EFFECT OF DIFFERENT SOLVENTS ON THE EXTRACTION OF SOYA BEAN OIL

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DECLARATION

I hereby declare that, except for references to other peoples' work which have been duly acknowledged, this report is the result of my own research work and has not, in part or whole, been presented for the award of a degree or diploma in any institution. I, however, take responsibility for any errors in this work.

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ABSTRACT

Extraction of soya bean oil is often by mechanical and solvent expression with mechanical expression efficiencies seldomly exceeding 80%, compared to over 98% achieved by solvent extraction. Solvent extraction, however, acquires its efficiency from the solvent hexane, which the government of the United States of America Clean Air Act has banned based on health and safety reasons for working staff and its explosive nature.

This research presents the findings of the use of alternate solvents (isopropyl alcohol and petroleum ether) as a replacement for hexane, conditions required to optimise maximum oil recoveries using parameters such as moisture content, duration, texture of samples and leaching rate per solid-liquid phase for each solvent and an assertion of the quality of soya oil extract through physicochemical analysis.

The results indicated that petroleum ether had higher oil recovery (fine = 95.4%, medium = 69.1%, and coarse = 55.6) than isopropyl alcohol (fine = 33%, medium = 32% and coarse = 18.3%) with texture of sample as a determinant. The physicochemical analysis of the oil indicated its high quality.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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CHAPTER ONE
INTRODUCTION

1.1 Background

There has been an increase in the world production of oilseeds over the last thirty years (Murphy, 1994); this would appear to be related to the increasing demand for oilseed products and by-products as oilseeds are primarily grown for their oil and meal. Vegetable oil is always at a higher price per ton than the cake, this is because the demand for oil is often higher than the cake.

Oils from most edible oilseeds are used in the food industry, though there is growing emphasis on industrial utilization as feedstock for several industries with about 80% of the world production of vegetable oils for human consumption. The remaining 20% utilization is between animal and chemical industries (Murphy, 1994). According to Rajagopal *et al.* (2005), bio-oils from oilseeds are used as Straight Vegetable Oil (SVO) or as biodiesel (transesterified oil) depending on type of engine and level of blend of the oil; soya bean oil is not an exception. This phenomenon has created a school of thought that it is better to use oilseeds as bio-fuel, which will lessen the competition for fossil fuels, which are not renewable. Fossil fuels are not only costly in terms of price but are also costly to the environment as they degrade land, pollute water and cause a general destabilization of the ecosystem with global warming as an end result. Furthermore, crude oil wields socio-political power that often dictates the pace of economic growth in specific locations, especially non-oil producing nations.

Nevertheless, the petroleum industry requires a greater quantity of oil to meet its demand. Demand, however, by the food industry alone is not secure for many

developing countries like Ghana that depend on imports of vegetable oil and fossil fuels.

In order to meet the required amounts needed by all industries, these fats and oils must be available in large quantities locally with an effective extraction process at an affordable cost.

The ability of a particular oilseed to fit into the growing industries depends on its utilisation potential, rate of production, availability and ease of the processing technology. Thus while some oilseeds are being largely utilised in the oil processing industries, quite a number of oilseeds are under-exploited.

Generally, oils and fats from seeds and nuts constitute an essential part of man's diet. Fats and oils, together with proteins, carbohydrates, vitamins and minerals, are the main nutrients required by the human body. Fats and oils are rich sources of energy, containing two and a half times the calories of carbohydrates (per unit weight). In addition to being a source of vitamins A, D, E and K, fats and oils also contain essential fatty acids. These essential fatty acids are not manufactured by the body and must be obtained from diets, with linoleic, oleic and linolenic acids as examples of unsaturated fatty acids (NRI, 1995).

Modern processing of vegetable oils yields valuable products such as oleo chemicals. Oleo chemicals are now largely being used in the manufacture of many industrial products, namely building auxiliaries, candles, detergents and cleaning agents, cosmetics, fire-extinguishing agents, flotation agents, food emulsifiers, insecticides,

lubricants, paints, paper, medicine and chemicals. The meal or cake is used in the formulation and preparation of livestock feeds and food additives.

The production of oil plants takes third place in the world production in terms of value, after starchy plants and fruits, and ahead of beverages and stimulants. Edible seeds and nuts noted for their oil contents include palm nut, coconut, soya bean, olive, groundnut, sunflower seed, and cottonseed, while non-edible seeds and nuts include jatropha seed, neem seed, and castor bean. Moreover, soya bean oil has strengthened its dominant role among fats and oils produced based on its quality and nutritional grade. Soya bean oil contains linoleic, oleic and linolenic acids that are found in many plant oils. Shortage of these fatty acids leads to deficiency symptoms especially in growing children and animals. Soya oil has the highest content of lecithin (1.1-3.2%) which is a surface-active compound used as an emulsifier in the food and pharmaceutical industries, and other industries (Sigmund and Gustav, 1991). The heat combustion of soya bean is 39 MJ/kg as compared to diesel fuel with 42 MJ/kg (Khan and Hanna, 1983[a]). This makes soya bean oil an effective competitor in the petroleum industry.

Among the industries that use oils and fats from oilseeds, apart from the food industry, are the beauty, pharmaceuticals, aromatherapies, building and construction, and the petroleum industry.

1.2 Problem Statement

Soya bean (*Glycine max*. L), is an important leguminous oilseed crop in the world, it is rich in protein about (40%) and oil (18-20%) depending on variety (Wolff, 1983). Global production of soya beans was 228.9 million tonnes during the year 2004-2005 (USDA, 2004) which was about 57% of the total oilseed production in the world (Soystats, 2005). Oil can be extracted from soya beans by using traditional methods of extraction (on a very small scale), mechanical expression (hydraulic and screw presses) which can be manual, semi-automated or automated, and solvent extraction (e.g. hexane, fluid carbon dioxide) or a combination of two of these methods. The efficiency of traditional methods of extracting soya bean oil is low (less than 50%), labour intensive, time consuming and possibly compromising quality and safety standards (Grace *et al*., 2008).

Mechanical pressing of oilseeds is the most common method of edible oil extraction used in the world (Mrema and McNulty, 1985). The main reason for this is that it provides a non-contaminated, protein-rich low-fat deoiled cake, an important by-product in many developing nations at a relatively low cost. However, mechanical presses do not have high extraction efficiencies, even when using hydrothermally treated oilseeds. Extraction efficiencies seldom exceed 80%, compared to over 98% achieved by the solvent extraction method (Bargale, 1997).

Solvent extraction is capable of removing virtually all of the oil available in the cells of the oilseed though the equipment required is generally too expensive to acquire and there is the inherent danger of fire explosion due to the chemicals used in the

extraction process (Singh *et al*., 1984). The process of solvent extraction, however, is faster and less expensive compared to mechanical extraction.

Conventionally, soya beans are subjected to solvent extraction because of the low oil content (about 18-20%) and relatively lower oil recoveries in mechanical expression systems. Solvent extraction, in general, implies the removal of miscellanea from an insoluble solid phase by dissolution in a liquid solvent.

In Ghana, literature on the exact quantities of oil extracted per seed conditioning and kind of solvent apart from hexane is unknown. Hexane however, is classified as being hazardous to working staff and based on this assertion has been banned by the government of the United States of America. It is therefore important to determine the extraction rates for other suitable solvents such as isopropyl alcohol and petroleum ether, which are available in Ghana at relatively cheaper prices.

1.3 Objectives of the Study

The main objective of the study was to find the effects of using Petroleum ether and Isopropyl alcohol for soya bean oil extraction.

The specific objectives of the study were:

1. To determine the optimum oil yield conditions for soya beans per solvent used, and
2. To determine the physicochemical qualities of the crude soya bean oil extract.

1.4 Importance of the Study

Increasing the extraction of oil to meet the rising demand for vegetable oils in different industries requires a suitable solvent which is readily available in the country at a relatively cheaper cost to replace hexane, which is considered hazardous, expensive and occasionally scarce based on demand and cost of petroleum. This study therefore, sought to provide a fair idea on a possible solvent replacement using isopropyl alcohol and petroleum ether for hexane in the extraction of soya bean oil giving a clear indication as to the best options and requirements for higher optimum oil recoveries from soya beans using these two solvents.

1.5 Structure of Thesis

This thesis is divided into five chapters: Chapter one states the background of the study as well as the reasons for the study. Chapter two reviews literature on soya beans, oil extraction methods, proximate composition and physicochemical properties, and characteristics of solvents used for the experiment. Chapter three describes the study sample as well as materials and methods adopted for conducting the research. Chapter four presents the results of the study on proximate composition of soya bean, extraction of soya oil, physicochemical analysis of the oil extracts and leaching effects. Chapter five draws conclusions from the study and presents recommendations for future studies.

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction

This chapter reviews previous work and studies on soya beans, vegetable oil extraction processes and solvent characteristics that are relevant to this study. The aim was to obtain information that would provide the context within which this study could be undertaken more comprehensively.

