ADDIS ABABA UNIVERSITY

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School of Chemical and Bio Engineering

Production and Characterization of Oil from *Brassica Nigra Seeds* Using the Solvent Extraction Method

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A Thesis

Submitted in Partial Fulfillment of the Requirement for the Degree of Master of Science in Chemical Engineering Under Process Engineering stream

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This is to certify that the thesis prepared by Getachew Chane, entitled: "**Production and Characterization of Oil from *Brassica Nigra Seeds* Using the Solvent Extraction Method**" and submitted in partial fulfillment of the requirements for Degree of Master of Sciences in Process Engineering that complies with the regulations of the university and meets the accepted standards with respect to the originality and quality.

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DECLARATION

I hereby declare that the work which is being present in the thesis entitle "Production and Characterization of Oil from *Brassica Nigra Seeds* Using the Solvent Extraction Method" is an authentic record of my own work in partial fulfillment for the requirement of the award of degree of master of science in Chemical Engineering under supervision of Dr. Eng. Abubeker Yimam(Assistant Professor) instructor in Addis Ababa University, Dean, School of Chemical and BioEngineering, Ethiopia. The matter embodied in this thesis has not been submitted by me for the award of any other degree of master or examination in any other universities. All relevant resource of information used or quoted in this thesis have been duly acknowledged

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ABSTRACT

The objective of this study was the production and characterization of oil from *Brassica Nigra seeds* using the soxhlet extraction method were undertaken. In the soxhlet extraction a n-hexane was chosen to determine the effect of extraction time (2,4 and 6 hours), extraction temperature (65,70 and 75℃) and solid to solvent ratio (0.08,0.1 and 0.12) on yield of extraction oil. Firstly, the oil extract was characterized by density, moisture content, specific gravity, viscosity and refractive index, saponification value, iodine value, acid value, peroxide value and the results were compared with literature. The results showed that there is no difference from the seed oil extract from the *Brassica nigra seeds* which was producing in other parts of the world. therefore, the extraction and physicochemical properties of seed oil from *Brassica nigra seed* using a soxhlet extraction method has been carried out. A Box-Behnken Design (BBD) was applied to extraction process using Design Expert software and linear regression model was obtained growing the individual effect of extraction time, solid to solvent ratio and extraction temperature as parameter and their interaction in the entire extraction process. For the soxhlet extraction, the maximum oil yield has been determined as 46.205% after the extraction time of 2 hours with solid to solvent ratio (0.12g/mL) at extraction temperature of 70℃ and the minimum oil yield 34.97% was found at the extraction time of 4 hours with extraction temperature of 65℃ at solid to solvent ratio (0.08 g/mL). Therefore, increasing solid to solvent ratio, extraction temperature and decreasing extraction time will increase the amount of oil extracted.the selected seed oil properties (specific gravity,viscosity, density, Saponification Value, Acid Value, Iodine Value, Peroxide Value and refractive index) of the extracted oil were determined and the result of characterization of the oil indicated that the viscosity at 21ºC (9.16*10^-6 m2/s) and specific gravity at 20℃ (0.89346), density(0.8891g/cm3), Saponification Value(172.788 mgKOH/gm), Acid Value(2.992), Iodine Value(95.26), Peroxide Value(2.14) and refractive index(1.45504) .

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LIST OF SYMBOLS AND ABBREVIATIONS

ACP	Africa caridean and pacific countries
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists’ Society
BBD	Box-Behnken Design
CAGR	Compounded Annual Growth
EFAS	Essential Fatty Acids
EU	European Union
EVOO	Extra Virgin Olive Oil
FAO	Food and Agriculture Organization of United Nations
FOSFA	Federation of Oil, Seeds and Fats Associations
HCL	Hydrochloric Acid
ISO	International organization for standardization
KOH	Potassium hydroxide
MFA	Mono Unsaturated Fatty Acid
PUFA	Poly Unsaturated Fatty Acid
PV	Peroxide Value
RSM	Response Surface methodology
SD	Standard deviation
SFA	Saturated Fatty Acid
SV	Saponification Value
WHO	World health Organization

CHAPTER ONE

1.Introduction

1.1. Background

Brassica nigra is an annual weedy plant cultivated for its seeds, which are commonly used as a spice. and species Brassicaceae is commonly called as black mustard, an annual weedy plant, 2-8' tall, usually grown for its seeds, which are used in seasoning and also taken as a tonic, appetite stimulant. The seeds are small, hard and vary from dark brown to black in color. The Mustard seed is often used in herbal medicine, especially as a rubefacient poultice. Oil prepared from mustard seed called as allyl isothiocyanate is used in commercial cat, dog repellent mixtures and in stimulating hair growth. Decoction of seeds are used in the treatment of liver, spleen indurations, cancers of throat, imposthumes, alopecia, epilepsy, snakebite, toothache. Mustard seed paste applied on the skin reduces rheumatism, congestion in internal organs by drawing the blood to the surface as in head afflictions, neuralgia, spasms and also exhibit antibacterial, antifungal, anti-tussive activity(Krishnaveni, 2016). Since, Brassica nigra seeds have valuable, industrial purpose, huge therapeutic applications it was decided to study its physio chemicals technically in order to validate.

Black mustard, once widely grown as a condiment mustard, has largely been replaced by B. juncea. It is still grown as a condiment crop in parts of Asia. Although little information is available, the occurrence of land races in Europe, the Mediterranean, and the Ethiopian plateau indicates that B. nigra probably originated in central and southern Europe. It is presumed to have been introduced into India relatively recently. *Brassica nigra,* has evolved separately from the other two diploid Brassica species, and numerous data sets (cytological, isozyme, nuclear and chloroplast DNA restriction site, and sequence data) have suggested a closer genetic relationship to the genus Sinapis, particularly the weed species S. arvensis (n = 9), than to B. rapa and B. oleracea (Suzanne , 2011)

Brassica is one of the most ancient spices. It has 3 varieties namely black, brown and white. Brown mustard is largely cultivated. Brown mustard plant produces tiny yellow colored flowers, which almost cover the plant. The black mustard plant normally grows to a height of 10 feet. White mustard is the mildest among all the varieties of mustard. Mustard Seed has a fresh aroma and slightly biting flavor but when the seeds are dried, they do give any fragrance. The leaves, the seeds, and the stem of this mustard variety are edible. The plant appears in some form in African, Indian, Chinese and Japanese(Shahzadi et al., 2015).

Mustard plant of different types has been widely cultivated and used as spice, condiment, medicine and source of edible oil since ancient time. Mustard oil is highly flavored and its seed has been used internally and externally since ancient times. Mustard oil obtains from the mustard seed by crushing process at high pressure and low temperature. The mustard seeds when crushed a yield around 33% oil and 67% protein meal is obtained for pet animals. The oil obtained from the mustard seed can be used as a lubricant, used as a protein meal for animal feed and used in making soap as well as for the cooking or frying the food. Mustard oil makes up about 24-40% of the mustard seeds, which is characterized by the presence of higher level of erucic acid and it has the lowest saturated fatty acids content among all the edible vegetable oils. Mustard oil is the mixture of various acids like, linoleic acid and linolenic acid which have beneficial properties. Though mustard oil provides many benefits it is banned in some countries and sold only for external use in the countries like United States, Canada and European Union as it is considered to be harmful for consumption in these countries. Mustard essential oil is totally different from mustard oil, in terms of the process of extraction, chemical composition and medicinal properties. Both of these oils are extracted from the seeds of mustard, which bears the scientific name Brassica Nigra (Black Mustard) or Brassica Hirta (White Mustard). Mustard oil is extracted at low pressure at low temperature (40-600). It contains 0.30-0.35% essential oil (Ally iso-Thiocynate) which act as preservative(Yadav & Kumari, 2015).

1.2. Statement of Problem

Processing *brassica nigra seed* oil industrially found in Ethiopia is very limited. Producing *brassica nigra seed* oil industrially results in a quality product and will be a source of income by exporting it to other countries and the demand for oil with balance in domestic and industrial purpose to increasing in our country and also it is important that the user or reader be aware of the difference investigation which will helps the local people to understand the full potential of the *brassica nigra seed oil* for several purposes and applications. The processing of oil seeds and oil-bearing seeds becomes more and more important because an increasing number of people need more and more oil for human nutrition and also for technical applications. A more convenient way is to produce seed oil from local available resource so as to reduce the demand of the society and looking for locally growing high seed oil content plants identification is crucial.

Difference studies showed that *Brassica Nigra seeds* oil processing industries have 31-33% oil contents which is an excellent source of an alternative to producing valuable oil product(lewkowitsch, 1904) and also difference scientific investigations reports that the process parameters such as extraction time, extraction temperature ,solid to solvent ratio, moisture contents, particle size have a significant effect on oil yield. Extraction time, extraction temperature, solid to solvent ratio are selected for this study based on results from previous work. Thus, this thesis attracts to investigate the optimum operating conditions for the production of oil from *Brassica Nigra seeds* that will maximize the oil yield.

1.3. Objectives

1.3.1. General Objective

The general objective of this study was the production and characterization of oil from *Brassica nigra seed* using the Soxhlet extraction method.

1.3.2. Specific Objectives

- To characterize the raw seeds of *Brassica nigra*
- To extract the oil from *Brassica nigra seeds* by soxhlet method
- To define optimum conditions (parameter) such as extraction temperature, extraction time and solid to solvent ratio for achieving the maximum result of the oil yield.
- To investigate the physicochemical properties of the oil extract from *Brassica nigra seeds* oil extracted.
- To compare the physicochemical property of the oil extracted at optimum operating condition with that of commercially available mustard seeds oil used for different application.

1.4. Significance of the Study

- The result of this study will provide an information about *Brassica nigra seed* locally grown in Ethiopia.
- The result will create awareness of the possibility of getting the oil for several applications and possible production.
- The experimental work will give the appropriate operating conditions for extraction of *Brassica nigra* oil.
- The result of this study will be used as baseline information for future study since the product has also an application in pharmaceutical industry and also used as edible oil.

CHAPTER TWO

2. Literature Review

2.1. Historical Background of Oil

Vegetable or plant oils which are often essential and non-essential oils have been known and used by human from pre-historic times. Indeed, apart from water, there is perhaps no liquid chemical but the common is more familiar with its uses in the home than vegetable oils. The major difference between these oils and fatty oils is their volatility. Vegetable or plant oils are used in many industries for diverse purposes which are necessities in advanced civilization, thereby contributing directly to health, happiness and general well-being. The various industries that use these products include pharmaceuticals, animal feeds, baked foods, canned foods, chewing gum, condiments and confectioneries, beverages, soft drinks, cosmetics, soaps, adhesives, insecticides, paints, paper and textile processing industries. Although the extraction and analyses of the chemical composition of vegetable oils has been the subject of numerous investigations, relatively very few reports contain comparative data on samples from different methods of extraction .Since vegetable (plant) oils are such important industrial (especially pharmaceutical) raw materials, there is need to evaluate and analyze the lipid content of this plan Vegetable oils are water-insoluble substances of plant origin which consist predominantly of long-chain fatty acids esters derived from the single alcohol, glycerol ($HOCH_2$ $CHOHCH_2$ OH), and are known as triglycerides. common usage considers as oils, triglycerides des that are liquid at room temperature and as fats, those that are solid or semi-solid under the conditions. This difference in their physical state arises from their chemical composition: oils being composed of low melting fatty acids (mostly unsaturated) while fats are famed from high-melting fatty acids (mostly saturated) However, at higher temperatures, fats (solid) become oils (liquid) so that both terms can , and will be used interchangeable (Aloh et al., 2015).

The mustard plant belongs to the Cruciferae (Brassicaceae) family. Mustard used in food is often a mixture of seeds from two or more species of Brassicaceae, for example, Sinapis alba L. (white or ‘yellow mustard), Brassica nigra (black mustard) and Brassica juncea L. (Brown or oriental mustard). Mustards are functional foods having beneficial physiological effects in humans. Sinapis alba can be used as a source for a wide range of active components including isothiocyanates, phenolics, dithiolthiones and dietary fiber. Flour from the yellow species (Sinapis alba) is used most commonly in Europe, while oriental mustard (Brassica juncea) is used most commonly in the United States and Japan. Mustard consumption in different countries varies according to local food habits. Mustard is principally grown as a source of condiment for the spice trade. Sinapis alba is commonly known as “white" or "yellow" mustard and contributes a "hot “principle which results in a sensation of sweetness and warmth. Brassica juncea, commonly called "brown" or “oriental" mustard, contributes the "pungent" principle. Mustard plant at different types have been widely cultivated and used as spice, medicine and a source of edible oil since ancient times. The mustard seed is rich in protein. The protein is of excellent nutritional quality, being rich in lysine with adequate amounts of sulfur containing amino acids-limiting amino acids in most of the cereals and oilseed proteins. The use of protein rich full-fat and defatted flour shows promises in improving the nutritive value of the final product as well as optimum utilization of the flour. Protein fortification of food is of current interest because of increasing awareness is consumer towards health and quality of food. Mustard is used on some meat products, such as hotdogs and burgers, but is very often an added ingredient in sauces, salads and other foods; for example, mayonnaise, salad dressing, barbecue and related products as well as ketchup, may contain mustard. Mustard is also used in various traditional remedies to stimulate appetite and as a laxative, expectorant and antiseptic agent for the treatment of various gastrointestinal, respiratory and skin disease(Ammar, 2011).

The mustard is used to describe several plants in the Brassica and Sinapis genera which are used as sources of food. There are a number of different types of mustard which are cultivated for different products, including greens and leaves. The incredible diversity and flexibility of mustard plants can cause them to pop up in a wide variety of places, from traditional American Southern cuisine to fiery Indian curries. B. nigra produces black seeds with a very strong and distinctive flavor. Black mustard is often used in Indian and Southeast Asian cooking, where it is incredibly popular; you may have encountered whole mustard seeds in marinades and curries if you eat a lot of Southeast Asian food. Black mustard can also be ground into condiment form. As a condiment, mustard is incredibly diverse. Mustard can be ground into a smooth puree or mixed with whole seeds for more texture. It can also be blended with things like horseradish for spicy mustard, which can be quite fiery, or sugar, for sweet mustard. Some cultures have a tradition of making mustard with beer or wine, creating a very distinctive, complex flavor which complements a range of food(Piri, 2012).

