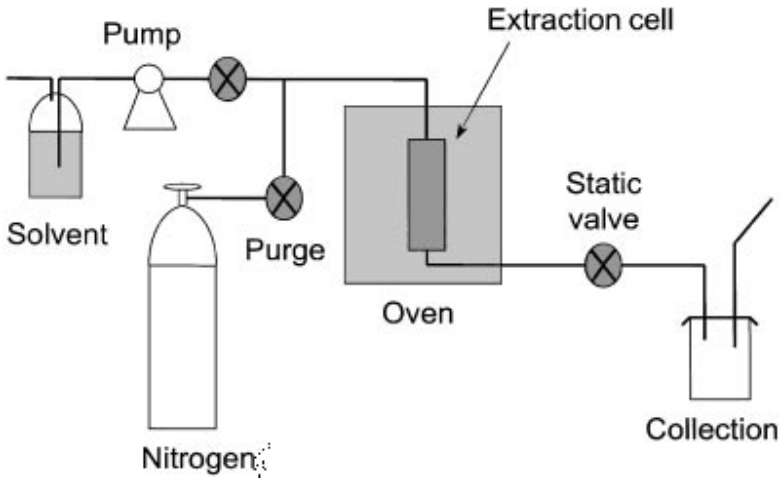

Figure 2.2: A schematic diagram of PFE process

(Richter *et al.*, 1996)

Microwave extraction has been used for isolating various components like essential oils (Bayramoglu *et al.*, 2008; Bousbia *et al.*, 2009; Deng *et al.*, 2006).

The MAE uses microwave radiation as the source of heating of the solvent–sample mixture. Due to the dipole rotation and ionic conductance, heating with microwaves is instantaneous and occurs in the heart of the sample which to leads to a very fast extraction. In most cases the extraction solvent is chosen to absorb microwaves but for thermo-labile compounds, the microwaves may be absorbed only by the matrix, resulting in heating of the sample and release of the solutes into the cold solvent. Microwave energy application to the samples may be performed using either closed vessels under controlled pressure and temperature or open vessels at atmospheric pressure. The two technologies are commonly named pressurized MAE (PMAE) or focused MAE (FMAE), respectively.

Ultrasound-assisted extraction is an extraction method based on ultrasonic waves. The ultrasound disintegration of cell structures (lysis) is used for the extraction of intra-cellular compounds. Sonicating liquids at high intensities possess sound waves that propagate into the liquid media resulting in alternating high-pressure known as compression and low-pressure. During the low-pressure cycle, high-intensity ultrasonic waves create small vacuum bubbles or voids in the liquid. Once the bubbles attain a volume at which they can no longer absorb energy, they collapse violently during a high-pressure cycle i.e. cavitations. Very high temperatures (approx. 5,000K) and pressures (approx. 2,000atm) are reached locally during the implosion. The implosion of the cavitations bubble also results in liquid jets of up to a velocity of 150m/s. The resulting shear forces improve material transfer by breaking the cell envelope mechanically (Mummery, 1978).

UAE has been widely applied for the extraction of nutritional material, such as bioactive compounds e.g., carotenoids (Sun *et al.,* 2006; Yue *et al.,* 2006), essential oils (Kimbaris *et al.,* 2006), flavonoids (Ma *et al.,* 2008; Zhang *et al.,* 2009), flavoring (Chen *et al.,* 2007; Da Porto *et al.*, 2009), lipids (Metherel *et al.,* 2009), proteins (Zhu *et al.,* 2009), polysaccharides (Iida *et al.,* 2008; Chen *et al.,* 2010; Wei *et al.,* 2010; Yan *et al.,* 2011), Saponins (Wu *et al.,* 2001) phenolic compound from strawberry (Herrara *et al.,* 2005) and dibenzylbutyrolactone lignans (Wang *et al.*, 2011).

Though SFE has some disadvantages, it is an excellent alternative method for seed oil extraction to replace conventional industrial methods most of which are mentioned in this section. It becomes the focus of attention (Chimowitz and Pennisi, 1986) due to its properties: Non-flammable, non-toxic and non-corrosive. Han *et al.,* 2009, in their work on extraction of

safflower seed oil by supercritical CO_2 reported that the extracts is of good quality and needs no refining operation. Thus, supercritical fluid technology has been applied to the extraction of oil and valuable product from a large number of materials.

Extraction rate varies from one method used to another. For supercritical fluid extraction which is used for the production of the corn in this work, there basically five parameters that affect the rate of extraction which are as follows:

1. Temperature and pressure
2. Particle size and amount
3. Extraction time
4. Carbon dioxide flow rate
5. Addition of Modifier

The two major parameters in supercritical extraction are temperature and pressure which are related in the sense that an increase in one may result in either an increase or decrease of the other depending on the solubility of the material in question. The extraction pressure is the main parameter that influences the extraction efficiency. It is established in the literature that an increase in pressure at a given temperature (especially at low pressure and temperature) results in an increase in the oil yield. This is due to the increase in the solubility of the oil components which is attributed to the increase of the CO_2 density.

Many researches abound in the literature on effect of temperature and pressure on the yield of supercritical extraction process. Some of these have been cited in various sections of this thesis.

Grosso *et al.,* 2008 in their work realized that temperature promotes the rapid release of the monoterpene hydrocarbons from the plant matrix. Zhang *et al.* (2010), on the other hand, studied supercritical fluid carbon dioxide extraction of seed oil from yellow horn. It was observed that the yield of oil significantly increases with the increase in pressure at a given temperature. Once the pressure reaches high levels, the oil yield slightly decreases. This is as a result of what is known as cross over phenomena which will be reviewed fully in section 2.7.1.

Addition of modifier is another powerful parameter that affects the yield of supercritical fluid extraction but it is not used in the present thesis. Modifier may be a complexing agent, an ion-pair reagent or a derivatization reagent which enhances the solvating power of the fluid. It reduces the extraction selectivity. The presence of the modifier changes the values of the critical pressure and temperature and is added to the polar solutes to enhance the extraction (Camel, 2001).

2.5 Supercritical Fluids

Fluids above their critical temperatures and pressures, called supercritical fluids (SFs), exhibit properties intermediate between those of gases and liquids. Consequently, each of these two boundary conditions shed insight into the nature of these fluids (Perry, 1997). A pure supercritical fluid (SF) is any compound at a temperature and pressure above the critical values (above the critical point). Above the critical temperature of a compound, the pure gaseous component cannot be liquefied regardless of the pressure applied. The critical pressure is the vapour pressure of the gas at the critical temperature. In the supercritical environment, only one phase exists. The fluid, as it is termed, is neither a gas nor a liquid and is best described as

intermediate to the two extremes. This phase retains solvent power approximating liquids as well as the transport properties common to gases.

A comparison of typical values for density, viscosity and diffusivity of gases, liquids, and SFs is presented in Table 2.3.

Table 2.1: Comparison of Physical and Transport Properties of Gases, Liquids, and SFs.

Property	Density (kg/m^3)	Viscosity (cP)	Diffusivity (mm2 /s)
Gas	1	0.01	1-10
SF	100-800	0.05-0.1	0.01-0.1
Liquid	1000	0.5-1.0	0.001

Source: Al-Damarki (2012)

2.5.1 Physico-chemical properties of a supercritical fluid

Unlike gases, SFs possess a considerable solvent strength and transport properties are more favourable (e.g., lower viscosities and higher diffusion coefficients) than in liquid solvents. In regions where a SF is highly compressible, its density and hence its solvent strength may be adjusted over a wide range with modest variations in temperature and pressure (Perry, 1997).

The behaviour of a fluid in the supercritical state can be described as that of a very mobile liquid. The solubility behaviour approaches that of the liquid phase while penetration into a solid matrix

is facilitated by the gas-like transport properties. As a consequence, the rates of extraction and phase separation can be significantly faster than for conventional extraction processes. Furthermore, the extraction conditions can be controlled to effect a selected separation. Supercritical fluid extraction is known to be dependent on the density of the fluid that in turn can be manipulated through control of the system pressure and temperature. The dissolving power of a supercritical fluid increases with isothermal increase in density or an isopycnic (i.e. constant density) increase in temperature. In practical terms, this means a SF can be used to extract a solute from a feed matrix as in conventional liquid extraction. However, unlike conventional extraction, once the conditions are returned to ambient, the quantity of residual solvent in the extracted material is negligible (http://sunny.vemt.bme.hu/sfe/angol/supercritical.html, 2012).

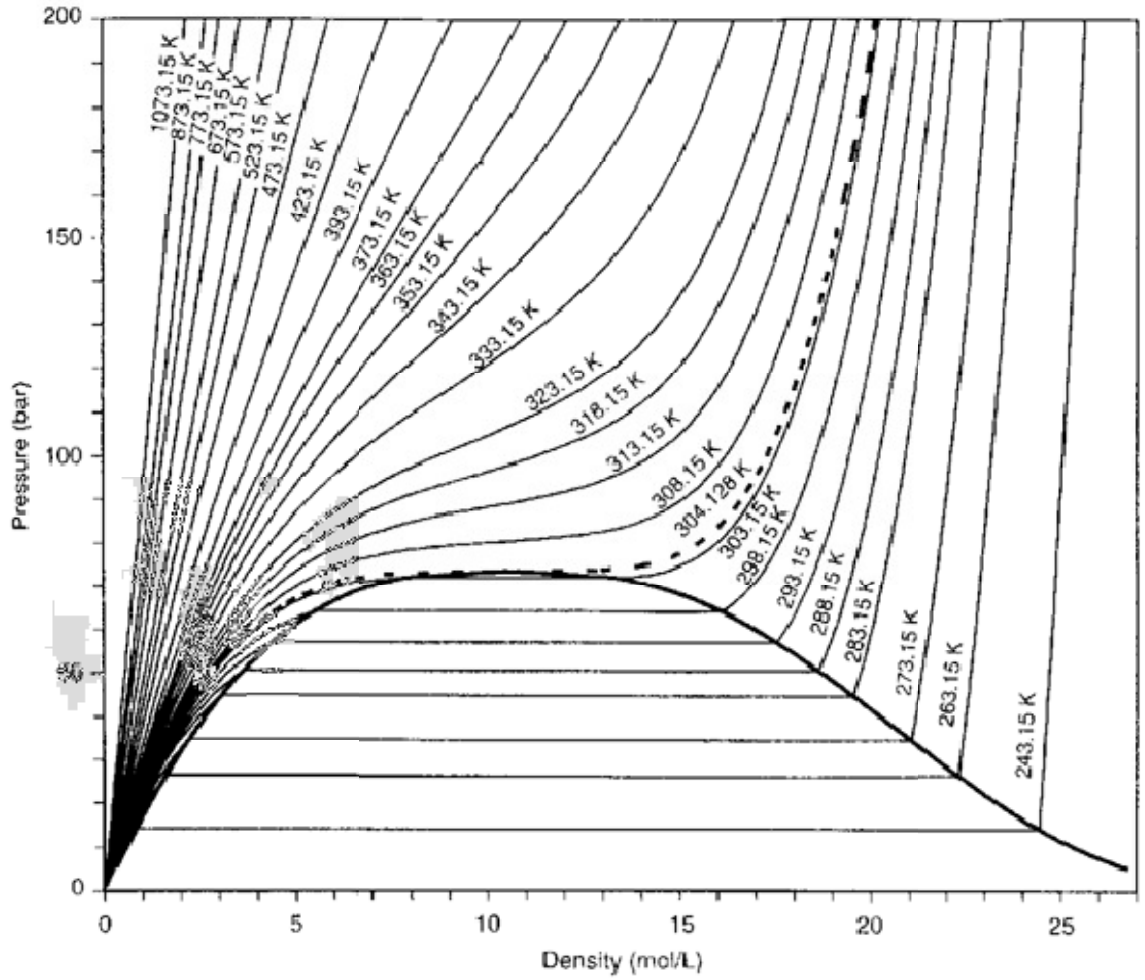

Figure 2.3: Chart for variation of CO_2 density with temperature and pressure

(Gupta and Shim, 2007)

Carbon dioxide is the most commonly used SF, due primarily to its low critical parameters (31.1 °C, 73.8 bar). Figure 2.3 variation of CO_2 density with temperature and pressure with the bold lines showing the saturated liquid line (left) and the saturated vapour line (right). Figure 2.4 is

Pressure-Temperature diagram of CO_2 with density as third factor. The points TP and CP in the Figure represent the triple and critical points respectively. The critical temperature (T_c) and critical pressure (P_c) are also given as 31.06 ^{0}C and 73.8 bar respectively.

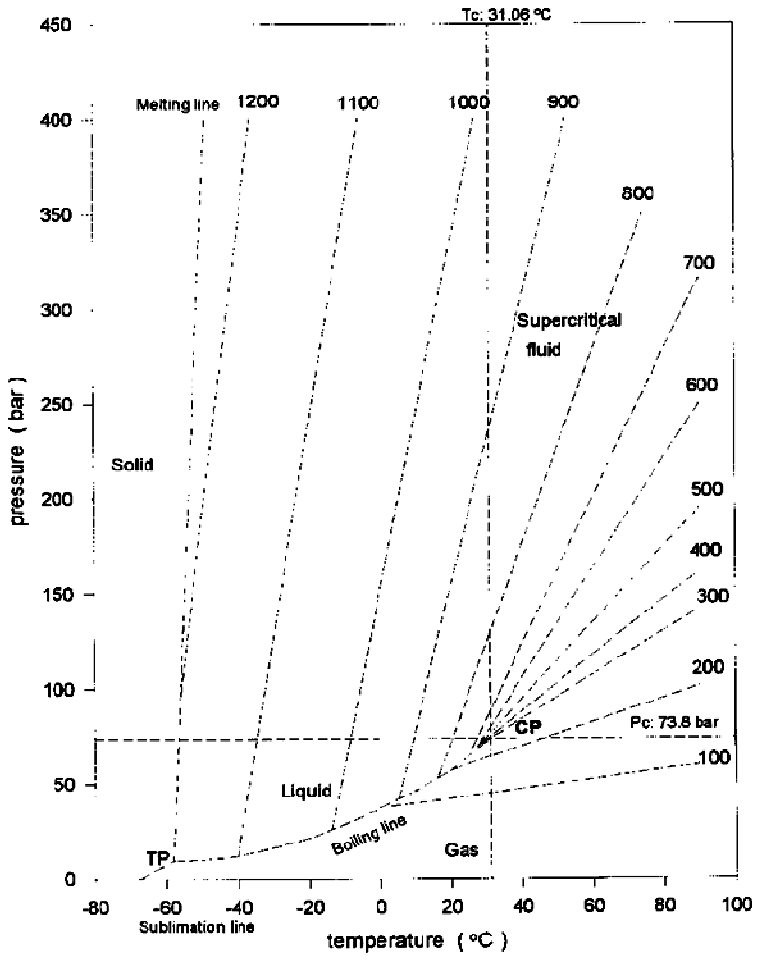

Figure 2.4: Pressure-Temperature diagram of CO_2 with density as third factor.

(Gupta and Shim, 2007)

Carbon dioxide is non-flammable, non-corrosive, non-toxic in low concentrations, readily available, inexpensive, and safe. Also, supercritical carbon dioxide has a relatively low viscosity

and high molecular diffusivity. Separation of carbon dioxide from the solute is often possible by simply reducing the extract pressure (Seadar and Henley, 1988). However, several other SFs can be used and they are listed in Table 2.2.

Table 2.2: Critical Conditions for Various Supercritical Solvents

Fluid	Critical Temperature (K)	Critical Pressure (bar)
Carbon dioxide	304.1	73.8
Ethane	305.4	48.8
Ethylene	282.4	50.4
Propane	369.8	42.5
Propylene	364.9	46.0
Trifluoromethane (Fluoroform)	299.3	48.6
Chlorotrifluoromethane	302.0	38.7
Trichlorofluoromethane	471.2	44.1
Ammonia	405.5	113.5
Water	647.3	221.2
Cyclohexane	553.5	40.7
n-Pentane	469.7	33.7
Toluene	591.8	41.0

Source: Al-Damarki (2012)

2.6 The use of CO_2 as a solvent

Carbon Dioxide (CO_2) is the king of extraction solvents for botanicals because it is an all-natural product which leaves no toxic residues behind. Its extraction properties can be widely and precisely manipulated with subtle changes in pressure and temperature. It is inexpensive and widely available. The capability of processing botanicals skilfully with CO_2 gives a company an added edge of status and prestige.

Figure 2.5 shows the phase diagram of CO_2 with the various extraction regions.

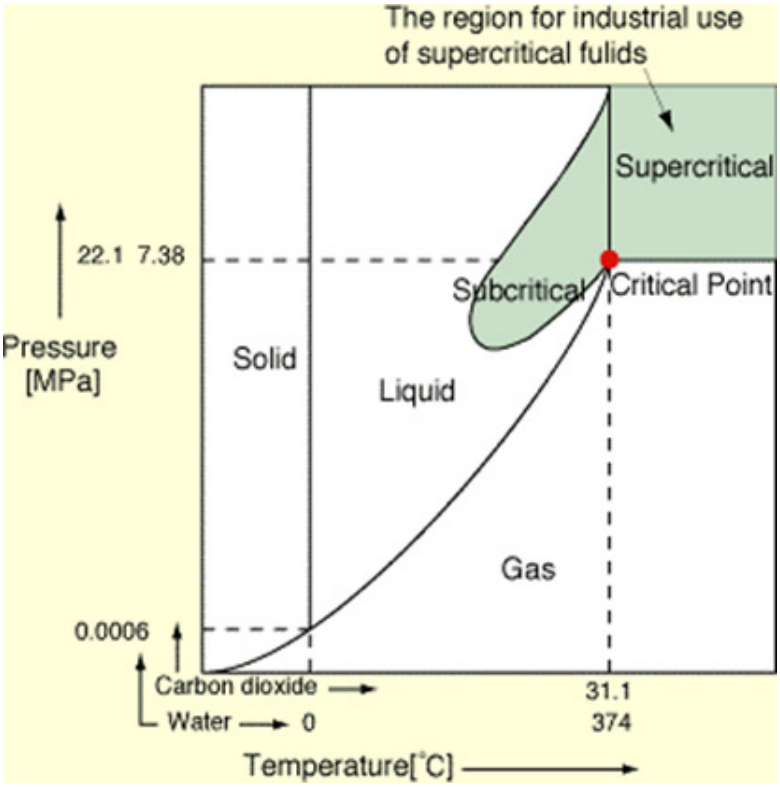

Figure 2.5: Phase diagram of CO_2

(Singh *et al.*, 2003)

2.7 Supercritical fluid extraction

High pressure extraction is the most effective and efficient way to extract valuable constituents from botanicals. The simplest way to explain this process is taking a plant material and putting it in a pressure vessel and pumping a particular liquefied gas or liquid solvent through it at a specific pressure and temperature. The pressure forces the solvent into the cell walls of the botanical and separates the desired constituent rapidly. The process of separating the extract from the solvent varies from one solvent to another.

The process of extraction with a solvent above its critical point is variably described as vapour phase or supercritical extraction or, as some authors prefer, supercritical distillative extraction, or even a "solvent-free" extraction. It is one of the more recent extraction and separation techniques (Panzner *et al.,* 1977).

Supercritical extraction and separation is attractive despite the high pressures involved for a number of reasons:

1. High boiling components can be gasified at relatively low temperatures.

2. Recovery of the solute and solvent is relatively easy from "supercritical" solutions.

3. At the lower temperatures, heat sensitive compounds are undamaged during the extraction process.

4. Separations can be achieved which are not possible by other techniques such as distillation or extraction.

5. If food components are to be separated, non-toxic solvents, such as carbon dioxide, may be used which leave no harmful residues.

6. In general terms, compressed gases are relatively cheap solvents.

Langenfeld (1995) stated that Supercritical fluid extraction (SFE) is becoming an attractive alternative to liquid solvent extraction (i.e., sonication) for organic compounds from environmental solids, as evident by number of publications.

2.7.1 Fundamental factors affecting supercritical fluid extraction

The cross over pressure is a fundamental factor in supercritical fluid extraction. Below the cross over pressure, solubility decreases with increasing temperature and above the cross over pressure, the opposite effect occurs.

Roy *et al.* (1996) in his work on extraction of ginger oil with supercritical carbon dioxide observed a cross over effect with respect to temperature and pressure. With respect to temperature and pressure effect, a crossover effect was observed where the higher temperature favoured the extraction at 24.5 MPa, while the lower temperature favoured the extraction at 10.8 MPa. According to King and Bott, 1993, the crossover phenomenon is due to the competing effects of reduction in density of SC-CO_2 and increase in the fatty acids volatility, which accompany the temperature rise. The crossover pressure of apricot kernel oil is between 200 and 300 bar (Özkal, 2004). This pressure is 350 bar for peanut oil (Goodrum and Kilgo, 1987), 300 bar for soybean oil (King and Bott, 1993) and 280-340 bar for pistachio nut oil (Palazoğlu and Balaban, 1998).

2.8 Research and Application of Supercritical Fluid Technology

This section is attempted to demonstrate the wide range of application of SFE based on the work of some researchers. Supercritical fluid technology finds application in the following areas:

2.8.1 Extraction of foods and pharmaceuticals as vegetable oil

Roy *et al.* (1996) reported the extraction rate of oil from freeze-dried ginger root with supercritical carbon dioxide as a function of CO_2 flow rate, particle size, temperature, and pressure. The extraction curves they obtained were found to be independent of CO_2 flow rate. Also, the extraction rate was found to increase as the particle size was decreasing. In the case of temperature and pressure effect, they observed a crossover effect where high temperature was discovered to favor the extraction at 24.5 MPa, while low temperature favored the extraction at 10.8 MPa. In addition, the shrinking core model was used to analyze the experimental results obtained taking the effective diffusivity and the solubility as the fitting parameters. The model was found to fit the experimental data successfully.

Reverchon *et al.* (1999) reported the supercritical CO_2 extraction of fennel seeds in two steps. The first one was at 90 bar and 50 °C to obtain the selective extraction of essential oil while the second one was at 200 bar and 40 °C and allowed the extraction of vegetable oil. All the experiments were performed using fractional separation of the extracts with three different CO_2 flow rates (0.5, 1.0, and 1.5 kg/h). From the extraction results and the analysis of the scanning electron microscopy (SEM) images of the vegetable matter obtained, the mathematical models of the two extraction processes were proposed. The extraction of fennel vegetable oil was modeled based on differential mass balances and on the concept of broken and intact cells as evidenced from SEM. In the model, only one adjustable parameter, the internal mass-transfer coefficient

(kt), was used and a fairly good fitting of the experimental data was obtained by setting kt = 8×10^{-8} m/s. Moreover, the fennel essential oil extraction process was modeled as desorption from the vegetable matter plus a small mass-transfer resistance. The same internal mass-transfer coefficient value used for vegetable oil extraction allowed a fairly good fitting of the essential oil extraction data.

Caredda *et al.* (2002) reported the supercritical carbon dioxide extraction of essential oil from *Laurus nobilis* at a pressure of 90 bar, temperature of 50 ^{o}C and carbon dioxide flow rate of 1.0 kg/h. The experimental set up consist of two separators, waxes were entrapped in the first separator that was set at 90 bar and -10 ^{o}C while the oil was recovered in the second separator working at 15 bar and 10 ^{o}C. Comparing the result obtained with those of hydrodistilled oil, reveal no significant difference. The main components of the oil obtained were found to be 1,8-cineole (22.8 %), linalool (12.5%), alpha-terpinyl acetate (11.4%), and methyleugenol (8.1 %).

Özkal *et al.* (2005) determined the solubility of hazelnut oil in supercritical carbon dioxide (SC-CO_2) at 15–60 MPa, and 40–60 °C. Extraction was made to occur in two periods (fast and slow). The released oil on the surface of the particles was extracted in the fast extraction period, and 39% of the initial oil was recovered at each condition. However, the duration of the fast extraction period was found to decrease with increases in pressure and temperature. The unreleased oil in the intact cells was extracted in the slow extraction period. The maximum recovery that was obtained was found to be 59% at 60 MPa and 60 °C, for 180 min of extraction.

Salgın *et al.* (2006) studied the extraction of sunflower oil from sunflower seeds (*Heliantus annuus L.*) using supercritical CO_2 by applying the shrinking core modeling approach to the modeling of the packed-bed extraction process studied. Extractions were conducted at different pressures, temperatures, CO_2 flow rates using various mean particle diameters. The developed model was found to fit the obtained experimental data satisfactorily.

Fiori (2009) carried out an experimental work on sunflower seed oil extraction using supercritical CO_2 utilizing a supercritical extraction equipment having a volumetric capability of 100 ml involving seeds milled to different particle sizes (mean diameter between 0.19 and 1.2 mm) and pressure range of 280-550 bar, while the temperature and the solvent flow rate were maintained constant at 40 °C and 10 g/min, respectively. The result obtained was used to develop a theoretical model. The reliability of the model used was demonstrated by the value of the effective diffusivity, resulting from the model optimization procedures, which was found to be similar for various experimental tests.

Corso *et al.* (2010) investigated the extraction of sesame seed (*Sesamun indicum L.*) oil using both supercritical carbon dioxide and compressed propane as the solvents. The extractions process was carried out on a laboratory scale unit using a temperature and a pressure range of 313–333 K and 19–25 MPa for carbon dioxide and 303–333 K and 8–12 MPa for propane extractions, respectively. 2^2 factorial experimental design with three replicates of the central point was used to organize the data collection for both solvents. The results obtained showed that solvent and density were important variables for CO_2 extraction, while temperature was found to be the most important variable for extraction yield with propane. The extraction with propane

was discovered to be much faster than that with carbon dioxide owing to the fact that propane is a better solvent for vegetable oils compared to carbon dioxide. Also, the characteristics of the extracted oils using the two solvents were found to be similar to each other. In addition, the developed mathematical model of the extraction kinetics using a second order kinetic was discovered to give good results for the extraction with both solvents.