2.2 Botany, Characteristics and Importance of Soya Beans

Soya bean has been known by many names scientifically, but in 1948, Ricker and Morse presented evidence that the correct scientific name should be *Glycine max*. (L.) (Ricker and Morse, 1948) and this conclusion is generally accepted and used. Soya bean is from the family *Fabaceae*, subfamily *Papilionoideae* and genus *Glycine*. Glycine comprises about 20 species distributed in the tropics and subtropics of Asia and Australia. The genus is further divided into three subgenera; Bracteata, Glycine (perennials) and Soja (annuals). The genera soja have two species; *Glycine soja* (wild type occurring in Eastern Asia) and *Glycine max* (cultivated type). *Glycine soja* is considered the wild ancestor of *Glycine max.* The two taxa hybridise easily and may be considered a single specie with two subspecies; *Glycine max* (L) and subspecies soja (Singh and Hymowitz, 1987). *Glycine max* and *Glycine soja* have diploid chromosome numbers of 40, crosses between them are made easily and F1 hybrids are fertile. However, *Glycine soja* has a twining growth habit, small hard seed and low productivity. Subgenus Bracteata contains one specie - *Glycine wightii*; this specie is subdivided into five subspecies. They are viny perennials used as tropical forages and have diploid chromosome numbers of 22 and 44; and are not hybridized with *Glycine max* (Hinson and Hartwig, 1977).

Soya bean is an arable, annual leguminous crop of native East Asia. It is unclear when the soya bean plant reached tropical Africa, but it is likely that it was introduced during the 19th century by Chinese traders who were active along the East Coast of Africa. The soya bean plant may grow prostrate, not higher than 20 cm or even up to 2 metres in height. Pods, stems and leaves are covered with fine brown or gray hairs; leaves are trifoliolate having three leaflets per leaf and the leaflets are 6-15 cm long and 2-7 cm broad, with leaves falling before the seeds mature. The small inconspicuous self-fertile flowers are borne in the axil of the leaf and are white, pink or purple with fruits growing in pods that are in clusters of 3-5. Fruits are slightly curved and usually compressed pods of 2.5-8.0 cm (Brink and Belay, 2006). Modern crop cultivars generally reach a height of around 1 m, and take 80–120 days from sowing to harvesting. Seeds exist in colours of yellow, black, green and red with the yellow being the most common and dominant (Boumans, 1985).

At present, the United States of America is the largest producer, accounting for about 32 percent of world production, followed by Brazil and Argentina (USDA, 2008; Soystats, 2008). The yield is between 1400 and 2000 kg/ha with high yielding varieties containing about 18 percent oil and 38 percent protein. The meal after the oil extraction is even more important as it is by far the biggest source of protein used in compound animal feed and human food additives. Soya beans, when unprocessed, have an undesirable bitterness and flavour; and contain the toxic proteins hemagglutinin and antitrypsin. These substances are inactivated by toasting the beans to make the beans palatable and digestible especially for animals. Products from soya beans include soya oil, soya milk, soya sauce, soya curd (*tofu*), yoghurt, soya concentrate and feed, defatted flours and grits, isolates, textured flours, textured

concentrates (commonly used as meat extender), soya sprouts, commercial synthesis of steroid hormones and other pharmaceuticals, and industrial products such as glue, synthetic fibre and foam (Brink and Belay, 2006). Mature soya seeds are boiled and used for relishes in tropical Africa, while the immature green beans are eaten as fresh vegetables. The plants are used as feed (fodder, silage) for animals, while some farmers allow the plant residue to decompose on their fields contributing to soil fertility.

2.3 Soil and Climatic Requirements of Soya Beans

Soya bean is grown from the equator to latitude 55^{o} N or 55^{o} S at altitudes from close to sea level up to 2000 m. Cultivation is successful in climates with hot summers, with optimum growing conditions in mean temperatures of 20°C to 30°C; temperatures below 20°C and over 40°C retard growth significantly. They can grow in a wide range of soils, with optimum growth in moist alluvial soils with good organic matter content and in an equable climate without excessive rainy periods; soya beans can only withstand a little water logging conditions (Norman *et al*., 1995). Soya beans, like most legumes, perform nitrogen fixation by establishing a symbiotic relationship with the bacterium *Bradyrhizobium japonicum* (synonymy: *Rhizobium japonicum;* Jordan 1982). However, for best results an inoculum of the correct strain of bacteria should be mixed with the soya bean (or any legume) seed before planting.

2.4 Proximate Composition of Foodstuffs

According to Pomeranz and Meloan (1987), the key parameters in oilseeds are fats and moisture. Proximate determination provides information on the basic chemical composition of foods/feeds. The components of food or materials are moisture, ash, fat, protein, crude fibre and carbohydrate. These components are fundamental to the assessment of the nutritive quality and quantity of foodstuff being analysed.

2.4.1 Moisture Content

Moisture content is the measure of water in a material. According to the Food Standards Committee (1979), the moisture content of foods is of great importance for many scientific, technical and economic reasons. Moisture determination is important in many industrial applications, for example, in the evaluation of material balance or processing losses. It is important to know the optimum moisture content when processing foods. The moisture content of food gives an indication of its shelf life and nutritive value, low moisture content is a requirement for long storage life. Compounds that volatilize under the same physical conditions as water also would be included; however, these are usually negligible (Aurand *et al*., 1987).

2.4.2 Ash Content

The ash content of a foodstuff is the inorganic residue remaining after the organic matter of the material has been burnt. The ash composition is not considered the same as the mineral content as there may be some loss due to volatilisation. It is a measure of the total mineral content in the foodstuff; the ash content can provide an estimate of the quality of the food product, since high levels may indicate contamination.

2.4.3 Crude Protein

Protein is composed of a large group of nitrogenous organic compounds that are essential constituents of living cells; they consist of polymers of amino acids and are essential for growth and repair of tissues. Generally, samples that do not contain unusually high concentrations of non-protein nitrogen-containing compounds may be analysed by simply determining the percentage of nitrogen (NH_3) and making the assumption that nitrogen was released from protein during digestion. Soya bean protein provides all the essential amino acids needed to fulfil human nutritional requirements for growth, maintenance or physical stress (Robert and Nemat, 1998).

2.4.4 Crude Fat

Crude fat is the term used to refer to the crude mixture of fat-soluble materials present in a sample (Aurand *et al*., 1987). The fat content (sometimes called the ether extract, neutral fat or crude fat), which may be considered as consisting of the free lipid constituents, comprising essentially neutral fats and free fatty acids. The total lipid content of food is commonly determined by organic solvent extraction methods (Nielsen, 1994).

2.4.5 Crude Fibre

Crude fibre is a measure of the quantity of indigestible cellulose, pentosans, lignin and other similar components found in food (Aurand *et al*., 1987). Crude fibre consists of hemicelluloses, cellulose and lignin. Lignin comprises polymers of phenolic acids and hemicelluloses consist of heteropolymers of polysaccharides. Crude fibre is the insoluble and combustible organic residue after the treatment of a sample. Treatments include consecutive dissolution of the sample with light

petroleum, boiling sample with dilute sulphuric acid and boiling sample with dilute sodium hydroxide (Kirk and Sawyer, 1991). Treatment with the solvents sulphuric acid (H_2SO_4) and sodium hydroxide (NaOH) removes all the protein and carbohydrate from the sample.

2.4.6 Carbohydrate

Carbohydrate is an essential structural component of living cells and source of energy; it includes simple sugars with small molecules as well as macromolecular substances. Classification of carbohydrate is based on the number of saccharide groups they contain as monosaccharide, oligosaccharides and polysaccharides (Pomeranz and Meloan, 1987). Carbohydrates are the most abundant and widely distributed food components in the world especially among roots and tubers, and cereals and legumes.

2.5 Physicochemical Characteristics of Vegetable Oil

The analytical characteristics and fatty acid compositions of fat, besides their value for reference purposes, serve as useful guides to oil and fat analysts in the determination of the components of unknown mixtures and for checking the specifications of suppliers and products (Cocks and Rede Van, 1966). Fats and oils from vegetable and animal sources do not have fixed compositions since they are subject to the effect of parameters such as geographical location and climatic conditions during the growth period in the case of vegetable oils, especially those derived from annual plants (Meara, 1980). Changes in agronomic practices and the introduction of new cultivars in recent years have been shown to affect tree nut

compositions (Asiedu, 1989). For the purpose of this study free fatty acid, iodine value, acid value, saponification value and unsaponifiable matter were reviewed.

2.5.1 Free Fatty Acid

Free fatty acid is the percentage by weight of a specified fatty acid (e.g. % oleic acid) (Nielsen, 1994). High concentrations of free fatty acids are undesirable in crude oils because they result in large losses of the neutral oil during refining. In crude fat, free fatty acids estimate the amount of oil that will be lost during refining steps designed to remove fatty acids (Kirk and Sawyer, 1991). High levels of free fatty acids especially linolenic acids are undesirable in finished oils because they can cause off-favours and shorten the shelf life of oils. The quantity of free fatty acid in oil is an indicator of its overall quality. They may be formed through hydrolysis or in the advanced stages of oxidation. An excessive amount of free fatty acids lowers the smoke point of an oil and will cause 'popping' of the oil during cooking. High quality oils are low in free fatty acids (Overhults *et al*., 1974). In refined vegetable oils, the lower the free fatty acid the more acceptable the oil is to man in terms of palatability. Free fatty acid is frequently expressed in terms of acid value instead of percentage free fatty acids. The acid value which is defined as the number of milligrams of potassium hydroxide necessary to neutralize 1g of the sample and is often calculated by multiplying the % free fatty acid by 1.99.