2.2. Description of *Brassica nigra* plants

Brassica nigra, black mustard, is an annual herbaceous plant. It grows up to 2 m (a little over 6 ft), with many branches. The lower leaves are dentate (toothed), pinnatifid (deeply lobed) or lyrate (deeply lobed, but with an enlarged terminal lobe and smaller lateral lobes), and are often hairy, at least on the underside. Upper leaves on flowering stems are narrow and oblong. In contrast to many Brassica species, the leaves are little if at all glaucous (waxy). The yellow, four-parted and cross-shaped flowers, occur in many racemes (spike-like cluster) and produce 4-sided siliques capsular fruit that dehisces (splits open) when mature that may be up to 2.5 cm (1 in) long. Each silique contains 2 to 12 or more reddish brown to black round seeds. A single plant may produce thousands of seeds, which must be harvested by hand or mechanically before they fully ripen, because the siliques spontaneously split and disperse the seeds when they are mature (Al-snafi, 2017).

(A), *brassica nigra Leaves* (B), *Brassica nigra fruit* (C),*Brassica nigra Flower*

Figure 2. 1 The three main part of Brassica nigra plant

2.3. Type of Mustard Seeds

There are three different kinds of mustard seeds: black mustard (*brassica nigra*), popular in the Middle East and parts of Asia; brown and oriental mustard (brassica juncea), whose origin is uncertain, with proposed sources between Eastern Europe, the Middle East or China and yellow mustard (Sinapis alba), which originated in the Mediterranean region and is broadly consumed around the world. Black mustard seeds are roughly globular with a diameter of 1 to 1.5 mm and a dark brown colour; the seed coat is pitted and when soaked in water the seeds produce a strong pungent odour. Brown mustard seeds are similar to black mustard seeds, their diameter is less than 2 mm and have a reddish brown to dark brown colour, it is primarily grown for the European market and has also become popular in North America as a replacement of yellow mustard. Oriental mustard seeds vary in colour from yellow to dark yellow and brown. It is mostly used in the Asian and Japanese markets as a condiment. These varieties have a pungent taste and contain about 28% of oil and 30% of protein (Heath 1981). Yellow mustard seeds, on the other hand, vary in colour from light creamy yellow to yellow and in some cases yellowish brown, have a roughly globular shape and have a diameter of 2 to 3 mm; the seed coat is minutely pitted, and seeds turn mucilaginous when soaked in water. Yellow mustard has a pungent taste and is low in starch, contains about 30% of oil and 25% of protein (Heath 1981) Mustard is a broad-leaved, yellow-flower plant that requires a short growing season, between 85to 95 days for yellow mustard seeds to reach maturity and between 95 to 105 days for the oriental and brown varieties to reach maturity. Crops require an annual precipitation of between 350 and 450 mm and give higher yields in temperate zones with a cool and dry weather. Mustard is capable of growing in a variety of soils from sandy loam to clay loam (Agroecommerce Network Private Ltd. 2002). Mustard seeds are considered more tolerant to frost, drought and heat than other crops like canola or flax, which makes the dry brown and dark soils, warm dry summers and cold dry winters in the southern Canadian prairies an ideal place for mustard growth(Soltero, 2013).

Table 2. 1The Four commonly known species of mustard

Species	Common Name	Type	%Oil in seed
Brassica Juncea	Indian Mustard	Canola like	35—40%
Brassica nigra	Black Mustard	Hardy canola type	38—42%
Sinapis Alba	White Mustard	Condiment Mustard	36—40%
Brassica carinata	Ethiopian Mustard	Canola like	38—42%

(A) Brown Mustard seed

(B) Yellow Mustard Seed

(C) Black Mustard seed

Figure 2.2 Three commonly known mustard seeds

The area and production of mustard seed have been increasing consistently. Being an important source of oil and protein meal, mustard seed is grown across the world. The area of mustard seed has risen from 24.68 million hectares to 30.06 million hectares with a Compounded Annual Growth of 3.02% while the production has increased from 37.32 million tons to 55.97 million tons with a CAGR of 5.70% during the period 2000-2011. European Union is the leading producer of mustard seed in the world accounting for 36% of the world production followed by Canada (24%), China (22%) and India (13%). EU, China and Canada all together accounts for 82% of the world mustard seed production. During the 2011, higher mustard seed production for Canada, Australia, China, and Ukraine more than offsets lower production for EU which was caused by the weather varieties. The purposes of both the rape seed and mustard seed is similar which makes it important to throw light on the major global producers of these two crops which give a clear idea about the global supplies (Grant Thornton ,2016).

Table 2.2 World's top ten mustard producers, 2012 – 2016(million tons), (Grant Thornton ,2016)

COUNTRIES	2012/13	2013/14	20114/15	2015/16
EU	9.424	9.495	10.251	9.671
CHINA	6.045	6.259	6.5	6.195
CANADA	2.95	3.16	3.23	3.07
INDIA	2.35	2.05	2.1	2.32
JAPAN	1.055	1.012	1.075	1.075
US	0.573	0.704	0.717	0.714
MEXICO	0.58	0.58	0.61	0.604
RUSSIA	0.416	0.478	0.49	0.45
PAKISTAN	0.395	0.415	0.474	0.454
UAE	0.294	0.293	0.345	0.345

Table 2.3 The most Competitors of mustard seed production in the world (metric tonnes), (Boersch et al.,2017).

Countries							
Years	Canada	France	Germany	Kazaksthan	Russian	Ukraine	Total
2000	202,200	1,164	10,418	1,680	46,440	8,000	269,902
2001	104,800	1,435	4,369	2,000	28,080	8,200	148,884
2002	154,300	2,033	4,593	1,327	35,040	27,100	224,393
2003	226,100	1,754	5,180	2,938	85,610	68,700	390,282
2004	286,700	2,042	4,060	1,312	55,300	148,100	497,514
2005	183,800	1,549	8,400	1,500	62,900	46,800	304,949
2006	108,200	2,300	9,522	1,060	63,990	21,100	206,172
2007	123,400	3,000	5,825	1,040	11,001	9,700	153,966
2008	161,000	3,500	5,489	610	29,050	38,800	238,449
2009	208,300	9,500	8,849	2,030	23,690	118,200	370,569
2010	186,400	16,016	9,750	1,170	36,408	64,400	314,144
2011	130,000	13,586	11,300	2,200	88,096	30,300	275,482

2012	118,600	13,959	10,500	2,310	41,506	30,980	217,855
2013	154,500	13,999	10,500	8,420	50,414	30,170	268,003
2014	198,000	13,999	8,157	16,198	93,159	79,440	408,778

2.4. The Role of Moisture and Temperature in Oil Extraction on Mechanical pressing

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. Researcher 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(LINDA, 2009).

2.5. Health Benefits of Oil Seeds

In the most of countries, the focus is on brassica crops, which includes canola, rape and mustard. The word rape as applied to oilseed crops is derived from the Latin word rapum that means turnip. Today turnip rapes and the similar, but, more common swede rapes are grown for their oil and widely recognized by their bright yellow flowers. The bright dandelion-yellow flowers of oilseed rape have been a familiar sight across farmland in spring season. Rapeseed oil is one of the highest yield oils, it has very black seeds, which are like poppy seeds, and they are 45% oil and the other 55% is high protein animal feed. The brassica oilseeds contain a high oil content which makes them a good candidate for producing feedstock oils for bio-diesel. For example, spring canola contains upwards of

42% oil as compared to an oil content of about 20% for soybean(Moghal et al., 2013). Edible fats and oils are similar in molecular structure; however, fats are solid at room temperature, while, oils are liquid. Fats and oils are essential nutrients, comprising about 40% of the calories in the diet of the average person. Edible vegetable oils are used as salad or cooking oils, or may be solidified (by a process called hydrogenation) to make margarine and shortening. These products supplement or replace animal products (butter, lard), supplies of which are inadequate to meet the needs of an increasing world population. While there are many uses for industrial vegetable oils, but, total world production is only about 3% of that of edible oils. Industrial applications are based on the properties of particular fatty-acid components of these oils. For example, flaxseed oil, rich in the unsaturated fatty acid linolenic, is a drying oil and is used in protective coatings (paints, varnishes). Vegetable oils are used in putty, printing inks, erasers, coating or core oils, greases, plastics, etc. The residue remaining after the oil has been extracted from oilseeds, is an important source of nutrients for farm animals. Oilseed meals from soybean, peanut, rapeseed and flaxseed are rich in protein; when mixed with other ingredients (cereal grains), and they provide nutritionally balanced feeds. Mustards are rich source of many health benefiting minerals. Calcium, manganese, copper, iron, selenium and zinc are some of the minerals especially concentrated in these seeds. Calcium helps to build bone and teeth. Manganese is used by the body as a co-factor for the antioxidant enzyme superoxide dismutase. Copper is required in the production of red blood cells. Iron is required for the red blood cell formation and cellular metabolism. Mustard seeds and its oil have traditionally been used to relieve muscle pain, rheuamtism and arthritic pain. The mustard oil is applied over scalp and is believed to stimulate hair growth. Its ground seeds act as a laxative, stimulant to gastric mucosa and increase intestinal secretion(Qadri et al., 2013).

Essential Fatty Acid deficiency and Omega 6/3 imbalance is linked with serious health conditions, such as heart attacks, cancer, insulin resistance, asthma, lupus, schizophrenia, depression, postpartum depression, accelerated aging, stroke, obesity, diabetes, arthritis, ADHD, and Alzheimer's Disease, among others. A primary function of Essential fatty acids is the production of prostaglandins, which regulate body functions such as heart rate, blood pressure, blood clotting, fertility, conception, and play a role in immune function by regulating inflammation and encouraging the body to fight infection. Essential fatty acid supports the cardiovascular, reproductive, immune, and nervous systems. The human body needs Essential fatty acids to manufacture and repair cell membranes, enabling the cells to obtain optimum nutrition and expel harmful waste products. Now a day's Mustard Essential Oil is attributed with its properties as a stimulant, irritant, appetizer, antibacterial, antifungal, insect repellant, hair vitalizer, cordial, diaphoretic, antirheumatic and tonic substance. Mustard essential oil is a very strong stimulant, just like mustard oil. It is particularly effective in stimulating circulation, digestion and excretion. It also stimulates the secretion of gastric juices and bile from the spleen and liver. This essential oil acts as an appetizer and boosts huger. It irritates the inner lining of the stomach and intestines, stimulates digestive juices, and creates a feeling of hunger(Lawal et al.,2016).

2.6. Mustard Seed Production

the production process of commercial mustard seed can be produces, after harvest, mustard seeds should be dried to an appropriate moisture content of 9% and then stored at temperatures lower than 20 °C. The drying process is essential to prevent mold growth, increase shelf life and guarantee long-time storage. Partial drying of the crop starts immediately on the farm and then is completed at the processing plants. Drying temperature should not be higher than 43 °C, otherwise it might damage the seed(Fahmi, 2016).

Prior to milling and processing the mustard seeds into various products such as flour, bran and ground mustard, the crop is usually partially deoleated to facilitate the crushing process. Mustard flour is a fine powder obtained from the seed kernel (endosperm). It is prepared by successive milling and sifting to remove the bran (testa and aleurone layer) from the interior part (embryo and cotyledons) of the seed. Mustard flours are used primarily to give mustard flavor to a product and for their functionality as emulsifiers. They are commonly used as binding agent in dressings, sauces, pickles and processed meat products, especially sausages. Mustard bran is the by-product from flour production, which is widely used as a natural thickener in sauces and dressings. The majority of the functional properties of mustard bran such as water-banding, emulsifying, and stabilizing are mainly attributed to the present of water-soluble polysaccharides in its structure. Ground mustard is another commercial product that is produced from grinding the whole mustard seed including the kernel and bran parts. It is widely used in processed meat products such as, salami, bologna, and frankfurters for flavoring, emulsifying, water-binding and also as a bulking agent for the inexpensive replacement of meat with vegetable protein sources. Ground mustard is also used in the production of pickles, sauces and dressings. Altogether, the mucilaginous compounds of the bran part play an important role in the functional properties of ground mustard and Mustard oil is cold extracted from mustard seeds, which depending on the mustard variety, contain between 29% and 36% fixed oil. Residues from the extracted seed kernels are known as "press cake". Well refined mustard oil has pleasurable flavour with a brownish yellow colour. In the European countries and North America where, mustard seed is primary used as condiment, only small portions are cold pressed for extracting the oil. However, in Asian countries such as India, mustard seed is more likely used to produce mustard cooking oil (Fahmi, 2016). The plant is believed to be native to the southern Mediterranean region of Europe and possibly South Asia where it has been cultivated for thousands of years. The spice is generally made from ground seeds of the plant, with the seed coats removed. The small (1 mm) seeds are hard and vary in color from dark brown to black. They are flavorful, although they have almost no aroma. The seeds are commonly used in Indian cuisine, for example in curry, where it is known as rai. The seeds are usually thrown into hot oil or ghee, after which they pop, releasing a characteristic nutty flavor. The seeds have a significant amount of fatty oil. This oil is used often as cooking oil in India. In Ethiopia, where it is cultivated as a vegetable in Gondar, Harar and Shewa, the shoots and leaves are consumed cooked and the seeds used as a spice.

Brassica nigra belongs to the botanical family Brassicaceae (Cruciferae). The plant is found in some parts of the world like southern Mediterranean region of Europe, south Asia, Canada, India, Ethiopia, North America, German, Ukraine, Myanmar, Russian, New Zealand. Brassica nigra originated in the Middle East(Danlami, 2016). The plant can grow from two to eight feet tall, with racemes of small yellow flowers. The leaves are covered with small hairs. Stem base, half way branched, quite erect, bluish lower part. Alternate, stalked basal bluish green leaves. Its seeds grow in long, slender pods. Each pod contains 10 – 12 brown or black seeds. Mustard seeds have been highly prized medicinal as well as culinary spice being in use since ancient times. The seeds are obtained from mustard plant (Brassica spp.) which also include cabbage, broccoli, brussels sprouts, etc. Mustard seeds have high energy content, having 28–32% oil with relatively high protein content (28–36%). Well refined mustard oil has a yellow to brown color and its smell and taste are enjoyable. Mustard oil has a special fatty acid composition: 20–28% oleic acid, 10–12% linolic, 9.0–9.5% linolenic acid and 30–40% erucic acid. Mustard seeds contain 28-32% protein by weight, although these values can vary slightly between varieties, growing regions and crop years. The amino acid composition of mustard protein is well balanced; it is rich in essential amino acids (Sharif et al.,2017).

