DECLARATION

I, Yusuf Bola Ramat with Matriculation No. 2003/15108EH declare that this research project report is my original work and has not been presented or submitted elsewhere to the best of my knowledge for the award of any degree. All materials consulted have and dully referenced.

.....

YUSUF BOLA RAMAT

08/11/2008

DATE

CERTIFICATION

This research project by Yusuf Bola Ramat has been examined and certified under the supervision of Engr. M. S. Galadima to be adequate in scope and quality for the partial fulfillment of the requirements for the award of Bachelor of Engineering (B.Eng) in Chemical Engineering.

M.S.G. ..... 08-11-2008 .....

ENGR. M.S. GALADIMA **DATE**

Project Supervisor

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DR. M. O. EDOGA **DATE**

Head of Department

....

External Examiner **DATE**

DEDICATION

I dedicated this project to Almighty Allah and to my lovely mother Alh. Mrs. Rafat Yusuf Iyadunni (YILATA), for her affectionate love, patience, understanding and motherly care. Mother, may you live longer to reap the fruit of your labour. Amen.

ACKNOWLEDGEMENT

My first acknowledgment goes to Almighty Allah ''the creator'' and ''the provider'' for his wonderful favour, protection, provision and guidance He showered on me to complete this work successfully.

I do acknowledge my outstanding supervisor Eng. M.S Galadima for his meticulous supervision, taken his time to read through the manuscript, and whose despite his tight schedules still found time to offer valuable corrections, comments, ideas and suggestions which has broadened my knowledge and increased my confidence in carrying out the practical aspect of this project. May Amighty God increase him in knowledge and crown his efforts (ameen).

I owe a debt of gratitude to my mentors in the department of chemical engineering in person of the Dean of Engineering and Engineering Technology. Prof. J.O Odigure, Prof. K.R Onifade, The Head of Department Dr. M.O Edoga, Eng.M.A Olutoye, and the entire staffs of Chemical Engineering Department for their efforts toward the completion of my course. I wish to thank Mallam Dikko, the laboratory Technician for giving me all technical assistance during the practical.

My appreciation is incomplete without appreciating the effort of my parents Alh.Yusuf O.A and Alh. Mrs Rafat Yusuf for their parental responsibility given to me ever since I was born till now. My sibling Mrs. Boluwadura T, Mr. Majeed Yusuf, Mrs. Bilkis Abdulraheem, Alhaja Sherifat(sugar), my lovely Kamal; you are the best! My gratitude also goes to Alhaji Adamu Adeyinka Ajayi for his kindness and generosity.

I give special thanks to my colleagues; Saheed Ige, Andrew, Nicholas, Kamal, Afolabi, Femi, Yemi, Mukaila, Bola and Yewande. I say kudos for the happy moments we shared together and the hard period we discussed over.

Unquantified appreciation to my love and finally to all that make my programme a success in one way or the other I pray almighty Allah will blessed them all (Amen)

ABSTRACT

This project researched into the extraction and characterization of garlic seed oil using n-hexane as the solvent. The soxhlet apparatus was used for the extraction and the yield of the garlic oil was found to be 13.93%. The oil obtained was then subjected to physical and chemical analyses.

On characterization, the oil was found to possess brown colour, has pungent oduor with specific gravity of 1.207 at 29^0C, pH of 5.6 at 29^0C, refractive index of 1.4470 at 29^0C, density of 1.353g/cm^3, viscosity of 3.65 x10^{-3} kg/ms. The chemical analysis revealed the free fatty acid content of the oil to be 56.4%, acid value as 1.128mg KOH/g, saponification of 224.40mgKOH/g and iodine value as 79.63mg of KOH. Though the quantity of the oil was very small but its extraction cannot be overlooked owing to its pharmaceutical importance.

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CHAPTER ONE

1.0 INTRODUCTION

Crude vegetable oil and fats are extracted from seeds and animal either by mechanical expression or by solvent extraction. Mechanical pressing involves pressing the material to obtain oil or fats where as solvent extraction involves the use of solvent to leach out the oil. Solvent extraction of oil is more economical due to the fact that large quantity of oil is achieved compare to the mechanical pressing.