2.8.2 Solar cells

Maniam *et al.* (2011) reported a new technique whereby a dye was deposited onto metal oxide surfaces using supercritical carbon dioxide (SC-CO_2) for use in solar cell applications. The technique was discovered to eliminate the need for hazardous organic solvents and waste solvents generated during the dyeing process. The solubility of a perylene anhydride dye in SC-CO_2 was enhanced by the incorporation of fluorinated alkyl substituents and the use of masked carboxylic binding groups, which allowed fast deposition of the dye onto the TiO_2 photo anode resulting in efficient photovoltaic performance.

2.8.3 Biotechnology for non thermal cell inactivation

Chen and Lin (1994) invented a method for the rupturing of the microbial cells in order to recover intracellular material in the cells by treating the cells with carbon dioxide under pressure sufficient to enter the cells for time sufficient to allow enough carbon dioxide into the cells to effect later rupture and, then, suddenly releasing the applied fluid pressure on the cells so that the outer wall or membrane of the cells would be ruptured by the expansion of the carbon dioxide within the cell. The authors separated and recovered the remaining intracellular material of the cells, and, also carried out the treatment in conjunction with lytic enzyme to increase rupture rates. The enzymes they used were found to remain active and the protein in the cells retained its

native state in the ruptured cell suspension. It was also discovered that the preferred time for treating was between one hour and fifteen hours. While the preferred pressure was from above about 500 psi gauge to about 5000 psi and a temperature of about 10 ^{o}C was found to improve rupture efficiency.

Nakamura ***et al.*** (1994) examined the disruption of microbial cells by rapid release of gas pressure under various conditions of pressure, temperature, treatment time and water content of the cells in order to develop a novel sterilization method for heat-sensitive materials by completely destroying wet cells of baker's yeast, and, after the microorganisms had been saturated with CO_2 gas at 40 °C and 40 atm for more than 3 h, the pressure was suddenly discharged. Furthermore, some dry cells were poorly killed under the same experimental conditions. It was discovered from the results that the death of the microorganisms was caused by mechanical breakage and/or physiological damage related to gas sorption and desorption by the cells.

Isenschmid ***et al.*** (1995) studied the effects of CO_2 on different yeast strains over a range of pressures and temperatures in an attempt to study the potential use of supercritical CO_2 extraction for the recovery of products from yeast cell cultures. They discovered that viability was dependent on temperature and dissolved CO_2 concentration, which could be described by a sigmoidal (S-shaped) curve. They also obtained from their work that cell death was mainly due to an "anaesthesia effect" rather than cell rupture. The differences in the sensitivity they observed for the strains studied had the following order of resistance: ***Kluyveromyces fragilis*** > ***Saccharomyces cerevisiae*** > ***Candida utilis***.

Kim and Hong (2001) studied the supercritical carbon dioxide (SC-CO_2) pretreatment of lignocellulose for enzymatic hydrolysis of cellulose. In these work, Aspen (hardwood) and southern yellow pine (softwood) with moisture contents in the range of 0–73% (w/w) were pretreated with SC-CO_2 at 3100 and 4000 psi and at 112–165 °C for 10–60 min. Each pretreated lignocellulose was hydrolyzed with commercial cellulase to assess its enzymatic digestibility. It was discovered that untreated aspen and southern yellow pine was able to produce final reducing sugar yields of 14.5±2.3 and 12.8±2.7% of theoretical maximum, respectively. Furthermore, when no moisture was present in the lignocellulose they pretreated, they found out that the final reducing sugar yield from the hydrolysis of the SC-CO_2-pretreated lignocellulose was similar to that of the untreated aspen.

Juhász ***et al.*** (2003) reported the production of thermo stable endoglucanase from ***Clostridium thermocellulum***. The recombinant ***E. coli*** was grown in a shake flask cultures, and the intracellular recombinant protein was extracted from the cells after applying supercritical CO_2 cell disruption. The supercritical CO_2 cell disintegration was optimized and then compared to the traditional ultrasonic cell disruption technique. With the supercritical cell disruption, the cellulase recovery was found to be approximately 17% lower than that of the one obtained with sonication.

Khosravi-Darani ***et al.*** (2004) carried out a research that focused on the disruption of the gram-negative bacterium ***Ralstonia eutropha*** cells by supercritical CO_2 for poly (R-hydroxybutyrate) (PHB) recovery by studying the variables such as drying strategy, type of modifier, and cultivation time, as well as operating pressure, temperature, and repeated release of supercritical CO_2 pressure, affecting cell disruption. The authors also investigated the effect of the disruption

technique on PHB molecular mass. Furthermore, using a combination of this method and chemical pretreatments, PHB recovery was examined. It was found that bacterial cells treated in growth phase exhibited less resistance to disruption than nutrient-limited cells in the stationary phase. The method they proposed in their work was found to be economical and comparable with other recovery methods in terms of percentage of PHB recovery and energy consumption, while it is also environmentally friendlier.

Yilmaz *et al.* (2011) carried out the supercritical carbon dioxide extraction of proantocyanidins by investigating the effect of different pressure, temperature and ethanol percentage using high performance liquid chromatography for the analysis of the compounds. It was found in the study that the most effective parameter on the extraction of the compound using supercritical carbon dioxide was the amount of the ethanol used.

2.8.4 Renewable energy

Sawangkeaw *et al.* (2011) evaluated a scale-up plug flow reactor for the continuous production of biodiesel from refined palm kernel oil (PKO) with supercritical methanol and optimized the process using response surface methodology. The effects of operating temperature (270–350 °C), pressure (15.0–20.0 MPa) and methanol:PKO molar ratio (20:1–42:1) at constant residence time of 20±2 min were considered. The analysis of variance of the developed model revealed that a modified quadratic regression model was able to give the best square of correlation coefficient (R^2) of 0.9615 and adjusted R^2 of 0.9273. The interaction terms in the regression model illustrated small synergistic effects of both temperature-pressure and temperature-methanol:PKO molar ratio. The optimal conditions were determined to be 325±5 °C for the temperature, 18.0±0.5 MPa for the pressure and a

methanol:PKO molar ratio of 42±2:1, which gave a maximum production rate of 18.0±1.5 g biodiesel/min with a fatty acid methyl ester content of 93.7±2.1%. The product obtained from the optimal conditions was found to have high cetane number, and, therefore, could be considered as a fuel additive for cetane number enhancement.

Al-Zuhair *et al.* (2012) developed and tested an integrated process for a continuous fat extraction from lamb meat followed by enzymatic production of biodiesel in supercritical CO_2. A system was used for the simultaneous the production of biodiesel and healthy low-fat lean lamb meat. The authors discovered that, for the enzymatic process to be feasible, lipase, which allows easy reuse, was preferred to be used in immobilized form. The system was operated at 200 bar and a SC-CO_2 flow of 0.5 ml min^{-1} with extraction and transesterification temperatures of 45 and 50 °C, respectively. The study also investigated the effects of methanol:fat (M:F) molar ratio and enzyme stability and discovered that using fresh enzyme, a M:F molar ratio of 10:1 was able to give the highest biodiesel production rate of 0.37 mg min^{-1} g-$enzyme^{-1}$ compared to only 0.09 mg min^{-1} g-$enzyme^{-1}$ using a M:F molar ratio of 5:1. In addition, when they used a M:F molar ratio of 10:1, they found the activity of the enzyme in the third meat replacement cycle to drastically drop to 18% of its original value, compared to 79% when a M:F molar ratio of 5:1 used.

2.8.5 Other Areas of Application of Supercritical Fluid Extraction

Kim and Hong, 2001 reported the use of SF for industrial waste treatment. Langenfeld *et al.,* 1995 reported the same method for polycyclic aromatic hydrocarbons removal from highly contaminated soil. SF has also been used in nanotechnology where Ru nanoparticles was

reported to be immobilized on metal–organic framework nano-rods (Zhao *et al.*, 2011), as in Figure 2.6.

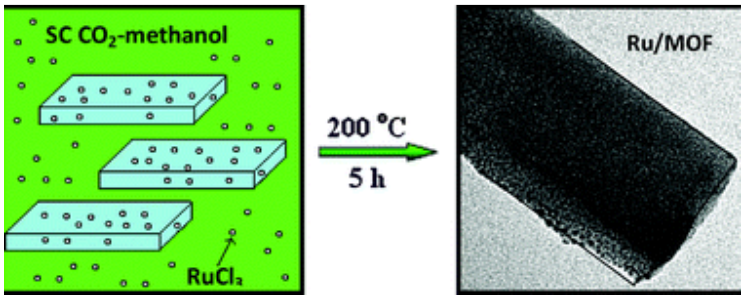

Figure 2.6: A schematic diagram of Ru nanoparticles immobilized on metal–organic framework nano-rods.

CHAPTER THREE

3 METHODOLOGY

3.1 Materials

The major raw material is corn germ obtained from winnowing Nigerian corn kernel. Industrially the germ is obtained using a de-germinator to separate it from the corn kernel.

3.2 Equipment used

The Equipment used for this research is given in Table 3.1.

Table 3.1: List of major equipment

Name	Manufacturer
Supercritical Extractor	Assembled in University of Birmingham, UK
Soxhlet	Assembled in University of Birmingham, UK
Air bath	Assembled in University of Birmingham, UK
Pressing Machine	SPECAC, England
Rotary evaporator	Rotavapor-RE, England

3.3 The Supercritical Extractor

The SC-CO_2 extractor was assembled in Chemical Engineering Department Laboratory, the University of Birmingham, UK using commercially available components as shown in Figure 3.1 with parts purchased from Baskerville Reactors Autoclaves and Swagelok, etc. The parts used to set up the rig are given in Table 3.2. The equipment is divided into three major parts, i.e. solvent delivery section, extraction section and separation sections.

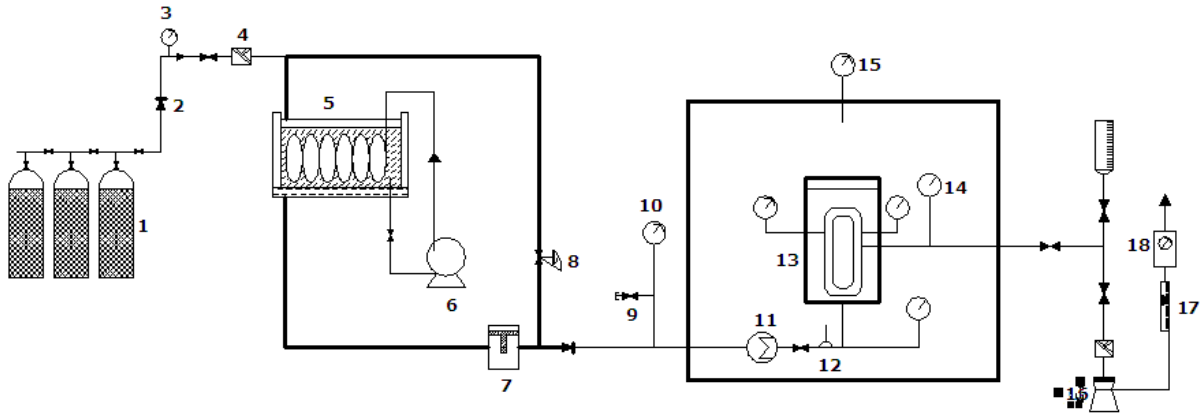

Figure 3.1: Schematic diagram of Sc-CO_2 extraction process

1. CO_2 Cylinder; 2. CO_2 inlet valve; 3. CO_2 Pressure valve; 4. 15 µm Particle filter; 5. Cold bath; 6. Chilling pump; 7. CO_2 air driven pump; 8. BPR; 9. Emergency relief valve; 10; Extraction pressure gauge; 11.Heat exchanger; 12. Safety bursting disk; 13. Extractor; 14.Vessel exit temperature; 15.Vessel temperature gauge; 16. Glass collector; 17. Gas totalyser; 18. Gas flow meter.

Table 3.2: List of major parts of supercritical extractor

Part label	Part	Manufacturer
5	Cold bath	Assembled in University of Birmingham, UK
6	Chilling pump	Assembled in University of Birmingham, UK
7	PowerStar4 air driven liquid pump Model 4F-64 (0-6400 psig)	Sprague Product, UK
8	Back pressure regulator Model UP 66	Go Product, UK

3.3.1 Details of solvent delivery

The solvent delivery section supplies carbon dioxide at the desired temperature and pressure to the extraction section. Liquid carbon dioxide withdrawn from the supply cylinders passes through a 15 µm particle removal filter (4) (Nupro, SF-4FT-15), and then through a bed of activated carbon for moisture removal. The dried carbon dioxide passes through a coil placed in the refrigerated bath 5, and its temperature is cooled to between 0 and 5 ^{0}C to ensure that it remained liquefied. It then passes through a filter and enters the air-driven pump 7 (Power Star 4, 4F 64) that compresses low compressible fluids. The pump operation is pneumatic; the air is filtered and the pump stroke rate regulated.

Carbon dioxide is compressed by the pump to the required experimental pressure displayed on the extraction pressure gauge (10). It then passes through heat exchanger (11) on its way to the extraction section. Some of the flow is recycled back to the cooling bath inlet through the back pressure regulator BPR (8) (Go Products, UP 66). The back pressure regulator acts as a relief valve which opens at just above the extraction pressure, and it closes at the extraction pressure. The regulator operates by balancing the force of a partially compressed spring against the system pressure so that when the pressure exceeds the force supplied by the spring, the relief valve stem lifts. As the valve has a large flow coefficient the pressure is quickly relieved, and the valve stem then reseals. The pressure can be controlled to within 0.5 bar using this unit. The pump stroke rate is adjusted to give a carbon dioxide flow from the pump in excess of that required further downstream, the excess being recycled via the regulator. This procedure enables the pressure at the pump outlet to be controlled at a level that does not depend on the flow rate in the downstream parts of the apparatus. The pump exit is protected from excessive pressure of 6000 psi by a bursting disc assembly.

3.3.2 Details of extraction section

The extraction section is housed in an air bath. It is operated in a “once through” mode for the extraction.

On entering the extraction section, the carbon dioxide stream first passes through the check valve CV (Autoclave Engineers, TWO 4400) and 15µm particle filter (Swagelok, AS-4IF-15) before passing through the vessel (13) (AS 884). Carbon dioxide is contacted with the materials to be extracted in the vessel, with mass transfer occurring as the carbon dioxide stream passes through

a bed of the particles. The extractable components enter the carbon dioxide stream and are carried out of this section into the separation section.

3.3.3 Details of separation section

In this section, solute is precipitated from the carbon dioxide stream by pressure-reduction and is collected in glass collection vessels, from which it may be recovered and weighed. The solute-free carbon dioxide leaves these vessels and is vented from the laboratory through a flow totalyser (18) and a flow meter (17).

Pressure reduction of the carbon dioxide stream, initially at the extraction pressure, is achieved by passing it through pressure-reducing valve and micro-metering valves MV where depressurization to atmospheric pressure takes place. The valve provides an intermediate pressure reduction stage, whilst the remainder of the pressure decreases to atmospheric across micro-metering valve MV (Swagelok, AS-31-AS4).

On leaving these vessels, the carbon dioxide stream, now solute-free, passes through the flow totalyser 17 (Alexander Wright) and the flow rate meter 18 (Rotameter Manufacturing Co., Size 7X) and is then vented from the laboratory.

3.3.4 Pressure vessels and internals used in the apparatus

The 500 ml O-ring closure pressure vessels AS 883 and 884 were supplied by Autoclave Engineers. The material of construction is 316 stainless steel and is designed to operate under a maximum allowable working pressure of 680 bar. They are fixed to an iron frame and are usually not moved during the experimental period. One port is located in the bottom of each vessel, and the other three in the vessel wall near the top of the vessel. These ports are tapped

with ¼ inch N.P.T. thread so that connecting piping can be screwed into them. Two of the ports on each vessel are used to insert a pressure transducer and a thermocouple respectively directly into the vessel. The vessel is sealed by inserting the cover with the O-ring it into the vessel. The main nut is screwed down by inserting a rod into the hole in it. This rod is gently tapped by hand until metal to metal contact is made. The vessel is now closed and ready for use.

To make the operation easy, the sample to be extracted, if this consists of a granular solid, is charged into a sample holder instead of into the vessel directly, and this is then lowered into the appropriate extraction vessel. The sample holder was designed in the Department (Lu, 1997) has a removable threaded top into which screw holes have been drilled to facilitate removal from the vessel. An O-ring is placed between the top and main body, to prevent carbon dioxide bypassing the sample. Samples are retained in the holder by a gauze strip. All O-rings were made of VITON material and those containing plasticisers such as “BUNA-N” or “Nitrile” should be avoided because they are extractable. The sample holder has internal diameter of 45mm and height of 95mm which correspond to the height and diameter of the bed form in it.

3.4 Moisture Content Determination

The oven was calibrated alongside as the moisture loss is been determined to ensure that the sample is not burnt in the oven. The moisture dishes were dried for 1 hour at 130 ^{0}C, cooled in the desiccator and the tare weight was obtained. Five moisture dishes labelled A-E were weighed and 2-3 g of the sample was added to each, covered and weighed at once. The dishes were uncovered, placed on the shelf of oven with the covers under each. After the oven has regained its temperature between 15-20 min, it was further heated from 60 to 300 min at a regular interval of 60 min. At the end of every 60 min, the moisture dish containing a dry sample was removed

and weighed. The loss in weight was determined to be the moisture content of the sample. The moisture loss can be calculated using the equation below:

$$\% \text{ Moisture loss } = \frac{A \times 100}{B} \quad (3.1)$$

Where,

A= Moisture loss, g

B= Original weight of sample, g

3.5 Sample Pretreatment (Sieve Shaking)

After collection of the sample, the first step was to carry out size reduction to remove the maize grain and dirt in the sample. This was done by arranging the 2.8 mm and 1.0 mm sieves in descending order of pore aperture. The pan was placed under the last sieve to collect residue. The sample was then poured on the topmost sieve and the sieves shaken at the amplitude of 2.1 mm for 4 sec using a sieve shaker to enable separation occur. The fractions of the sample retained on the various sieves and the pan were collected and placed in different labelled containers but only the sample that passed 1.0mm sieved was used throughout in this research.

3.6 Solvent Extraction (Soxhlet)

An empty and clean thimble was weighed and recorded as (W_1). About 20 g of the sieved sample was put into the empty thimble. The thimble containing the sample was then weighed and recorded as (W_2). The sample in the thimble was subsequently covered with glass wool and placed in the sohxlet apparatus. 200 ml of n-hexane was measured and poured into a round-

bottomed flask. The sohxlet apparatus was mounted on this flask and fixed under a condenser, which was already clamped to a retort stand. In addition, the condenser was already connected to 2 pipes, one pipe connected to a water supply (tap) and the other removes water from the condenser. The electro thermal heating mantle was switched on and temperature set at 60 ^{0}C.

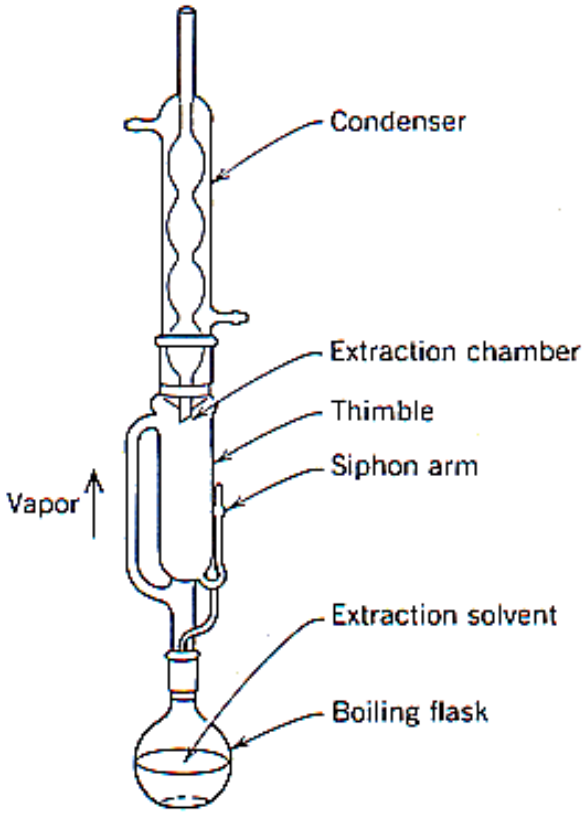

Figure 3.2: Schematic diagram of a soxhlet extractor

Extraction was carried out for 4-14 hours to establish the optimum extraction time. After the required extraction time, the heating mantle was switched off. The soxhlet apparatus was disconnected from the condenser with the thimble removed and the solute (oil + solvent) dried using the rotary evaporator (a rotary-evaporator (Rotavapor-RE, Buchi Orme Scientific Ltd.,

England) set on temperature of 60 ^{0}C. The schematic diagram of the soxhlet extractor used is as shown in Figure 3.2.

At the end of the extraction, an empty evaporator flask was weighed and the weight noted as (W_3) before the solute is poured. The flask containing the solute was also weighed. Drying continued with the weight of the flask containing the solute being weighed at interval until a constant weight (W_4) is recorded. The amount of oil extracted for all the runs were calculated using the equation below:

$$\%\ weight\ of\ oil\ extracted = \frac{W_4 - W_3}{W_2 - W_1} \times 100 \qquad (3.2)$$

3.7 Supercritical Fluid Extraction

The supercritical extraction of the oil was carried out in the following sections.

3.7.1 Sample pretreatment (pelletizing)

Some samples were pressed into pellets (35 mm in diameter) using the SPECAC pressing machine to compress the sample so that the oil matrix can be easily reached by the CO_2. About 7 g of sample is poured into a die of 35 mm diameter and placed into the pressing machine. The samples were pressed with a force of 1, 2 and 5 tonnes and held for 30 sec in each case. Each pellet made was removed and crushed using a mortar and pestle.

3.7.2 Apparent solubility test

The test was carried out using SC-CO_2 at temperature of 40 to 50 °C, with pressure of 80 to 300 bar. The flow rate of CO_2 was fixed at 4 L/min in all experiments. The sample holder was

cleaned with methanol. 'O' rings were placed between the top and main body of the sample holder to avoid leakage. The sample holder was then filled with corn germ and placed inside the extractor. Mass of the germ charged in was noted at the beginning of the experiment. The CO_2 inlet valve was fully opened and the pump was started using the compressed air valve. The BPR and the pump were regulated as required until the desired pressure is obtained. When the desired temperature and pressure is reached, the collection valve was fully open and the micro-metering valve was open gradually controlling the CO_2 flow rate. Samples were taken at time intervals (15-30 min). After taking each sample, the collection flask was immediately covered to avoid evaporation of water until the wet sample is weighed. Finally, the mass of the dry sample was measured and the apparent solublity computed.

$$\text{Apparent solubility (g/kgCO2)} = \frac{final\ mass\ of\ oil - initial\ mass\ of\ oil}{final\ mass\ of\ CO_2\ used - initial\ mass\ of\ CO_2\ used} \qquad (3.2)$$

3.7.3 Supercritical Extraction

Batch extraction was used throughout the experiments. In this study, corn germ oil was extracted using SC-CO_2 at temp of 25 to 60 °C, with pressure of 150 to 300 bar. The flow rate of CO_2 was fixed at 4 L/min in all experiments.

Figure 3.1 shows the schematic diagram of SC-CO_2 extraction system. An air bath was first used to maintain the temperature of the whole extraction system at ±0.5 °C before placing the sample inside the extraction vessel. Corn germ (25 g) was then charged into the vessel. Mesh filters were filled on both top and bottom of vessel with corn germ placed in the middle to avoid any material loss in the extractor. Liquid CO_2 was then drawn from cylinder (1), as solvent. After passing through a check valve and a 15 µm filter (4), the CO_2 was cooled to a temperature of 0-5 ºC in a

cold bath (5) located between the cylinder outlet and the air driven liquid pump. This low temperature condition was used to ensure the CO_2 was in liquid state hence preventing the pump from cavitations. The CO_2 was then pumped into the system using an air driven liquid pump (7) until the required pressure (±5 bar) was obtained. A back pressure regulator (8) from Go Products, model UP 66 is used to set the system pressure. When the extraction conditions reached the desired temperature and pressure, the SC-CO_2 flow was provided continuously through the extractor, contact with packed bed and extract the extractable material from the bed.

The CO_2 loaded with extracts flowed to the collector through valves and was separated from the extracts by dropping the pressure to ambient. Corn germ extracts were collected in collector (16).

3.8 Transmission Electron Microscopy (TEM)

Transmission Electron Microscope in Figure 3.3 was used to obtained the surface morphology of the corn germ. In order to prepare the samples for the analysis, primary fixation was carried out using 2.5% of glutaraldehyde for three days, after which chemical fixation was carried out by adding 1% osmium tetroxide for 1 hour. This was followed subsequently by dehydration of the sample using 50, 70, 90 and 100% methanol for 30 minutes in each case.

Propylene oxide was embedded into the resin at a ratio of 1:1 for 45 min (on a rotator in a fume cupboard). The resulting resin was further placed on a rotator in a fume cupboard for another 1 hour. The samples were then placed under the surface of the resin in an embedding mould, and vacuum was pulled for 30 min, after which it was allowed to return to atmospheric pressure. The resin was polymerized at 60 °C for 16 hours resulting into a block sample. A microtome was used to take ~1 μm of the resin for light microscope examination by mounting it on slides and

staining it with toluidine blue. An electron microscope grids with a lacy carbon coat was prepared. Using a diamond knife an ultra-thin section (50-150 nm) was cut and placed on the grids. The sample was then stained with uranyl acetate and lead citrate before the Transmission Electron Microscope operation. A clear picture was achieved by varying the magnification using magnification knob. After photographing the desired picture, the carbon grid was discharged from the machine and subsequent samples were analysed by using the same procedure and micrographs obtained.