According to Mecit *et al*. (2005), oleic acid, which is a monounsaturated fatty acid, reduces the risk of high blood pressure, fights cholesterol by reducing bad cholesterol, demonstrates preventive impact on cardiovascular diseases, reduces insulin needs of diabetic patients, and it has preventive power on cancer.

2.5.2 Iodine Value

Iodine value is a measure of the unsaturation of fats and oils, and is expressed in terms of the number of centigrammes of iodine absorbed per gramme of the sample (% iodine absorbed) during oxidation, which consumes the double bonds resulting in a reduction in iodine. It is an indicator for double bindings in the molecular structure, which influences the long term stability properties of the oil (important for storage). Oils having high iodine number are polyunsaturated indicating the degree of unsaturation and are desired by oil processors, while a lower iodine number is indicative of lower quality (Overhults *et al*., 1974).

The determination of the iodine value is important in the classification of fats and oils (Pomeranz and Meloan, 1987). The classification is as follows:

a) Drying fats and oils: oils with an iodine value of 130-200,
b) Semi drying fats and oils: oils with an iodine value of 100-130, and
c) Non-drying fats and oils: oils with an iodine value lower than 100.

Soya bean oil is generally classified among drying fats and oils. Oils and fats do not contain iodine as a property, but the test measures the amount of iodine which can be absorbed by the unsaturated acids in the fat. It is a measure of fat stability and resistance to oxidation (Asiedu, 1989).

2.5.3 Acid Value

Acid value is the number of milligrammes of potassium hydroxide necessary to neutralize the free acids in one gram of oil sample. The samples that contain virtually no free acids other than fatty acids, the acid value may be directly converted by means of a suitable factor to percent free fatty acids. Where vegetable oils are used as lubrication products, the acid value can affect the properties of the lubrication oil, if larger quantities reach the oil sump.

2.5.4 Saponification Value

Saponification is the chemical reaction in which an ester is heated with an alkali (especially the alkaline hydrolysis of a fat or oil to make soap). Saponification value is however, the amount of alkali necessary to saponify a definite quantity of oil sample. It is expressed, as the number of milligrammes of potassium hydroxide required to saponify one gramme of the oil sample under specified conditions. It is a measure of the average molecular weight (or chain length) of all the fatty acids present. As most mass of a fat is in the three fatty acids, it allows for comparison of the average fatty acid chain length. The smaller the saponification value, the longer the average fatty acid chain (Nielsen, 1994). In combination with acid values, saponification values are useful in providing information as to the quantity, type of glycerides and mean weight of the acids in a given sample of oil. Saponification is only of interest if the oil is for industrial purposes, as it has no nutritional significance. But due to the fact that each fat has within the limits of biological variation, a constant fatty acid composition determination of the saponification value is a reasonable means of characterising the fat (Asiedu, 1989).

2.4.5 Unsaponifiable Matter

Unsaponifiable matter indicates the sum of all components stable in bases, insoluble in water and soluble in fats, and not volatile at 100^{o}C. It consists of fat-like substances such as hydrocarbons, alcohols and sterols, and non-fatty constituents like mineral oil (Boekenoogen, 1964). Most fats and oils of normal purity contain less than two percent of unsaponifiable matter. Adulteration of fats and oils with paraffin hydrocarbons appears in the unsaponifiable matter. Thus high values of unsaponifiable matter may indicate adulteration and contamination (Kirk and Sawyer, 1991). The determination of unsaponifiable matter is based on the fact that fat is often converted into soap by the process of saponification. During this process, the solution is diluted with water and the unsaponifiable matter extracted using diethyl ether (Cocks and Rede Van, 1966).

2.6 Concept of Vegetable Oil Extraction

Vegetable oil is a group of liquid edible fats and oils that are obtained from plants and seeds, and the extraction of vegetable oil is the processing of oilseeds and plants to remove oil for human consumption and industrial purposes. Edible vegetable oils and fats are components of foodstuffs, which are composed primarily of glycerides of fatty acids obtained only from plant sources. They may contain small amounts of other lipids such as phosphatides, unsaponifiable constituents and of free fatty acids naturally present in the fat or oil (Codex Alimentarius, 1999). Methods often used for the extraction process are mechanical and solvent methods. In most cases, the process involves two or more combinations of these methods to ensure optimum oil recovery. Traditionally, oil is extracted though oil recoveries are very low and are

often for home consumption. Among the factors enhancing optimum oil yield in oilseeds are, moisture content and temperature.

2.6.1 The Role of Moisture and Temperature in Oil Extraction

Oil and water can each wet the solid components of oilseeds, though the two differ in affinity for the hydrophilic surfaces of particles. Water has a higher affinity and wets the surface of particles at a faster rate than oil due to its polarity and absorption ability. As such, the surface tension on the particles and water interface is insignificant while that of the oil interface is considerable. Research has proven that particles are selectively wetted by liquids with lower surface tension at the interface; hence water will tend to displace oil from the surface of particles. At a certain moisture content all the surface of the particles will be saturated by water and the oil will flow freely from the molecular forces. Thus, moisture increases the flow of oil through the pores of the press cake, hence reducing the amount of oil entrained in the cake and increasing the oil yield mostly in mechanical expression. High moisture content stops oil flow possibly because the structures of the finely milled particles have been altered (high aggregation). Moisture lubricates the pulp during pressing and causes a slower pressure increase and reduces oil yields (Sefah, 2006).

Temperature is increased for oilseeds after pre-treatments such as cracking, dehulling, and milling by heating, roasting and steaming of oilseeds prior to extraction and is termed thermal treatment of oilseeds. Better extraction is achieved by heating, which reduces the oil viscosity and releases oil from intact cells, and also reduces moisture in the cells. Temperature plays an active role in the seed treatment for mechanical extraction and ensures an effective solvent process by heating the

solvent which hastens the extraction process. At the right temperature and moisture content, the individual oil droplets unite to form a continuous phase and flow out maximising oil yield.

2.6.2 Traditional Extraction of Vegetable Oil

Traditionally, the commonest way of oil extraction is the water flotation process; oilseeds are thermally treated, crushed and milled into slurry (paste). With the aid of simple domestic utensils, oil is extracted by hand kneading. Water is added to the slurry and the mixture stirred and kneaded by hand until the oil separates to the top and sides of the utensils being used for the kneading. Water plays a vital role in hydrolyzing the paste, which displaces oil from hydrophilic surfaces in the slurry.

Under the traditional method, there are two ways of extracting oil; wet and water assisted extractions. This method is used, however, on a small scale, as it is labour-intensive, slow and tedious in operation compared to other methods but is assumed to produce high oil quality. In the wet extraction method, water is used in relatively large amounts to suspend the oilseeds such that the extracted oil floats on the top of the suspended oilseeds. Hot water flotation method of edible oil extraction is traditionally used in the rural areas of many developing countries. The water-assisted method involves the addition of small quantities of water to the slurry before the oil is extracted by manual kneading. The slurry is suspended in boiling water and boiled for at least 30 min with liberated oil floating on the surface. Further quantities of water are added after boiling to replace the lost water that occurred during evaporation, and to facilitate the floatation of the oil to the surface. The oil is carefully scooped from the surface of the water and boiled.

2.6.3 Mechanical Expression of Vegetable Oil

The main applications of mechanical expression are in the extraction of oils and juices. Expression is often combined with size reduction to maximise the yield of product. Components are extracted from plant parts either for direct use or for use in subsequent processing such as refining. In oil-bearing seeds, the oil is found inside cells in small droplets (10-80μm) in diameter (Fellows, 1998). However, a single type of equipment is not suited to all oilseeds owing to variation in oil content, moisture content, porosity and solidity of the material, applied pressure, heating temperature, heating duration, particle size and shape, storage and handling practices, and the proportions of hulls in different oilseeds are factors influencing yield and quality of vegetable oil expressed (Weiss, 2000).

Expression is achieved either in two stages (size reduction to produce pulp or slurry, followed by separation in a press) or in a single stage, which both rupture the cells and express the oil. In general, the single-stage operation is more economical, permits higher throughputs and has lower capital and operational cost but not suitable for hard nuts as the two stage of expression is more effective. The degree of effectiveness varies with the kind of oilseed and method of oil expression (Akinoso, 2006).

In mechanical expression of soya oil, pieces of equipment often used are presses and expellers. These equipment are operated manually, semi-automated or automated. Often in mechanical expression, hydraulic presses are usually employed due to their lower initial and maintenance costs. The method of oil extraction employed depends on the scale of operation and technology available within the location of use.

According to Singh *et al*., (1987), though simple, they are inefficient and are no longer in use for soya bean oil processing. Screw expellers are observed to be more efficient in oil extraction. This is due to their ability to combine size reduction (milling) and oil expression at the same time (Khan and Hanna, 1983[b]). As mentioned however, mechanical expression gives relatively low oil yields in soya beans. Deoiling of soya beans follows pre-treatments such as dehulling, cracking, grinding or flaking, heating or steaming to enhance the quantity of oil extracted.