Mustard and its oil have been used as a topical treatment for rheumatism and arthritis, as a foot bath for aching feet, and in the form of plasters over the back and chest to treat bronchitis and pneumonia. Mustard oil contains a high amount of selenium and magnesium, which gives it anti-inflammatory properties. It also helps stimulating sweat glands and helps lowering body temperature. In traditional medicines, it is used to relieve the pain associated with arthritis, muscle sprains and strains. Mustard is not just edible oil also an important medicine in the indigenous Ayurveda system of healthcare. It is used for therapeutic massages, muscular and joint problems. Oil with garlic and turmeric is used for rheumatism and joint pains. Mustard oil is also used as a mosquito repellent (Ammar, 2011).

Vegetable oils in human diet constitute an important source of energy and have considerable importance in human health. Besides being the gastronomic delights and a source of energy, edible vegetable oils provide fat-soluble micronutrients and essential and nonessential fatty acids, which perform various functions. Besides the fatty acids, unsaponifiable matter is a major chunk of edible vegetable oils. This includes various micronutrients such as tocopherols, tocotrienols, α carotene, oryzanol, squalene etc. depending on the type of the edible oil. The micronutrients in vegetable oils have various functions. Tocopherols, for example, are recognized for the antioxidant activity, and have been observed to be useful in degenerative diseases like cancer, cataract, cardiovascular disease and ageing. Tocopherols in oil act through several mechanisms such as inhibiting the lipid peroxidation, chain termination, singlet oxygen quenching, and radical scavenging to deactivate free radicals that are produced during the oxidation of biomolecules. Mustard oil is extracted from the seeds of mustard plant (Brassica campestris L.), belonging to the cruciferous family of plants. It is originally from the Mediterranean regions, but it grows easily in many parts of the world. It has a characteristically pungent flavor and aroma. Though this oil is nutty tasting it is good for heart and also has many other benefits. Mustard oil contains a high amount of selenium and magnesium, which gives it anti-inflammatory properties. It also helps stimulating sweat glands and helps lowering body temperature. In traditional medicines, it is used to relieve the pain associated with arthritis, muscle sprains and strains. Seed paste applied on wounds whereas paste of leaf said to heal cattle wounds. Mustard oil consists of fatty acids like omega alpha 3 and omega alpha 6 which have beneficial properties. Specialty oils having high amounts of a specific fatty acid are of immense importance for both nutritional and industrial purposes. Oil high in oleic acid has demand in commercial food-service applications due to a long shelf-life and cholesterol-reducing properties. Both linoleic and linolenic acids are essential fatty acids; however, less than 3% linolenic acid is preferred for oil stability. High erucic acid content is beneficial for the polymer industry, whereas low erucic acid is recommended for food purposes. Therefore, it is important to undertake systematic characterization of the available gene pool for its variable fatty acid profile to be utilized for specific purposes. Mustard belongs to the Brassica plants. With about 30-35% the fraction of proteins in mustard seed is very high. Some of these proteins are known for being allergenic, predominantly heat resistant making them stable to different production processes. In addition to brown mustard (Brassica juncea) and black mustard (Brassica nigra) primarily yellow mustard (Sinapsis alba) is used as an ingredient in many foods and food preparations. For mustard allergic persons hidden mustard allergens in food are a critical problem. Already very low amounts of mustard can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, mustard allergic persons must strictly avoid the consumption of mustard or mustard containing food. Cross-contamination, mostly in consequence of the production process, is often noticed. The sausage production process is a representative example. This explains why in many cases the existence of mustard residues in food cannot be excluded. For this reason sensitive detection systems for mustard residues in foodstuffs are required (Arabia, 2013).

Brassica nigra (L.) synonym- Sinapis nigra, B. sinapioides, commonly known as black mustard, brown mustard, mustard noire and senf. is an annual herb native to most parts of

Europe, the Mediterranean region, and other parts of north Africa and has been naturalized in Great Britain and North America, and in the Canterbury and Otago regions of New Zealand. Use of the active constituent, allyl isocyanate, of B. nigra and spp., either internally or externally, is not recommended for therapeutic purposes in Australia due to the irritant effect of the chemical. In Australia, black mustard oil is utilized for production of soap and for medicinal remedies; the seeds are valued as a stimulant, irritant, emetic and to treat bronchitis. Regular consumption of black mustard seeds is reported to improve the body's biological defense mechanisms against cancer development and studies have shown that it can reduce the rate of colon, bladder and lung carcinogenesis It relieves congestion by drawing the blood to the surface as in head afflictions, neuralgia and headaches. In another Australian study it was suggested that the antioxidant activity and polyphenolic content of *Brassica nigra* can inhibit pancreatic α- amylase enzyme resulting in improved glucose tolerance in diabetics. The α-amylase enzyme is one of the key enzymes in the small intestine for the digestion of starch, breaking it down to glucose and maltose which leads to increased postprandial glucose levels and inhibition of this enzyme is one of the pathways to manage diabetes(Rahman, 2018).

2.7. Importance of Mustard Oil Seeds

Brassicas play an important role in the world agriculture as oil seeds, vegetables, forage and fodder, green manure and condiments. Indian mustard, is predominately play important role the oil seed economic. A large proportion of mustard oil is used directly in cooking, the oil is also used in the manufacture of salad dressage and table oils, confectionery fats and shortenings which is two are used in making cakes, biscuits, pastries and many other products. It is also of great importance in the manufacture of margarine. The fatty acids and their derivatives are widely used for industrial purpose. Oils may not be used directly in the preparation of paints, linoleum or inks, due to its semidrying nature. However, its derivatives and fatty acid can be used for this purpose. It is also used in production of rubber, tanning industries, as lubricants, and manufacture of soaps, detergents and bonding compounds. It seems likely that many new uses will be found for oil in future, such as medium for pesticide application, as herbicide additives and as fuel in the form of biodiesel. Oilseed cake or meal is a byproduct during the extraction of oil from the seeds. It is an important source of protein for animals and it's currently being considered as a potential supplementary source for human beings. The cake has 35 to 40percent crude protein depending upon the variety as well as condition of growing and processing. The amino acid content is comparable well with soybean meal, but it is richer in Sulphur amino acids and poorer in lysine. The fiber content in meal is very higher, as compared to soybean meal, which lead to a lowering of the metabolically and digestible energy values and decrease in the bioavailability of minerals. The low glucosinolate meal is good food for young ruminants and lactating cows, piggery and poultry purposes. The economically most important product is oil. The mustard oil contains substantial amount of unsaturated fatty acid and the low concentration (around 7 percent) of saturated fatty acid. In unsaturated fatty acid it contains oleic acid (8-40percent), lenolenic acid (5-10 percent), linoleic acid (10-29 percent), eicosinoic acid (5-12 percent) and erucic acid (40-55 percent). The Brassica seed meal (oil cake) contains: protein (36-38 percent), carbohydrate (14-16 percent), fiber (10-15 percent), moisture (6-8 percent), ash (4-6 percent), mineral (3-4 percent), vitamins (0.7-0.9 percent) glucosinolate (2-3 percent), phytic acid (3-6 percent), sinapine (1-1.5percent) and 1.6-3.1 percent of tannin (Misra, 2008). Specialty oils having high content of a specific fatty acid are of immense importance for both nutritional and industrial purposes. Oil rich in oleic acid has high demand in commercial food-service applications due to its long shelf-life and cholesterol-reducing properties(Misra, 2008).

Table 2.4 Characteristics of mustard oi*l*(Asad Hasan, 2003)

Characteristic	Value
Specific gravity 25°C	0.906 - 0.910
Refractive index 30°C	1.469 - 1.485
iodine value	87 – 122
Saponification value	170 – 200
Colour	Yellow-Brown
Acid value	0.6 mg/KOH per gram
Peroxide value	10

2.8. Frying Properties of Mustard Oil

mustard oil for frying and for making oil-based pickles. The unrefined oil has a unique, strong flavor. Mustard: High smoke point makes it ideal for frying. When oil is to be used for frying it has to be refined variety which one should use. Like Extra Virgin Olive Oil (EVOO) kachi ghani Mustard Oil is also cold pressed oil. The virgin or cold compressed oils are delicate and hence get denatured on heating. When we compare mustard, soya and sunflower for their smoking point's mustard is the most stable of all. Mustard oil contains SFA, MUFA, PUFA 13%, 60%, 21% respectively and Smoking point- 254◦C (489◦ F) can be used in Cooking, frying, deep frying, salads, and dressings. There is a major difference between the PUFA percentage of olive oil (extra virgin) to mustard oil- saturated fats 14%/ 13%, MUFA 73%/ 60%, PUFA 11%/ 21%. High PUFA of mustard oil can be attributed to its being rich in Omega-3 and Omega-6 and thus makes it a better choice for daily cooking. For salad dressings, low heat applications it will be better to use olive oil. By blending different types of oils, the consumer can be offered a better-quality product with respect to flavour, frying quality and nutritive value. The oil used for frying must have good flavor and oxidative stability in order to achieve good shelf life for the products fried. To meet today's consumer demands the frying oil must be low in saturated fat, linolenic acid, and have good flavor, high oxidative stability(Kumari, 2015).

Table 2.5 Proximate analysis of Brassica nigra seeds(Panchal et al.,2014)

Components	Values (%) (n = 3)
Moisture (%)	4.16±0.5
Ash (%)	5.14±0.4
Crude fat (%)	30.30±0.5
Crude fibre (%)	0.30±1.0
Crude protein (%)	24.70±0.3
Carbohydrate (%)	35.40±1.0

2.9. Application of Mustard Seed Oil

Mustard is a condiment made from the seeds of a mustard plant (white or yellow mustard, Sinapis hirta; brown or Indian mustard, Brassica juncea; or black mustard, Brassica nigra). The whole, ground, cracked, or bruised mustard seeds are mixed with water, salt, lemon juice, or other liquids, and sometimes other flavorings and spices, to create a paste or sauce ranging in color from bright yellow to dark brown. Mustard has been used medicinally since the time of the Greek physician Hippocrates (460—375 BC). The Romans ate the whole seed as a spice during meals, but mustard was not milled for use at table until the eighteenth century. Today, mustard is number one in the world spice trade in terms of volume. That is perhaps a little-known fact, as most people think of spices in terms of nutmeg, mace, cinnamon, ginger and the other tropical spices and condiments. The action of mustard as a condiment is due to three qualities. These are its ability to stimulate appetite and salivation and so hasten the first stage of digestion, its ability to break down indigestible fats and meat fibers, and its ability to stimulate digestive juices to complete the digestive process. Many people find the taste itself adds to their enjoyment, so aiding good digestion. Mustard contains an essential oil (allyl isothiocyanate) which, when applied to the outside of the body, increases the circulation and so helps the elimination of poisons. Two or three tablespoons of mustard powder can be used in the bath to ease chills, relax tired muscles and promote sleep(Allwyn et al., 2014).

Mustard oil consists of fatty acids like omega alpha 3 and omega alpha 6 which have beneficial properties. Specialty oils having high amounts of a specific fatty acid are of immense importance for both nutritional and industrial purposes. Oil high in oleic acid has demand in commercial food-service applications due to a long shelf-life and cholesterol-reducing properties. Both linoleic and linolenic acids are essential fatty acids; however, less than 3% linolenic acid is preferred for oil stability. High erucic acid content is beneficial for the polymer industry, whereas low erucic acid is recommended for food purposes. Therefore, it is important to undertake systematic characterization of the available gene pool for its variable fatty acid profile to be utilized for specific purposes(Arabia et al., 2013).

2.10. Erucic Acid

Erucic acid constitutes about 30–60% of the total fatty acids of mustard seed. Erucic acid is the major and characteristic component occurs extensively in seed oils of the mustard family (Cruciferae) and Tropaeolaceae. It distinguishes other vegetable oils which generally contain fatty acids with 16 and 18 carbon atoms. Erucic acid, also known as cis–13- docosenoic acid, is an unbranched, monounsaturated fatty acid with a 22– carbon chain length and a single double bond in the omega 9 position. Erucic acid, as a fatty acid, is digested, absorbed and metabolized, for the most part, like other fatty acids. [http://www.foodstandards.gov.au]. As erucic acid is a compositional fatty acid, many reviews have concentrated on the general physiological processes for the absorption, digestion and metabolism of lipids and fatty acids. The High erucic acid content is beneficial for the polymer industry, whereas low erucic acid is recommended for food purposes (Danlami,et al., 2016)

Table 2.6 Application of Erucic acid(Nath, 2008)

Application	Units (Equivalent × 10^3 metric tons of 90% erucic acid)
Surfactants	1.38
Detergents	0.75
Plastic additives	7.96
Food additives	0.77
Cosmetics	1.76
Pharmaceuticals	0.59
Personal care products	0.84
Ink additives	0.30
Textiles	0.94
Lubricants	0.50
Fuel additives	0.24

2.11. Essential Fatty Acid

Mustard oil is extracted from the black mustard seeds, which have been macerated in warm water by steam or water distillation. Although mustard seeds and powder do not contain allyl isothiocyanate, it is formed when the seeds came in contact with water and the essential oil is formed when a glycoside decomposes due to enzymatic action. The essential oil is not present in the fresh seeds or the powder, and so preparations made of these do not contain Allyl isothiocyanate. White mustard seeds are not containing any essential oil. Essential Fatty Acids are the "good fats" and recently it became research topic of great interest. Fatty Acids (EFAs) are necessary fats that humans cannot synthesize, and must be obtained through diet. EFAs are long-chain polyunsaturated fatty acids derived from linolenic, linoleic, and oleic acids. There are two families of EFAs: Omega-3 and Omega 6. The number following "Omega-" represents the position of the first double bond, counting from the terminal methyl group on the molecule. Omega-3 fatty acids are derived from Linolenic Acid, Omega-6 from Linoleic Acid, and Omega 9 from Oleic Acid. A desirable n- 6/n-3 ratio is in the range of 5 –10. A ratio above 50 is injurious to health. Further, health agencies such as WHO and American Heart Association recommends that fats and oils should not supply more than 30% energy of diet and that the fatty acid composition in oil and fats should have a SFA, MUFA, PUFA ratio of 1: 1.5: 1. A higher MUFA in oils and fats is recommended for health benefits. Health agencies, therefore, recommend that human should consume more ω-3 fatty acids(Danlami et al ., 2016).

Table 2.7 Fatty Acid Composition of Mustard oil(Yadav & Kumari, 2015)

Fatty Acid Composition of Mustard oil		
No	Name of Acid	Range
1	Palmitic	1-3%
2	Stearic	0.4-3.5%
3	Arachidic	0.5-2.4%
4	Behenic	0.6-2.1%
5	Lignoceric	0.5-1.1%
6	Oleic	12.24%
7	Eicosenoic	3.5-11.6%
8	Erucic	40-55%
9	Linoleic	12-16%
10	Linolenic	7-10%

Table 2.8 Chemical structure of common fatty acids(Muhammad Mahbubul Alam, 2014).