Figure 3.3: Jeol 1200 EX Transmission Electron Microscope

CHAPTER FOUR

4 DISCUSSION OF RESULTS

The results of the experimental work are present in the sections below. The results of moisture content are presented in section 4.1. This was followed by the result and discussion for oil content of the samples in section 4.2, which forms the basis for the available oil in sample for the SFE. Sections 4.3 and 4.4 focused on the results and discussion for the SFE of the corn germ. Transmission electron microscopy was used to study the morphology of the sample before and after the solvent extraction so that the difference could be observed and the micrographs presented in section 4.5. Sources of error in this work could be due to sampling, impurities and sample analysis.

4.1 Moisture Content

According to Onwuka (2005) moisture content is of great importance to food processors for the fact that most biochemical reactions and physiological changes depend on it, thereby affecting the stability and quality of food. In this work, the moisture content of raw materials was evaluated from the water evaporation loss measured by standard oven method. To ensure the temperature does not exceed the limit (100-115 °C), the oven was calibrated prior the tests.

The moisture content of three corn germ samples from different sources was measured. The results are given in the Figure 4.1 to Figure 4.3 below.

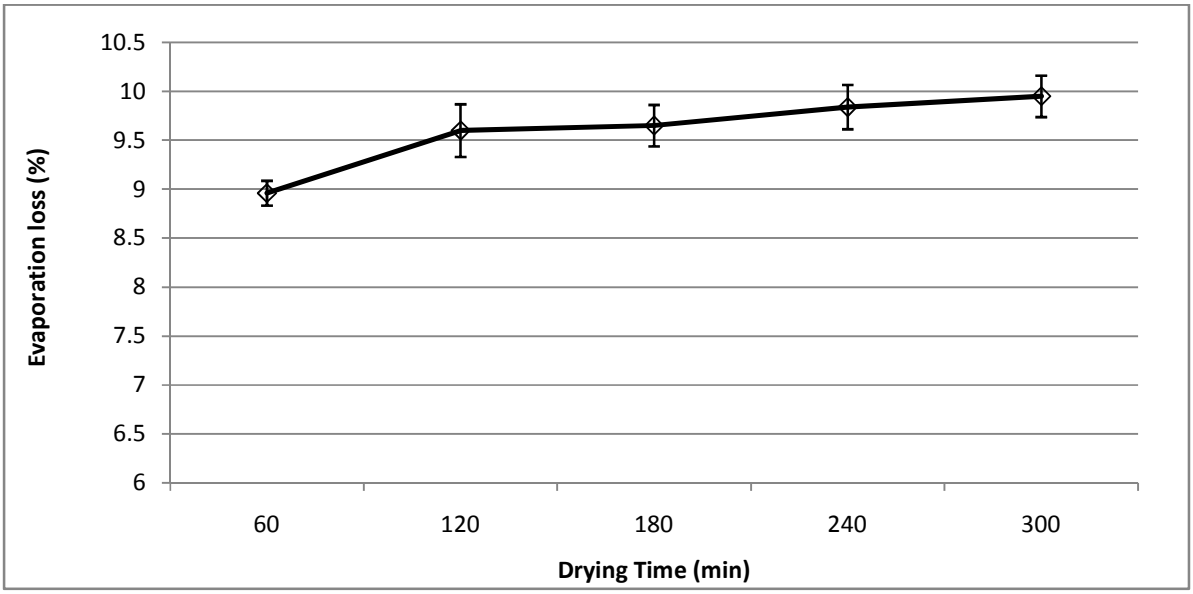

Figure 4.1: Evaporation loss of the United Kingdom (UK) corn germ sample 1.

Figure 4.1 presents the graph of Evaporation loss of UK sample 1 for different time interval at 105°C. It is clear that the mass loss was increasing with time until it became constant at 240 minutes, where the evaporation loss in the sample was 9.9%, and hence a minimum drying time of at least 240 minutes is needed.

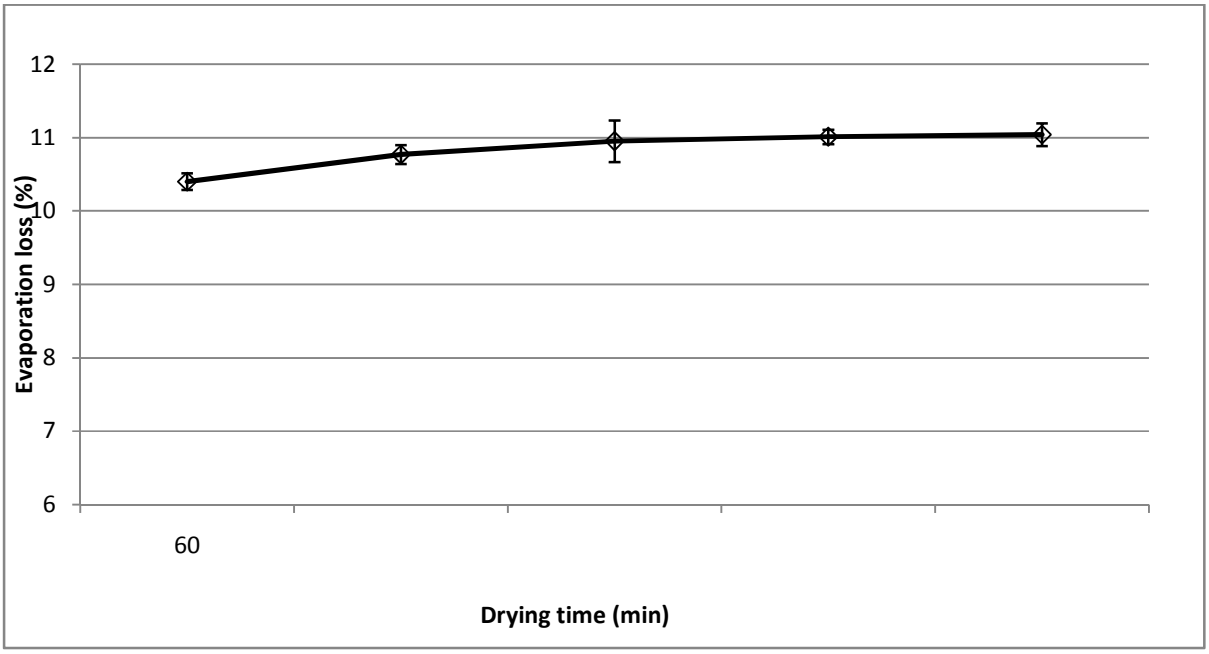

Figure 4.2: Evaporation loss of the UK corn germ sample 2.

Figure 4.2 is the result of UK corn germ sample 2. It shows a similar trend as Figure 4.1, with the evaporation loss also increasing with time until when it became constant at 180 minutes, resulting in an evaporation loss of 11.0%. Figure 4.3 presented the results for Nigerian (NGN) sample, with the temperature fluctuating between 99 ^{o}C and 106 ^{o}C. The evaporation loss of NGN sample was 12.1%.

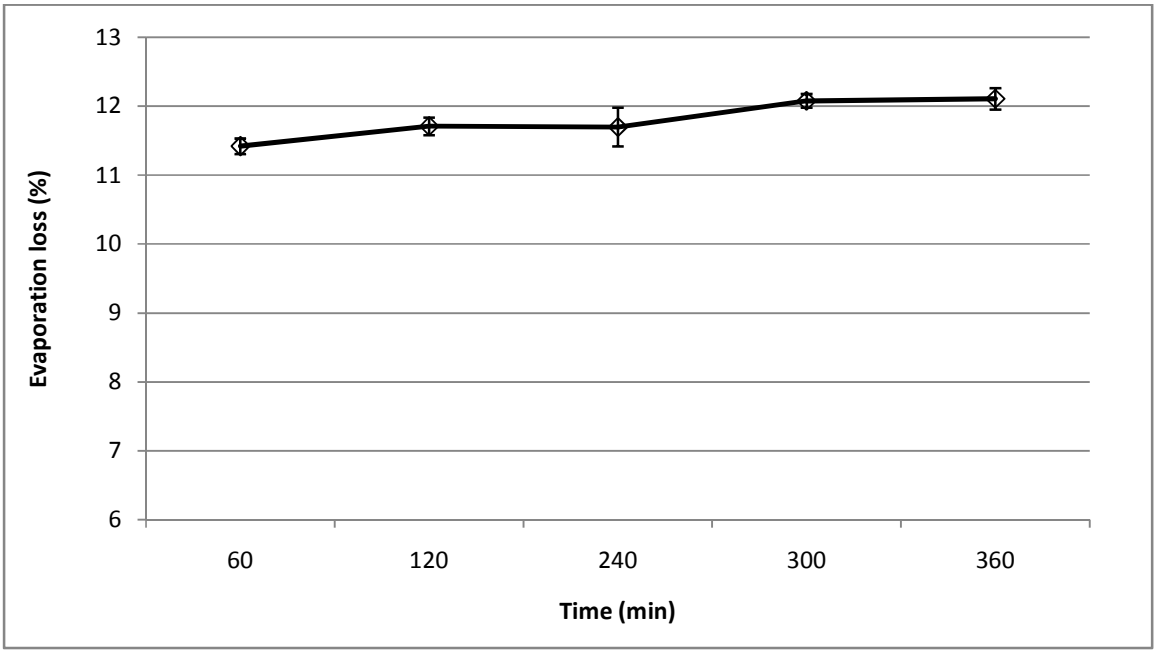

Figure 4.3: Evaporation loss of the Nigerian (NGN) corn germ sample.

The moisture content of corn is a very important parameter because it affects the oil content of corn germ. Corn seed with moisture up to 20-25%, can cause structural damage and rupture to the germ which could result in lack of oil in the germ due to liquids flow out of the endosperm. In the same way, a germ sample with moisture content up to 20% is an indication of lack of oil in the sample as a result of rupture of the seed.

4.2 Solvent Extraction

The results for the Soxhlet extraction of the samples are presented in this section. The summary of the oil contents of the samples is shown in Table 4.1 while the extraction curve is shown in Figure 4.4 to Figure 4.6.

Table 4.1 gives the summary of oil content of all the samples used as calculated using equation 3.2. UK sample 1 has the lowest oil content of 9.00%, UK sample 2 has the highest oil content of

16.37% and NGN sample has oil content of 12.10%. Although, Abdulkadir and Isah (2010) were able to extract 18.00% oil from product of winnowing corn (improvised method) using similar materials after 6 hours, the raw materials were not defined because at the time various grains were processed by the winnowing machine.

Table 4.1: Oil content of samples by solvent extraction.

S/NO.	Sample Type	Average Oil Content (%)
1	UK Sample 1	9.00
2	UK Sample 2	16.37
3	NGN Sample	12.10

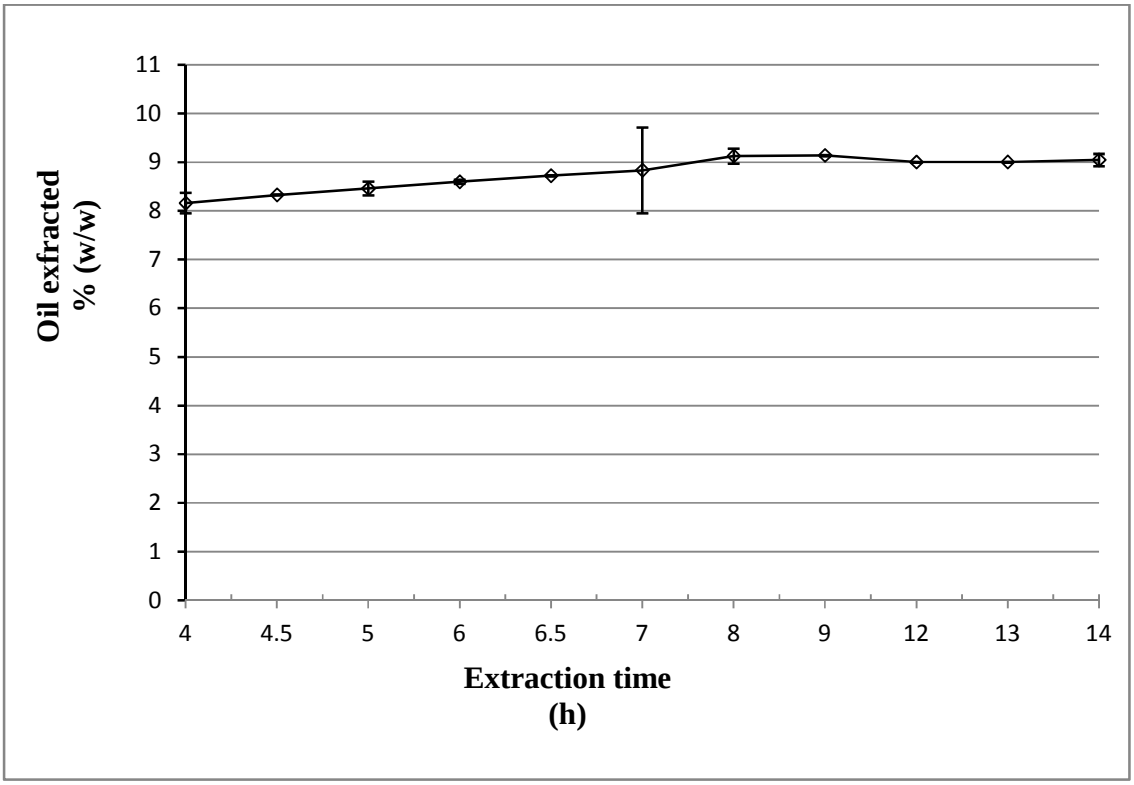

Figure 4.4: Oil content of UK sample 1.

In Figure 4.4, the extraction was carried out starting at 4 hours to determine the oil content of the sample to find out the optimum time for the extraction of oil from corn germ. The result show that increase in extraction time result in a corresponding increase oil obtained until at about 9 hours when the oil extracted remain constant with time signifying that virtually all the oil have been extracted from the germ. The finding from this work differs from the work of Kaya, ***et al.*** 2009. The author reported that the oil content from oil peanut could be determined using soxhlet extraction after 5 hours. Zia-Ul-Haq ***et al.,*** 2008 and Mabaleha ***et al.*** 2007 used mixture of n-hexane/2 propanol (3:1, v:v) in soxhlet apparatus for 6 hours to determine the oil content of four mungbean cultivars oil and melon seed oil, respectively. Figure 4.5 and Figure 4.6 present the result of oil content determination for UK sample 2 and NGN sample which followed the same

trend with that of UK sample 1. The extractions were started at 9 hours due to fact that from the preliminary investigation in Figure 4.4, the optimum time for extraction of oil from corn germ is 9 hours.

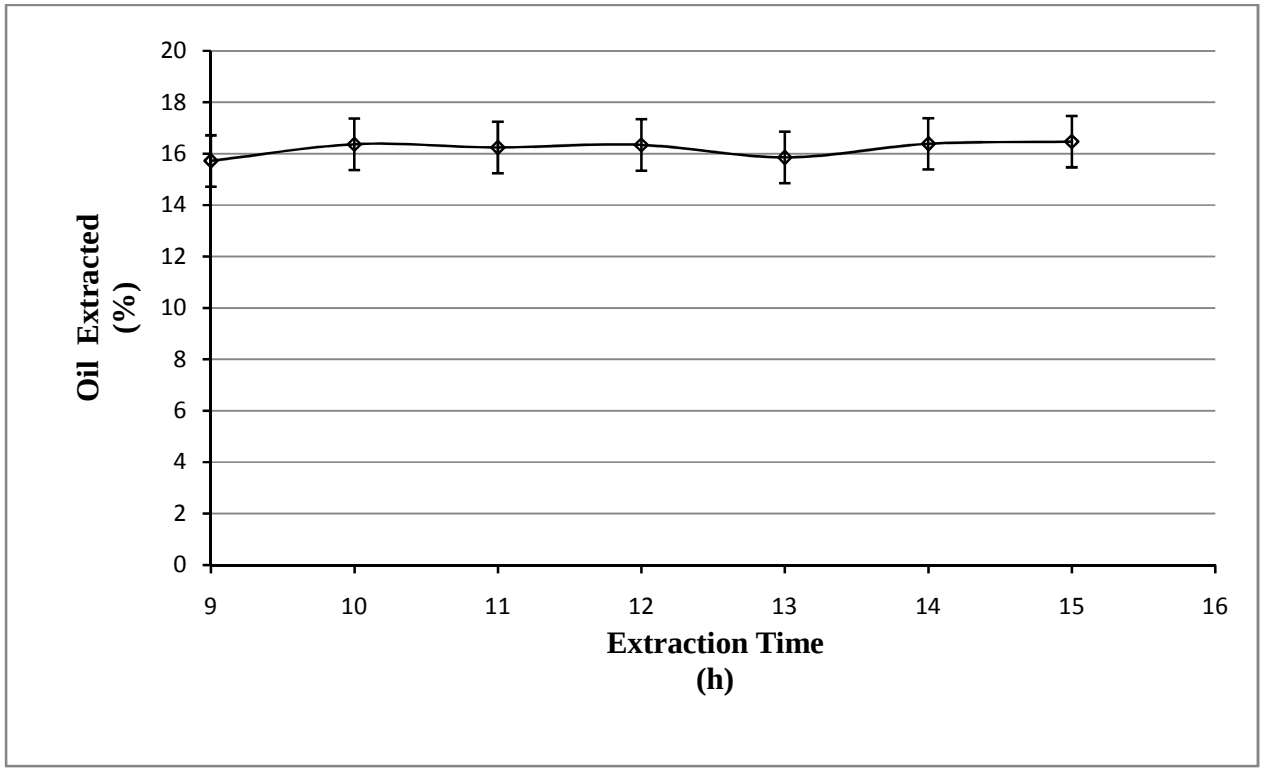

Figure 4.5: Oil content of UK sample 2

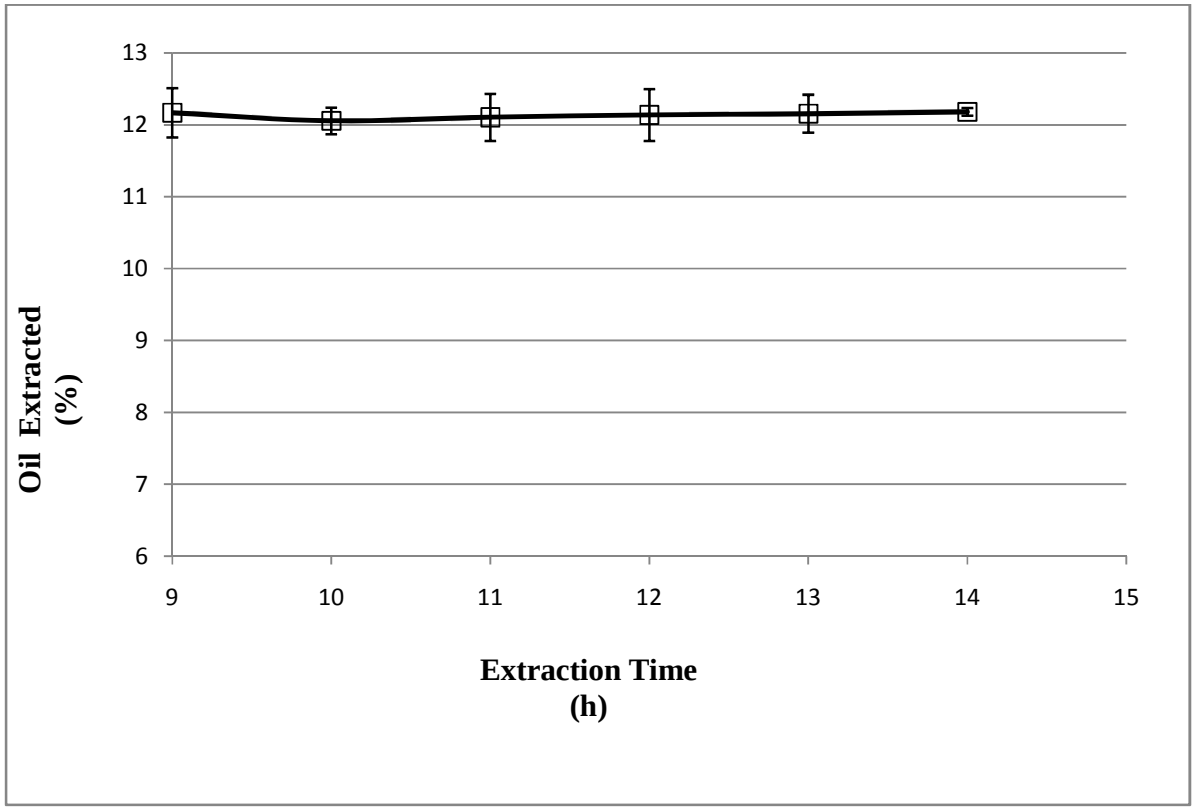

Figure 4.6: Oil Content of NGN sample.

The oil content of commercially processed dry-milled germ using Beall's degerminator (MIAG, Braunsweig, Germany) was 15.00% on dry matter basis (Djerdj, 1992). The UK sample 1 is a commercially processed corn germ powder but has just 9.00% oil, compared to the NGN, which is processed from the product winnowing corn in Nigeria and still has an oil content of 12.10%. UK sample 1 was the sample the experiment was started with and as usual, immediately the sample was received, the moisture and oil contents were determined.

During these preliminary tests, the sample was noticed to have impurities such as stone, stick and even some corn seeds. This forms the basis for pretreating the sample using 2.8 mm and 1.00 mm aperture sieves to remove the impurities and the corn seeds in the sample. After sieve

analysis, only samples that pass through 1.00 mm sieve were used because most of the impurities were larger than that aperture. Subsequently, all samples used for both the soxhlet extraction and SFE were pretreated using the same method. It was observed during the sieve analysis of the corn germ powder and the work of Abdulkadir and Isah (2010) that series of experiments carried out using different sieve apertures will result into having different oil contents. It was reported by Sniegowski and Baldwin (1954) that higher oil content is of economic interest to users of corn, therefore it will be worthwhile to carry out a particulate analysis of NGN corn sample so that high yield of oil can be obtained from the product.

Alexander, *et al.* (1967) described the procedure of nuclear magnetic resonance for determination of oil content and was used by Curtis *et al.* (1989). Taylor, *et al.* (1993) used analytical supercritical fluid extraction to determine the oil content of oilseeds and compared his result with official method of American Oil Chemist. The two results are quite close.

4.3 Apparent Solubility Tests

The detailed experimental data for the solubility tests are given in Appendix A with the formula and sample calculations given in Appendix B. The plot of apparent solubility of corn oil against pressures for 40 ^{o}C and 50 ^{o}C is given in Figure 4.7.

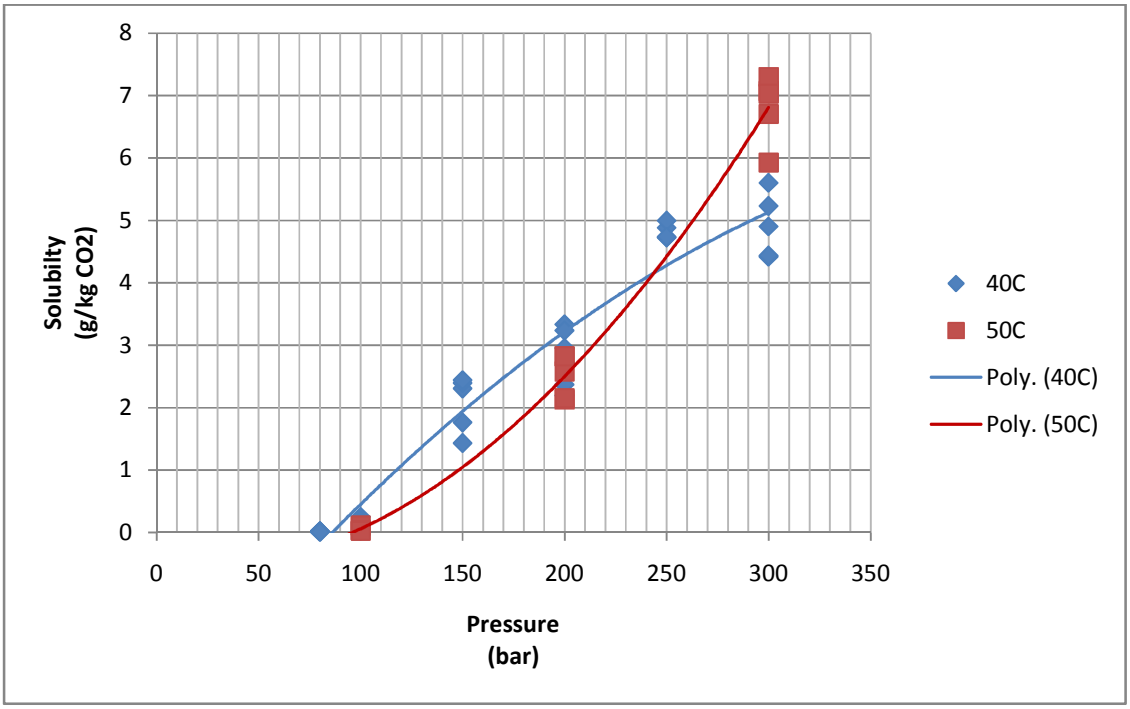

Figure 4.7: Apparent solubility test chart for un-pretreated UK corn germ Sample 2

Christianson *et al*. (1984), in their work outlined that the straight line portions of a plot of oil recovered against CO_2 consumed shows the apparent solubility of the oil in CO_2. Appendix A shows results of the solubility tests with the highest correlation coefficient R^2 (0.9999), after drawing a trend line on all the results. The apparent solubility values obtained for the plots with the highest R^2 were plotted against pressure at temperatures of 40 ^oC and 50 ^oC. The points obtained were joined with a trend line so that the crossover temperature can be estimated. From Figure 4.7 the crossover pressure is 240 bar. Below this pressure, the apparent solubility tends to decrease with increase in temperature. However, above this pressure, the opposite effect occurs. The result from this study shows appreciable consistency with the work of Özkal *et al.,* (2005) who stated that in the supercritical carbon dioxide extraction of oil from hazelnut the solubility increased with increase in temperature above the crossover pressure. Gupta and Shim (2007)

added that high solubility is usually required in supercritical extraction processes of seed oil. It is on the basis on this premises that it is recommended to carry out the supercritical extraction of oil from corn germ above 240 bar.