2.6.4 Solvent Extraction of Vegetable Oil

Solvent extraction is the use of chemicals as solvents in the extraction of oil from oilseeds. Solvent extraction is known for its high yielding oil output, ease and swiftness to carry out; relatively cost effective, high overhead cost, and hazardous effects during and after operations. The use of this method requires a complete refining process to ensure traces of the solvents are removed totally. Solvent extraction of cleaned, cracked, dehulled and conditioned thin soy flakes (0.25-0.30 mm) with hexane is commercially practised to extract oil (Becker, 1971; 1978, Galloway, 1976). Commercial solvent extraction does not include any pre-pressing operation due to the relative disadvantages of low oil content and slower oil recoveries. Becker (1978), and Johnson and Lusas (1983) indicated that hexane, a petroleum-derived product has been extensively used as solvent for the oil extraction of soya beans and other oilseeds because of its low vapourisation temperature (boiling point 63^{o}-69^{o}C), high stability, low corrosiveness, low greasy residual effect, and better aroma and flavour productivity for the milled products.

Hexane, however, is listed as a hazardous air pollutant by the United States Clean Air Act. Its use in the oilseed extraction plants can adversely affect workers' central nervous system, while its vapour ignites spontaneously with air at 25^{o}C (Becker, 1978; Johnson and Lusas, 1983; Lusas *et al*., 1991; Gandhi *et al*., 2003). Hexane is occasionally scarce and its price fluctuates depending on the supply and demand for gasoline (Johnson and Lusas, 1983). Narain and Singh (1988) also reported that the use of hexane in small-capacity plants makes the processing expensive due to high operational losses. Research, however, is being conducted to find solvents that are safer and readily available than hexane. The ideal solvent must be easily removed from the meal and oil, non-flammable, stable, non-reactive with oil; meal or equipment, pure, only slightly soluble in water and readily available at low prices (Johnson and Lusas, 1983).

2.6.4.1 Solvent Characteristics

In the search for a replacement for hexane, isopropyl alcohol and petroleum ether were chosen for this research. These solvents were selected on the basis of their availability, ability to extract oil from soya beans with as much recovery as hexane, and affordability. Below are the descriptive characteristics of the solvents.

2.6.4.1.1 Isopropyl Alcohol

Isopropyl alcohol (IPA, isopropanol, propan-2-ol, 2-propanol, C_3H_8O) produced from the combination of water and propene, like acetone, dissolves a wide range of non-polar compounds. It is relatively non-toxic and dries quickly. Isopropyl alcohol vapour is heavier than air and is highly flammable with a very wide combustible range. Isopropyl alcohol has a density of 0.786 g/cm^{3}, melting point of -89^{o}C, a

molar mass of 60.10 g/mol, and is miscible in water. It should, however, be kept away from heat and open flame. When mixed with air or other oxidizers, it can explode through deflagration and is reported to form explosive peroxides (Wikipedia, 2008[a]). Isopropyl alcohol is described as an efficient and advantageous extraction solvent for soya beans and other oilseeds as an attractive alternative to extraction grade hexane (Sullivan *et al*., 1982; Baker and Sullivan, 1983; Lusas *et al*., 1991; Seth *et al*., 2007).

IPA extraction produces high quality oil that requires less refining, produces high quality meal that requires less toasting, uses less energy and is safe, and is less toxic (Baker and Sullivan, 1983; Gandhi *et al*., 2003). IPA has a higher vaporization temperature (boiling point 82.3^{o}C) than hexane, only a small portion of the total IPA in the system requires vaporization and energy savings should result. Owing to the significant amount of trypsin inhibitor inactivation achieved, the desolventising-toasting (D-T) cycle is considerably shorter, producing a high quality meal. Gandhi *et al.* (2003) obtained the highest degree of purity in soya bean oil for extraction with IPA and reported that IPA extraction process is equally effective when compared with hexane.

2.6.4.1.2 Petroleum Ether

Petroleum ether (benzine, naphtha, petroleum naphtha, petroleum spirit, X4 or ligroin), is a group of various volatile, highly flammable and irritant, liquid hydrocarbon mixtures used chiefly as non-polar solvents. Petroleum ether is obtained from petroleum refineries as the portion of the distillate which is intermediate between the lighter naphtha and the heavier kerosene. Petroleum ether has a specific

gravity of between 0.625 - 0.660 g/cm^3 depending on its composition and is not water soluble (Wikipedia, 2008[b]). It has a boiling point of 20^{o}C - 75^{o}C, melting point of -73^{o}C, and a molar mass of between 87-90 g/mol. Petroleum ether being a petroleum product also faces occasional scarcity and fluctuation in price depending upon supply and demand of gasoline (Johnson and Lusas, 1983, Seth *et al*., 2006).

2.6.4.1.3 Mechanism of Mass Transfer in Solvent Extraction

The transfer of soluble material from a particle by the actions of a solvent is termed leaching or percolation. Leaching is a complex mechanism involving the transfer of the solvent to the surface of the solid particles, penetration of the solvent into the solid, dissolution of the solute into the solvent, diffusion of the solute into the solvent and transfer of the solute to the bulk solvent (Adu-Amankwa, 2006). Based on the different phenomena for the leaching process, it becomes virtually impractical to use any one theory to explain or describe the leaching activity. The dissolution of a sample from the solid to the liquid phase depends on the rate of mass transfer from the solid surface to the solvent as the controlling factor.

The mass transfer rate of a solute A being dissolved in a solvent of volume V (m^3) is

$$N_A = k_L a\left(C_{AS} - C_A\right) \qquad (1)$$

where,

N_A is the kg/mol of A dissolving to the solution per second,

a = inter-surface area of the particles (m^2),

k_L = mass transfer coefficient (m/s),

c_{AS} = saturation solubility of the solute (kg/mol/m^3)

c_A = time dependent concentration of the solute.

The rate of accumulation of A into the solvent by material balance is:

$$\frac{Vd_{CA}}{dt} = k_L a\left(C_{AS} - C_A\right) \qquad (2)$$

By integration of equation (2) for t = 0, $c_A = c_{A0} = 0$, $t = t_f$, $c_{A=} c_{AS,}$ we have;

$$\frac{C_{AS} - C_A}{C_{AS} - C_{AO}} = \ell^{-(k_L)t} \qquad (3)$$

Or

$$\ell_n \frac{C_{AS} - C_A}{C_{AS} - C_{AO}} = -\left(k_L\right)t \qquad (4)$$

These equations aid in the understanding and calculation of the process of leaching of oil or liquid from a solid substance using a solvent.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Introduction

This chapter describes the materials and methods used in the process of data collection as well as appropriate formulae for data analysis and presentation.

3.2 Study Sample

“Jenguma” variety of soya bean, developed and released by the Savannah Agricultural Research Institute (SARI), Tamale, was obtained from the Grains and Legumes Board (GLB) of the Ministry of Food and Agriculture (MoFA) for the purpose of this research. The sample was milled (broken) using a laboratory blender. The samples were sieved and categorized into fine (brokens retained on sieve size 180 µm), medium (brokens retained by sieve size 710 µm), and coarse (brokens retained on sieve size 1.40 mm). The samples were conditioned to obtain samples at moisture contents of 7% and 4.5%.

3.3 Equipment and Data Collection

Data of various types were collected for the study and these included proximate composition data, flow quantity of oil per time, moisture and solvent; and physicochemical properties of the oil extract. Equipment used for the study included a Soxhlet apparatus, desiccator, drying oven, weighing scale, furnace chamber, laboratory equipment, chemicals and reagents, and computer with appropriate software (Microsoft Excel and Statistical Package for Agricultural Scientists – GenStat discovery edition 3, 2008) for data entry, organisation and analysis. For oil extraction, a complete randomised design (CRD) with a two way treatment structure was used. All treatments were in triplicate.

To ensure the objectives of the study were achievable, the following data were collected.

3.4 Proximate Determination of Samples

For the purpose of this study, the parameters for which data were collected for analysis included moisture content, ash, crude protein, crude fat, and crude fibre and carbohydrate.

3.4.1 Moisture Content Determination

Moisture content was determined using the procedure as follows: 2 g of the milled sample was weighed using an analytical balance, placed in a crucible and dried in a thermostatically controlled oven at 105^{o} C for 5 h. The sample was removed and placed in a desiccator and cooled to room temperature. The sample and crucible were weighed repeatedly until a constant weight was obtained. Loss in weight of the sample was reported as moisture content.

$$\%\ moisture\ content = \frac{Loss\ in\ weight}{Weight\ of\ sample}\ x\ 100$$

3.4.2 Ash Content Determination

The following procedure was used for the determination of ash content. 2 g of the milled sample was transferred into a previously ignited and weighed crucible and placed in a muffle furnace (preheated to 600^{o} C) for 2 h. The sample with the crucible was transferred directly from the furnace into a desiccator, and allowed to cool and the weight taken.

$$\%\ ash = \frac{Weight\ of\ ash}{Weight\ of\ sample}\ x\ 100$$

3.4.3 Crude Protein Determination

The Kjeldahl method was used to determine crude protein. 2 g of the milled sample, half of selenium based catalyst tablet and a few anti-bumping agents were placed into a digestion flask. 25 ml of concentrated sulphuric acid ($H_2SO_{4)}$ was added to the sample and the flask shaken vigorously to obtain a wet and well mixed mixture. The flask was placed on a digestion burner and heated slowly until the boiling ceased and a clear solution was obtained. The solution was cooled to room temperature and the digested sample transferred into a 100 ml volumetric flask. For distillation of the sample, the apparatus was flushed out before use. 25 ml of 2% boric acid was pipetted into a 250 ml conical flask and 2 drops of mixed indicator added.