Fatty acids	Systematic name	Structure x: y	Formula
Lauric	Dodecanoic	12:0	$C_{12}H_{24}O_2$
Myristic	Tetra decanoic	14:0	$C_{14}H_{28}O_2$
Palmitic	Hexadecenoic	16:0	$C_{16}H_{32}O_2$
Stearic	Octadecanoic	18:0	$C_{18}H_{36}O_2$
Arachidic	Eicosanoic	20:0	$C_{20}H_{40}O_2$
Behenic	Docosenoic	22:0	$C_{22}H_{44}O_2$
Lignoceric	Tetracosanoic	24:0	$C_{24}H_{48}O_2$
Oleic	Cis-9-octadecenoic	18:1	$C_{18}H_{34}O_2$
Linoleic	Cis-9,cis-12-octadecadienoic	18:2	$C_{18}H_{32}O_2$
Linolenic	Cis-9,cis-12,cis-15-octadecatrienoic	18:3	$C_{18}H_{30}O_2$
Erucic	Cis-13-docosenoic	22:1	$C_{22}H_{42}O_2$

The Vegetable oils are extracted from plants, the basic constituents of which are triglycerides. Triglycerides are esters of 3 long chain acids, commonly called fatty acids with a glycerol. Vegetable oils contain 90 to 98% triglycerides and small amount of mono and diglycerides. Fatty acids vary in their carbon chain length and in the number of double bonds. The fatty acids which are commonly found in vegetable oils are stearic, palmitic, oleic, linoleic and linolenic. The chemical formulae of common fatty acids are given in Table 2.8. In the structure column x: y indicates x number of carbon atoms in the fatty acid chain with y number of double bonds. Due to their varying compositions, the properties of vegetable oil differ from each other and therefore their performance as engine fuel also differs((Muhammad Mahbubul Alam, 2014).

2.12. Methods for Extraction of Oil

2.12.1. Traditional Extraction of 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(LINDA, 2009).

2.12.2. Mechanical Extraction

The technique for oil extraction by mechanical presses is the most conventional one among the other methods. In this method, either a manual ram press or an engine driven screw press was used. the design of mechanical extractor is very important in conventional mechanical presses techniques since the oil yield is affected by the type of mechanical extractor is used(Ashwath et al .,2015). pressing of oil-bearing materials for the production of oil has been known for several hundred years and only after World War II this process was replaced by solvent extraction. Today, more than 98% of the oil production worldwide is carried out by solvent extraction, but for specialty oils, such as extra virgin olive oil or virgin rapeseed oil, oils produced in rural areas, or for pre-pressing before solvent extraction this old technique is still in use. The aim of the pressing process is to separate the oily phase from the solid phase of the seed material(Matthäus, 2012). Mechanical expression results in high quality oil, but has a relatively low yield. Generally, it is only used for smaller capacity plant speciality products or as a pre-press operation in a large-scale solvent extraction plant. The mechanical pressing of oilseed is the common method of edible oil extraction used in the world(Sumaya, 2009).

2.12.3. Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but it can also be from liquids. SFE can be used as a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. Extraction conditions for supercritical CO_2 are above the critical temperature of 31°C and critical pressure of 74 bars. Addition of modifiers may slightly alter this. Supercritical extraction mostly uses carbon dioxide at high pressure to extract the high value products from natural materials. Unlike other processes, the extraction process leaves no solvent residue behind. More over the CO_2 is non-toxic, nonflammable, odorless, tasteless, inert, and inexpensive. Due to its low critical temperature 31°C, carbon dioxide is known to be perfectly adapted in food, aromas, essential oils and nutraceutical industries (Bhatbhage et al., 2010).

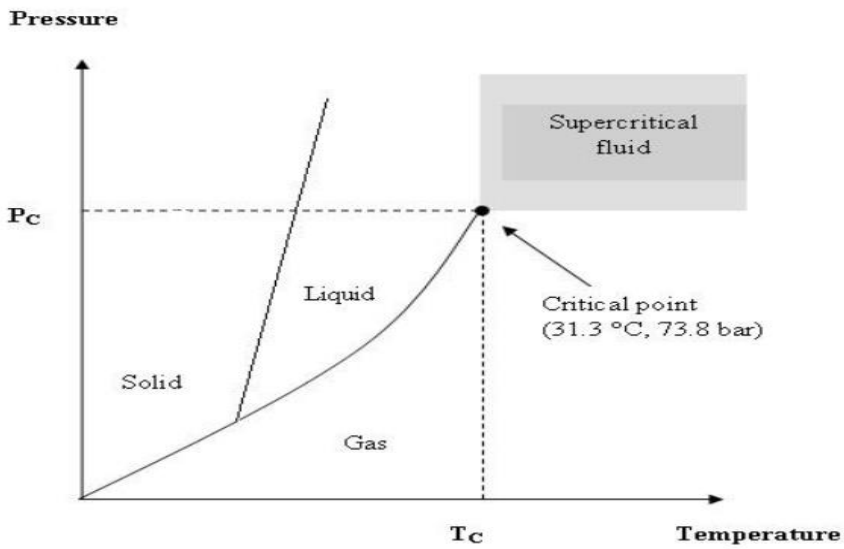

Figure 2. 3 Phase Diagram of CO2

Table 2.9 The advantages and disadvantages of the SFE process

Advantages	Disadvantage
1.Enhanced extraction efficiency	1. High capital investment
2. Tunability of the solvent strength	2. Large number of variables to optimize
3. Low organic solvent consumption	3. Strong dependence on matrix analyte interactions
4. Preservation of bioactive properties and organoleptic properties of the extracts	4. Difficulties in scale-up and technology transfer
	5. Difficulty in implementing continuous extraction processes
5. In-line integration with sample preparation and detection method	6. Difficulty of extracting more polar compounds

2.12.4. Solvent Extraction

Extraction by solvent is recommended if it is necessary to reduce the oil content in the raw material to lower than 2%. This means the aim of extraction by solvent is to remove as much oil as possible from the oilseed. From an economical point of view In most cases, n-hexane is used as solvent, because it is cheap, has good oil solubility at relatively low temperature, has an appropriate boiling temperature, is noncorrosive to metal, does not react chemically with the oil, is stable under the process conditions, is not mixable with water, which eases the separation of water from the seeds, and it is easily and completely removed from the residue with low energy input and without impairment of the raw oil. Nevertheless, this solvent also has some disadvantages with regard to its potential danger from which results high requirements concerning the equipment. Hexane is highly flammable and mixtures of air and hexane are explosive(Matthäus, 2012). Solvent extraction is the transfer of solutes from a solid, usually in particulate form, to contiguous liquid, the extract. If the solute is uniformly dispersed in the solid, the material near the surface will be dissolved first, leaving a porous structure in the solid residue. The solvent will then have to penetrate this outer layer before it can reach further solute, and the process will become progressively more difficult and the extraction rate will fall. If the solute forms a very high proportion of the solid, the porous structure may break down almost immediately to give a fine deposit of insoluble residue, and access of solvent to the solute will not be impeded. Generally, the process can be considered in three parts: first the change of phase of the solute as it dissolves in the solvent, secondly its diffusion through the solvent in the pores of the solid to the outside of the particle, and thirdly the transfer of the solute from the solution in contact with the particles to the main bulk of the solution. Any one of these three processes may be responsible for limiting the extraction rate (J. F. Richardson,2002).

Table 2.10 Advantages and disadvantages of n-Hexane as solvent for Extraction of Brassica nigra seed oil.

Advantages	Disadvantages
1.Repeatable and reproducible results and process	1.Less sought after than virgin oil
2.High oil yields	2.High potential for solvent contamination
3.Relatively simple and quick	3.Safety issues and environmental concerns
4.Suitable for bulk oil extraction	4.Very costly if the hexane cannot be recovered
5.Low capital investment	5.High n-hexane requirement.
6.No especial equipment required	
7.Hexane can be recovered and reused, reducing cost significantly	

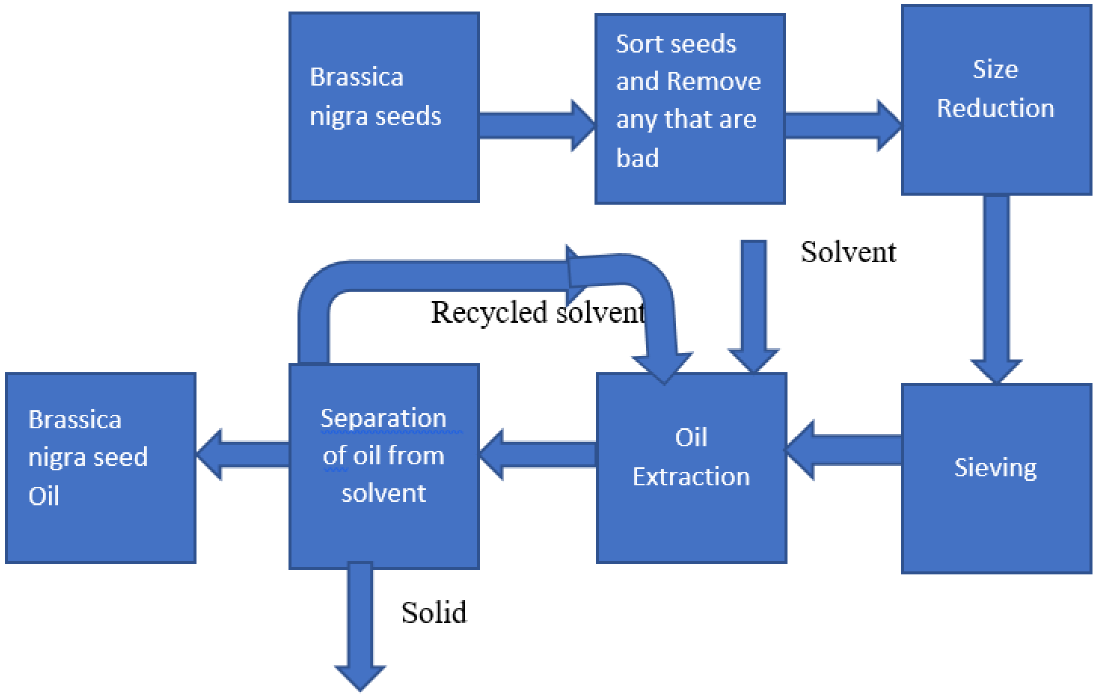

Figure 2.4 Solvent extraction *of Brassica nigra seed* oil

2.12.4.1 Soxhlet Apparatus

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. On the laboratory scale, the Soxhlet apparatus is used. It consists of a flask, a Soxhlet extractor and a reflux condenser. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. Mustard seed powder is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapor travels up a distillation arms and floods into the chamber housing the thimble of mustard seed powder. The condenser ensures that any solvent vapor cools, and drips back down into the chamber housing of the seed powder. The chamber containing the seed powder slowly fills with warm solvent. Some of the oil will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any seed powder to the still pot. This cycle may be allowed to repeat many times, over hours or days. There are several advantages of Soxhlet extraction. The most important are that the sample is repeatedly brought into contact with fresh portions of the solvent. This procedure prevents the possibility of the solvent becoming saturated with extractable material and enhances the removal of the analyte from the matrix. Moreover, the temperature of the system is close to the boiling point of the solvent. This excess energy in the form of heat helps to increase the extraction kinetics of the system. Soxhlet extraction has several disadvantages, including it requires several hours or days to perform; the sample is diluted in large volumes of solvent, and due to the heating of the distillation flask losses due to thermal degradation and volatilization have been observed(Abdurahman H et al ., 2016).

Table 2.11 Advantage and disadvantage of Soxhlet extractor

Advantage	Disadvantage
- The displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix. - Maintaining a relatively high extraction temperature with heat from the distillation flask. - Long experience of use - No fltration of the extract is required. - Simple to operate - High extraction temperature enables exhaustive recovery of interest - Economical	- Agitation is not possible in the Soxhlet device. - Considerable solvent consumption - The possibility of thermal decomposition of the target compounds cannot be ignored as the extraction usually occurs at the boiling point of the solvent for a long time. - Non-selective extraction - Risk of thermal decompositions as the extraction is conducted at the boiling point of the solvent - Only temperature and solvent type can be Varied

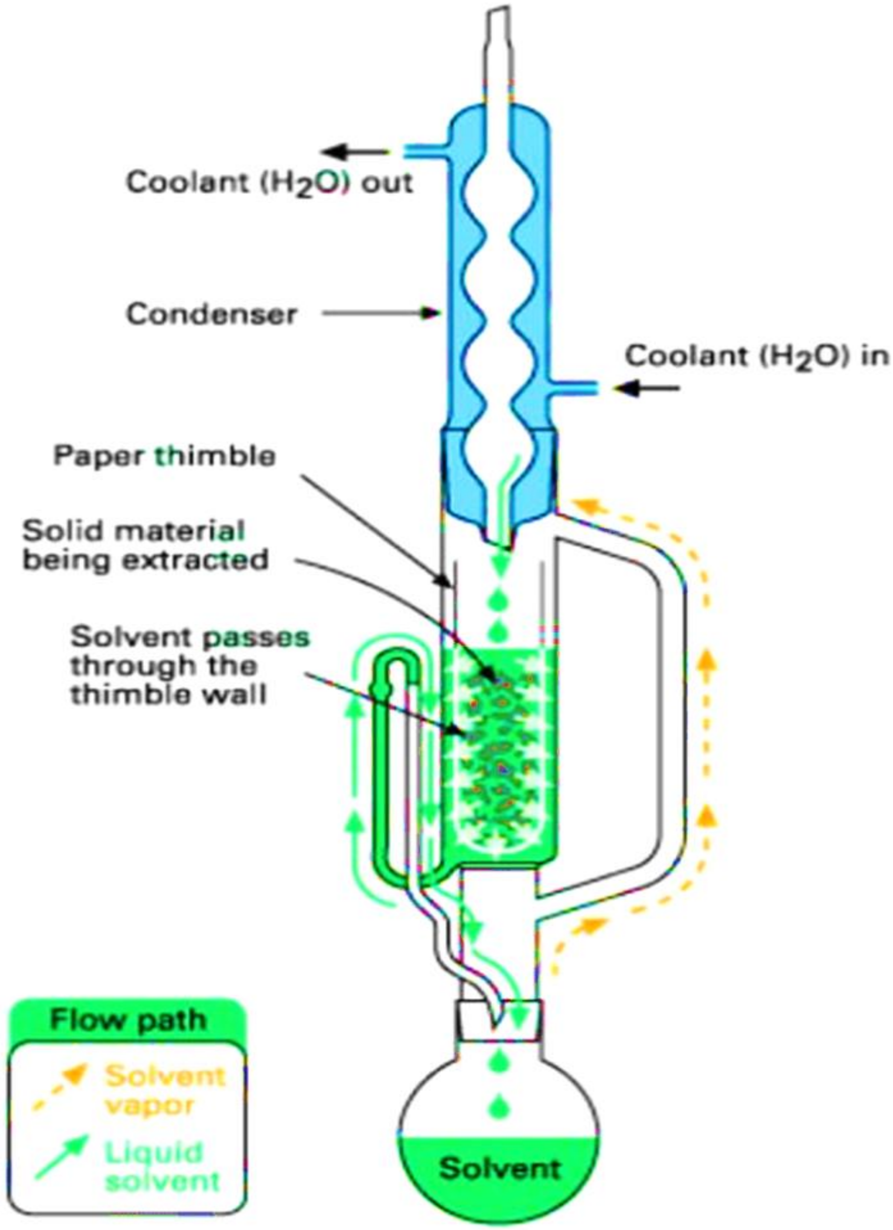

Figure 2.5 Soxhlet extractor (YAHAYA, 2013)

2.12.4.2 Factors Affecting the Rate of Solvent Extraction

The efficiency of solvent extraction of oil from oil seeds can be influced by different factors such as particle size, solvent type used, solid to solvent ratio, temperature and extraction time, geographical variation that the seed is originated (Genetic variation), moisture content of the seed.