4.4 Supercritical Fluid Extraction

The results of the supercritical fluid extraction at different conditions are given below with the detailed results given in Appendix C. SFE is a diffusion-based process which requires that the solvent to diffuse into the matrix, and the extracted material to diffuse out of the matrix into the solvent. Diffusivities are much faster in supercritical fluids than in liquids, and therefore extraction can occur faster. Also, there is no surface tension and viscosities are much lower than in liquids, so the solvent can penetrate into small pores within the matrix inaccessible to liquids. Both the higher diffusivity and lower viscosity increase the speed of the extraction.

4.4.1 Effect of pretreatment on supercritical extraction of corn oil

Since it has been established in section 4.3 that above 240 bar the supercritical extraction is favoured, at any pressure above the crossover pressure, the effect of pretreatment was studied at 250 bar and a temperature of 40 ^{0}C, which is just slightly above the critical value of CO_2. In Figure 4.8, the oil extracted for non-pretreated (NPT), 1 tonne and 5 tonnes pretreated samples were plotted against the CO_2 used for the extraction. The amount of oil recovered increased with increase in the pretreatment from 1 tonne to 5 tonnes. Pretreatment of 5 tonnes has the highest oil extracted and so the 5 tonnes pretreatment was used to study the effect of temperature and flow rate. The oil recovered for 5 tonnes was not high enough due to some oil lost while pressing the sample with that force but could be improved with some little modification to the Pressing Machine.

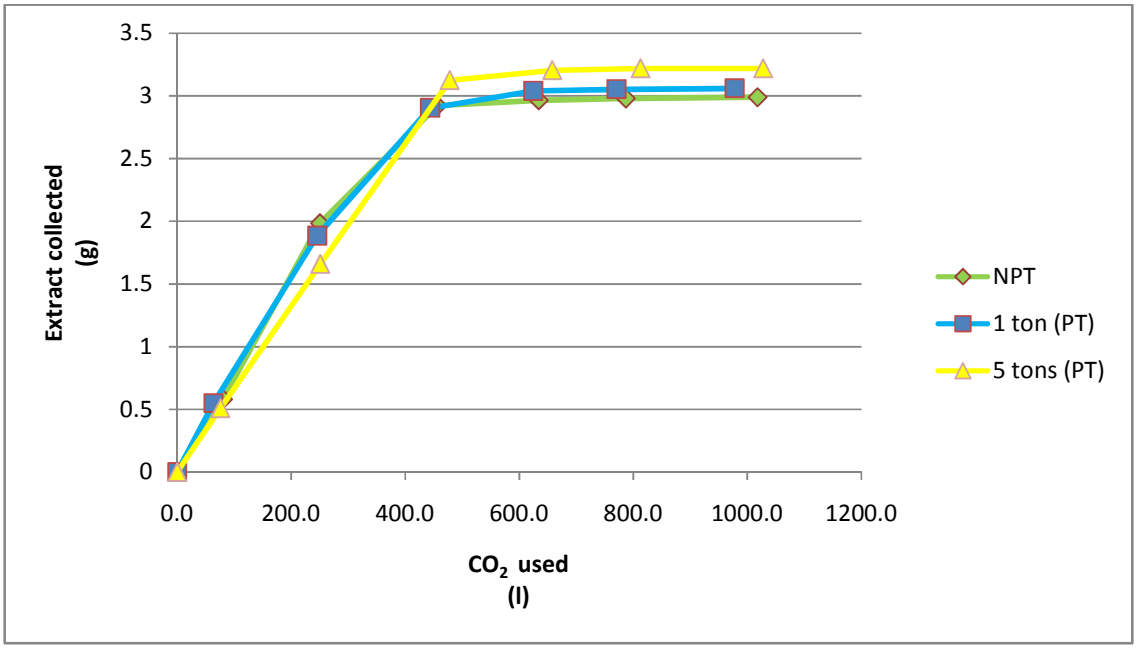

Figure 4.8: **Effect pretreatment of sample on supercritical fluid extraction for UK corn germ sample 2 at 250 bar and 40 ºC**

4.4.2 Effect of pressure on supercritical extraction of corn oil

Supercritical extraction processes are enhanced by high solubility at the pressures above the crossover pressure. In this work, despite the crossover pressure being 240 bar, a pressure of 150, 200 and 300 bar were used to confirm the trend. A temperature slightly above the critical point is usually started with and in this case, 40 ^{o}C is used, but a temperature (22 ^{o}C) below the critical temperature was also tried.

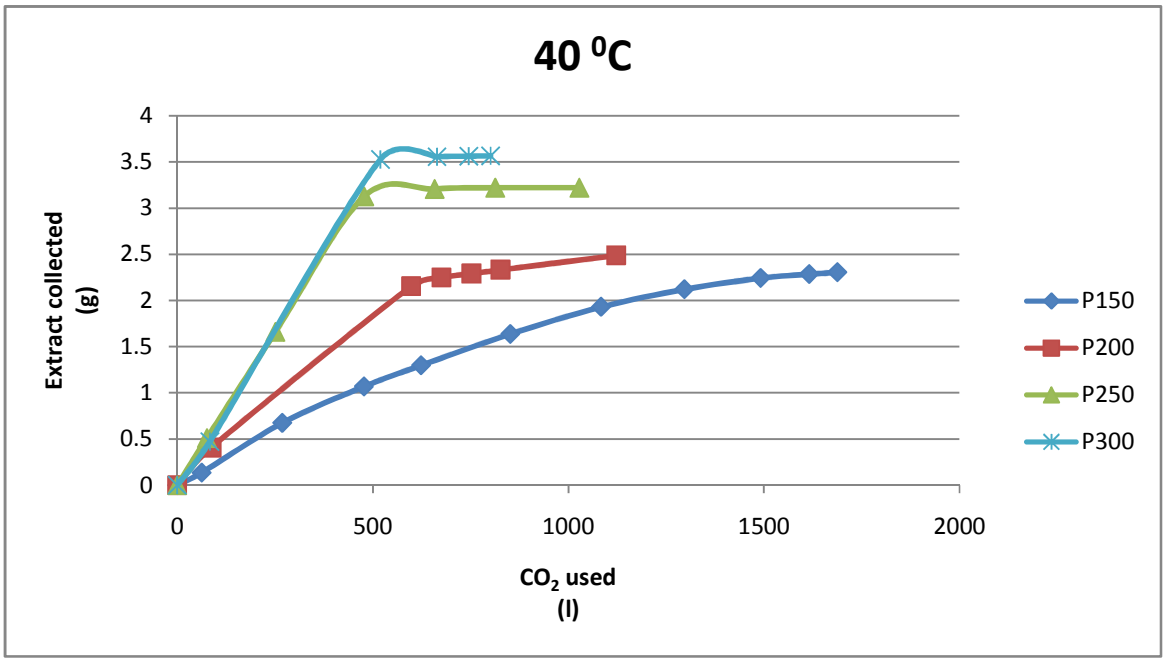

Figure 4.9: Effect of pressure on supercritical fluid extraction for the UK 2 corn germ sample at 40 °C

Figure 4.9 shows the graph of the effect of pressure on the extraction of corn oil using the 5 tonnes pretreated UK corn germ sample 2 at 40 ^{0}C. From the graph, an increase in the pressure from 150 bar to 300 bar results in a corresponding increase in the amount of oil extracted. This is clearly attributed to the increase in CO_2 density and consequently it's dissolving ability. This result agreed well with the work of Singh *et al*. (2003) and Lu (1997) who reported the extraction of oil from sarawak black pepper and rosemary respectively. From the result above, since the extraction does not deviate from the literature, the minimum pressure of 200 bar and the maximum pressure of 300 bar were used. Del Valle *et al*. (2008) found their crossover pressure to be 150-200 bar but realized that extraction at 35-50 ^{o}C did not affect the yield of garlic oil at 300 bar.

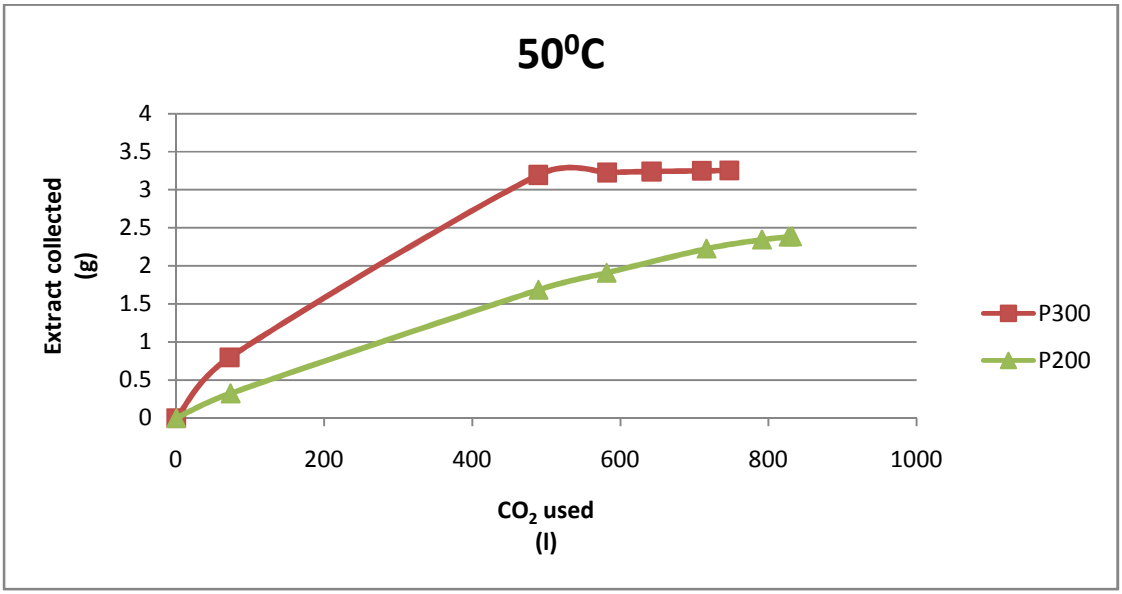

Figure 4.10: Effect of pressure on supercritical fluid extraction for the UK 2 corn germ sample at 50 ^{o}C

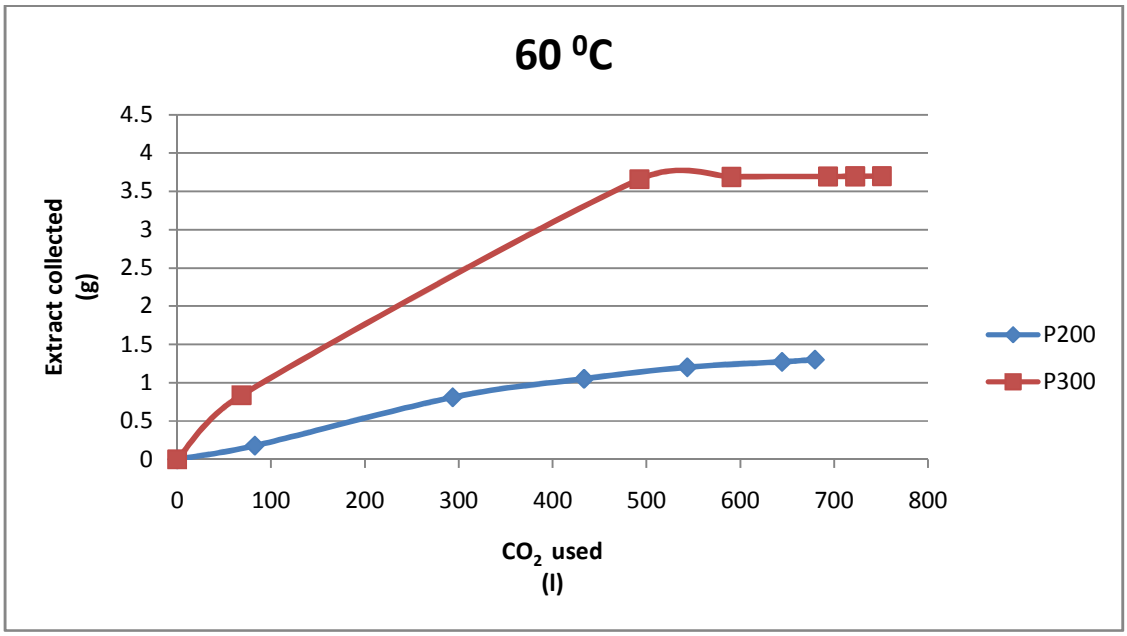

Figure 4.11: Effect of pressure on supercritical fluid extraction for the UK 2 sample at 60 ^{o}C

Figure 4.10 and Figure 4.11 represent the effect of pressure on the oil extracted at 50 ^{0}C and 60 ^{0}C respectively. The trend is similar to what was observed in Figure 4.9. At room temperature the extraction curve for the UK sample corn germ 2 is represented in Figure 4.12 and here too an increase in the pressure increases the amount of oil extracted.

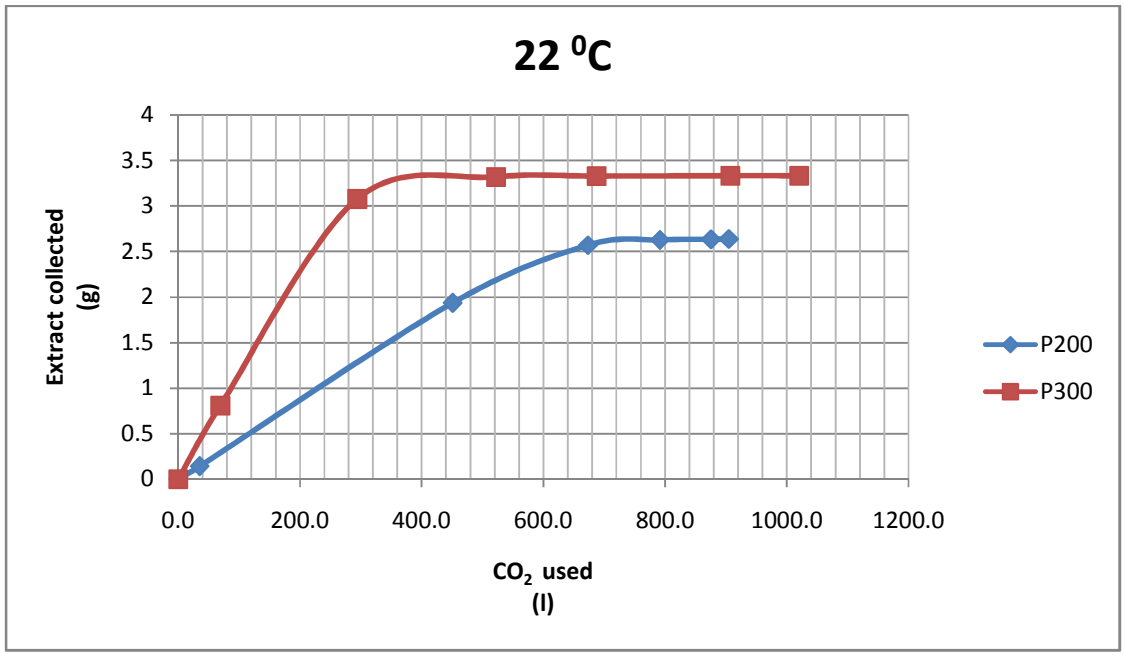

Figure 4.12: Effect of pressure on liquid CO_2 extraction of the UK sample at 22 ^{o}C

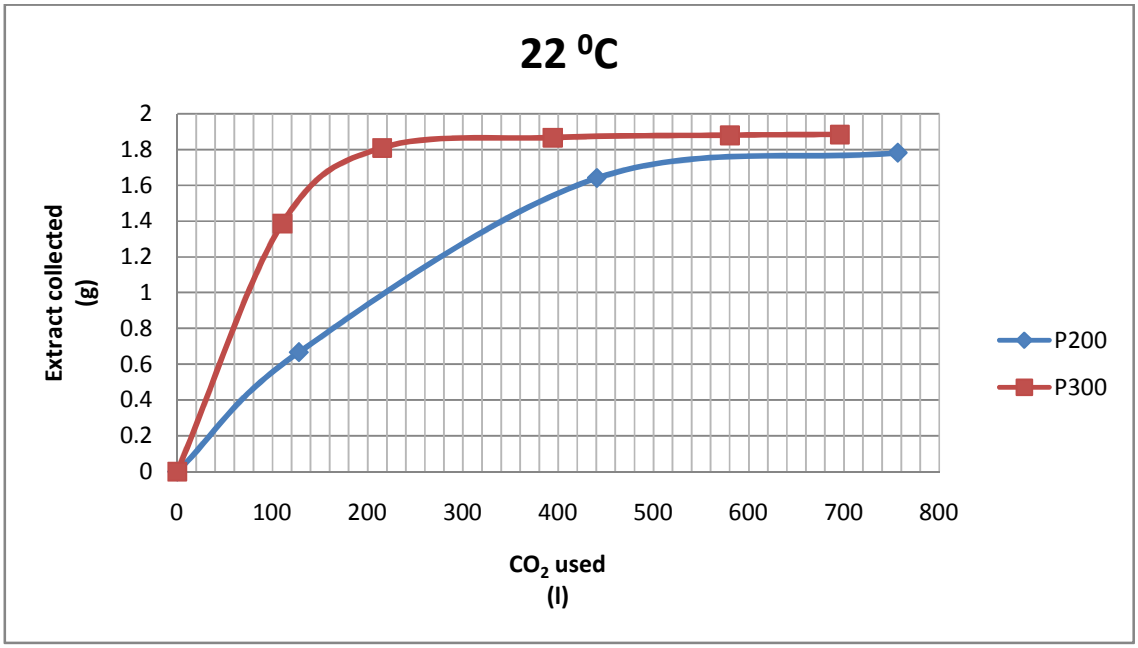

Figure 4.13: Effect of pressure on liquid CO_2 extraction of the NGN sample at 22 ^oC

4.4.3 Effect of Temperature on Supercritical Extraction of Corn Oil

The tests were undertaken at pressure of 200 bar (below the cross over pressure; 240 bar) and 300 bar (above the cross over pressure) to confirm in this research the observation made by Roy et al; (1996) that below the cross over pressure the yield increases with increase in temperature while above the cross over pressure, the yield increases with decrease in Temperature.

Figure 4.14 shows the effect of temperature on the oil extracted at 200 bar. Increase in temperature from room temperature to 60^0C resulted in a reduction in the oil extracted. The most plausible reason for this decrease is the resulting decrease in the solvent density, whose effect seems to dominate over the increase of the solute vapor pressure. It is important to add that the effect of temperature on SF strongly depends on the sample used. According to Lu (1997) in the report of oil extraction from thyme and rosemary, the oil yield increases with increase in

temperature for rosemary, but for the thyme the curve for low and high temperature overlap which implies that there is no noticeable difference in oil yield. Similarly, Djerdj, *et al.* 1992 on modelling of oil extraction from corn germ stated that there is no practical effect of temperature on yield.

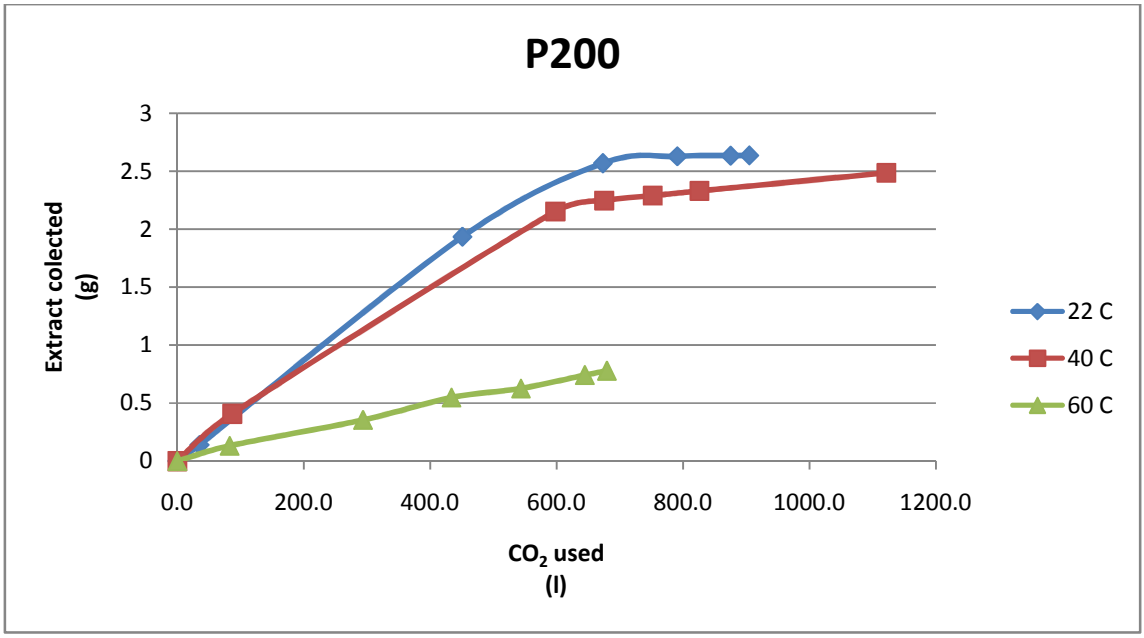

Figure 4.14: Effect of temperature on supercritical fluid extraction of UK 2 sample at 200 bar

Figure 4.15 shows the effect of temperature on the yield of oil from UK sample 2 at 300 bar. This is opposite of what was observed when the pressure was below the crossover pressure. The two results agree with what is available in the literature with respect to crossover pressure. The yield increased from room temperature through 40 ^{0}C to the highest at 60 ^{o}C.

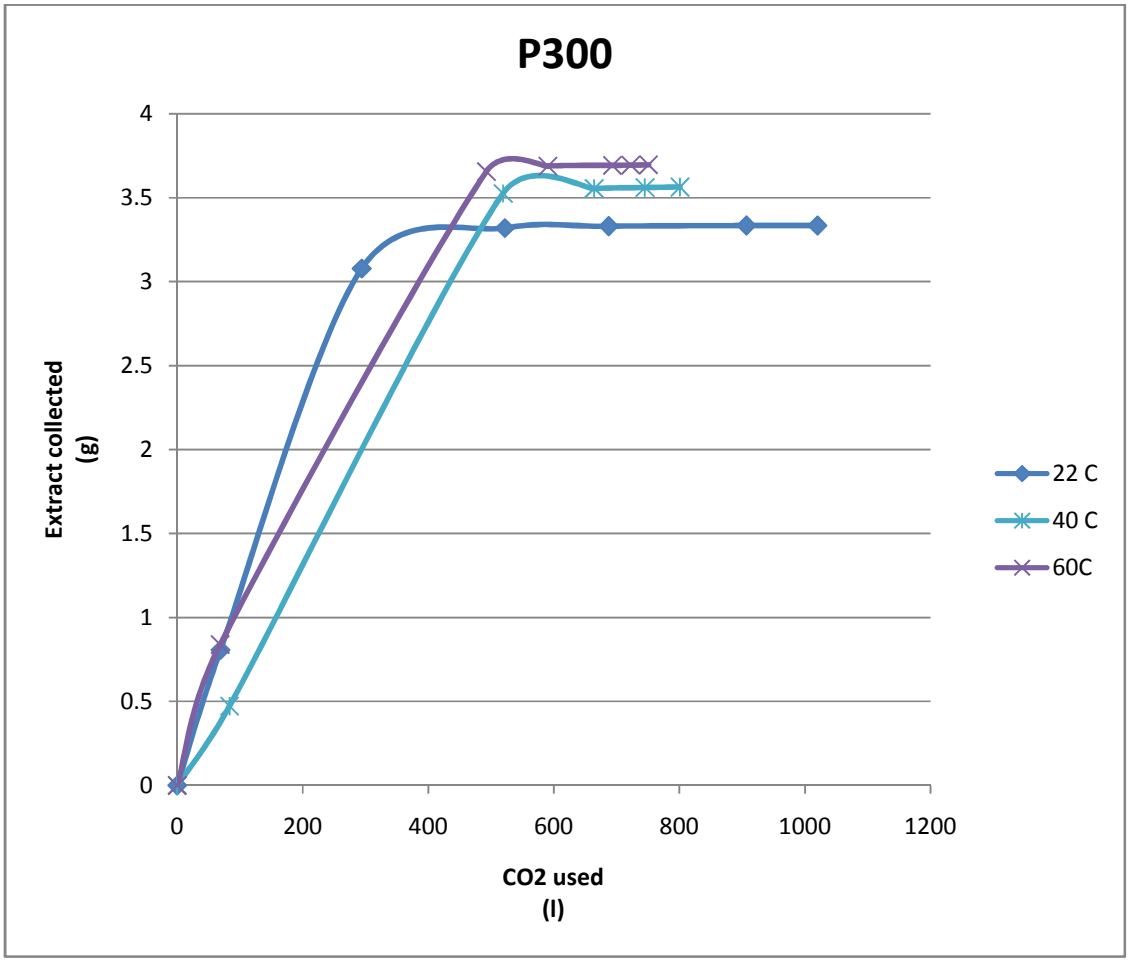

Figure 4.15: Effect of temperature on supercritical fluid extraction of UK 2 sample at 300 bar

4.4.4 Effect of Flow Rate

Figure 4.16 shows the effect of flow rate on liquid CO_2 extraction of UK 2 corn germ sample at 300 bar and room temperature. The flow rates used were 4 L/min, 8 L/min and 12 L/min of CO_2. From the Figure shown below the extractions occur in two stages known as fast and slow extraction periods. This is due to kinetic and mass transfer control during the extraction. The oil on the surface of the particles was extracted in the fast extraction period while the remainder in the intact cell of the corn germ was extracted in the slow extraction period. In the fast extraction

period, the yields of oil were 3.2 g, 2.3 g and 2.1 g for 4 L/min, 8 L/min and 12 L/min respectively. While from beginning of the extraction to the end of the slow extraction period, the yields were 3.3 g, 3.2 g and 3.3 g for 4 L/min, 8 L/min and 12 L/min respectively. Though in reality, an increase in flow rate should result in increased yield because the amount of CO_2 is increasing. However in this case this was not realistic as some of the oil were blown off with the CO_2 but trapped using glass wool. Findings from this work stipulate that a flow rate of 4 L/min is the most appropriate for SFE of corn germ.