Liquid was drained from the steam trap while leaving the stop cork which drains the steam trap opened. The conical flask with its content was placed under the condenser in a position where the tip of the condenser was completely immersed in the solution. 10 ml of the digested sample was measured and added to the decomposition flask. 40% of NaOH (about 20 ml) was also added to the decomposition flask. Distillation was allowed to continue for about 5 minutes and the burner removed from the steam generator. The sample was titrated with 0.1N hydrochloric (HCl) solution until the sample solution became colourless.

$$\%\ nitrogen = \frac{100\ x\ (V_A - V_B)\ x\ N_A\ x\ 0.01401\ x\ 100\ x\ 6.25}{W\ x\ 10}$$

where,

V_A = volume of standard acid (ml)

V_B = volume of standard acid in the blank (ml)

N_A = normality of HCl

W = weight of sample (grams)

F (6.25) = non-protein (urea) nitrogen factor

3.4.4 Crude Fat Determination

The following procedure was used for the determination of crude fat. Moisture determined samples were transferred into 22 x 80 mm paper, sealed and placed into a thimble with cotton wool to prevent loss of the sample. An anti-bumping granule and 150 ml petroleum spirit was weighed into a 250 ml round bottom flask and fluxed for 4 hours on high heat. The flask was removed and evaporated on a steam bath and the oil dried for 30 minutes in an oven at 103^{o} C. The oil was cooled to room temperature and weighed.

$$\%\ crude\ fat = \frac{Weight\ of\ fat}{Weight\ of\ sample}\ x\ 100$$

3.4.5 Crude Fibre Determination

The following procedure was used in the determination of crude fibre. The defatted sample for crude fat determination was transferred into a 750 ml Erlenmeyer flask and approximately half gram of asbestos added. 200 ml of boiling 1.25% H_2SO_4 was added and the flask immediately set up on a hot plate and connected to a condenser. The content came to a boil within 1 min and agitated frequently until the sample was thoroughly wetted. The flask was removed in 30 min and the sample filtered through a linen cloth in a funnel. The boiled sample was washed continuously until there was no indication of the presence of an acid in the sample. The charge and asbestos were washed into a flask using 200 ml boiling 1.25% sodium hydroxide (NaOH) solution

and the condenser connected and set back on the hot plate. At the end of 30 min the content was filtered through a linen cloth and thoroughly washed with boiling water. The residue was transferred into a crucible and washed with approximately 15 ml alcohol. The content was dried in an oven at 100^{o}C for one hour; the content was transferred into a desiccator and weighed. The crucible with content was ignited in furnace between 500^{o}C - 600^{o}C for 30 min, cooled and weighed. The weight loss was reported as crude fibre.

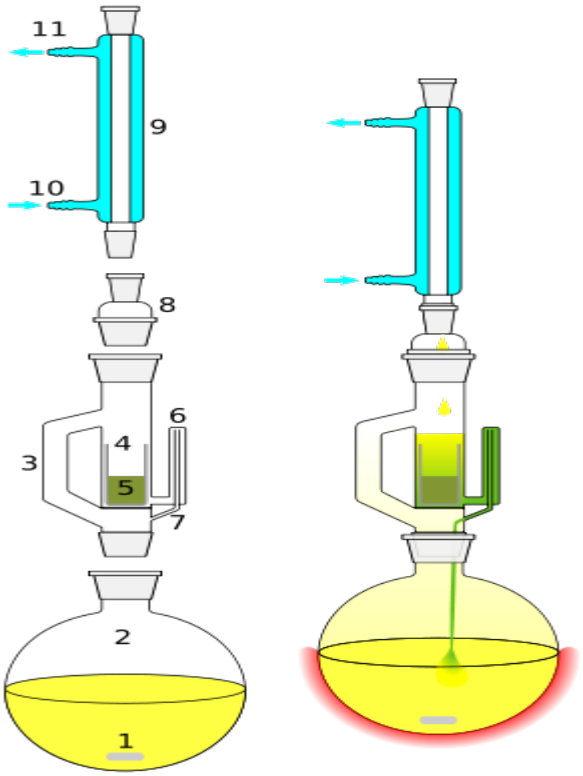
3.5 Method used in Oil Extraction

The following procedure was used in the extraction of samples. 100g of the milled soya bean sample (for each replicate) conditioned at moisture contents of 7.0% and 4.5% were bagged and used for the extraction process; using a Soxhlet apparatus. 150ml of each solvent was measured into 250ml flat bottom flask and heated at a constant temperature of 50^{o} C to reflux. The heat caused the solvent to vaporise through the thimble containing the sample as the solvent boiled in the flask; the vapour was trapped and cooled by the condenser above the thimble. The cooling turned the vapour into warm liquid which hydrolysed the sample in the thimble. When the thimble was filled with the drops of the warm solvent from the condenser, the solvent (which contained traces of the oil) was poured out into the flat bottom flask beneath the thimble containing either petroleum ether or isopropyl alcohol automatically through a siphon arm. The process was continued for the durations of 8 and 22 h for each replicate.

At the end of each extraction process, the milled sample was removed from the thimble and the extraction process repeated, but this time for solvent recovery from

the oil sample. This is done for an unspecified time depending on the quantity of oil and solvent contained in the flask from extraction. The oil was poured into a beaker and placed on a steam bath, and finally dried in the oven for 30 minutes at 103^{o} C; the amount of oil extract was determined by pouring the oil into a measuring cylinder.

For the leaching determination, the sample was milled and sieved to obtain a particle size of 180 micrometers and bagged into 100g for extraction using the same process adopted for oil extraction above. The extraction of oil was conducted continuously for a durational interval of 1 - 12 h for the determination of mass flow by leaching for soya bean oil using both solvents at temperature 60^{o}C. At the end of each extraction, the weight of the extract was divided by the original mass of the sample (100g) to give the mass fraction and used in plotting a graph against time.



Source: Wikipedia, (2008[c]) ***Source: L. Dari***

Figure 3.1 Schematic description and pictorial setup of the Soxhlet apparatus

1: Stirrer bar/anti-bumping granules
2: Still pot (extraction pot) - still pot should not be overfilled and the volume of solvent in the still pot should be 3 to 4 times the volume of the soxhlet chamber.
3: Distillation path **4:** Soxhlet Thimble **5:** Extraction solid (residue solid)
6: Siphon arm inlet **7:** Siphon arm outlet **8:** Expansion adapter **9:** Condenser
10: Cooling water in **11:** Cooling water out

3.6 Physicochemical analysis of soya bean oil

Several indices are used for the determination of oil quality. For the purpose of this experiment; free fatty acid, iodine value, acid value, saponification value and unsaponifiable matter were determined.

3.6.1 Free Fatty Acid (% Oleic)

The free fatty acid was determined according to the Official Method Ca 5a-40 of American Oil Chemists' Society (AOCS) (1993). About 2 g of the extracted oil sample was weighed into a 250 ml Erlenmeyer flask using an analytical balance. 20 ml of 95% neutralized ethanol was added to the flask. The solution was heated slightly at 20^{o}C to aid the dissolution of the fat in the alcohol. 2 drops of phenolphthalein solution was added as indicator.