2.12.4.3 Particle Size of seeds

Particle size affects the extraction rate in a number of ways. The smaller the size, the greater is the interfacial area between the solid and liquid and therefore, the higher is the rate of transfer of material and the smaller is the distance the solute must diffuse within the solid phase On the other hand, the surface may not be so effectively used with a very fine material if circulation of the liquid is impeded, and separation of the particles from the liquid and drainage of the solid residue are made more difficult (J. F. Richardson,2002). For most efficient extraction, the particle size should be reduced during the procedure by micro grinding. A sufficiently small particle size is difficult to achieve with a single grinding step(Barthet et al. , 2004).

The phenomenon could be attributed to the fact that smaller particles have larger amount of surface area as well as an increased number of ruptured cells resulting in a high oil concentration at the particle surface. Little diffusion into the particles surface takes place; therefore, the amount of oil available for extraction is proportional to the surface area. larger particles present smaller contact surface areas and are more resistant to solvent entrance and oil diffusion. Therefore, less amount of oil will be transferred from inside the larger particles to the surrounding solution compared to the smaller one(Ebewele et al., 2010).

2.12.4.4 Extraction Temperature of seeds oil

Temperature generally affects both the equilibrium and mass transfer rate of the extraction process. In the former, a higher temperature results in greater solubility of compounds in the solvent, resulting in a larger value. In the latter, the higher the temperature, hence increasing rate of extraction. In conventional solid-liquid extraction processes, temperature is limited by the boiling point of the solvent. Increasing the temperature would not only enhance extraction of the bioactive compound but also other soluble compounds as well and at times, reduce the selectivity of the process for the compound of interest. It is also important to bear in mind that increasing the extraction temperature may also potentially degrade thermolabile bioactive compounds. Thus, an optimized balance has to be determined when selecting the extraction temperature(CHEAH LI CHIN, 2009).

2.12.4.5 Extraction Time

In general, a prolonged extraction time results in an increased yield of seed oil compound until equilibrium is reached. Thereafter, the concentration of seed oil compound will not increase further but there will have greater liability for degradation. Prolonged extraction time is also not desirable from an economic standpoint of labor and energy requirements; therefore, it is best to find out the modern extraction technologies

2.12.4.6 Choice of Solvents for seeds oil extraction

Successful determination of biologically active compound from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to complex or dissociate(Shrivastava et al. , 2010).

Solvent extraction was developed because it allows a more complete extraction at lower temperatures. It begins to be economically attractive where large quantities of seed can be processed (at least 200 tons per day for continuous-feed processes); where storage, transportation, power, water, and solvent supply are adequate; and where occupational safety and training standards can be enforced but Solvent extraction using hexane has several drawbacks including high capital equipment cost and operational expenditures, the perpetual hazard of fire and/or explosion as well as the residual solvent associated with both oil and meal.

A high solid-to-solvent ratio was found to be favorable in extraction of antioxidant compounds. These results were consistent with mass transfer principles where the driving force for mass transfer is considered to be the concentration gradient between the solid and the solvent. A high solid-to-solvent ratio could promote an increasing concentration gradient, resulting in an increase of diffusion rate that allows greater extraction of solids by solvent

The most commonly used solvents for food processing are water, aqueous solutions of acids and nontoxic salts, commercial hexane, and in some cases other alkanes, ethanol and to a lesser extent the other lower alcohols, methylene chloride, methyl ethyl ketone, and acetone. The use of alcohols and alcohol-water mixtures for extracting vegetable oil has attracted attention recently. These solvents can provide greater selectivity than hexane, which is currently used for most vegetable oil extractions. The use of solvents and soxhlet extraction process for the extraction of *Brassica nigra seed* oil is generally preferred choice. This is due to very high oil yield and less turbid oil obtained than from mechanical pressing. Due to the relatively low operating cost compared to supercritical extraction. The solvent n-hexane is the most commonly used, as well as preferred choice in extraction of oils from seed, even in *brassica nigra seed* oil solvent extraction. This is largely due to its availability at a reasonable cost and its suitable functional characteristics for oil extraction. Amongst such characteristics is its high solvent power for triglycerides at fairly low temperatures, non-reactivity with oil and oil with equipment.

CHAPTER THREE

3.Materials and Methods

3.1 Materials and equipment

The experimental work has been done in School of Chemical and Bio Engineering laboratory at Addis Ababa Institute of Technology

Materials used during the experiment were *Brassica nigra Seeds*, n-hexane (99.9%), Absolute ethanol (99.5%), potassium hydroxide (85%), hydrochloric acid (35.4%) of concentration, phenolphthalein indicator powder, filter paper, distilled water, ethyl alcohol, diethyl ether, glacial acetic acid, chloroform solution, potassium iodide, sodium thiosulphate solution, starch powder and Wij's solution. All the chemicals and reagents were purchased from Edulab Trading PLC and obtained from School of Chemical and Bio Engineering.

The equipment used were Soxhlet apparatus, Graduated cylinder (10,50,100 mL), Electronic weighing balance, Beaker (50, 250, 500, 1000 mL), Erlenmeyer Flask (250, 500,1000 mL), Analytical balance, water bath, condenser, oven, Vibro Viscometer, flask, density meter and coffee miller beaker, test tubes, sieve,

3.2. Raw Material Preparation

The raw material was purchased from Shola market and came from Mekele, Then Sort seeds and remove any moldy or rotten and the *Brassica nigra seeds* was dried in the oven at 43^0C for 8 hours for the size reduction of seeds.

3.3. Sample analysis

3.3.1. Determination of moisture content of the Brassica nigra seeds

50gram of the brassica nigra seeds were weighed and dried in an oven at 106℃ and the weight was measured every two hours interval for 8 hours. The procedure was repeated until a constant weight was obtained and the percentage moisture content of the seeds was determined.

3.3.2. Size Reduction and Sieve Analysis

Brassica nigra seeds were crushed using a coffee mill with sieve size 0.5mm. The sample was sieved using vibrated shaker with a set of sieve sizes arranged. This helps to investigate the effect of particle size on yield and quantity of the oil. From the result using 0.5mm sieve size was more effective and gives a greater amount of oil. After the moisture was removed by placing in an oven at 43^0C for 8 hours and the dried Brassica nigra Seeds were milled in coffee miller with a sieve size of 0. 5mm, then the sample was sieved using vibrating shaker for 10 minutes.

3.3.3. Oil Extraction

150mL of normal hexane was poured into round bottom flask. 12g,15g and18g of the sample were placed in the thimble and was inserted in the centre of the extractor. The Soxhlet was heated to at 65℃,70℃ and 75℃. This was allowed to continue for two, four and six hours. The experiment was repeated by placing the same amount of the sample into the thimble. The weight of oil extracted was determined for each run hours. At the end of the extraction, the resulting mixture containing the oil was heated to recover solvent from the oil.

3.3.4. Determination of Percentage of Oil Extracted

The yield of oil extracts was expressed as a percentage of the weight of extracts obtained from extraction relative to the weight of Brassica nigra seeds used for extraction.

$$\text{Yield of oil extraction} = \frac{weight\ of\ oil\ extracted}{weight\ of\ Brassica\ nigra\ seeds\ used} * 100\% \text{------------} (3.1)$$

3.3.5. Characterization of the Physical Properties of Oil

3.3.5.1. Determination of Moisture of Oil

0.509gm of oil was weighted and put in a dried dish and then dried in an oven at 100^0C for 10 minutes. The process was measured in digital equipment (moisture tester) that was measured the desirable value at maximum oil yield and the result was displayed on the screen after a time completed, then sample containing dish was removed from the digital moisture tester and the moisture of the oil was determined.

3.3.5.2. Determination of Kinematic Viscosity of Oil

A kinematic viscosity of the oil was measured indirectly using Vibro Viscometer which is available in laboratory at School of Chemical and Bio engineering. Initially, a sample was heated at a temperature of 70^0C. A sample of 35 mL oil was measured and fed to a sample holder of the Vibro Viscometer. A sensor of the Viscometer was immersed in 35 mL of oil and then a dynamic Viscosity of oil was displayed on the vibro viscometer screen at a temperature of 25^0C. and then the Kinematic Viscosity was calculated. The kinematic viscosity is then equal to the ratio of dynamic viscosity to the density of the oil.

$$\text{kinematic viscosity, mm}^2\text{/s } (\mu) = \frac{dynamic\ viscosity(mPa.\text{sec})}{density\ of\ the\ oil} \quad \text{-------(3.2)}$$

Where μ=kinematic viscosity (mm^2/s)

ν=dynamic viscosity (mPa.sec) and ρ=density (kg/m^3)

3.3.5.3. Determination of the Specific Gravity

The specific gravity of oil was determined using digital specific gravity measurement. The machine was cleaned with fresh distilled water and Again cleaned by the sample was injected using syringe into the density meter and 5ml of oil yield at 20^0C was filled with the maximum oil yield and the injected oil waited for 10 minutes, with in the same operating temperature then, the results were displayed on the screen of the machine and the specific gravity was determined (A.O.A.C official method, DMA 4100 model).

3.3.5.4. Density Determination

To determine the density of the oil extracted, the measured value of oil yield was determined using digital density meter measurement machine and It was calibrated by using fresh distilled water, then 5ml of the oil was poured into it and the results was noted on the screen. The sample was brought to a specified temperature and a test portion was transferred into the digital machine, therefore the density of the oil calibrated automatically at 20 ^oC was determined by taking the a few minutes, then after the density of the oil was determined (A.O.A.C official method, DMA 4100 model).

3.3.5.5. Determination of Refractive Index

The refractive index of the sample was determined using refractometer at Addis Ababa Institute of Technology in analytical testing laboratory before the sample was placed on refractive index tester was calibrated by its own reagent then, A few drops of the maximum oil yield result were placed on the face of the refractive index and allowed to gently spread, closed and tightened for some time for the oil yield and the equipment was attaining at room temperature, then refractive index value of oil was determined

3.3.6. Characterization of the Chemical Property of Oil

3.3.6.1. Determination of Saponification Value

2.5gm of the sample was taken and added into a 250 ml flask. 25 ml of alcoholic potassium hydroxide solution was added into the flask. The flask was connected to reflux condenser and kept in the water bath and boiled gently at the 70 ^oC for half an hour. After the flask and the condenser were cooled, the inside of the condenser was washed with 10 ml of hot ethyl alcohol. Then three drops of alcoholic phenolphthalein indicator were added and the excess potassium hydroxide was titrated with 0.5 N hydrochloric acid to the end point, until the pink color of the indicator just disappears. The same procedure was conducted for the blank (without oil) and the saponification value (SV) expressed as the number of milligrams of KOH required to saponify 1 gm of oil was calculated.

$$\text{Saponification Number} = \frac{56.1*(Vb-Va)*N}{W} \quad \text{--(3.3)}$$

Where: W= weight of oil taken in gram.

N= normality of HCL solution

Va= volume of HCL solution used in the test in milliliter.

Vb= volume of HCL solution used in blank (without oil sample) in milliliter.

3.3.6.2. Determination of Acid Value

2.5gm of the sample was weighed and put into a 250 ml conical flask and 25ml of Absolute ethanol (99.5%) was mixed with 25ml of diethyl ether (equal volume) and 1ml of phenolphtlialein indicator (1% in ethanol) was added. The mixture was boiled for about 5 minutes and titrated while hot with a standard aqueous solution of potassium hydroxide (0.1N KOH) while shaking vigorously during the titration until a pink colour appear and then the acid value was determined.

Acid Number = $\frac{56.1*V*N}{w}$ --(3.4)

Where: V is the volume expressed in milliliter of 0.1N solution of ethanolic KOH

W is mass in gram of the test at the maximum oil yield

N is concentration of ethanolic KOH

Free fatty acid = $\frac{Acid\ Value}{2}$--(3.5)

3.3.6.3. Determination of Peroxide Value

Peroxide value (PV) is the most frequent measurement of lipid oxidation. Hydroperoxides have no flavor or odor of their own, but they are not stable and split up rapidly to other products such as aldehydes that have a strong, disagreeable flavor and odor. Peroxide value is one of the most widely used test for oxidative rancidity in oils. It is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Generally, the peroxide value should be less than 10 mg/g oil in the fresh oils(Kittiphoom, 2013)

Testing of peroxide value of Brassica nigra seed oil has been conducted at JIJE Analytical testing service laboratory PLC.

5.135gm of the oil was dissolved in 30ml of a solvent mixture consisting of 60% glacial acetic acid and 40% chloroform (3:2) and 0.5ml of 15% saturated solution of potassium iodide was added. The flask was shaken and after which 30ml of distilled water was added. The mixture was then titrated with 0.01N of $Na_2S_2O_3$ solution using 2ml of 1% starch solution as indicator and the end point of titration, until the disappearance of dark blue Colour that was formed and it became light yellow.