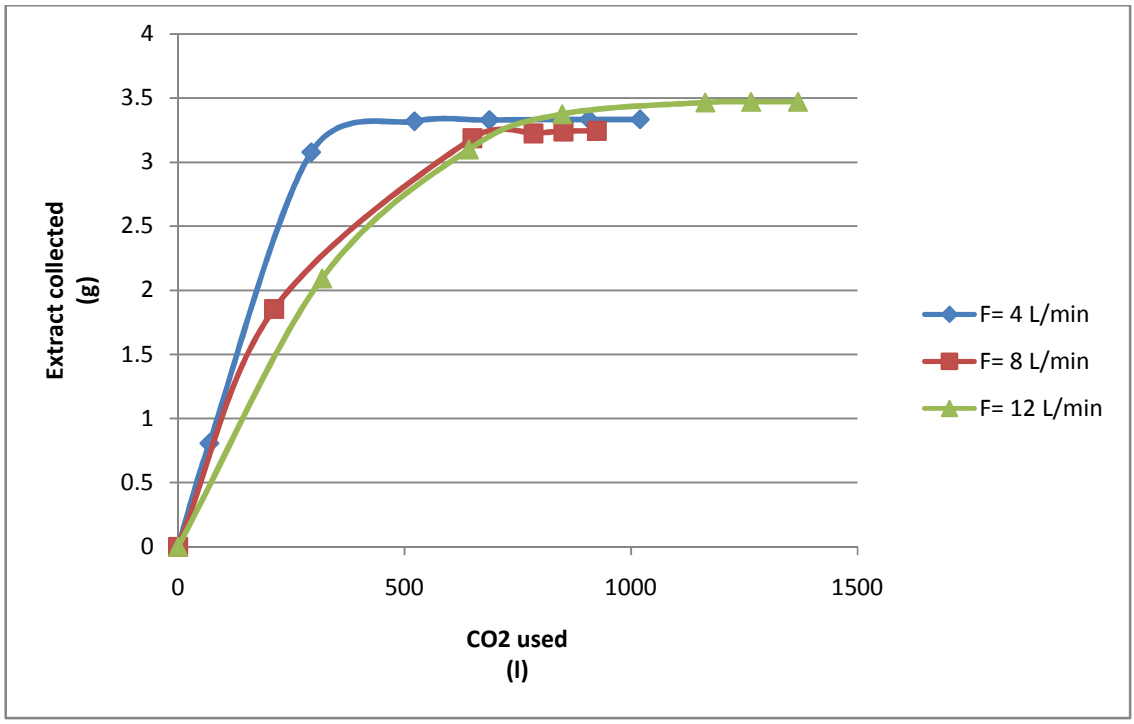

Figure 4.16: Effect of flow rate on liquid CO_2 extraction of UK 2 at 300 bar and 22 ^{0}C

4.4.5 The Best Operating Parameters

Figure 4.14 shows the effect of temperature on the yield of oil at 200 bar. It can be seen that the best temperature is room temperature (22 ^{o}C). In Figure 4.15 for the yield of 300 bar, the maximum yield in the slow extraction period at 22 ^{o}C, 40 ^{o}C and 60 ^{o}C are 3.3 g, 3.6 g and 3.7 g respectively. In this process difference between the yield at room temperature and the highest yield is just 0.4g. The yields in the fast extraction period after using 280 litres of CO2 are 3.0 g, 1.9 g and 2.3 g respectively. Since the oil extracted in the slow extraction period is negligible with respect to the amount of CO_2 used, it is also better to carry out the extraction at room temperature for 300 bar.

Based on the yield of oil at room temperature with pressures of 200 and 300 bar, the best operating parameter was 300 bar for the UK corn germ sample 2. This agrees with the conclusion reached by Del Valle *et al.* (2008) in their work on extraction of garlic with supercritical CO_2 and conventional organic solvent. Oil was also extracted from the NGN sample at 200 and 300 bar to enable comparison. Figure 4.13 shows the result of extraction of oil from NGN sample at 200 and 300 bar with oil yield of 1.68g and 1.82g respectively. For both the UK sample 2 and NGN sample, the extraction was best at 300 bar.

Figure 4.12 and Figure 4.13 show the percentage oil recovery of the UK sample 2 and NGN samples are 81.4% and 62.2% respectively based on the available oil in the corn germ. Considering the amount of oil extracted, the UK sample was discovered to be better.

4.5 Transmission Electron Microscope (TEM)

Figure 4.17-4.19 shows the transmission electron microscope (TEM) of the corn germ samples used in this work. Transmission electron microscopy (TEM) is a microscopy technique in which

a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera.

TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small de Broglie wavelength of electrons. This enables the instrument's user to examine fine detail, even as small as a single column of atoms, which is thousands of times smaller than the smallest resolvable object in a light microscope. It is as a result of the high magnification that TEM is used to study the morphological structure of the sample so that the difference can be observed and conclusion drawn.

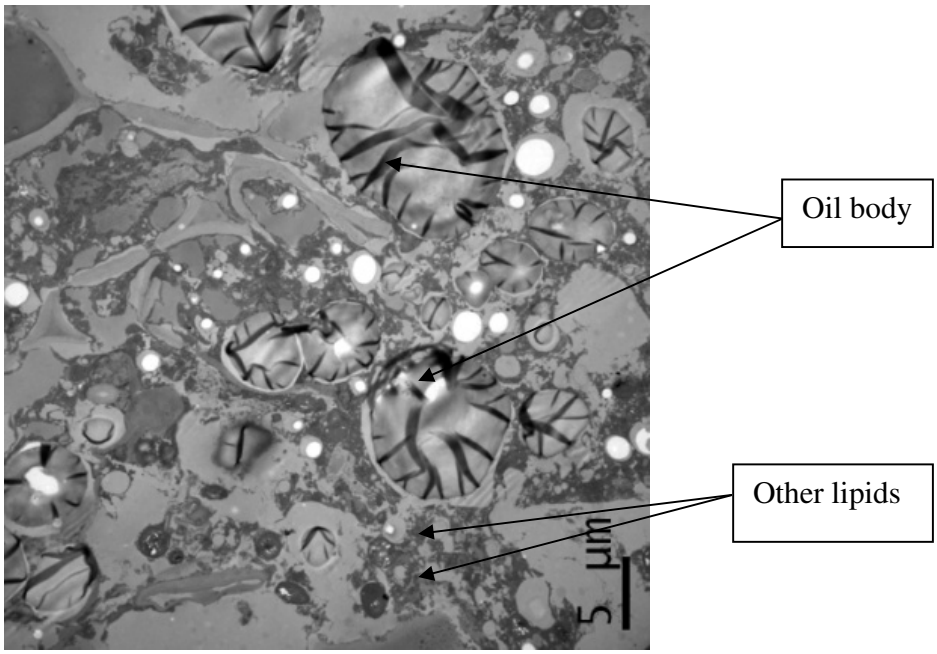

Figure 4.17: TEM of the raw material of UK corn germ sample 2

Careful observation of Figure 4.17 shows that the black jelly spot represent the oil body while other parts in the micrograph are resins in the particles.

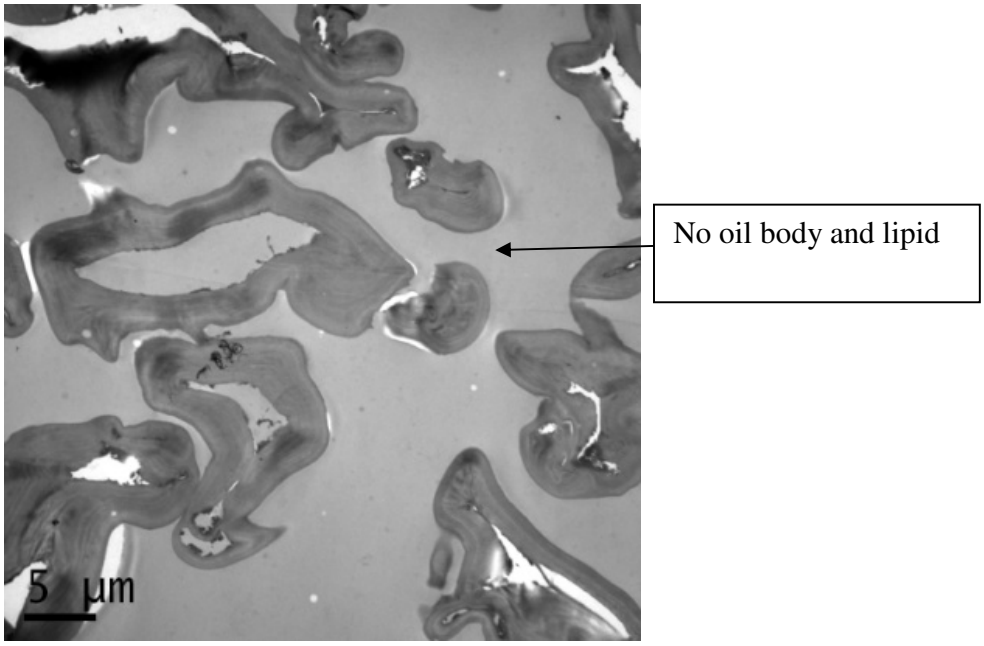

Figure 4.18: TEM of the raw material for UK sample 2, after 12 h of Soxhlet extraction

The oil body is shown in the portion labeled in Figure 4.17. In all the subsequent Figures the oil body is similar to the shape in this micrograph. In Figure 4.19 after a short time (less than 1 hour) it can be clearly observed that it was just small oil body that was left on the micrograph. Also based on the selectivity of CO_2 most of other resins (lipid) in the sample still remain. The difference between Figure 4.17 and 4.18 clearly shows that after 12 hours extraction most of the oil body have been removed from Figure 4.18 during the extraction process.

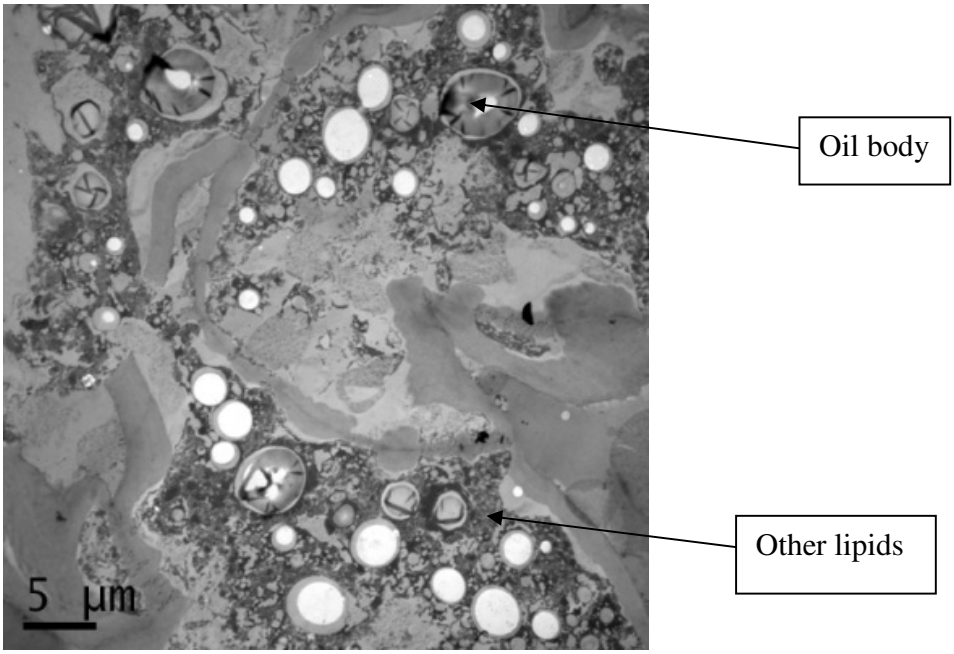

Figure 4.19: TEM of the raw material for UK sample 2, after solubility test

Solubility test of the corn germ is carried within a short time interval and so only small amount of the lipids in the particles are removed as can be seen by observing the difference in the micrograph of Figure 4.17 and Figure 4.19.

4.6 Summary of Results

The experiment was started with the determination of moisture content of sample to ascertain the state of the sample for the SFE of corn oil but also oil content was determined to ensure the presence of the oil in the sample to be used. Supercritical fluid extraction was carried out successfully for the three samples and the results presented in this Chapter. TEM though not used by most researchers was used to observe the samples before and after extraction for the removal of resins from the sample which is confirm to be corn oil. Conclusions and recommendation to the findings are summarised in Chapter five.

CHAPTER FIVE

5 CONCLUSIONS AND RECOMMENDATIONS

The conclusions and recommendations to this work on the production of edible oil from corn germ using supercritical CO_2 as solvent are presented below.

5.1 Conclusion

The moisture content of corn germ was less than 20%, which implies that structural damage of the grain has not occurred. This is an indication that the corn germ samples used in this research contain some oil. This parameter is very important at the beginning of an experiment to help in making decision as whether to use a sample or not.

The Soxhlet extraction was used to ascertain the possibility of extracting oil from the samples as well as to establish the oil content to be used as the basis for the supercritical fluid extraction. Of all three used samples, UK sample 1 had the lowest oil content of 9.00%, UK sample 2 has the highest oil content of 16.37% and NGN sample has oil content of 12.10%. Despite the fact that UK sample 1 is a commercially processed germ, it contains just 9.00% oil, compared to the NGN, which is processed from the by-product of winnowing germ in Nigeria and still has an oil content of 12.10%. Since higher oil content is of economic interest to users, it is therefore viable to conclude that UK sample 2 has high economic potentials followed by NGN sample.

The crossover pressure of corn oil in SC-CO_2 at different temperature is 240 bar, above which the solubility increases with increasing temperature. Extraction of corn oil occurred in two stages namely slow and fast extraction periods. The oil recovery in the fast extraction period was higher

than that of the slow extraction period. In the fast extraction period, the 'free' oil which was released by pretreatment process was extracted while in the slow extraction period, the unreleased oil was extracted. The oil recovered in the slow extraction period is negligible as compared to that recovered in the fast extraction period. Therefore for economic reason it is not necessary to continue the extraction in the slow period. The pelletizing of sample in a 35 mm diameter die with a force of 5 tonnes under a controlled environment yielded highest amount oil from the supercritical extraction of corn germ.

Generally, increasing extraction pressure from 150 to 300 bar at isothermal temperatures of 22 ^{o}C, 40 $^{\circ}$C, 50 ^{o}C and 60 ^{o}C resulted in an increase in the yield of corn oil. At isobaric pressure of 200 bar, increase in temperature from room temperature to 60 ^{o}C resulted in a decrease of corn oil yield. On the other hand, at isobaric pressure of 300 bar, the yield was lowest at 40 ^{o}C to the highest at 60 ^{o}C. Though, in theory an increase in flow rate should result into higher oil yield from the corn germ, it can be limited by the equipment. When CO_2 flow rate exceeded a certain value (4 L/min) in this work, some oil is blown out of the collector with CO_2. Based on the results, it is therefore suitable to carry out the test at a flow rate of 4 L/min.

For both UK sample 2 and the NGN samples, extraction at 300 bar has a higher oil recovery of 81.4 % and 62.2 % respectively in the fast extraction period based on the available oil in the corn germs. However lower quantity of CO_2 (200 litres) used for the NGN as against 280 litre for the UK sample which is an industrially processed corn germ.

5.2 Recommendations

Particulate analysis of the sample using different sieve sizes to enable the study of effect of particle on oil recovery from corn germ could be carried out. Full solubility study could be carried out including modeling the extraction process using mass transfer models and possible fractionation of constituents from corn oil. Empirical optimization of the effect of operating parameters using response surface methodology (RSM) should be carried out using appropriate statistical software to enable the development of model equation that will show the relationship between the oil recovery and these fundamental operating parameters. The design and fabrication of an indigenous SCF plant for the production of corn oil should be carried out.

In course of this work, some modifications were done to the rig but the rig still needs some more modification. The separation section of the rig could be modified to use two or more separators in series such that during extraction at high flow rate, oil loss would be minimized. The rig should also be modified to recycle CO_2 instead of venting off to the environment as it is presently

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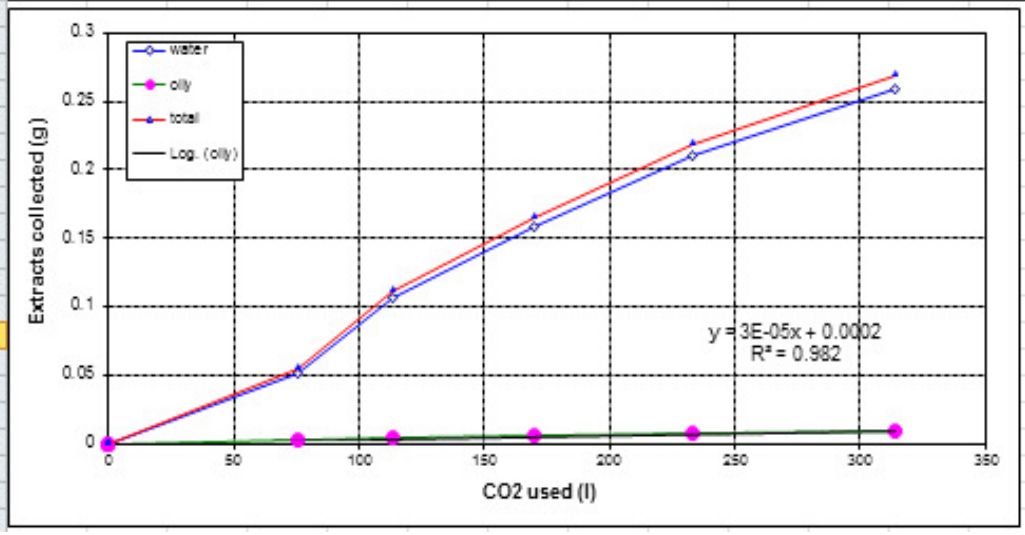
APPENDIX A
SOLUBILITY TEST RAW DATA

The raw data for the solubility test are given below:

1	solubility-8					Extract from corn germ																24-Jun	2010	
2		Extraction conditions =		P		80	bar	T	40	°C	F	3.5	rotary-meter		Time	100	min							
3			sample:	100% passed BS410 1mm aperture sieve, and has a oil content of								8.33	%											
4			sample charged in =			82.22	g																	
5			Residue =			80.07	g			Room T	27.2	C												
6			bed conditions =	D:	45 mm, H:	95 mm																		
7			available oil =			6.849	g																	
8																								
9		data readed								Sampling			data calculated											
10		time	P	temperature C				CO_2	Nº:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts						
11	hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total				
12	18:10	0	0	29.4	29.4	30.6	34.9	83.0		0	0	0	0	0	0	0	0	0	0	0				
13	18:15	5	62.7	31.1	30.7	31.8	37.5	83.0		0	0	0	0.00	0	0	0	0	0	0	0				
14	18:25	15	72.5	36.8	34.0	36.3	40.9	83.0		0	0	0	0.00	0	0	0	0	0	0	0				
15	18:40	30	79.5	37.7	35.0	33.5	41.5	83.0		0	0	0	0.00	0	0	0	0.0	0	0.000	0				
16	19:00	50	80.8	39.4	36.3	35	41	158.7	1	130.392	130.34	130.34	3.79	0.054	0.051	0.003	75.7	0.051	0.003	0.054				
17	19:20	70	81.6	39.4	37.1	35.8	40.9	196.8	2	113.762	113.71	113.705	1.91	0.057	0.056	0.001	113.8	0.107	0.004	0.111				
18	19:40	90	80.8	39.5	37.9	37	40.7	253.2	3	127.050	127	127	2.82	0.054	0.052	0.002	170.20	0.159	0.006	0.165				
19	20:00	110	79.6	39.5	38.3	37.4	40.8	316.0	4	130.579	130.53	130.53	3.14	0.053	0.051	0.002	233.00	0.21	0.008	0.218				
20	20:10	120	81.1	39.4	38.7	37.7	40.8	396.8	5	126.556	126.51	126.51	8.08	0.050	0.049	0.001	313.80	0.259	0.009	0.268				

* 14mg oily extracts found on the tube and cap



average flow rate (volume):	3.83 l/min
(mass):	0.410 kg/h
total oily extracts mass:	0.003 g
yield of oily extracts:	0.01 %
mass balance(out/in)	97.7 %

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
75.7	0.051	0.003	0.054
113.8	0.107	0.004	0.111
170.2	0.159	0.006	0.165
233.0	0.21	0.008	0.218
313.8	0.259	0.009	0.268

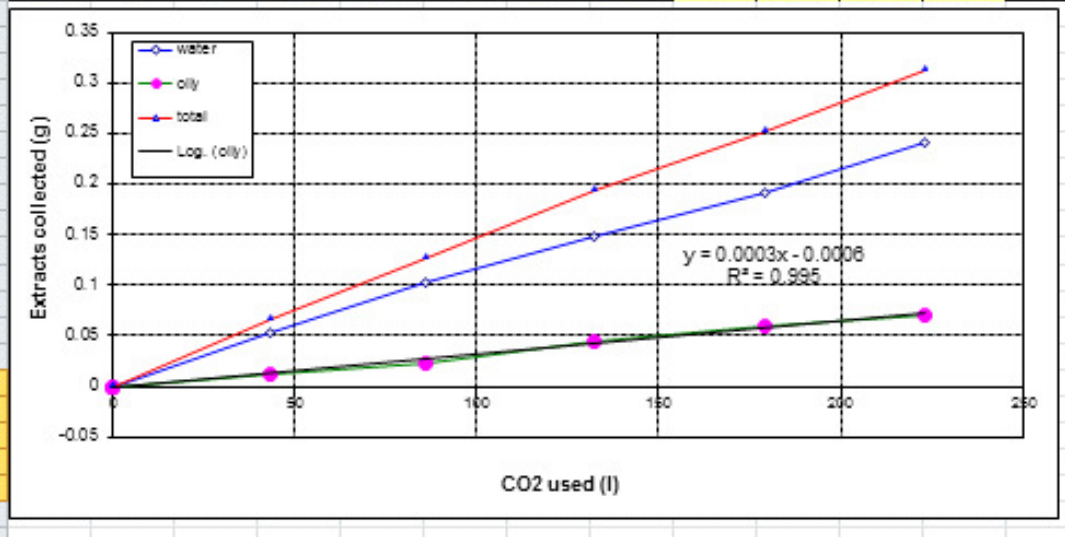
solubility	
CO_2 (kg)	g/kg CO2
0.000	0.000
0.000	0.000
0.000	0.000
0.135	0.022
0.203	0.015
0.304	0.020
0.416	0.018
0.560	0.007

Plate A1: Solubility test at pressure of 80 bar and Temperature of 40 ^{0}C.

solubility-21b					Extract from corn germ												8888	2010	
Extraction conditions =		P		100	bar	T	40	°C	F	5	rotary-meter		Time	60	min			
sample:	100% passed BS410 1mm aperture sieve, and has a oil content of									8.33	%							
sample charged in =				82.53	g													
Residue =				76.3	g			Room T	20.9	C								
bed conditions =	D:	45 mm, H:	95 mm															
available oil =				6.355	g													

	data readed							Sampling				data calculated							
	time	P	temperature C				CO_2	Nº:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
14:20	0	0	39.9	37.9	36.8	38.9	142.5		0	0	0	0	0	0	0	0	0	0	0
14:40	20	51.2	39.8	38.0	36.9	38.8	142.5		0	0	0	0.00	0	0	0	0	0	0	0
14:50	30	94.7	39.9	38.7	35.8	38.8	142.5		0	0	0	0.00	0	0	0	0	0	0	0
15:10	40	101.5	39.9	38.4	36.6	38.9	142.5		0	0	0	0.00	0	0	0	0.0	0	0.000	0
15:20	60	102.5	38.5	38.5	36.7	38.9	185.5	1	130.414	130.36	130.35	2.15	0.066	0.054	0.012	43.0	0.054	0.012	0.066
15:30	70	102.9	38.9	38.6	36.8	38.8	228.5	2	113.77	113.72	113.713	4.30	0.060	0.049	0.011	86.0	0.103	0.023	0.126
15:40	80	101.5	38.7	38.7	36.9	38.8	275	3	127.07	127.03	127.01	4.65	0.067	0.046	0.021	132.50	0.149	0.044	0.193
15:50	90	101.3	38.7	38.6	36.9	38.7	321.5	4	130.59	130.55	130.54	4.65	0.058	0.042	0.016	179.00	0.191	0.06	0.251
16:00	100	101.5	38.5	38.6	36.9	39.0	365.9	5	126.58	126.53	126.515	4.44	0.061	0.050	0.011	223.40	0.241	0.071	0.312

* 14mg oily extracts found on the tube and cap



average flow rate (volume):	3.60	l/min
(mass):	0.394	kg/h
total oily extracts mass:	0.071	g
yield of oily extracts:	0.09	%
mass balance(out/in)	92.8	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
43.0	0.054	0.012	0.066
86.0	0.103	0.023	0.126
132.5	0.149	0.044	0.193
179.0	0.191	0.06	0.251
223.4	0.241	0.071	0.312