The obtained yellowish solution was titrated with 0.1N standard sodium hydroxide solution while shaking the solution vigorously. The colour of the solution turned pink and at the point when the pink colour persisted for 30 s was termed the end point. The percentage of free fatty acid in the oil was calculated as oleic as follows:

$$\%\ free\ fatty\ acid\ (\%\ oleic) = \frac{V\ x\ N\ x\ 28.2}{Weight\ of\ sample}$$

where,

V = average volume of NaOH (ml)

N = normality of NaOH (0.1)

3.6.2 Iodine Value

The iodine value was determined according to Official Method Cd 1-25 of AOCS (1993). 0.20 g of the filtered oil sample cooled at a temperature of 68^{o} – 71^{o} C was weighed into a 500 ml flask. 15ml of tetrachloride was added to the sample and swirled to ensure that the sample was completely dissolved in the tetrachloride. 25 ml of Wijs solution was dispensed into the flask containing the sample and swirled to ensure an intimate mixture. The flask with content was immediately kept in a dark place at a temperature of about 25^{o} – 30^{o} C for two hours. The flask was removed and 20 ml of 10% potassium iodide (KI) solution added followed by an addition of 150 ml of distilled water. The solution was titrated with 0.1 N thiosulphate ($Na_2S_2O_{3)}$ solution while shaking it constantly and vigorously until the yellow colour had almost disappeared. 1.5 ml starch indicator was added and the titration continued until the blue-black colour just disappeared. A blank was preformed alongside.

$$Iodine\ value = \frac{(V_2 - V_1)\ x\ N\ x\ 12.69}{Weight\ of\ sample}$$

where,

V_2 = Titration of $Na_2S_2O_3$ blank (ml)

V_1 = Titration of $Na_2S_2O_3$ sample (ml)

N = Normality of $Na_2S_2O_3$ solution (ml)

3.6.3 Acid Value

The acid value was determined using the procedure recommended by Kirk and Sawyer (1991). 5 g of the oil sample was weighed into a flask, 25 ml of 95% alcohol and diethyl ether each was added to the sample in the flask. 1 ml of phenolphthalein was added and the solution mixed thoroughly. Upon complete dissolution, titration

was carried out using 0.1N potassium hydroxide (KOH) and the sample shaken vigorously while titrating to obtain the first permanent pink colour of the same intensity. The pink colour persisting for 30 second was recorded as the end point. A blank was performed along side.

$$Acid\ value = \frac{(A - B)\ x\ (N)\ x\ 56.1}{W}$$

where,

A = KOH used in titration (ml)

B = KOH used in the blank (ml)

N = normality of KOH

W = weight of sample (g)

3.6.4 Saponification Value

The saponification value was determined using AOCS (1993) Official Method Cd 3-25. 2 g of the dried and filtered oil sample was weighed into a 250 ml Erlenmeyer flask. 25 ml N potassium hydroxide was added using a pipette and the pipette was allowed to drain for some time. A blank was conducted simultaneously where all reagents were added with the exception of the oil sample. The air condenser was connected and the sample boiled gently, but steadily for 45 min. After the flask and condenser have cooled but not sufficiently to forming gel, the inside of the condenser was washed with a small quantity of distilled water and the condenser disconnected. 1 ml of phenolphthalein indicator was added to the sample and the sample titrated with 0.5N hydrogen chloride (HCl) until the pink colour just disappeared and the volume of the HCl recorded.

$$Saponification\ value = \frac{(B - S)\ x\ N\ x\ 65.1}{W}$$

where,

B = 0.5N HCl required to titrate blank (ml)

S = 0.5N HCl required to titrate sample (ml)

N = normality of HCl solution (ml)

W = weight of sample (g)

3.6.5 Unsaponifiable Matter

The unsaponifiable matter was determined using Official method Ca 6a-40 of AOCS (1993). 2.5 g of the oil sample well mixed was weighed into a 250 ml Erlenmeyer flask with ground glass-joint. 25 ml of about 95% ethyl alcohol and 1.5 ml of 50% potassium hydroxide solution was added. The solution was boiled gently but steadily under reflux with occasional swirling for 30 min until completely saponified. The saponified sample was transferred into an extraction cylinder using a total of 50 ml water and the flask washed with 50 ml of diethyl ether and added to the cylinder. The content of the cylinder was cooled to room temperature (20 – 25^{o}C). The cylinder was corked with a stopper, shaken for at least one minute, and allowed to settle until both layers could be clearly identified. A glass siphon was used to remove the upper layer completely as possible without including any of the lower portions. The diethyl ether layer was transferred into a 250 ml separation funnel. The extraction was repeated twice using 50 ml portion of diethyl ether each time and shaking vigorous with each extraction. The combined diethyl ether extract was rotated gently with 20 ml of water after which the layers were allowed to separate completely and the lower aqueous layer drawn off. The diethyl ether layer was washed twice using 20 ml of

water each time, while shaking gently and discarding the lower aqueous layer after separation.

The combined extract was washed in a separation funnel thrice using 20 ml portion of 0.5N potassium hydroxide while shaking vigorously. After each alkali was washed, washing was re-conducted using 20 ml of water. After the third washing of the alkali, the diethyl ether was washed with successive 20 ml of water until the washings did no longer appear pink on addition of phenolphthalein, indicating that the sample was no longer alkaline. The diethyl ether was transferred into a tarred beaker. The separation funnel with its edges were rinsed with diethyl ether and added to the content of the beaker. The diethyl ether was evaporated to dryness on a water bath using a gentle stream of clean, dry nitrogen. After all the diethyl ether had been evaporated 2-3 ml of acetone was added and all traces of solvent removed with the aid of a stream of nitrogen. The drying was completed to a constant weight in a vacuum oven at 75 – 80^{o}C and an internal pressure of not more than 200 mm of mercury. The residue was recorded as A. A reagent blank was determined without fat and recorded as B.

$$\%\ unsaponifiable\ matter = \frac{(A-B)-C\ x\ 100}{Weight\ of\ sample}$$

where,

A = weight of residue
B = weight of fatty acid
C = weight of blank

CHAPTER FOUR
RESULTS AND DISCUSSIONS

4.1 Introduction

This chapter presents the results and discussions of the proximate composition of the sample, oil extraction, physicochemical analysis of the oil and the leaching rate of soya bean oil using the solvents, isopropyl alcohol and petroleum ether. Comparison of the results with literature is done to point out areas of direct relationship.

4.2 Proximate Composition Results

The result from the proximate analysis is shown in figure 4.1. The protein content was highest in the soya bean sample followed by carbohydrate and hulls, fats and oils, and ash respectively. This conforms to the general literature that soya bean has a higher protein proportion of about 40%, and 18% - 20% oil. The 48% protein measured in this experiment could be attributed to agronomic practices and varietal difference of the sample.

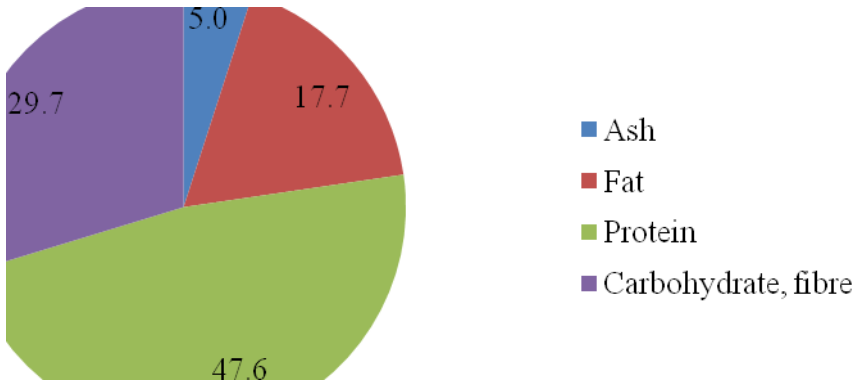


Figure 4.1. Proximate compositions of soya beans (*Jenguma*)

4.3 Oil Yield of Extracts

Comparing oil extracts in graphs below with the analysis of variance of oil extracts from table 4.1 below, there were significant differences between mean yields from the solvents; isopropyl alcohol and petroleum ether. Petroleum ether gave relatively higher oil yields whiles in trying to evaporate the solvent isopropyl alcohol from the extracted oil resulted in the formation of a jelly like composition on the surface of the oil, contributing to the low oil yields from isopropyl alcohol. The total oil extraction of soya bean were, fine 95.4%, medium 69.1%, coarse 55.6% for petroleum ether and fine 33%, medium 32%, and coarse 18.3% for isopropyl alcohol. With an average of 73.4% and 27.8% for petroleum ether and isopropyl alcohol respectively, petroleum ether yielded relatively higher oil compared with hexane, used by Seth *et al*., (2007) which yielded 75.5% of oil at a sample moisture content of 12.4% with a particle size of 2 mm for extraction duration of 24 h.

On acquisition of the solvents for this experiment, isopropyl alcohol was more costly than petroleum ether in terms of price. The price of isopropyl alcohol was twice the price of petroleum ether for the same quantity. Isopropyl alcohol could therefore be used for the extraction of oil for consumption, whiles petroleum ether could be used for the extraction of oil for biodiesel and other industrial use since petroleum ether is not a food grade solvent.

From table 4.1, there were significant differences ($p \leq 0.05$) between the textures of the milled soya used for the experiment, with the fine texture yielding more oil followed by medium and coarse respectively. This indicates that for better oil recoveries, the milled sample must have a texture of between 180 - 710 µm to ensure

proper cell rupture of samples. Othmer and Agarwal (1955) reported that the inability to express oil from whole and half soybeans clearly indicated that cell walls must be broken by a flaking operation to allow the oil to be removed from otherwise impervious cells. The highest extract was 22ml/100g and thus confirms the assertion by Rosenthal *et al*. (2001) that '' Soybean oil is present in the range of 22.5–27 ml oil/100 g soybean'' and that solvent extraction of soybean meal gave a value of 25 ml oil/100 g soybean.

There were no significant differences ($p \leq 0.05$) between the moisture contents. This suggests that drying samples beyond the recommended storage specifications will only increase production cost and not necessarily have an enhanced effect in the processed output.

There were no significant differences ($p \leq 0.05$) between extraction times 8 and 22. It was however observed that a greater amount of oil was extracted within the interval of 2-8 h signifying that extraction can be successfully optimised within 8 h (fig. 4.6).