The peroxide value was calculated using the formula

$$PV = \frac{(S-B)*N*1000}{\text{weight of oil sample}} \quad \ldots\ldots (3.6)$$

Where, S = Volume of sodium thiosulphate used in sample

B = Volume of sodium thiosulphate used in blank

N = Normality of sodium thiosulphate

W = Weight or mass of oil sample

3.3.6.4. Determination of Iodine Value

The Iodine Number gives a measure of the average degree of unsaturation of oils and fats. the higher the iodine value, the greater the number of C=C double bonds. By definition, the Iodine Number is expressed in terms of milligram of iodine per gram of sample (weight percent of absorbed iodine). One of the most commonly used methods for determining the iodine value of oils and fats

Testing of Iodine value of Brassica nigra seed oil has been conducted at JIJE Analytical testing service laboratory PLC.

0.381g of oil was taken into 250 ml conical flask. 15 ml of glacial acetic acid to cyclohexane (1:1) was added to oil the sample dissolves this oil after 25 ml of Wij's solution was added and to allow it for one hour in a dark place. Then 20 ml of 15% potassium iodide excess solution was added to oils containing solution and 150 ml water were added to each bottle and titrated against 0.1N $Na_2S_2O_3$ solution using 1ml of 1% starch solution as the indicator when the color was starting to change and at end point of titration, the color of the solution becomes colorless. Dark blue color disappears, which indicates the end point. At the same time and under similar conditions carry out a blank titration (without oil) sample. Iodine Value was calculated using the formula:

$$IV = \frac{(B-S)*N*12.69}{W} \quad \text{------}(3.7)$$

Where, B= Volume of sodium thiosulphate used in blank

S =Volume of sodium thiosulphate used in sample

N = Normality of sodium thiosulphate and

W = Weight or mass of oil sample

3.3.6.5. Experimental Design

The experiment was designed using Design Expert version 6.0.8 software where a Box-Behnken experimental design was employed in order to optimize the extraction of oil from Brassica nigra seeds. The tendency of any experiments that the result of each and every experiment must be optimized. So, there is necessarily needed to design some input parameters for optimal results. The results of an experiment can be optimized by minimize or maximize some parameters. Out of these methodologies, response surface technique is the best to get optimized results. Because in the response surface methodology, we must have to examine the various process parameters that affect the whole process, and collect the different mathematical and statistical method for optimize results. Response surface methodology also provides a better relationship between optimum input feedstock parameters and optimum yield output parameter. The experiment was designed on three levels, three factors that generated 17 experimental runs. The three independent factors are extraction time, extraction temperature, and seed to solvent ratio. To reduce design points. In general, the number of design points of the BBD's was calculated based on the tabulated equation of 2K (K-1) + NC is where K is the number of factors and NC is the number of center points. The suitability for multiple variables can reveal possible interactions between variable and because of its reasonable design and excellent outcomes

Table 3.1 the experimental design Summery

				Level		
Factor	Name	Unit	Type	-1	0	1
A	Extraction temperature	^oC	Numerical	65	70	75
B	Solid to solvent ratio	g/ml	Numerical	0.08	0.1	0.12
C	Extraction Time	Hrs.	Numerical	2	4	6

3.3.6.6. Optimization of Oil Yield Using Response Surface Methodology

Response surface methodology has been used to study the optimization of chemical processes and products. In general Response surface methodology (RSM) is stated as an efficient and effective way to optimize the parameters that affect the process and also RSM technique is used in order to find the optimal conditions when a number of factors are involved in the process and the response is affected by them. RSM has many benefits such as it reduces process cost and process time.

RSM is used to optimize the process parameters for the solvent extraction of Brassica nigra seed oil and to study the effect of process parameters on oil yield, such as seed to Solvent ratio, extraction time and extraction temperature base on this the response surface methodology was used to investigate the optimum process parameters for the extraction of oil from Brassica nigra seed using the solvent extraction. The factors considered to be solvent ratio, extraction temperature, and extraction time, among others, for optimal oil yield. A three level, three-factor Box-Behnken Design (BBD) was employed using Design Expert 6.0.8 software to examine the optimum conditions of extraction of oil from Brassica nigra seed using the solvent extraction.

CHAPTER FOUR

4.Results and Discussions

4.1. Yield of Oil Extracted from Brassica nigra Seeds

The percentage oil yield was calculated by using equation 3.1 and the result is summarized in the table 4.1 below.

Table 4.1 Experimental design layout using Box-Behnken design.

Runs	Factor A Extraction Temperature (°C)	Factor B Seed/solvent Ratio (g/ml)	Factor C Extraction time (h)	Response Oil yield (%)
6	65.00	0.08	4.00	34.97
8	75.00	0.08	4.00	34.985
7	65.00	0.12	4.00	39.528
4	75.00	0.12	4.00	45.89
10	65.00	0.10	2.00	35.35
5	75.00	0.10	2.00	38.975
16	65.00	0.10	6.00	38.76
11	75.00	0.10	6.00	39.944
14	70.00	0.08	2.00	35.27
9	70.00	0.12	2.00	46.205 *
1	70.00	0.08	6.00	35.935
2	70.00	0.12	6.00	45.79
17	70.00	0.10	4.00	38.925

3	70.00	0.10	4.00	37.395
12	70.00	0.10	4.00	37.498
15	70.00	0.10	4.00	38.294
13	70.00	0.10	4.00	37.967

The result of the so extraction of oil from *brassica nigra seed* oil is presented in Table 4.1. The varying oil yield values are indications that the oil yield from the extraction of oil from Brassica nigra seed was affected by the different process parameters or conditions, therefore, the extraction parameters considerably affect the oil yield. The maximum oil yield of 46.205% was obtained from the extraction of the corresponding seed to solvent ratio of 0.12, extraction temperature of 70ºC, and extraction time of 2 hours.

4.2. Analysis of Data for Extraction of Oil from *Brassica nigra Seeds*

The statistical analysis for oil extraction from Brassica nigra seeds was done using analysis of variance (ANOVA). As shows in table 4.2 from the ANOVA results of oil extraction. The multiple regression analysis of the experimental data gives a second-order polynomial equation, which was modified to discard the insignificant AC, and BC model terms. The reduced quadratic model developed shows the interaction between the dependent oil yield (Y) and the coded values of the independent variables A, B, and C (seed/solvent ratio. Extraction temperature, and Extraction time). The Model equation of this work represented by Final Equation in Terms of Actual Factors:

$$Y = -35.66507 + 3.73984 * T - 1699.69375 * R + 3.03786 * t - 0.034306 * T^2 + 4212.75 * R^2 + 0.27477 * t^2 + 15.8675 * T * R - 0.061025 * T * t - 6.75 * R * t$$

Where Y represents a response variable oil yield measured in %.

T=the difference of extraction temperature from the overall average

t= the difference of extraction time from the overall average

R= the difference of solid to solvent ratio from the overall average

The significance and adequacy of the model were tested by using ANOVA. It was observed that from the table 4.2 the linear interaction effects are due to the Coded Factor the extraction temperature(A), corresponding to seed to solvent ratio(B), which is a significant factor. The quadratic effects of solid to solvent ratio (B^2), the effects of extraction temperature and seed to solvent ratio (AB), and seed to solvent ratio(B) are all significant beings less than 0.05, while the values greater than 0.05 means not significant. The ANOVA result shows that the factors of extraction temperature with extraction time(AC), solid to solvent ratio with extraction time(BC), The quadratic effects of extraction time($C^{2)}$, and extraction time(C) are not significant because Extraction time plays a great role on the percentage yield of *Brassica nigra seeds* oil and the interaction between the effects of solid to solvent ratio and extraction temperature on the yield when the maximum amount of extractable oil is obtained, the oil yield level remains invariable even by extending the reaction time. So that in the soxhlet extraction the maximum oil yield could be finding at an extraction time of 2 hours and below. The interaction effects on oil yield were investigated using the interaction plot and 3D surface plot of Response.

. Regressional model equation

The following table shows analysis of variance (ANOVA) obtained from Design expert software, which tells as the significance of different factors.

Table 4.2 Analysis of variance (ANOVA) for extraction of oil from Brassica nigra seed (partial sum of squares*)*.

Source	Sum of Square	Degree of Freedom	Mean Square	F Value	Prob > F	Remark
Model	213.69	9	23.74	22.24	0.0002	Significant
A	15.31	1	15.31	14.34	0.0068	
B	164.29	1	164.29	159.90	<0.0001	
C	2.54	1	2.54	2.38	0.1667	

A^2	2.99	1	2.99	2.80	0.1381	
B^2	11.74	1	11.74	11.00	0.0128	
C^2	5.23	1	5.23	4.90	0.0626	
AB	10.07	1	10.07	9.43	0.0180	
AC	1.35	1	1.35	1.26	0.2984	
BC	0.29	1	0.29	0.27	0.6173	
Residual	7.47	7	1.07			
Lack of Fit	5.91	3	1.97	5.05	0.0758	Not significant
Pure Error	1.56	4	0.39			
Cor Total	221.16	16				
Std. Dev.	1.03		R-Squared	0.9662		
Mean	38.93		Adj R-Squared	0.9228		
C.V.	2.65		Pred R-Squared	0.5613		
PRESS	97.03		Adeq Precision	15.442		

4.3. Interaction Effect of the three Factors

From design expert software 6.0.8 output, interaction effect between;

- Solid to solvent ratio and extraction time at 70^{o}C
- Solid to solvent ratio and extraction Temperature at 2 hours and
- Extraction time and Extraction Temperature at solid to solvent ratio

The yield of oil (%) are shown in Figure below:

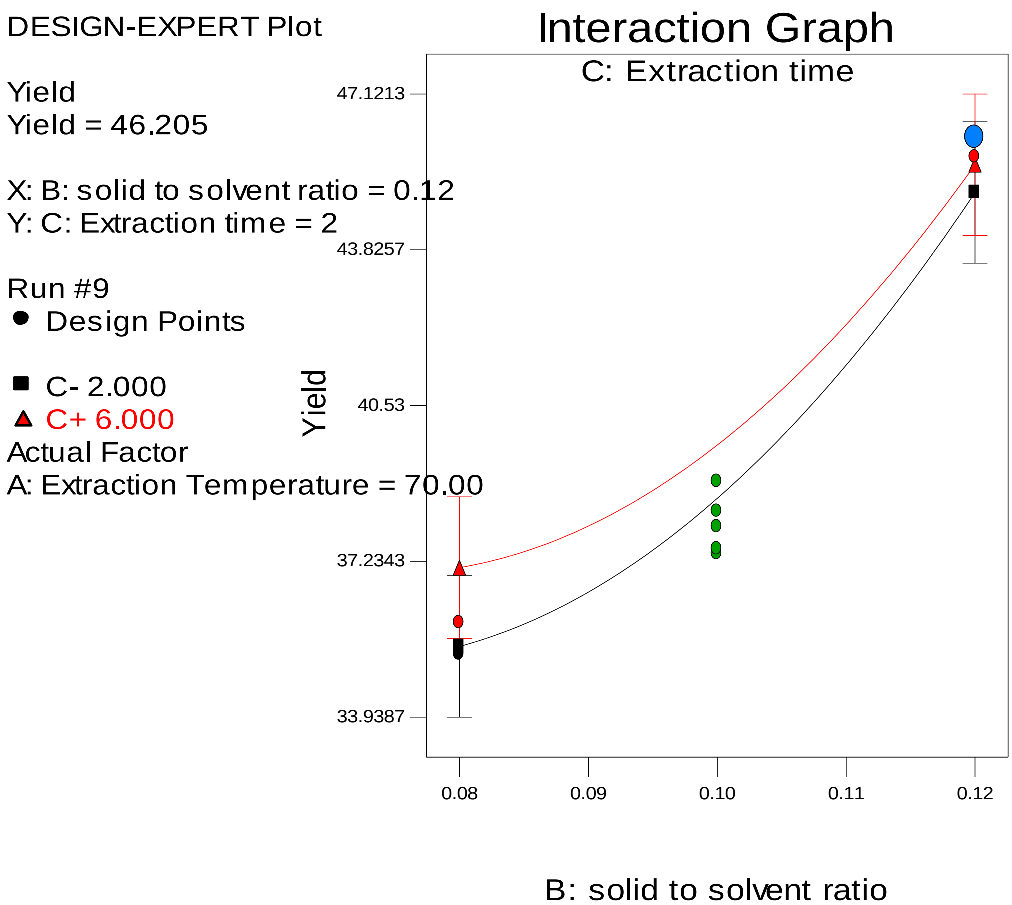

Figure 4.1 Interaction effect of solid to solvent and extraction time on oil yield

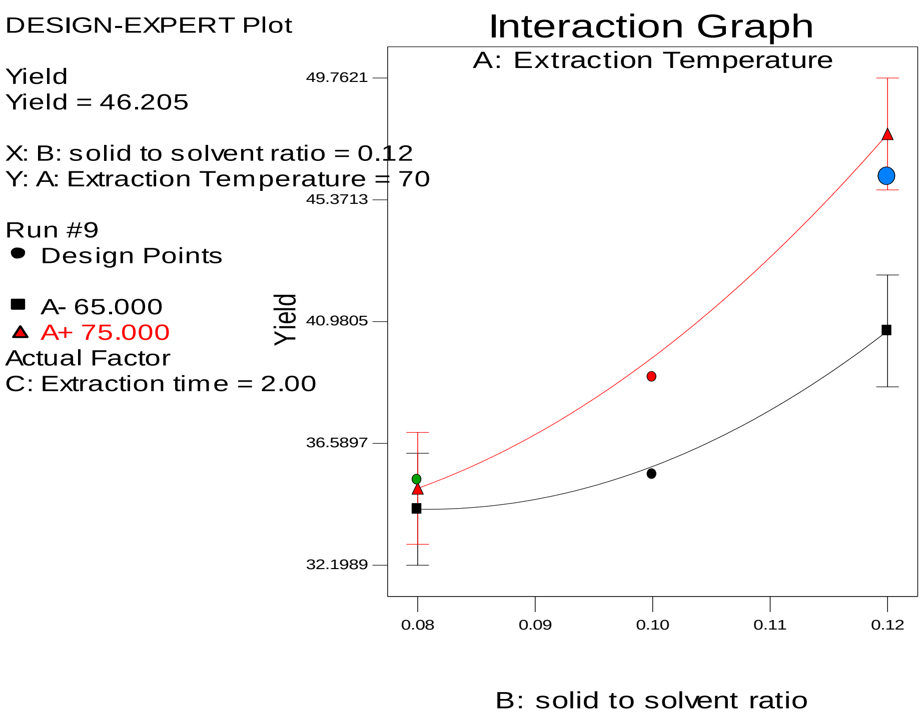

Figure 4.2 interaction effect of Solid to solvent ratio and extraction Temperature on Oil Yield