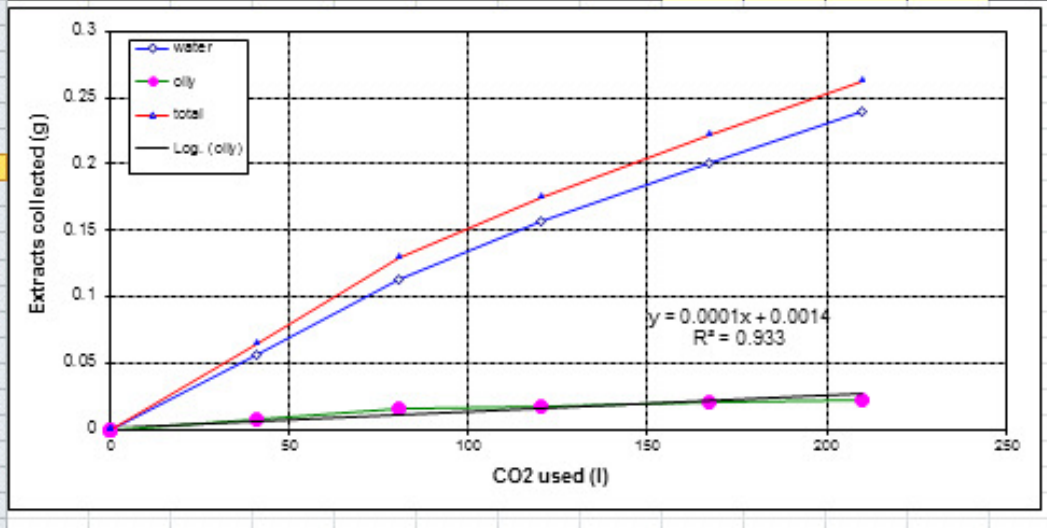
solubility	
CO_2 (kg)	g/kg CO2
0.000	0.000
0.000	0.000
0.000	0.000
0.078	0.153
0.157	0.140
0.242	0.248
0.327	0.189
0.408	0.136

Plate A2: Solubility test at pressure of 100 bar and Temperature of 40 ^{0}C.

solubility-27a					Extract from corn germ												7-Oct	2010	
Extraction conditions =		P	100	bar	T	50	°C	F	5	rotary-meter		Time	45	min				
sample:	100% passed BS410 1mm aperture sieve, and has a oil content of								8.33	%								
sample charged in =			83.05	g														
Residue =			73.87	g				Room T	21.9	C								
bed conditions =	D:	45 mm, H:	95 mm															
available oil =			6.153	g														

	data readed							Sampling				data calculated							
	time	P	temperature C				CO_2	No:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
10:20	0	0	52.4	49.0	46.3	52.2	939		0	0	0	0	0	0	0	0	0	0	0
10:30	10	50.3	50.9	48.4	45.7	49.6	939		0	0	0	0.00	0	0	0	0	0	0	0
10:32	12	53.3	50.7	48.4	45.7	49.6	939		0	0	0	0.00	0	0	0	0	0	0	0
10:35	15	100.9	49.1	48.5	45.1	49.6	939		0	0	0	0.00	0	0	0	0.0	0	0.000	0
10:40	20	101.9	47.7	48.6	44.5	49.4	979.5	1	130.412	130.36	130.35	8.10	0.065	0.057	0.008	40.5	0.057	0.008	0.065
10:50	30	102.3	48.8	49.5	45.2	51.2	1019.5	2	113.78	113.72	113.715	4.00	0.064	0.056	0.008	80.5	0.113	0.016	0.129
11:00	40	101.3	49.4	49.7	45.3	51.1	1059.5	3	127.05	127.01	127.01	4.00	0.046	0.044	0.002	120.50	0.157	0.018	0.175
11:10	50	102	49.6	49.8	45.5	51.2	1106	4	130.58	130.54	130.53	4.65	0.046	0.043	0.003	167.00	0.2	0.021	0.221
11:20	60	101.9	47.9	48.9	45.3	49.6	1149.2	5	126.55	126.51	126.511	4.32	0.041	0.039	0.002	210.20	0.239	0.023	0.262

* 14mg oily extracts found on the tube and cap



average flow rate (volume):	9.55	l/min
(mass):	1.042	kg/h
total oily extracts mass:	0.023	g
yield of oily extracts:	0.03	%
mass balance(out/in)	89.3	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
40.5	0.057	0.008	0.065
80.5	0.113	0.016	0.129
120.5	0.157	0.018	0.175
167.0	0.2	0.021	0.221
210.2	0.239	0.023	0.262

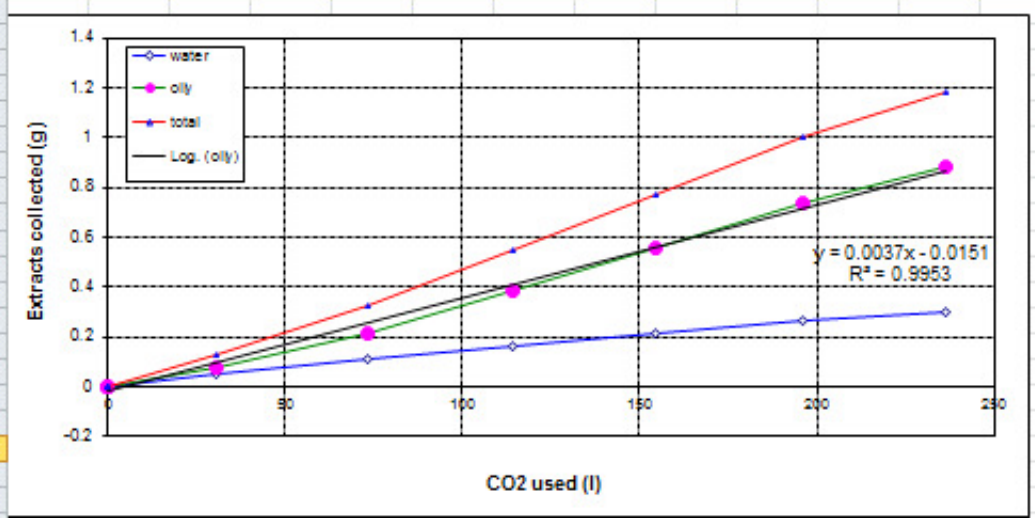
solubility	
CO_2 (kg)	g/kg CO2
0.000	0.000
0.000	0.000
0.000	0.000
0.074	0.109
0.146	0.110
0.219	0.028
0.304	0.035
0.382	0.025

Plate A3: Solubility test at pressure of 100 bar and Temperature of 50 ^{0}C.

solubility-15						Extract from corn germ											16-Jul	2010	
Extraction conditions =		P		150	bar	T	40	°C	F	4	rotary-meter	Time	50	min					
sample:	100% passed BS410 1mm aperture sieve, and has a oil content of									8.33	%								
sample charged in =				73.714	g														
Residue =				69.64	g			Room T	25.5	C									
bed conditions =	D:	45 mm, H:	95 mm																
available oil =				6.140	g														

	data readed							Sampling				data calculated							
	time	P	temperature C				CO_2	Nº:	Total	Dry	Bottle	flow	indvidual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
15:40	0	16.4	24.5	23.9	26.1	28.0	157.4		0	0	0	0	0	0	0	0	0	0	0
15:50	10	151.2	28.7	30.9	34.2	39.5	157.4		0	0	0	0.00	0	0	0	0	0	0	0
16:20	40	149.1	31.5	35.2	40.8	40.9	157.4		0	0	0	0.00	0	0	0	0	0	0	0
17:00	120	152.4	35.1	36.9	37.8	40.8	157.4		0	0	0	0.00	0	0	0	0.0	0	0.000	0
17:10	140	150.6	33.8	37.3	36.2	40.0	187.7	1	124.853	124.8	124.72	1.52	0.130	0.052	0.078	30.3	0.052	0.078	0.13
17:20	150	150.9	34.8	37.8	36.3	40.6	230.6	2	124.4	124.35	124.211	4.29	0.193	0.057	0.136	73.2	0.109	0.214	0.323
17:30	160	151.1	34.7	38.2	36.7	40.8	271.4	3	123.25	123.19	123.02	4.08	0.227	0.058	0.169	114.00	0.167	0.383	0.55
17:40	170	151.3	34.7	38.4	37.0	40.9	311.8	4	124.58	124.53	124.36	4.04	0.221	0.047	0.174	154.40	0.214	0.557	0.771
17:50	180	151.4	34.7	38.7	37.2	40.8	353.5	5	124.47	124.42	124.23	4.17	0.234	0.051	0.183	196.10	0.265	0.740	1.005
18:00	200	150.9	34.4	38.6	37.2	40.0	393.8	6	124.22	124.19	124.05	2.02	0.177	0.034	0.143	236.40	0.299	0.883	1.182

* 14mg oily extracts found on the tube and cap

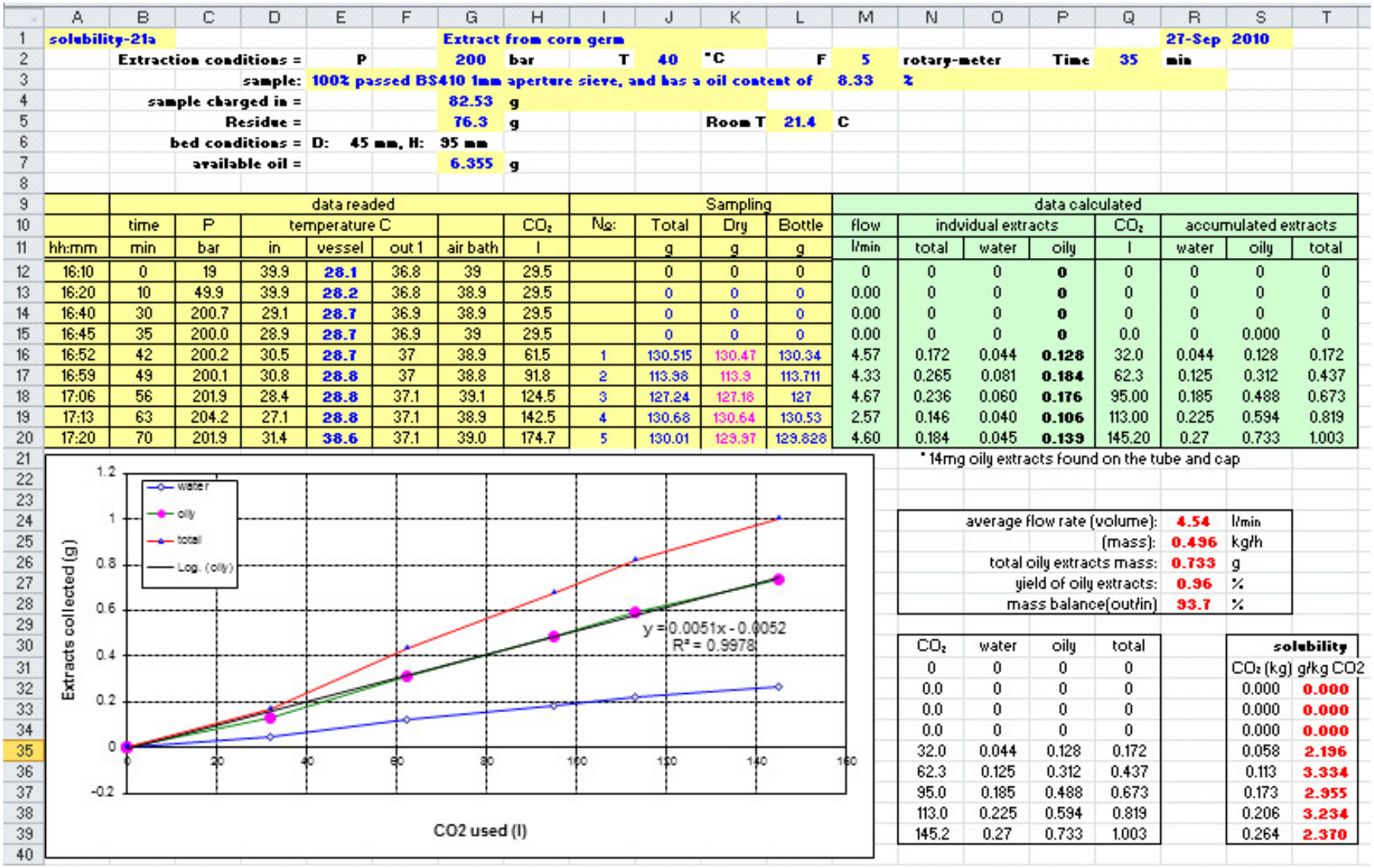


average flow rate (volume):	1.38	l/min
(mass):	0.143	kg/h
total oily extracts mass:	0.883	g
yield of oily extracts:	1.27	%
mass balance(out/in)	95.8	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
30.3	0.052	0.078	0.13
73.2	0.109	0.214	0.323
114.0	0.167	0.383	0.55
154.4	0.214	0.557	0.771
196.1	0.265	0.74	1.005
236.4	0.299	0.883	1.182

solubility	
CO_2 (kg)	g/kg CC
0.000	0.000
0.000	0.000
0.000	0.000
0.054	1.433
0.131	1.765
0.205	2.306
0.277	2.398
0.352	2.443
0.425	1.975

Plate A4: Solubility test at pressure of 150 bar and Temperature of 40 ^{0}C.



solubility-21a						Extract from corn germ												27-Sep	2010	
	Extraction conditions =			P		200	bar	T	40	°C		F	5	rotary-meter		Time	35	min		
	sample:	100% passed BS410 1mm aperture sieve, and has a oil content of											8.33	%						
	sample charged in =					82.53	g													
	Residue =					76.3	g			Room T		21.4	C							
	bed conditions =	D:	45 mm, H:	95 mm																
	available oil =					6.355	g													

	data readed							Sampling				data calculated							
	time	P	temperature C				CO_2	№:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
16:10	0	19	39.9	28.1	36.8	39	29.5		0	0	0	0	0	0	0	0	0	0	0
16:20	10	49.9	39.9	28.2	36.8	38.9	29.5		0	0	0	0.00	0	0	0	0	0	0	0
16:40	30	200.7	29.1	28.7	36.9	38.9	29.5		0	0	0	0.00	0	0	0	0	0	0	0
16:45	35	200.0	28.9	28.7	36.9	39	29.5		0	0	0	0.00	0	0	0	0.0	0	0.000	0
16:52	42	200.2	30.5	28.7	37	38.9	61.5	1	130.515	130.47	130.34	4.57	0.172	0.044	0.128	32.0	0.044	0.128	0.172
16:59	49	200.1	30.8	28.8	37	38.8	91.8	2	113.98	113.9	113.711	4.33	0.265	0.081	0.184	62.3	0.125	0.312	0.437
17:06	56	201.9	28.4	28.8	37.1	39.1	124.5	3	127.24	127.18	127	4.67	0.236	0.060	0.176	95.00	0.185	0.488	0.673
17:13	63	204.2	27.1	28.8	37.1	38.9	142.5	4	130.68	130.64	130.53	2.57	0.146	0.040	0.106	113.00	0.225	0.594	0.819
17:20	70	201.9	31.4	38.6	37.1	39.0	174.7	5	130.01	129.97	129.828	4.60	0.184	0.045	0.139	145.20	0.27	0.733	1.003

* 14mg oily extracts found on the tube and cap

average flow rate (volume):	4.54	l/min
(mass):	0.496	kg/h
total oily extracts mass:	0.733	g
yield of oily extracts:	0.96	%
mass balance(out/in)	93.7	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
32.0	0.044	0.128	0.172
62.3	0.125	0.312	0.437
95.0	0.185	0.488	0.673
113.0	0.225	0.594	0.819
145.2	0.27	0.733	1.003

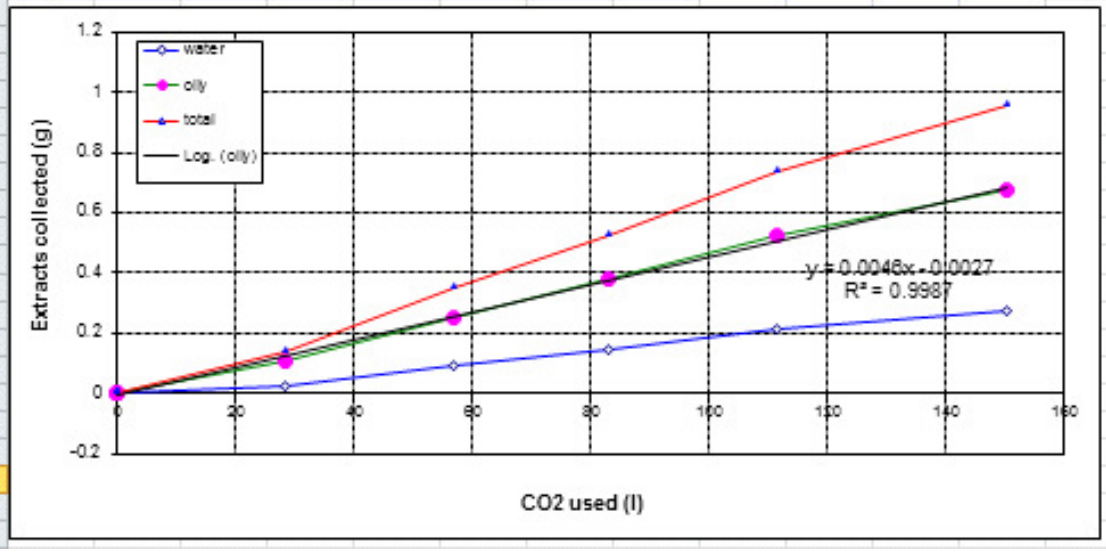
solubility	
CO_2 (kg)	g/kg CO2
0.000	0.000
0.000	0.000
0.000	0.000
0.058	2.196
0.113	3.334
0.173	2.955
0.206	3.234
0.264	2.370

Plate A5: Solubility test at pressure of 200 bar and Temperature of 40 ^{0}C.

solubility-27b					Extract from cora germ												7-Oct	2010	
Extraction conditions =		P		200	bar	T	50	°C	F	5	rotary-meter		Time	35	min			
sample:	100% passed BS410 1mm aperture sieve, and has a oil content of									8.33	%							
sample charged in =				83.05	g													
Residue =				73.87	g			Room T	22.5	C								
bed conditions =	D:	45 mm, H:	95 mm															
available oil =				6.153	g													

	data readed							Sampling				data calculated							
	time	P	temperature C				CO_2	No:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
11:20	0	101.9	47.9	48.9	45.3	49.6	1149.2		0	0	0	0	0	0	0	0	0	0	0
11:30	10	150.9	40.9	48.9	45.3	49.6	1149.2		0	0	0	0.00	0	0	0	0	0	0	0
11:40	20	200.3	39.1	49.0	45.2	49.5	1149.2		0	0	0	0.00	0	0	0	0	0	0	0
11:50	30	201.2	39.5	48.9	45.3	49.5	1149.2		0	0	0	0.00	0	0	0	0.0	0	0.000	0
11:57	37	200.9	41	48.9	45.4	49.3	1177.7	1	124.860	124.83	124.72	4.07	0.136	0.026	0.11	28.5	0.026	0.11	0.136
12:04	44	200.5	41.5	48.9	48.9	49.6	1206	2	124.42	124.36	124.210	4.04	0.212	0.067	0.145	56.8	0.093	0.255	0.348
12:11	51	201.5	41.8	48.9	45.5	49.6	1232.5	3	124.53	124.48	124.35	3.79	0.179	0.055	0.124	83.30	0.148	0.379	0.527
12:18	58	201.6	42.1	49.0	45.5	49.3	1261	4	123.23	123.16	123.02	4.07	0.210	0.064	0.146	111.80	0.212	0.525	0.737
12;25	65	201.1	42.2	48.9	45.5	49.3	1299.9	5	124.45	124.39	124.234	5.56	0.218	0.066	0.152	150.70	0.278	0.677	0.955

* 14mg oily extracts found on the tube and cap



average flow rate (volume):	5.58	l/min
(mass):	0.608	kg/h
total oily extracts mass:	0.677	g
yield of oily extracts:	0.92	%
mass balance(out/in)	90.1	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
28.5	0.026	0.11	0.136
56.8	0.093	0.255	0.348
83.3	0.148	0.379	0.527
111.8	0.212	0.525	0.737
150.7	0.278	0.677	0.955

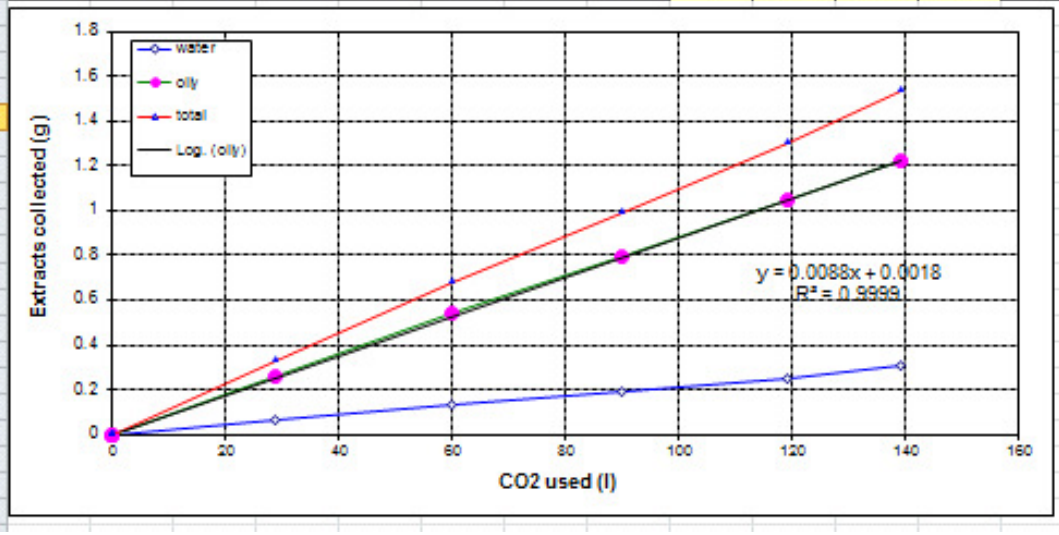
solubility	
CO_2 (kg)	g/kg CO2
0.000	0.000
0.000	0.000
0.000	0.000
0.052	2.127
0.103	2.824
0.151	2.579
0.203	2.823
0.273	2.154

Plate A6: Solubility test at pressure of 200 bar and Temperature of 50 ^{0}C.