Table 4.1 Analysis of variance of mean yields

Chemicals	**Yield**	**Moisture content (%)**	**Yield**	**Time**	**Yield**	**Texture**	**Yield**
Isopropyl Alcohol	6.11^{a}	7.0	11.36^{a}	22	11.42^{a}	Fine (180 µm)	14.28^{a}
Petroleum ether	16.23^{b}	4.5	10.98^{a}	8	10.92^{a}	Medium (710 µm)	11.12^{b}
						Coarse (1.40 mm)	8.10^{c}
Standard error of difference (s.e.d)	0.656	s.e.d	0.656	s.e.d	0.656	s.e.d	0.804

**Different superscripts represent significant difference among means*.

Fig. 4.2 – 4.5 show the results of the mean yields based on texture, moisture content, time, and solvent. The graphs indicate the amount of oil recovered per extraction.

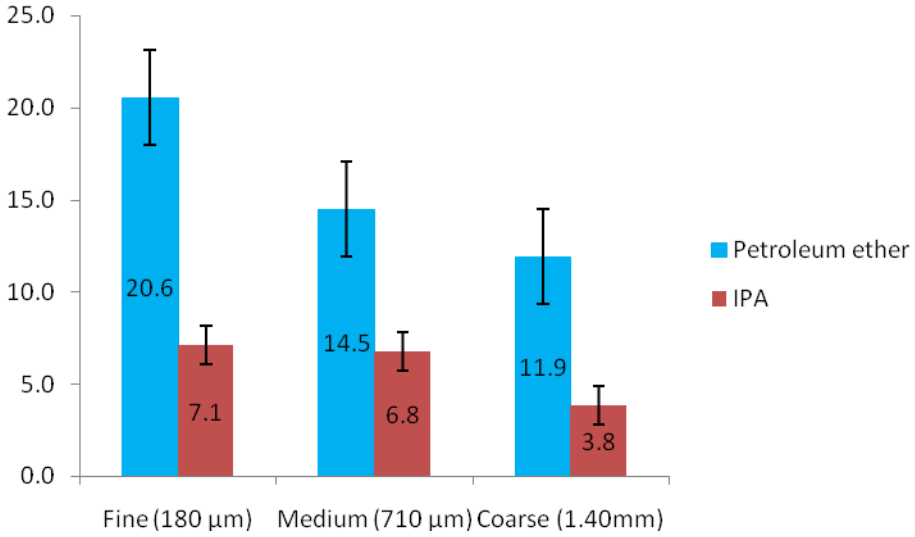


Figure 4.2. Oil yield of soya at 7.0% moisture content extraction duration of 8 h (Isopropyl alcohol and Petroleum ether). Bars represent standard error of difference.

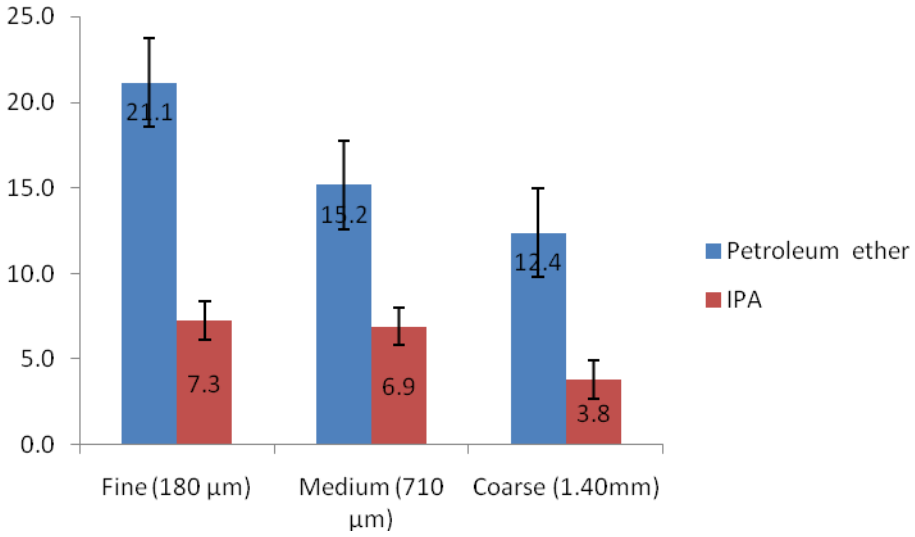


Figure 4.3. Oil yield of soya at 4.5% moisture content extraction duration of 8 h (Isopropyl alcohol and Petroleum ether). Bars represent standard error of difference.

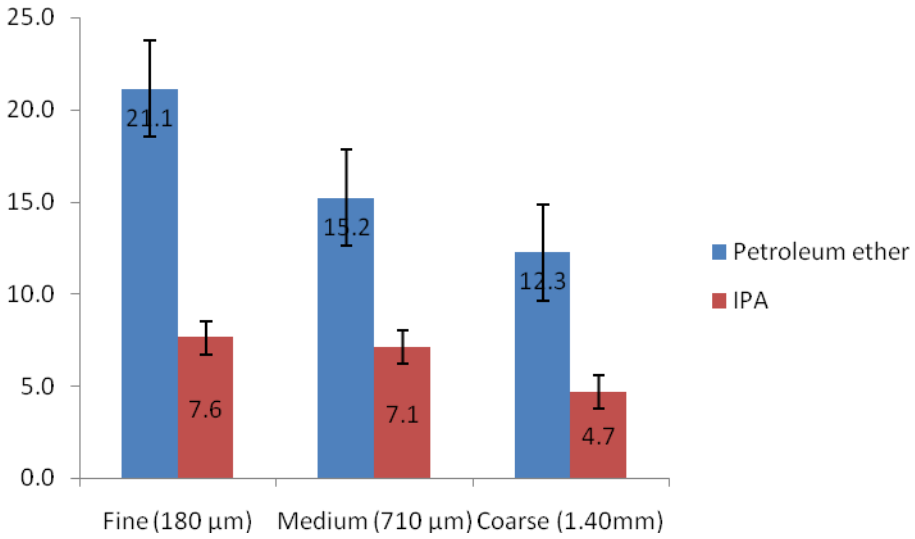


Figure 4.4. Oil yield of soya at 7.0% moisture content extraction duration of 22 h (Isopropyl alcohol and Petroleum ether). Bars represent standard error of difference.

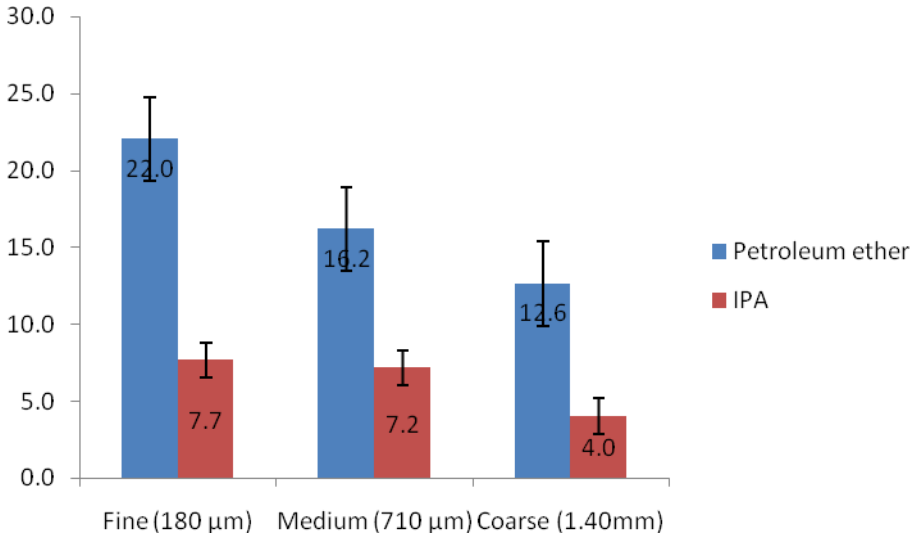


Figure 4.5. Oil yield of soya at 4.5% moisture content extraction duration of 22 h (Isopropyl alcohol and Petroleum ether). Bars represent standard error of difference.

It was observed from the extraction results that the liquid solvent from the thimble that poured into the flask contained a trace of the oil (yellowish) in the solution. This yellowish coloration faded with continuous extraction, and the solution became colourless. During leaching for the extraction period from fig. 4.10, it was observed that extractions beyond 8 hours did not yield further oil indicating that extraction is best accomplished between 2-8 h. The rate of leaching of the solution from the solid state to liquid state using petroleum ether as solvent was 4.01×10^{-4} ml/min as compared to isopropyl alcohol of 2.85×10^{-4} ml/min, indicating that petroleum ether has a relatively higher ability to leach oil from the sample better than isopropyl alcohol.