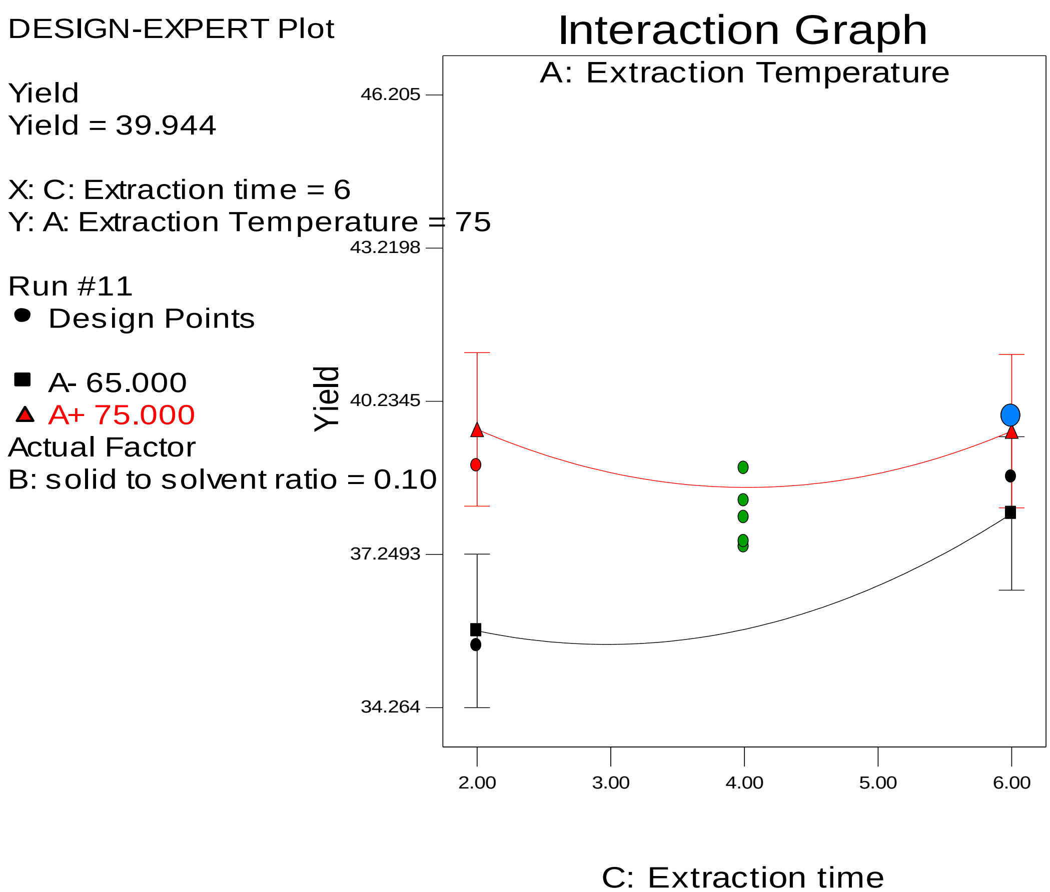

Figure 4. 3 interaction effect of Extraction time and extraction Temperature on Oil Yield

There was no interaction effect between solid to solvent ratio and extraction time as shown by the similar shape of the two parallel curves in the Figure 4.1. This implies that the extraction process of Brassica nigra seed oil can be completed within lower extraction time. The experiment results have been observed that the oil extraction rate was quick at the starting of the extraction process before reaching the steady state. This is because the driving force for transfer of oil from the solid phase to the liquid phase is higher in start of the process. In the other word, the difference of oil concentration between the solid phase and solvent phase is greater in the initial extraction process. Therefore, the oil diffuses rapidly from brassica nigra seed powder to the n-hexane(solvent) and the maximum amount of extractable oil was transferred. The oil yield unchanged even after prolongation the time of the extraction process. The optimum extraction time is about 2hours for all the extraction Temperature. At the lower extraction time and at modern extraction temperature can give higher yield. Therefore, the extraction process depends on solvent temperature as can be noticed from Figures 4.2 and 4.3 there was an interaction effect between solid to solvent and extraction Temperature has been high interaction effect and also the interaction between lower extraction time and extraction temperature respectively. In general, there was a high oil yield with in the interaction effect between solid to solvent ratio and extraction Temperature.

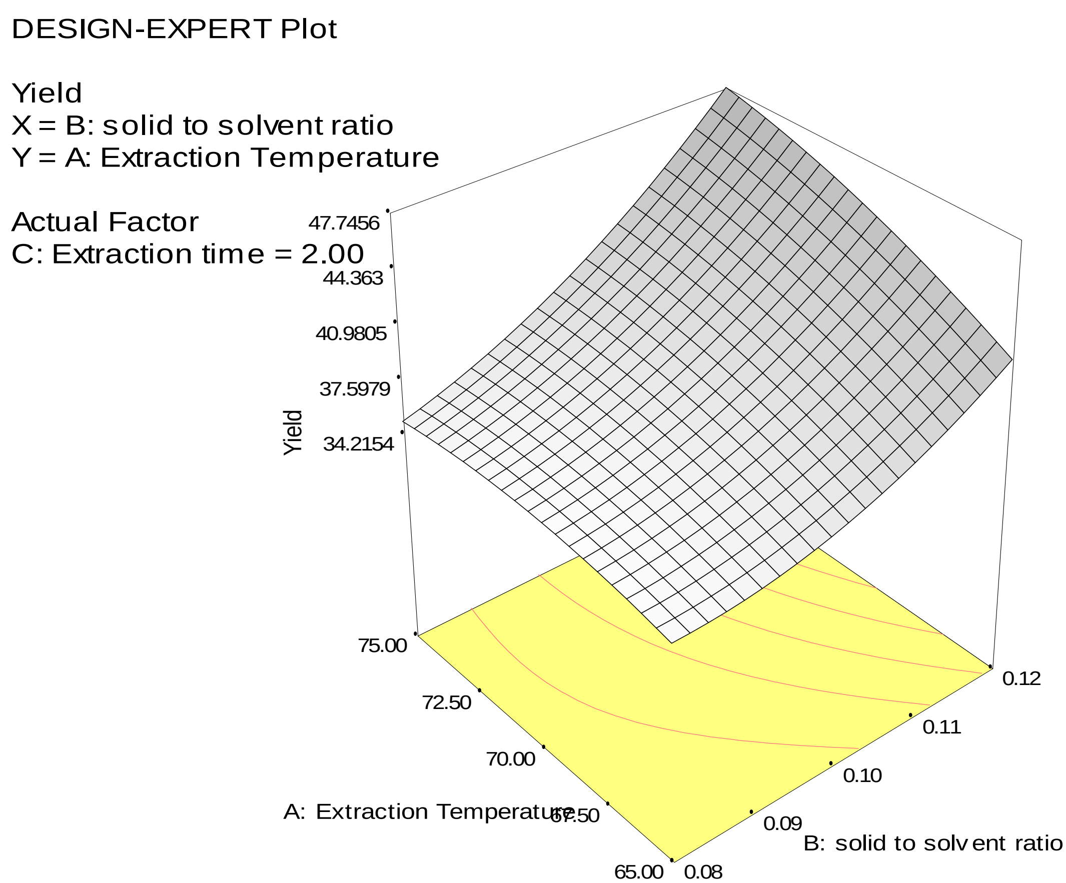

Figure 4.4, 3D Response surface plot showing the effect of extraction Temperature and seed to solvent ratio on oil yield.

From figure 4.4 the combined effect of extraction Temperature and seed to solvent ratio was studied using the interaction plot of RSM and it was observed that the oil yield increased with increase in extraction Temperature until boiling point of solvent and seed to solvent ratio. Also, it can be deduced from this figure 4.4 that increase in seed to solvent ratio and extraction temperature favors the extraction rate as much solvent is needed to ensure that vapor rose through the vertical monitor tube into the condenser at the top just as the solvent boiled. However, the oil yield was observed to decrease with higher temperature and higher extraction time, particularly at the temperature higher than the boiling temperature of the solvent. This is because at this temperature higher than the boiling temperature of the solvent, evaporation of the solvent will take place as a result of the increased temperature. The most advantage of n-hexane as solvent may be indicative of relatively lower temperature and a more economical solvent when considering heating cost among other requirements for solvent extraction. Beyond the boiling temperature of the solvent was applied it caused the rancidity and the losses of oil yield. The use of high temperatures during extraction of oil over a longer period of time is the damage to enzymatic components.

From Table 4.2, standard deviation of 1.03, mean of 38.93, C.V of 2.65, R^2 of 0.9662, adj. R^2 of 0.9228, and Adeq. precision of 15.442 were obtained for different industrial application yield. The fitness of the polynomial model was expressed with the coefficient of determination of R^2 and the coefficient of adjusted R^2, which were obtained at 0.9662 and 0.9228, respectively. It is suggested that these values should be at least 0.80 for the best fit of the model. Therefore, the R^2 value of 0.9662 and the adjusted R^2 value of 0.9228 indicate that the regression model is acceptable. The "Lack of Fit F-value" of 5.05 implies that the Lack of Fit is not significant relative to the pure error. There is only 7.58% chance that a “Lack of fit F-value” this large could occur due to noise. Significant lack of fit is bad because we want the model to be fit.

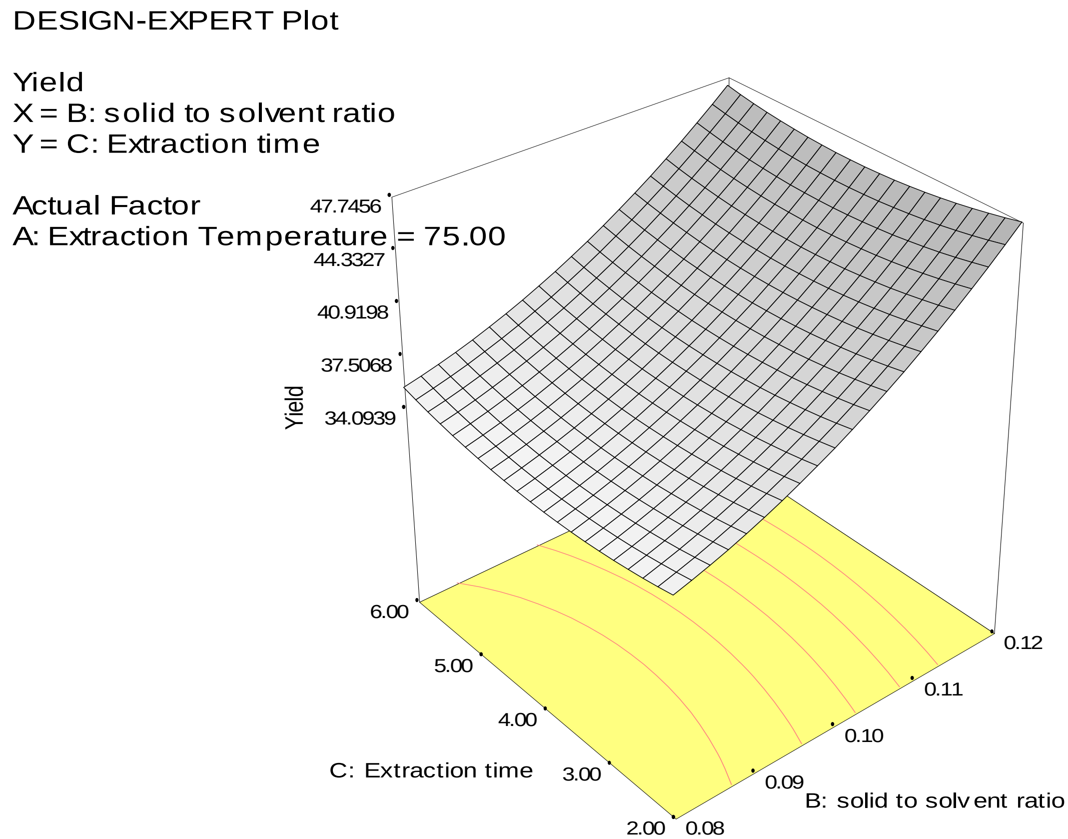

Figure 4. 5, 3D Response surface plot showing the effect of extraction time and solid to solvent ratio on the oil yield.

Effect of the solid to solvent ratio and extraction time on the oil yield of Brassica nigra seed. The 3D surface plot interaction for Design Expert presented in Figure 4.5 represents the effects of solid to solvent ratio and extraction time. Their interactions on the oil yield of extraction process were investigated. It was observed that increase in solid to solvent ratio favors the extraction rate within fixed solvent is needed for extraction, while the oil yield increases gradually as extraction Temperature increases until the boiling temperature of the solvent is reached, when the oil yield starts declining. The curved nature of the surface plot in Figure 4.5 shows that a mutual interaction between at the lower level extraction time and solid to solvent ratio. The effect of interaction between the extraction temperature and lower extraction time had a direct significant effect on the yield of oil. It was noticed that increase in at the lower level extraction time favored the extraction of oil until it got close to the moderate extraction Temperature. Increased extraction time above this level tends to produce lower oil yields. The oil yield was observed to decrease with higher temperature particular at the temperature higher than the boiling point of the solvent. This is because at this temperature level higher than the boiling temperature of the solvent, evaporation of the solvent will take place as a result of the increased until moderate extraction Temperature. The combined effect of these two variables or factor at high-level experimental process will obviously result in a decrease in oil yield as recorded

4.4. Characterization of Physical properties of Brassica nigra Seed Oil

4.4.1. Determination of Brassica nigra Seeds Moisture Content

The moisture content of Brassica nigra seeds was known factor that affects the processing and yield of oil, preservation and storage of food and herbal products. High moisture content renders plant products susceptible to microbial attack and thus leads to spoilage and a lowered shelve life. The moisture content of the seed should be known that it can be stored for a long period time without spoilage. 50 grams of Brassica nigra Seeds was taken and the moisture content of the sample was obtained (table 4.3)

Table 4.3 Moisture content determination of *Brassica nigra Seeds*

Time (Hrs.)	0	2	4	6	8
Weight of sample(g)	50	48.2	47.6	47.3	47.3

Moisture content of seed $=\frac{2.7gram}{50gran}$*100% = 5.4%

4.4.2. Determination of Moisture Contents of Oil

The results were obtained read on screen displayed was 7.04 at a temperature of 100℃.the digital moisture tester need pure oil but in this study the amounts of normal hexane could not remove totally as result the tester consider the n-hexane as moisture so it has been slightly differenced from literature, therefore the studies were good for long period of time to preserve oil.