1	solubility-26a						Extract from corn germ												5-Oct	2010
2		Extraction conditions =		P			250	bar	T	40	°C	F	5	rotary-meter		Time	33	min		
3			sample:	100% passed BS410 1mm aperture sieve, and has a oil content of									8.33	%						
4			sample charged in =				78.38	g												
5			Residue =				70.280	g			Room T	21.4	C							
6			bed conditions =	D:	45 mm, H:	95 mm														
7			available oil =				5.854	g												

		data readed							Sampling				data calculated							
10		time	P	temperature C				CO_2	No:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
11	hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
12	9:30	0	0	39.5	35.1	36.4	38.9	651		0	0	0	0	0	0	0	0	0	0	0
13	9:55	25	52.6	39.3	35.7	36.5	38.8	651		0	0	0	0.00	0	0	0	0	0	0	0
14	10:10	40	228.9	31	36.3	336.2	38.9	651		0	0	0	0.00	0	0	0	0	0	0	0
15	10:18	48	249.9	28.1	26.3	35.6	39	651		0	0	0	0.00	0	0	0	0.0	0	0.000	0
16	10:25	55	250.1	29.1	36.5	34.9	38.9	679.9	1	130.671	130.61	130.35	4.13	0.323	0.066	0.257	28.9	0.066	0.257	0.323
17	10:32	62	250.9	29.1	36.7	35	38.1	711	2	114.07	114	113.714	4.44	0.352	0.069	0.283	60.0	0.135	0.54	0.675
18	10:39	69	250.0	29.2	36.8	35.1	38.7	741	3	127.32	127.26	127	4.29	0.315	0.057	0.258	90.00	0.192	0.798	0.99
19	10:46	76	250.4	29.4	37.0	35.4	38.7	770.5	4	130.85	130.79	130.53	4.21	0.315	0.061	0.254	119.50	0.253	1.052	1.305
20	10:51	81	246.9	29.7	36.9	35.3	38.6	790.2	5	126.75	126.69	126.517	3.94	0.228	0.058	0.17	139.20	0.311	1.222	1.533

* 14mg oily extracts found on the tube and cap



average flow rate (volume):	3.24	l/min
(mass):	0.354	kg/h
total oily extracts mass:	1.222	g
yield of oily extracts:	1.74	%
mass balance(out/in)	91.6	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
28.9	0.066	0.257	0.323
60.0	0.135	0.54	0.675
90.0	0.192	0.798	0.99
119.5	0.253	1.052	1.305
139.2	0.311	1.222	1.533

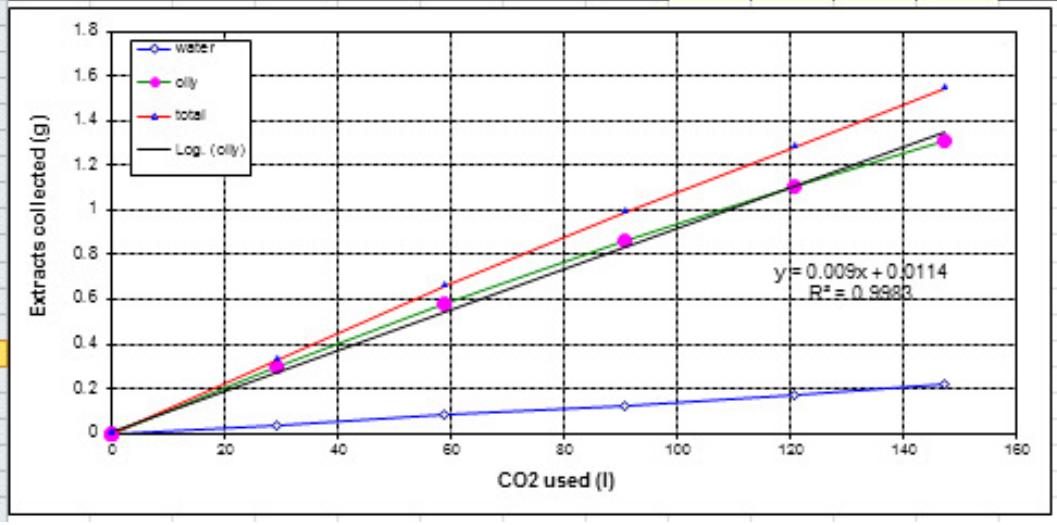
solubility	
CO_2 (kg)	g/kg CO2
0.000	0.000
0.000	0.000
0.000	0.000
0.053	4.883
0.109	4.997
0.164	4.722
0.218	4.728
0.254	4.738

Plate A7: Solubility test at pressure of 250 bar and Temperature of 40 ^{0}C.

solubility-23b					Extract from corn germ											####	2010		
	Extraction conditions =		P		300	bar	T	40	°C	F	5	rotary-meter		Time	31	min		
	sample:	100% passed BS410 1mm aperture sieve, and has a oil content of									8.33	%							
	sample charged in =				81.16	g													
	Residue =				73.7	g			Room T	23.2	C								
	bed conditions =	D:	45 mm, H:	95 mm															
	available oil =				6.140	g													

	data readed								Sampling				data calculated							
	time	P	temperature C				CO_2		Total	Dry	Bottle	flow	indvidual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l	№	g	g	g	l/min	total	water	oily	l	water	oily	total
18:30	0	200.1	30.7	37.7	36.3	38.9	8.5		0	0	0	0	0	0	0	0	0	0	0
18:50	20	300.3	25.9	27.9	27.9	28.9	8.5		0	0	0	0.00	0	0	0	0	0	0	0
18:51	21	300.3	25.9	27.9	27.9	28.9	8.5		0	0	0	0.00	0	0	0	0	0	0	0
18:53	23	299.2	26.6	37.8	36.8	38.7	8.5		0	0	0	0.00	0	0	0	0.0	0	0.000	0
19:00	30	299	27.8	39.9	36.7	39	37.5	1	125.051	125.01	124.72	4.14	0.331	0.037	0.294	29.0	0.037	0.294	0.331
19:07	37	299.1	28.0	37.9	36.7	38.7	67.5	2	124.54	124.43	124.207	4.29	0.330	0.046	0.284	59.0	0.083	0.578	0.661
19:14	40	299.6	28.0	27.9	36.7	38.8	99.5	3	124.68	124.63	124.35	10.67	0.327	0.043	0.284	91.00	0.126	0.862	0.988
19:21	47	299.1	28.1	38.0	36.8	38.9	129.5	4	123.3	123.26	123.02	4.29	0.289	0.049	0.24	121.00	0.175	1.102	1.277
19:28	54	299.1	28	37.9	36.8	38.7	156.0	5	124.5	124.45	124.233	3.79	0.262	0.049	0.213	147.50	0.224	1.315	1.539

* 14mg oily extracts found on the tube and cap



average flow rate (volume):	9.22	l/min
(mass):	1.001	kg/h
total oily extracts mass:	1.315	g
yield of oily extracts:	1.78	%
mass balance(out/in)	92.7	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
29.0	0.037	0.294	0.331
59.0	0.083	0.578	0.661
91.0	0.126	0.862	0.988
121.0	0.175	1.102	1.277
147.5	0.224	1.315	1.539

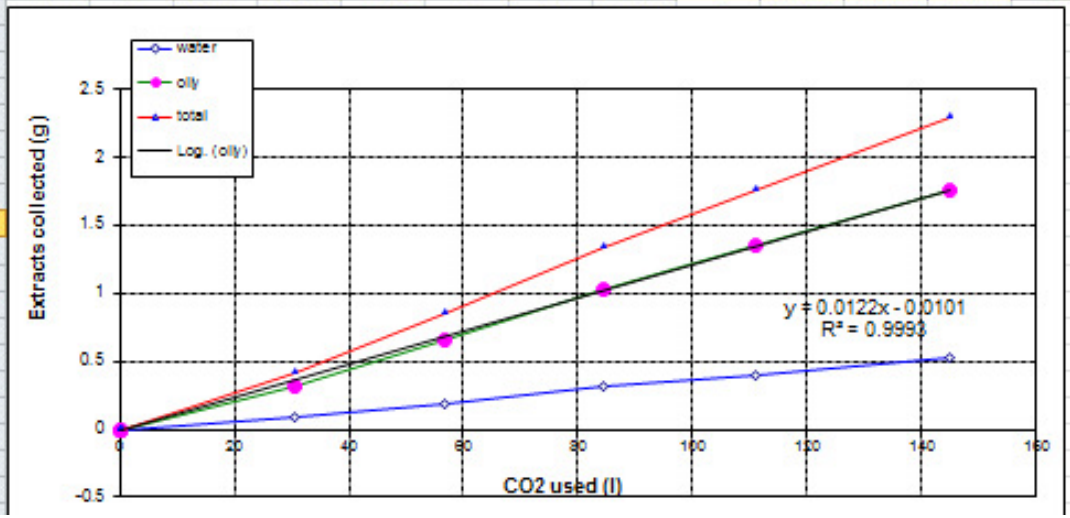
solubility	
CO_2 (kg)	g/kg CO2
0.000	0.000
0.000	0.000
0.000	0.000
0.052	5.601
0.107	5.230
0.165	4.903
0.219	4.420
0.267	4.440

Plate A8: Solubility test at pressure of 300 bar and Temperature of 40 ^{0}C.

1	solubility-27d					Extract from corn germ											7-Oct	2010	
2		Extraction conditions =		P		300	bar	T	50	°C	F	5	rotary-meter		Time	35	min		
3			sample:	100% passed BS410 1mm aperture sieve, and has a oil content of								8.33	%						
4			sample charged in =			83.05	g												
5			Residue =			73.87	g			Room T	25.2	C							
6			bed conditions =	D:	45 mm, H:	95 mm													
7			available oil =			6.153	g												

	data readed							Sampling				data calculated							
	time	P	temperature C				CO_2	No:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
15:10	0	106.5	47	48.3	43.3	49.6	436.6		0	0	0	0	0	0	0	0	0	0	0
15:25	15	153.5	46.6	48.7	44.8	49.5	436.6		0	0	0	0.00	0	0	0	0	0	0	0
15:30	20	210.4	42.7	48.6	45.4	49.6	436.6		0	0	0	0.00	0	0	0	0	0	0	0
15:40	30	300.1	39	48.6	45.6	49.6	436.6		0	0	0	0.00	0	0	0	0.0	0	0.000	0
15:47	37	301.1	40.1	48.6	45.5	49.5	467.0	1	125.134	125.05	124.73	4.34	0.409	0.085	0.324	30.4	0.085	0.324	0.409
15:54	44	302.4	41.3	48.6	45.5	49.3	493.5	2	124.65	124.55	124.211	3.79	0.440	0.103	0.337	56.9	0.188	0.661	0.849
16:01	51	301.2	42.1	48.6	45.6	49.4	521	3	124.84	124.72	124.35	3.93	0.484	0.123	0.361	84.40	0.311	1.022	1.333
16:08	58	302.1	42.2	48.6	45.6	49.4	547.5	4	123.44	123.35	123.02	3.79	0.426	0.091	0.335	110.90	0.402	1.357	1.759
16:15	65	301.6	42.9	48.6	45.6	49.6	581.5	5	124.76	124.65	124.235	4.86	0.528	0.118	0.41	144.90	0.52	1.767	2.287

* 14mg oily extracts found on the tube and cap



average flow rate (volume):	5.37	l/min
(mass):	0.579	kg/h
total oily extracts mass:	1.767	g
yield of oily extracts:	2.39	%
mass balance(out/in)	91.7	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
30.4	0.085	0.324	0.409
56.9	0.188	0.661	0.849
84.4	0.311	1.022	1.333
110.9	0.402	1.357	1.759
144.9	0.52	1.767	2.287

solubility	
CO_2 (kg)	g/kg CO2
0.000	0.000
0.000	0.000
0.000	0.000
0.055	5.928
0.102	7.073
0.152	7.301
0.199	7.031
0.261	6.707

Plate A9: Solubility test at pressure of 300 bar and Temperature of 50 ^{0}C

APPENDIX B
APPARENT SOLUBILITY CALCULATIONS

Volumetric flow rate of CO_2

$$F_{CO_2} = \frac{V_f - V_i}{t_f - t_i} \quad (1)$$

Where V_i = volume of CO_2 at time t_i

V_f = volume of CO_2 at time t_f

For example at 250 bar pressure and 40^{o}C,

$$F_{CO_2} = \frac{674.6 - 611.0}{132 - 112} = 3.18 \text{ L/min}$$

For individual extracts,

Total mass of extract (g) = mass of bottle + extract – mass of empty bottle (2)

Mass of water (g) = (mass of bottle + extract + water) – (mass of bottle + extract) (3)

Mass of oil (g) = (mass of bottle + extract) – (mass of empty bottle) (4)

For accumulated extracts,

Accumulated mass of oil (g) =

Total mass of last extracts + mass of current oil extract (5)

Accumulated volume of CO_2 used =

Last total volume of CO_2 used – initial volume of CO_2 recorded (6)

Average volumetric flow rate of CO_2,

$$F_{CO_2}(avg) = \frac{Total\ accumulated\ volume\ of\ CO2\ used}{t_f - t_i} \quad (7)$$

For example at 250 bar pressure and 40°C,

$$F_{CO_2}(avg) = \frac{977.6}{392-112} = 3.49 \text{ L/min}$$

Mass flowrate of CO_2,

$$Qco_2 = \frac{MM_{CO2} \times F_{CO2} \times 60}{1000\ RT} \quad (8)$$

For example at 250 bar pressure and 40°C in which

MM_{CO_2} = Molar mass of CO_2= 44.02

F_{CO_2} (avg) = 3.49 L/min

R = Universal Gas Constant

T = Room temperature = 22.5 ^{o}C

$$Qco_2 = \frac{44.02 \times 3.49 \times 60}{1000 \times 0.08206 \times (22.5 + 273.15)} = 0.380 \text{ kg/h}$$

Oil Yield

$$\text{Oil yield} = \frac{total\ oily\ extract}{Mass\ of\ sample\ charged\ in} \times 100\ \% \quad (9)$$

Mass Balance

$$\text{Mass balance} = \frac{mass\ of\ residue + accumulated\ mass\ of\ extract}{Mass\ of\ sample\ charged\ in} \times 100\% \quad (10)$$

Apparent solublity of oil in CO_2

$$\text{Apparent solublity (g/kg CO2)} = \frac{final\ mass\ of\ oil - initial\ mass\ of\ oil}{final\ mass\ of\ CO2\ used - initial\ mass\ of\ CO2\ used} \quad (11)$$

APPENDIX C
SFE DATA

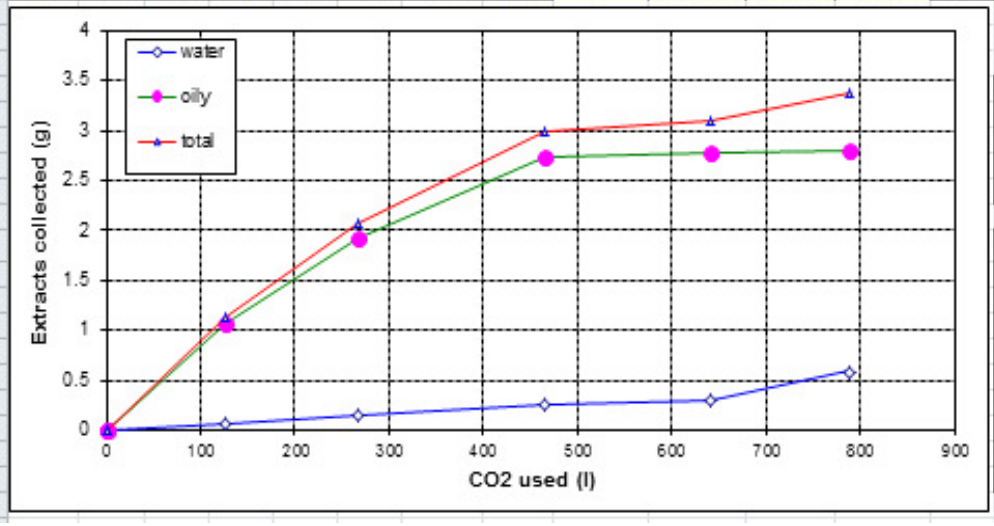
The data for the supercritical fluid extraction of the corn germ is given below:

Extraction-1a — **Extract from corn germ** — **2011**

Extraction conditions =		**P**	**250**	**bar**	**T**	**40**	**°C**	**F**		**rotary-meter**	**Time**	**210**	**min**						
sample:	**100% passed BS410 1mm aperture sieve, and has a oil content**								**16.4**	**%**									
sample charged in =			**25.000**	**g**															
Residue =			**19.16**	**g**					**Room T:**		**C**								
bed conditions =	**D: 45 mm, H: 95 mm**																		
available oil =			**4.093**	**g**															

	data readed								Sampling				data calculated							
	time	P	temperature C				CO_2	No:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
11:50	0	52.3	26.1	**25.1**	26.7	30.2	576.5		0	0	0	0	0	0	**0**	0	0	0	0
12:00	10	95.3	35.4	**30.1**	31.7	37.9	576.5		0	0	0	0.00	0	0	**0**	0	0	0	0
12:10	20	166.3	38	**31.7**	34.3	39.9	576.5		0	0	0	0.00	0	0	**0**	0	0	0	0
12:30	40	255.0	39.2	**34.8**	37.2	41.5	576.5		0	0	0	0.00	0	0	**0**	0.0	0	0.000	0
12:50	60	254.1	41.7	**37.9**	36.2	43.7	702.7	1	131.465	131.409	130.335	6.31	1.130	0.056	**1.07**	126.2	0.056	1.074	1.13
13:40	110	254.4	41.2	**38.7**	37	42.6	844.5	2	114.648	114.549	113.702	2.84	0.946	0.099	**0.85**	268.0	0.155	1.921	2.076
14:40	170	255.4	39.4	**39.0**	37.5	40.7	1043	3	127.91	127.8	126.994	3.30	0.916	0.110	**0.81**	466.00	0.265	2.727	2.992
15:20	210	255.6	39.3	**39.4**	37.9	40.9	1218	4	130.62	130.58	130.523	4.38	0.097	0.040	**0.06**	641.00	0.305	2.784	3.089
16:00	250	256.2	39.4	**39.6**	38.1	40.9	1364.5	5	126.806	126.524	126.509	3.68	0.297	0.282	**0.02**	788.00	0.587	2.799	3.386

* 14mg oily extracts found on the tube and cap



average flow rate (volume):	**3.72**	l/min
(mass):	**0.438**	kg/h
total oily extracts mass:	**2.799**	g
yield of oily extracts:	**14.61**	%
mass balance(out/in)	**90.2**	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
126.2	0.056	1.074	1.13
268.0	0.155	1.921	2.076
466.0	0.265	2.727	2.992
641.0	0.305	2.784	3.089
788.0	0.587	2.799	3.386

solubility	
CO_2 (kg)	g/kg CO2
0.000	**0.000**
0.000	**0.000**
0.000	**0.000**
0.248	**4.333**
0.526	**3.042**
0.915	**2.073**
1.259	**0.166**
1.548	**0.052**

Plate C1: Supercritical Fluid Extraction of un-pretreated UK sample 2 at 250 bar and 40 ^{0}C

Extraction 2a — **Extract from corn germ** — **28-Feb 2011**

Extraction conditions = P **250** bar T **40** °C F **4** rotary-meter **Time 280** min

sample: 100% passed BS410 1mm aperture sieve, and has a oil content **16.4** %

About 7.0 grams sample was pressed in a 35mm die to 1 ton for 30 seconds to make a pellet and re-smashed into powder by using a pestle and mortar bef

sample charged in = **25** g

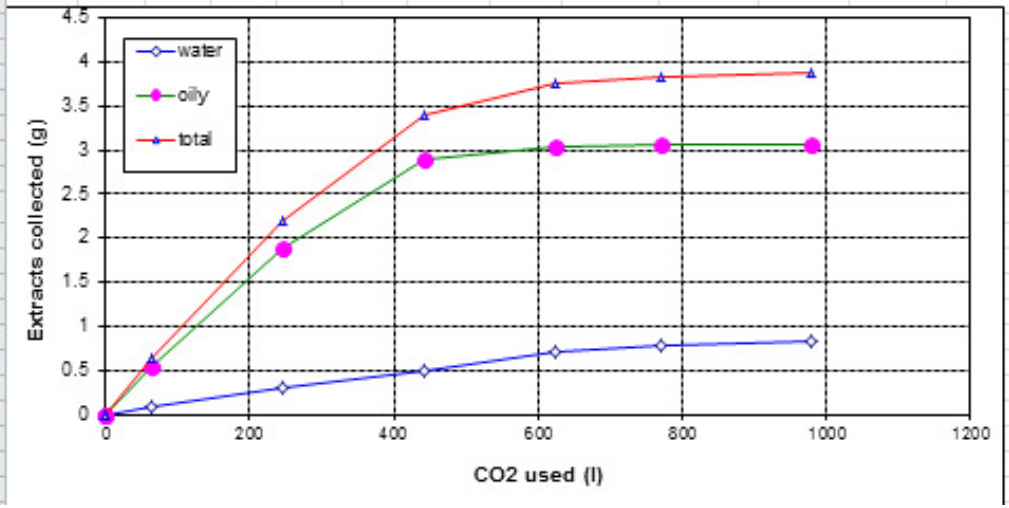
Residue = **18.95** g — **Room T** **22.5** C

bed conditions = D: 45 mm, H: 95 mm — **Chiller T** **-3.0** C

available oil = **4.093** g

	data readed							Sampling				data calculated							
	time	P	temperature C				CO_2	No:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
11:30	0	0	22.1	**20.9**	23.3	26.5	611.0		0	0	0	0	0	0	**0**	0	0	0	0
13:00	90	57.7	44.3	**38.2**	40.4	43.6	611.0		0	0	0	0.00	0	0	**0**	0	0	0	0
13:10	100	249.3	42.3	**37.4**	37.1	39.8	611.0		0	0	0	0.00	0	0	**0**	0	0	0	0
13:22	112	256.7	38.3	**36.8**	36.8	39.8	611.0		0	0	0	0.00	0	0	**0**	0.0	0	0.000	0
13:42	132	253.0	38.5	**37.5**	36.0	39.9	674.6	1	130.973	130.886	130.338	3.18	0.635	0.087	**0.55**	63.6	0.087	0.548	0.635
14:32	182	252.4	38.2	**38.2**	36.8	39.8	856.5	2	115.258	115.037	113.701	3.64	1.557	0.221	**1.34**	245.5	0.308	1.884	2.192
15:32	242	251.8	38.7	**38.5**	37.4	39.9	1055	3	128.208	128.013	126.993	3.30	1.215	0.195	**1.02**	443.50	0.503	2.904	3.407
16:22	292	250.9	38.9	**38.8**	37.7	39.9	1236	4	130.872	130.659	130.524	3.62	0.348	0.213	**0.13**	624.50	0.716	3.039	3.755
17:02	332	250.4	38.9	**38.9**	37.8	40.0	1381.5	5	126.571	126.512	126.498	3.65	0.073	0.059	**0.01**	770.50	0.775	3.053	3.828
18:02	392	250.2	38.7	**38.8**	37.9	39.9	1588.6	6	129.869	129.822	129.815	3.45	0.054	0.047	**0.01**	977.60	0.822	3.060	3.882

* 14mg oily extracts found on the tube and cap



average flow rate (volume):	**2.62**	l/min
(mass):	**0.309**	kg/h
total oily extracts mass:	**3.060**	g
yield of oily extracts:	**12.24**	%
mass balance(out/in)	**91.1**	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
63.6	0.087	0.548	0.635
245.5	0.308	1.884	2.192
443.5	0.503	2.904	3.407
624.5	0.716	3.039	3.755
770.5	0.775	3.053	3.828
977.6	0.822	3.06	3.882

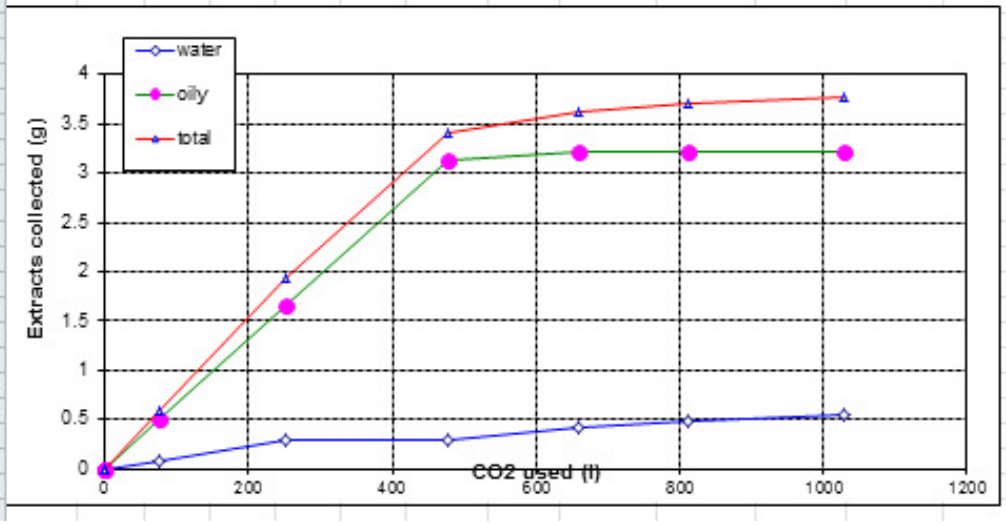
solubility	
CO_2 (kg)	g/kg CO2
0.000	**0.000**
0.000	**0.000**
0.000	**0.000**
0.115	**4.749**
0.445	**4.048**
0.805	**2.839**
1.133	**0.411**
1.398	**0.053**
1.774	**0.019**

Plate C2: Supercritical Fluid Extraction of 1 ton pretreated UK sample 2 at 250 bar and 40 ^{0}C

Extraction 2b						**Extract from corn germ**												**2-Mar**	**2011**	
Extraction conditions =			**P**			**250**	**bar**		**T**	**40**	**°C**		**F**	**4**	**rotarg-meter**		**Time**	**270**	**min**	
sample:	**100% passed BS410 1mm aperture sieve, and has a oil content**													**16.4**	**%**					
About 7.0 grams sample was pressed in a 35mm die to 5 tons for 30 seconds to make a pellet and re-smashed into powder by using a pestle and mortar b																				
sample charged in =						**25**	**g**													
Residue =							**g**				**Room T**	**23.4**	**C**							
bed conditions =	**D:**	**45 mm,**	**H:**	**95 mm**							**Chiller T**	**-3.1**	**C**							
available oil =						**4.093**	**g**													

	data readed							Sampling				data calculated							
	time	P	temperature C				CO_2	No:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
14:00	0	0.0	43.9	**37.2**	40.6	43.6	588.5		0	0	0	0	0	0	**0**	0	0	0	0
14:10	10	57.1	44.2	**37.8**	40.6	43.6	588.5		0	0	0	0.00	0	0	**0**	0	0	0	0
14:26	26	250.0	44.3	**38.1**	38.4	43.5	588.5		0	0	0	0.00	0	0	**0**	0	0	0	0
14:36	36	251.6	39.5	**397.1**	37.1	40.7	588.5		0	0	0	0.00	0	0	**0**	0.0	0	0.000	0
14:56	56	252.0	40.2	**38.0**	38.0	40.7	664.5	1	130.929	130.844	130.335	3.80	0.594	0.085	**0.51**	76.0	0.085	0.509	0.594
15:46	96	250.2	39.9	**38.7**	37.1	40.9	839.5	2	115.051	114.85	113.700	4.38	1.351	0.201	**1.15**	251.0	0.286	1.659	1.945
16:46	156	250.1	39.0	**38.8**	37.3	39.8	1066.5	3	128.458	128.458	126.992	3.78	1.466	0.000	**1.47**	478.00	0.286	3.125	3.411
17:36	206	250.0	39.0	**38.9**	37.4	39.8	1246.5	4	130.738	130.6	130.52	3.60	0.218	0.138	**0.08**	658.00	0.424	3.205	3.629
18:16	246	250.3	**38.9**	**38.9**	37.4	39.9	1401.5	5	126.563	126.511	126.497	3.88	0.066	0.052	**0.01**	813.00	0.476	3.219	3.695
19:16	306	150.0	38.9	**38.7**	37.1	39.7	1616.6	6	129.883	129.812	129.812	3.59	0.071	0.071	**0**	1028.10	0.547	3.219	3.766