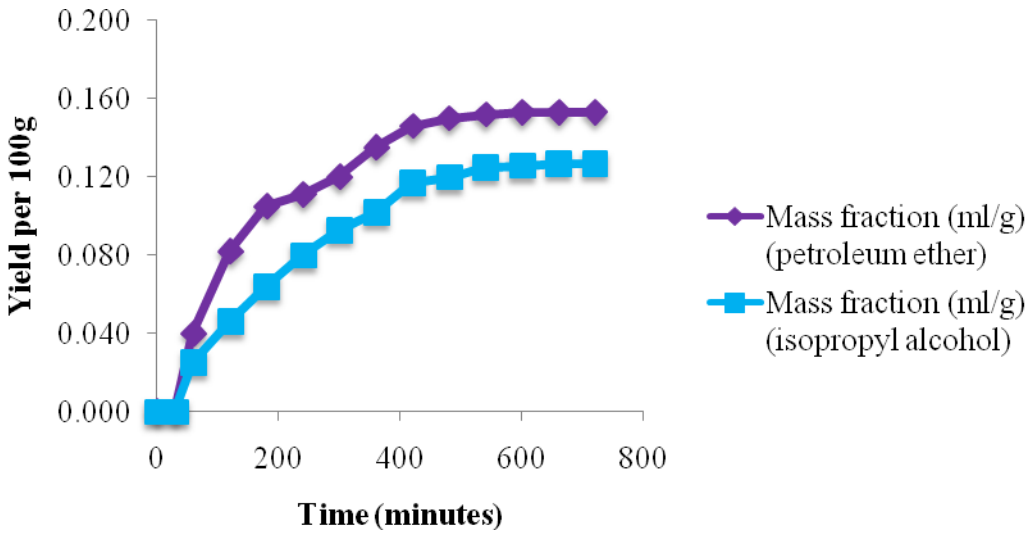


Figure 4.6. Concentration time graph for leaching of crude soya oil

4.4 Physicochemical Results of Soya Bean Oil

Several indices are used for the determination of oil quality. For the purpose of this experiment, the following parameters were analysed:

4.4.1 Free Fatty Acid (% Oleic) and Iodine Value

From the analysis, the free fatty acid (% oleic) content was 0.3 which is within the acceptable limits of 0.3-0.7 for crude soya bean oil (Pryde, 1980; American soybean Association, 1981), signifying the oil's stability and quality. From the results, the iodine value was 67 ml/g and falls within the expected iodine value range (51-100) for all oils according to AOCS recommended method Cd 1-25. It thus differs from the expected range of 124-139 stipulated by Codex Alimentarius for crude soya bean oil, and can be attributed to the method of analysis. Since the result meets the range outlined by the method used, it can be considered that the oil extracted has good stability.

4.4.2 Acid Value, Saponification Value and Unsaponifiable Matter

Saponification value in combination with the acid value provides information on the quantity, type of glycerides and mean weights of the acids in a given sample. Saponification value is of interest if the oil is for industrial purposes, as it has no nutritional significance (Asiedu, 1989). The larger the saponification number, the better the soap making ability of the oil (Nielsen, 1994). From the analysis in table 4.2, the saponification value gives an indication that the extracted oil sample is not only suitable for the food industry but is also suitable for other industries (e.g. soap manufacture), as it meets the standards of Codex Alimentarius for crude soya bean oil . Suitable oils for industrial purposes must have low acid values especially for the

petroleum industry as high acid values cause destruction to the sump of engines. The acid value from the analysis gave soya bean oil an added advantage for use in the petroleum industry.

Unsaponifiable matter was 5 g/g compared to estimated range of less than or equal to 15 in Appendix 1. The unsaponifiable matter in this analysis could be linked to the presence of substances like pigments, phosphatides, carbohydrates, gum and protein contained in the extracted fat without refining. The figure is within the estimated range based on the observed hygienic practices during this study.

Table 4.2 Physicochemical results of Soya bean oil extract

Properties	**Value**
Free fatty acid (% Oleic)	0.30 ± 0.01
Iodine value	67.00 ± 0.06
Saponification	190.18 ± 0.10
Acid value	0.56 ± 0.01
Unsaponifiable matter	5.0 ± 0.05

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This chapter draws on the various discussions made in relation to the results in arriving at conclusions and recommendations for the study.

5.2 Conclusion

The proximate result of the sample prior to the extraction process indicated the sample had all the compositional requirements of soya beans outlined in the literature. The high crude protein content of 48% can be attributed to varietal differences, agronomic practices during growth of sample and the method of analysis.

The results of the study showed that various parameters contributed to the extraction optimization of soya bean oil as the petroleum ether gave higher oil yields as compared to isopropyl alcohol. Lower oil extracts with isopropyl alcohol could be attributed to the reaction between the solvent and the oil extract which resulted in the jelly-like formation on top of the oil during the solvent recovery stage. The mass transfer rates were 4.01×10^{-4} ml/min and 2.85×10^{-4} ml/min for petroleum ether and isopropyl alcohol respectively.

From the solvent characteristics, both solvents are considered to be less hazardous compared with hexane though isopropyl alcohol has a higher boiling point than hexane, whiles petroleum ether has a lower boiling point of 20^{o}C and an upper boiling point of 75^{o}C. Isopropyl alcohol is a better choice than petroleum ether as its

supply does not depend on the availability of petroleum and may not suffer the scarcity associated with petroleum and petroleum products.

The texture of the milled sample had a great impact on the quantity of oil extract, as samples that were finely milled had a greater yield regardless of the solvent used, followed by medium and coarse textures respectively. From the analysis, there were significant differences between all three textures. The results confirm the assertion that for optimum oil recoveries, seed cells must be ruptured finely enough to enable solvents leach out the oil effectively.

Results based on the pre-determined moisture contents had no significant effect on oil output, indicating that once seeds are dried to the recommended moisture content (10-13%) further drying will not be necessary. Further drying of seeds will only increase the cost of production which will not affect yield of the extracts, leading to a loss in production.

Analysis based on the extraction duration showed that time was not significant in the oil extraction process. From the mass transfer and oil yield graphs, extraction of oil is optimised within 2-8 h.

According to the physicochemical analysis of the oil extract, the free fatty acid content was within the acceptable levels for the oil to be considered stable and not prone to rancidity. The iodine value was relatively high indicating the level of unsaturation which is indicative of the high quality desired by processors. The acid value was also minimal; meaning that when refined, it could reduce the quantity making it suitable for use in the petroleum industry. Saponification value was high and within the recommended range specified by Codex Alimentarius for crude soya

bean oil. This enhances the oil extract usage industrially, especially in the soap manufacturing industry. Results of unsaponifiable matter in the extract were lower and within the expected range signifying the level of the extract purity and reduced contamination based on method of analysis and hygienic practices observed during the extraction period.

5.3 Recommendations

The study could not consider certain parameters such as extraction with more solvents to aid in the search for a suitable replacement of hexane and other physicochemical analysis due to lack of requisite reagents and facilities. It is therefore suggested that;

1. A further study should be conducted to find out the cause of the jelly-like formation on the surface of the isopropyl alcohol extract.
2. A further study should be conducted using other food grade solvents.

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APPENDICES

Appendix 1: Chemical and Physical Characteristics of Crude Vegetable Oils (Codex Alimentarius, 1999), extract for soya bean oil.

Parameters	Range
Saponification value (mg KOH/g oil)	189-195
Iodine value	124-139
Unsaponifiable matter (g/kg)	≤15

Appendix 2: Analysis of variance

Variate: Yield_ml

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Chemical	1	1846.294	1846.294	950.97	<.001
M.C (%)	1	2.722	2.722	1.40	0.241
Texture	2	457.639	228.819	117.86	<.001
Time (hrs)	1	4.601	4.601	2.37	0.128
Residual	66	128.138	1.941		
Total	**71**	**2439.393**			

Appendix 3: Standard errors of differences of means

Table	Chemical	M.C (%)	Texture	Time (hrs)
rep.	36	36	24	36
d.f.	66	66	66	66
s.e.d.	0.328	0.328	0.402	0.328

Appendix 4: Least significant differences of means (5% level)

Table	Chemical	M.C (%)	Texture	Time (hrs)
rep.	36	36	24	36
d.f.	66	66	66	66
l.s.d.	0.656	0.656	0.803	0.656

Appendix 5: Table of means

Variate: Yield (ml) Grand mean 11.17

Chemical	Mean yields
Isopropyl alcohol	6.11
Petroleum ether	16.23
Moisture content	
7.0%	11.36
4.5%	10.98
Time	
22 hours	11.42
8 hours	10.92
Texture	
Fine	14.28
Medium	11.12
Coarse	8.10

Appendix 6: Subject stratum

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Chemical	1	644.31	644.31	28.82	<.001
M.C (%)	1	0.44	0.44	0.02	0.888
Texture (µm)	2	228.82	114.41	5.12	0.009
Time (h)	1	441.70	441.70	19.76	<.001
Residual	66	1475.42	22.35	1.14	

Appendix 7: Time stratum

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time	1	63.73	63.73	3.26	0.076
Time/Chemical	1	1251.98	1251.98	63.97	<.001
Time/M.C %	1	2.78	2.78	0.14	0.708
Time/Texture	2	228.82	114.41	5.85	0.005
Time/Time h	1	323.40	323.40	16.52	<.001
Residual	66	1291.72	19.57		
Total	**143**	**5953.13**			

d.f. correction factor 1.0000