4.4.3. Determination of the Specific Gravity

The result was obtained from digital density meter reading the specific gravity of oil was 0.8935 at a temperature of 20ºC. The common physical properties of such oils are that they float on water, but are not soluble in it, they are greasy to touch, and have lubricating properties.

4.4.4. Determination of the Density

The result was obtained from digital density meter reading the density of oil was $0.8891 g/cm^3$

4.4.5. Determination of Kinematic Viscosity of Oil

Dynamic viscosity of oil, which was read from Vibro Viscometer on screen displayed, was 8.14 mPa.s at a temperature of 25^0C. the kinematic Viscosity was calculated using the equation (3.2) from section 3.3.5.2

Kinematic viscosity = $9.16*10^{\wedge -6}\ m^2/s$

Viscosity is the measure of the internal fraction in the oil and is the important index of the study of oil and their inter molecular forces and its useful criterion for degradation or depolymerization.

4.4.6. Determination of Refractive Index

The result was obtained from refractive index, refractive index value of 1.45504 at a temperature of 20^0c. Refractive index indicates the purity of the oil and to control hydrogenation and isomerization processes.

4.5. Characterization of Chemical properties of Brassica nigra Seed Oil

4.5.1. Determination of Saponification Value

The determination of saponification value found using equation 3.3 and the results obtained was shown in table 4.4:

Table 4.4 Saponification value of Brassica nigra seed oil

Run	Volume of HCL solution used in blank	volume of HCL solution used in the test	weight of oil taken for test(gram)	SV
1	83	67.5	2.5	173.91

2	83	66.4	2.5	186.252
3	83	68.9	2.5	158.202
Mean ±S. D (n=3)				172.788±11.479

SD $=\frac{(\sqrt{(SV-s\bar{v})}}{n}$

An average result of the saponification value of Brassica nigra seed oil was 172.788±11.479. High saponification value showed that the molecular weight of proportion of fatty acid is low. The High saponification value of the Brassica nigra seed oil will suggest the use of the oil in the production of liquid soap, shampoos and also oils could be generally used in confectionery, cosmetics, pharmaceuticals, food supplements, varnishes and oil paints therefore, in general Brassica nigra seed oil is favorable to industrial purpose.

4.5.2. Determination of Acid Value

Acid value indicates the amount of free acid present in the oil. It can be calculated using equation 3.4 and the results are summarized in the following table 4.5

Table 4.5 Acid value was summarizing in table below

Run	volume of KOH solution used in the titration	weight of oil taken for test (Gram)	AV
1	1.3	2.5	2.9172
2	1.2	2.5	2.6928
3	1.5	2.5	3.366
Mean ±SD			2.992±0.4848

Acid value is an important indicator of vegetable oil quality and Acid value is a measure of the extent to which the glycerides in the oil have been decomposed by lipase action. The result of acid value as compare with Brassica juncea an acid value of 3.9 ± 0.32 was reported by (Nwokonkwo. D.C, 2016).but the result of this study was 2.992, therefore the acid value of Brassica nigra seed oil is favorable to industrial purpose as indicating, the low acidity of an oil is an indication of oil which is free from hydrolytic rancidity.

Effects of temperature on Acid value

Temperature can affect both the yield and the quality of *Brassica nigra seeds* oil. The quality of oil can be determined studying the effect of temperature on Acid value. The result Indicated that as temperature increase acid value increase this is because the extraction temperature influences the hydrolysis of oil into free fatty acids and glycerol. The decomposition of the glycerides in the oil is also affected by the heat treatment. As transition theory states' increasing of the reaction temperature has affected the production of fatty acids which clearly showed an increase in conversion of fats in to free fatty acids. Lipase enzyme hydrolyzes oil become free fatty acid and glycerol. The decrease oil quality is due to increased activities of lipase enzyme at the lower temperature. Therefore, as temperature increase oil quality decrease since temperature affects the active ingredient.

4.5.3. The determination of Free Fatty Acid value

The determination of Free Fatty Acid value was calculated using equation (3.5)

Free fatty Acid value =1.496±0.2424

4.5.4. Determination of Peroxide Value

The determination of Peroxide value was calculated using equation (3.6)

PV =2.13799056, Without standard solution.

Hence, Peroxide value expressed as milli equivalent of peroxide oxygen per kg sample (meq/kg). The result of peroxide value in this work was 2.14 therefore, it was less than 10 meq/kg and also it indicates the Fresh oils usually have peroxide values well below 10 meq/kg(Standards Authority Of India Ministry Of Health, 2015). Peroxide value is one of the most widely used testing for oxidative rancidity in oils. It is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. And also, an acceptable (low) peroxide value which shows that the oil was relatively stable, an indication of little or no unsaturation.

4.5.5. Determination of Iodine Value

The Determination of Iodine value can be calculated by using equation (3.7)

IV =95.25826772 without standard solution measurement.

The result indicated that the *Brassica nigra seed* oil has low iodine value, which also indicates high resistance to oxidation and have longer shelf life. The oil can be classified as a non-drying oil since its iodine value is lower than 100 (Adebayo et al.,2012). The oil is a semi-drying oil with low degree of unsaturation which will make it stable against oxidation when exposed to the atmosphere.

Effects of temperature on Iodine value

Iodine value can affect by extraction temperature on which Unsaturated compounds contain molecules with double or triple bonds, which are very reactive toward iodine. This suggests higher temperature results the loss of unsaturation (double or triple bonds) in the fatty acids of the triacylglycerols as a result, decreased in iodine value due to loss of double and triple bonds in the oil. The more iodine is attached, the higher is the iodine value, and the more reactive, less stable, softer, and more susceptible to oxidation and rancidification. Decrease in iodine value indicates lipid oxidation and this might be due to metallic ions present among other factors, which enhances or promotes oxidation.

Table 4.6 The comparisons of Brassica nigra seeds oils physicochemical properties within the commercially available specification are summarized in the table below

Oil properties	Measured value	Commercial((LEWKOWITSCH, 1904)
Moisture (%)	7.04	-
Specific gravity	0.9835	0.916-0.92
Density	0.889g/cm^3	-
Refractive index	1.45504	1.4672
Viscosity	At 25°C (9.16*10^$^{-6}$)	At 70°F (379.3)
Saponification value (mgKOH/g)	172.788	172.1-180.1
Acid value(mgKOH/g)	2.99	7.35
Peroxide value (meq /kg)	2.14	10
Iodine value (wij's g/100g)	95.26	96

The physicochemical properties of the oil were compared within commercially available specification except the acid value which was lower. The Density and moisture content of the oil were not compared due to the lack of data under this specific property. The Brassica nigra seed oil was shown to be good source of extractable vegetable oil and the physical properties showed that the oil was liquid at room temperature and non-volatile. therefore, in general Brassica nigra seed oil is the best for favorable to industrial purpose

CHAPTER FIVE

5. Conclusions and Recommendations

5.1. Conclusions

In this research oil was extracted from *Brassica nigra seed* using the soxhlet method. It can be seen that seeds analyzed have appreciable oil contents and the results therefore, suggest the suitability of the oils for domestic and industrial uses. Solid to solvent ratio, extraction Temperature and extraction time were the considered parameters for optimization investigation. Under this investigation seed to solvent ratio (0.08, 0.1and 0.12 g/ml), extraction temperature (65, 70 and 75ºC), and extraction time (2, 4 and 6 hrs.) were considered. For the soxhlet extraction, the maximum oil yield has been determined as 46.205% after the extraction time of 2 hours with solid to solvent ratio (0.12 g/mL) and the minimum oil yield 34.97% was found at the extraction time of 4 hours with extraction temperature of 65℃ at solid to solvent ratio (0.08 g/mL. Therefore, increasing solid to solvent ratio, extraction temperature and decreasing extraction time will increase the amount of oil extracted

The effect on all parameters was found to be significant ($p<0.002$) for extraction temperature and solid to solvent ratio on oil yield except at a higher extraction time. It can be concluded that the extraction process of *Brassica nigra seed* oil found to be completed within lower extraction time. The experiment results have been observed that the oil extraction rate was quick at the starting of the extraction process before reaching the steady state. This is because the driving force for transfer of oil from the solid phase to the liquid phase is higher in start of the process. In the other word, the difference of oil concentration between the solid phase and solvent phase is greater in the initial extraction process. Therefore, the oil diffuses rapidly from *brassica nigra seed* powder to the n-hexane(solvent) and the maximum amount of extractable oil was transferred. The oil yield unchanged even after prolongation the time of the extraction process. The optimum extraction time is about 2hours for all the extraction Temperature. At the lower extraction time and at modern extraction temperature can give higher yield. Therefore, the extraction process depends on solvent temperature.

After the optimum operating condition for maximum oil yield was determined, using the optimum operating condition, the oil was extracted and the physicochemical properties of the oil Like viscosity($9.16*10^{-6}$ m^2/s), specific gravity(0.9835), density(0.889g/cm^3), Saponification Value(172.788), Acid Value(2.99), free fatty acid value(1.496), Iodine Value(95.26), Peroxide Value(2.14), and refractive index(1.45504) were determined for characterization and quality analysis. The results were compared with commercially available Black mustard seed oil specifications except the acid value which is slightly lower.

Generally, the result revealed that the extract Brassica nigra seed oil used for industrial application.

5.2. Recommendations

The available varieties of the *Brassica nigra seed* in Ethiopia at different region like Gojam, Mekele, Shewa should be studied and it may become economically competitive. The people need to be fully aware of Brassica nigra seeds oil uses for their specific purpose.

The total oil content of oil extracted from *Brassica nigra seed* is very interesting to consider it as potential human edible oil source and industrial application. But to do so further the oil should be also studied and Improving oil extraction operations as well as finding other ways more efficient.

Additionally, I recommend further studies to be performed if necessary, in the production of the oil in large scale and exporting its valuable oil product to other country.

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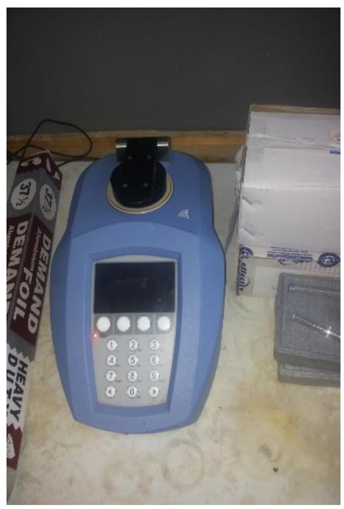
APPENDICES

APPENDIX A: photo taken all sample needs for Oil Extraction

Laboratory setup for Brassica nigra seed oil extraction

Laboratory setup at Lower extraction Temperature

After extraction, oil plus n-hexane and Oil obtained during saponification Titration end Point



density meters at AAIT thermal laboratory service and Refractometer at AAIT in Analytic laboratory service

APPENDIX B: Photo taken at working in JIJE laboratory analytic service


Wiji solution at JIJE laboratory service for Iodine value, two solution with oil sample and without oil for Iodine Value

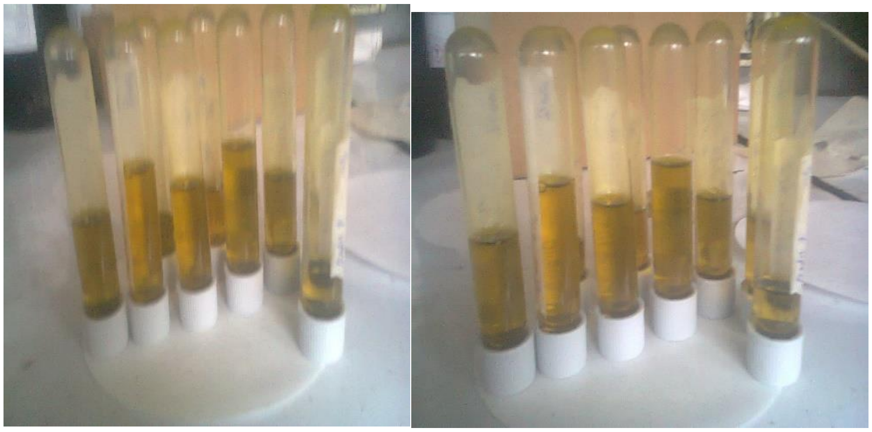
The two solution with and without oil sample for iodine value

APPENDIX C: color change during titration

Color change during titration for Iodine value

color change during titration for peroxide value

APPENDIX D: extracted oil sample



Samples of Brassica nigra oil seeds

Seeds, Seeds powder and Oil of Brassica nigra

JIJE LABOGLASS Pvt. Limited Company	JIJE Analytical *Testing* Service Laboratory	Doc. No: JATSL/F5.10-3	Page 1 of 1	
			Ver. No: 04	Effective Date: July 08, 2017
	Analytical Test Report			
Customer Name	Addis Ababa University	Test Report No	022	
Contact Person:	Getachew Chane	Reported date:	08/10/18	
Sample Type:	Brassica Niger Seed Oil	Test Request No:	Not Specified	
Sample Source:	5 kilo University	Tel:	+251913626495	
Sample collected by:	Getachew Chane	Fax:	Not Specified	
Sample Collected Date:	14/1/2010	E-mail:	gech.chane@yahoo.com	
Sample Received Date:	01/10/18	Tested by:	LN-05	
Sample Condition	Normal	Date tested:	01/10/18 to 06/10/18	

S/N	Lab No	Customer Id.	IV (g/100g)	PV (meq/kg)
1	J-0143/0110/19	189/150 ml	95.26	2.14

S/N	Tests	Test Methods
1	Iodine Value (IV)	AOAC Official Method 993.20 - Wijs (Cyclohexane - Acetic Acid Solvent) Method
2	Peroxide value (PV)	AOAC Official Method 965.33

Remark:

1. This test report is only for specific sample(s) which has been tested by JIJE Analytical *Testing* Service Laboratory.

Verified by			*Authorized by*		
Name	**Signature**	**Date**	**Name**	**Signature**	**Date**
Henok Shiferaw	[signature]	08/10/18	**Mulugeta Terefe**	[signature]	08/10/18
Technical Signatory			*Laboratory Manager*		

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The results of peroxide value and Iodine value at standard value from JIJE laboratory service