* 14mg oily extracts found on the tube and cap

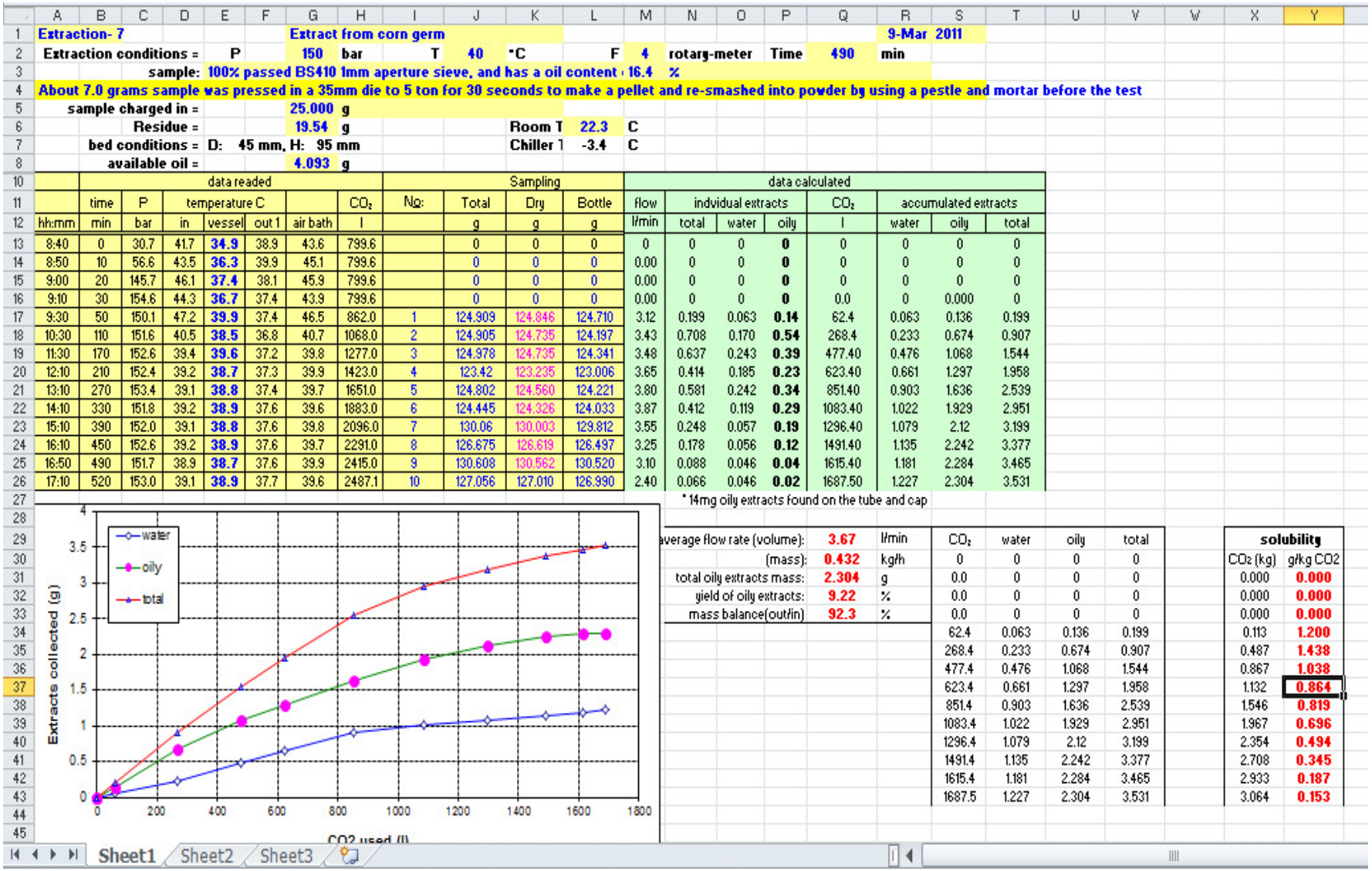


average flow rate (volume):	**3.91**	l/min
(mass):	**0.461**	kg/h
total oily extracts mass:	**3.219**	g
yield of oily extracts:	**12.88**	%
mass balance(out/in)	**14.8**	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
76.0	0.085	0.509	0.594
251.0	0.286	1.659	1.945
478.0	0.286	3.125	3.411
658.0	0.424	3.205	3.629
813.0	0.476	3.219	3.695
1028.1	0.547	3.219	3.766

solubility	
CO_2 (kg)	g/kg CO2
0.000	**0.000**
0.000	**0.000**
0.000	**0.000**
0.137	**3.702**
0.454	**3.633**
0.865	**3.570**
1.190	**0.246**
1.471	**0.050**
1.860	**0.000**

Plate C3: Supercritical Fluid Extraction of 5 ton pretreated UK sample 2 at 250 bar and 40 ^{0}C



Extraction- 7						Extract from corn germ											9-Mar	2011
Extraction conditions =		P		150	bar	T	40	°C		F	4	rotary-meter	Time	490	min			
sample:	100% passed BS410 1mm aperture sieve, and has a oil content =										16.4	%						
About 7.0 grams sample was pressed in a 35mm die to 5 ton for 30 seconds to make a pellet and re-smashed into powder by using a pestle and mortar before the test																		
sample charged in =				25.000	g													
Residue =				19.54	g			Room T	22.3	C								
bed conditions =	D:	45 mm,	H:	95 mm				Chiller T	-3.4	C								
available oil =				4.093	g													

	data readed							Sampling				data calculated							
	time	P	temperature C				CO_2	No:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
8:40	0	30.7	41.7	34.9	38.9	43.6	799.6		0	0	0	0	0	0	0	0	0	0	0
8:50	10	56.6	43.5	36.3	39.9	45.1	799.6		0	0	0	0.00	0	0	0	0	0	0	0
9:00	20	145.7	46.1	37.4	38.1	45.9	799.6		0	0	0	0.00	0	0	0	0	0	0	0
9:10	30	154.6	44.3	36.7	37.4	43.9	799.6		0	0	0	0.00	0	0	0	0.0	0	0.000	0
9:30	50	150.1	47.2	39.9	37.4	46.5	862.0	1	124.909	124.846	124.710	3.12	0.199	0.063	0.14	62.4	0.063	0.136	0.199
10:30	110	151.6	40.5	38.5	36.8	40.7	1068.0	2	124.905	124.735	124.197	3.43	0.708	0.170	0.54	268.4	0.233	0.674	0.907
11:30	170	152.6	39.4	39.6	37.2	39.8	1277.0	3	124.978	124.735	124.341	3.48	0.637	0.243	0.39	477.40	0.476	1.068	1.544
12:10	210	152.4	39.2	38.7	37.3	39.9	1423.0	4	123.42	123.235	123.006	3.65	0.414	0.185	0.23	623.40	0.661	1.297	1.958
13:10	270	153.4	39.1	38.8	37.4	39.7	1651.0	5	124.802	124.560	124.221	3.80	0.581	0.242	0.34	851.40	0.903	1.636	2.539
14:10	330	151.8	39.2	38.9	37.6	39.6	1883.0	6	124.445	124.326	124.033	3.87	0.412	0.119	0.29	1083.40	1.022	1.929	2.951
15:10	390	152.0	39.1	38.8	37.6	39.8	2096.0	7	130.06	130.003	129.812	3.55	0.248	0.057	0.19	1296.40	1.079	2.12	3.199
16:10	450	152.6	39.2	38.9	37.6	39.7	2291.0	8	126.675	126.619	126.497	3.25	0.178	0.056	0.12	1491.40	1.135	2.242	3.377
16:50	490	151.7	38.9	38.7	37.6	39.9	2415.0	9	130.608	130.562	130.520	3.10	0.088	0.046	0.04	1615.40	1.181	2.284	3.465
17:10	520	153.0	39.1	38.9	37.7	39.6	2487.1	10	127.056	127.010	126.990	2.40	0.066	0.046	0.02	1687.50	1.227	2.304	3.531

* 14mg oily extracts found on the tube and cap

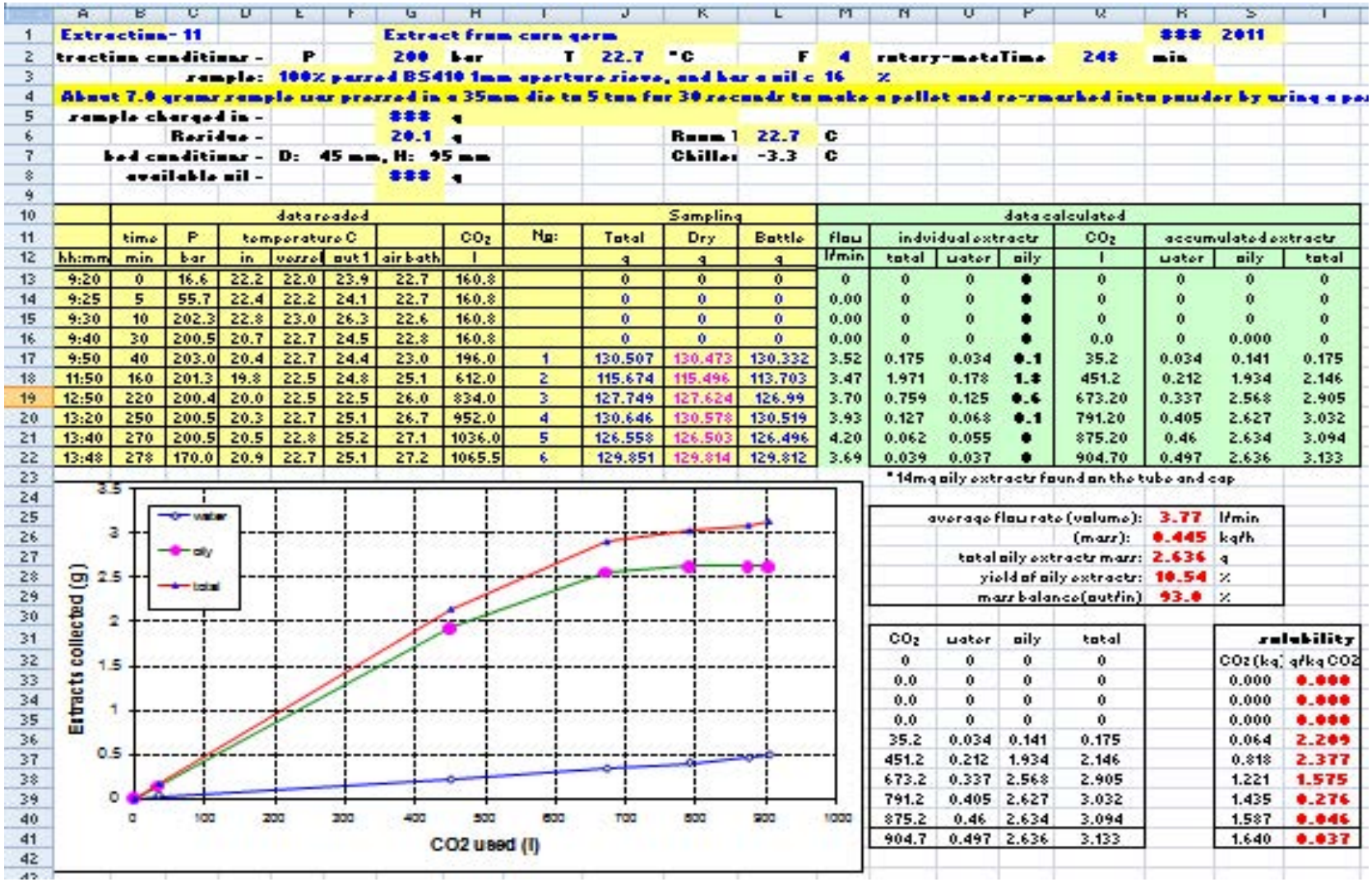
average flow rate (volume):	3.67	l/min
(mass):	0.432	kg/h
total oily extracts mass:	2.304	g
yield of oily extracts:	9.22	%
mass balance(out/in)	92.3	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
62.4	0.063	0.136	0.199
268.4	0.233	0.674	0.907
477.4	0.476	1.068	1.544
623.4	0.661	1.297	1.958
851.4	0.903	1.636	2.539
1083.4	1.022	1.929	2.951
1296.4	1.079	2.12	3.199
1491.4	1.135	2.242	3.377
1615.4	1.181	2.284	3.465
1687.5	1.227	2.304	3.531

solubility	
CO_2 (kg)	g/kg CO2
0.000	0.000
0.000	0.000
0.000	0.000
0.113	1.200
0.487	1.438
0.867	1.038
1.132	0.864
1.546	0.819
1.967	0.696
2.354	0.494
2.708	0.345
2.933	0.187
3.064	0.153

Sheet1 Sheet2 Sheet3

Plate C4: Supercritical Fluid Extraction of 5 ton pretreated UK sample 2 at 150 bar and 40 ^{0}C



Extraction- 11 — Extract from corn germ — ### 2011

extraction conditions - P 200 bar T 22.7 °C F 4 rotary-meter Time 240 min

sample: 100% passed BS410 1mm aperture sieve, and bar & oil c 16 %

About 7.0 grams sample was pressed in a 35mm die to 5 tons for 30 seconds to make a pellet and re-smashed into powder by using a pe

sample charged in - ### g

Residue - 20.1 g — Room T 22.7 C

bed conditions - D: 45 mm, H: 95 mm — Chiller -3.3 C

available oil - ### g

	data readed							Sampling				data calculated							
	time	P	temperature C				CO_2	No:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
9:20	0	16.6	22.2	22.0	23.9	22.7	160.8		0	0	0	0	0	0	●	0	0	0	0
9:25	5	55.7	22.4	22.2	24.1	22.7	160.8		0	0	0	0.00	0	0	●	0	0	0	0
9:30	10	202.3	22.8	23.0	26.3	22.6	160.8		0	0	0	0.00	0	0	●	0	0	0	0
9:40	30	200.5	20.7	22.7	24.5	22.8	160.8		0	0	0	0.00	0	0	●	0.0	0	0.000	0
9:50	40	203.0	20.4	22.7	24.4	23.0	196.0	1	130.507	130.473	130.332	3.52	0.175	0.034	●.1	35.2	0.034	0.141	0.175
11:50	160	201.3	19.8	22.5	24.8	25.1	612.0	2	115.674	115.496	113.703	3.47	1.971	0.178	1.8	451.2	0.212	1.934	2.146
12:50	220	200.4	20.0	22.5	22.5	26.0	834.0	3	127.749	127.624	126.99	3.70	0.759	0.125	●.6	673.20	0.337	2.568	2.905
13:20	250	200.5	20.3	22.7	25.1	26.7	952.0	4	130.646	130.578	130.519	3.93	0.127	0.068	●.1	791.20	0.405	2.627	3.032
13:40	270	200.5	20.5	22.8	25.2	27.1	1036.0	5	126.558	126.503	126.496	4.20	0.062	0.055	●	875.20	0.46	2.634	3.094
13:48	278	170.0	20.9	22.7	25.1	27.2	1065.5	6	129.851	129.814	129.812	3.69	0.039	0.037	●	904.70	0.497	2.636	3.133

* 14mg oily extracts found on the tube and cap

average flow rate (volume):	3.77	l/min
(mass):	0.445	kg/h
total oily extracts mass:	2.636	g
yield of oily extracts:	10.54	%
mass balance (out/in)	93.0	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
35.2	0.034	0.141	0.175
451.2	0.212	1.934	2.146
673.2	0.337	2.568	2.905
791.2	0.405	2.627	3.032
875.2	0.46	2.634	3.094
904.7	0.497	2.636	3.133

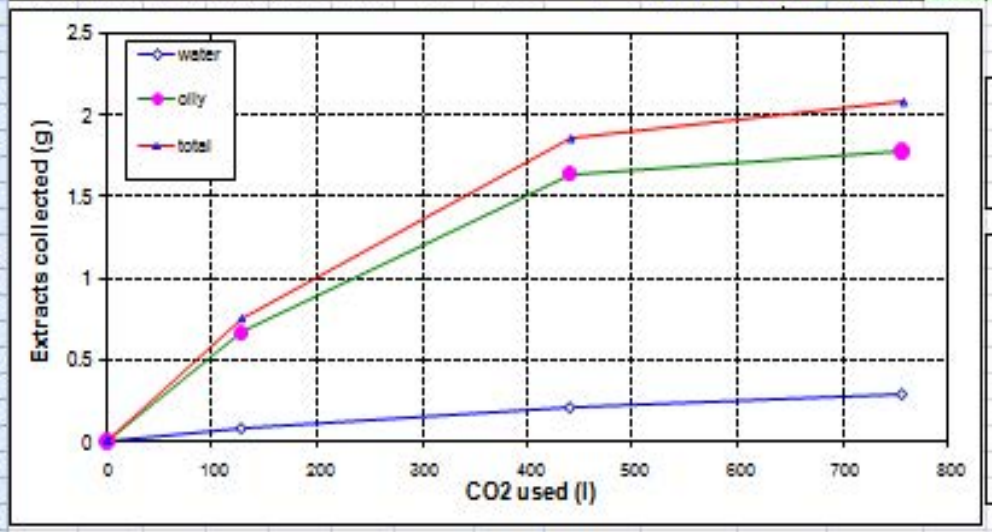
solubility	
CO2 (kg)	g/kg CO2
0.000	●.●●●
0.000	●.●●●
0.000	●.●●●
0.000	●.●●●
0.064	2.209
0.818	2.377
1.221	1.575
1.435	●.276
1.587	●.●46
1.640	●.●37

Plate C5: Supercritical Fluid Extraction of 5 ton pretreated UK sample 2 at 200 bar and RT

1	**Extraction-20**					**Extract from corn germ**												**####**	**2011**
2	**Extraction conditions =**		**P**			**200**	**bar**	**T**	**26.2**	**°C**		**F**		**rotary-meter**	**Time**	**180**	**min**		
3	**sample:**	**100% passed BS410 1mm aperture sieve, and has a oil conte**											**11**	**%**					
4	**sample charged in =**					**###**	**g**												
5	**Residue =**					**21.4**	**g**				**Room T**	**26.4**	**C**						
6	**bed conditions =**	**D:**	**45 mm, H:**	**95 mm**								**-3.5**							
7	**available oil =**					**2.750**	**g**												

		data readed							Sampling				data calculated							
10		time	P	temperature C				CO_2	No:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
11	hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
12	12:00	0	0	20.3	21.9	24.6	26.2	548		0	0	0	0	0	0	**0**	0	0	0	0
13	12:30	30	0	20.5	21.9	24.7	26.3	548		0	0	0	0.00	0	0	**0**	0	0	0	0
14	12:35	35	55.3	23	22.6	25.7	27.1	548		0	0	0	0.00	0	0	**0**	0	0	0	0
15	12:50	50	202	21	23.6	27	27.2	548		0	0	0	0.00	0	0	**0**	0.0	0	0.000	0
16	13:20	80	###	19.7	23.5	25.8	27.5	676.0	1	131.081	131	130.333	4.26	0.748	0.081	**0.7**	127.7	0.081	0.667	0.748
17	14:35	155	204	19.0	23.8	26.2	28.2	989	2	114.801	114.67	113.699	4.17	1.102	0.129	**1**	440.7	0.21	1.64	1.85
18	15:50	230	204	19.5	24.0	26.4	29.0	1305	3	127.213	127.13	126.99	4.21	0.223	0.082	**0.1**	756.70	0.292	1.781	2.073

* 14mg oily extracts found on the tube and cap



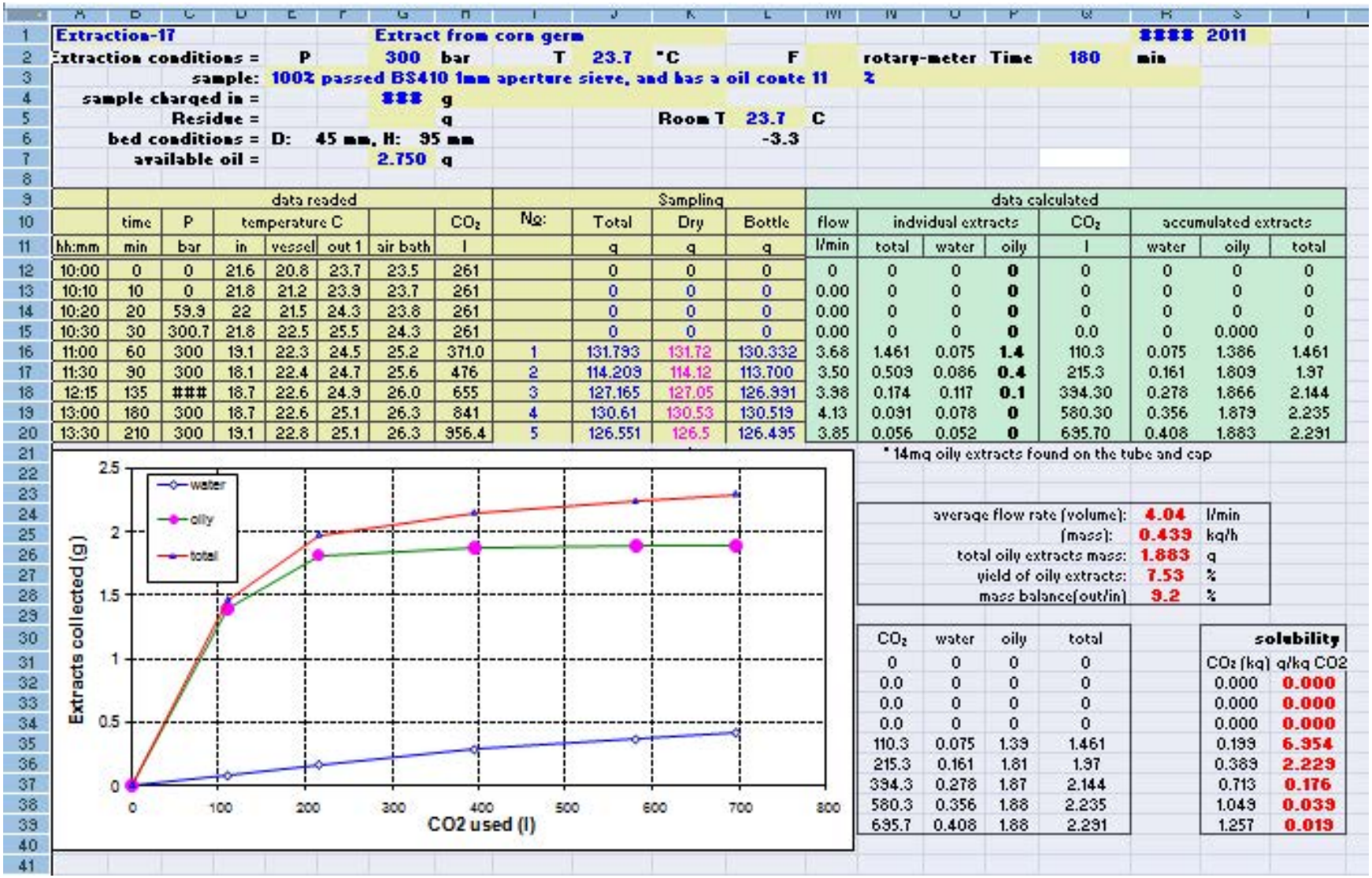
180

average flow rate (volume):	**4.20**	l/min
(mass):	**0.452**	kg/h
total oily extracts mass:	**1.781**	g
yield of oily extracts:	**7.12**	%
mass balance(out/in)	**94.1**	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
127.7	0.081	0.67	0.748
440.7	0.21	1.64	1.85
756.7	0.292	1.78	2.073

solubility	
CO_2 (kg)	g/kg CO2
0.000	**0.000**
0.000	**0.000**
0.000	**0.000**
0.229	**2.917**
0.789	**1.736**
1.355	**0.249**

Plate C6: Supercritical Fluid Extraction of NGN sample at 200 bar and Room Temperature



Extraction-17						Extract from corn germ													
Extraction conditions =			P			300	bar		T	23.7	°C		F		rotary-meter	Time	180	####	2011
sample:	100% passed BS410 1mm aperture sieve, and has a oil conte 11 %																		
sample charged in =						###	g												
Residue =							g					Room T	23.7	C					
bed conditions =	D: 45 mm, H: 95 mm												-3.3						
available oil =						2.750	g												

	data readed								Sampling				data calculated							
	time	P	temperature C				CO_2	No:	Total	Dry	Bottle	flow	individual extracts			CO_2	accumulated extracts		
hh:mm	min	bar	in	vessel	out 1	air bath	l		g	g	g	l/min	total	water	oily	l	water	oily	total
10:00	0	0	21.6	20.8	23.7	23.5	261		0	0	0	0	0	0	0	0	0	0	0
10:10	10	0	21.8	21.2	23.9	23.7	261		0	0	0	0.00	0	0	0	0	0	0	0
10:20	20	59.9	22	21.5	24.3	23.8	261		0	0	0	0.00	0	0	0	0	0	0	0
10:30	30	300.7	21.8	22.5	25.5	24.3	261		0	0	0	0.00	0	0	0	0.0	0	0.000	0
11:00	60	300	19.1	22.3	24.5	25.2	371.0	1	131.793	131.72	130.332	3.68	1.461	0.075	1.4	110.3	0.075	1.386	1.461
11:30	90	300	18.1	22.4	24.7	25.6	476	2	114.209	114.12	113.700	3.50	0.509	0.086	0.4	215.3	0.161	1.809	1.97
12:15	135	###	18.7	22.6	24.9	26.0	655	3	127.165	127.05	126.991	3.98	0.174	0.117	0.1	394.30	0.278	1.866	2.144
13:00	180	300	18.7	22.6	25.1	26.3	841	4	130.61	130.53	130.519	4.13	0.091	0.078	0	580.30	0.356	1.879	2.235
13:30	210	300	19.1	22.8	25.1	26.3	956.4	5	126.551	126.5	126.495	3.85	0.056	0.052	0	695.70	0.408	1.883	2.291

* 14mg oily extracts found on the tube and cap

average flow rate (volume):	4.04	l/min
(mass):	0.439	kg/h
total oily extracts mass:	1.883	g
yield of oily extracts:	7.53	%
mass balance(out/in)	9.2	%

CO_2	water	oily	total
0	0	0	0
0.0	0	0	0
0.0	0	0	0
0.0	0	0	0
110.3	0.075	1.39	1.461
215.3	0.161	1.81	1.97
394.3	0.278	1.87	2.144
580.3	0.356	1.88	2.235
695.7	0.408	1.88	2.291

solubility	
CO_2 (kg)	g/kg CO2
0.000	0.000
0.000	0.000
0.000	0.000
0.199	6.954
0.389	2.229
0.713	0.176
1.049	0.039
1.257	0.019

Plate C7: Supercritical Fluid Extraction of NGN sample at 300 bar and Room Temperature