On industrial scale, extraction is carried out either in batch or in continuous process, ranging from single cylindrical drum extractors to rotor cells. At the laboratory scale, several researches have been carried out with equipment designed to suit the purpose of finding out ways of improving the efficiency of extraction process (Kariya, 1979)

Garlic plant of Botanical name *Allium Sativum* is a common plant used as food in all part of the World. It is also used as ointment since ancient times. In the past decade, there has been a renewed research interest in the therapeutic uses of garlic. The following properties have also been reported: insecticidal, antibacterial, antifungal, anti-tumor, hypoglycemic, antiseptic and anti-atherosclerotic (Adamu etal, 1984)

Garlic, from the history is believed to be originated from Central Asia, being distributed at an early date to the Mediterranean. It has been in cultivation for more than 30 years ago. It is grown in Western and Eastern part of Africa such as Ethiopia and Kenya. It was first brought to Nigeria in the 18^{th} century and more cultivated in the North than any other part of the country. This is partly due to the environmental and vegetation support of the Northern region. The relative temperature of $30^{o}C$ is required for the optimum growth of the garlic and the cooler condition at later and early period of growth respectively is highly favored in the North.

Garlic seed oil is extracted by the method of solid-liquid extraction otherwise known as leaching i.e. separation of one or more soluble constituent(s) from sole phase by extraction with solvent. The solvent is later separated by collecting the pure oil separately.

1.1 Cultivation of Garlic

- **Soil and Preparation:** It can best be grown on an organically soft loamy soil, cultivated to a depth of 8- 10inches into the ground.
- **Method of Propagation:** The cloves are separated and planted in a shallow drill at the surface until almost covered; it is planted in firm soil at the later part of the rain.
- **Planting and Spacing**: The rows are spaced from 12 to 18 inches apart for the effective germination and the plants are 4 to 6 inches next to each other laterally.
- **Cultivation and Maturity**: Weeding, regular irrigation during growing period is normally practiced during growth until maturity. It matures in 10 to 15 weeks after planting.
- **Harvesting:** Bulbs are lifted when leaves turn brown and dry. Leaves used for flavouring are removed with sharp knife above top of the bulb can be stored for one year in a well ventilated condition (Vanketel and Dehaan, 1978).

1.2 Aims and Objectives

Since garlic oil has been reported to enhance the cure of hypertension and other related artery diseases, this research work aimed at the followings:

1. Extraction of garlic oil using solvent extraction method.
2. Examine both the physical and chemical characterization of the garlic oil.

1.3 Significant of Study

The review of literature revealed that garlic oil contains some special group of ester, alcohol, and amine groups which serves as the major ingredients in the hypertensive, diabetes drugs (Atueyi I.1992). It also enhances cure for heat attack, stroke, common cold as well as other associated illness.

As a result of the afore mentioned application of garlic oil, there is a need for research work on garlic oil extraction to fully exploit its potentials

1.4 Scopes and Limitation of the Work

Many researchers are still underway to discover the potentials and the unprecedented health benefits of garlic and its oil to human consumption. For instance, the relevance of garlic and anti-cancer, anti-infection and cardiovascular and among others,

The non-availability of ultra modern equipments and computers required for the extraction and analysis of the relevant constituents has a major impediment for the detailed research on these tests.

Also because of the huge amount of money involved in carrying out these micro-researches, the scope of this work is hence reduced to the extraction of the oil and analysing the physical and chemical constituents and characteristics.

CHAPTER TWO

2.0 LITERATURE REVIEW

The literatures reviewed in this work are presented in this section.

2.1 History

The word garlic comes from Old English garleac, meaning "spear leek." Dating back over 6,000 years, it is native to Central Asia, and has long been a staple in the Mediterranean region, as well as a frequent seasoning in Asia, Africa, and Europe. Egyptians worshipped garlic and placed clay models of garlic bulbs in the tomb of Tutankhamen. Garlic was so highly-prized; it was even used as currency. Folklore holds that garlic repelled vampires, protected against the Evil Eye, and warded off jealous nymphs said to terrorize pregnant women and engaged maidens. And let us not forget to mention the alleged aphrodisiacal powers of garlic which have been extolled through the ages.

Surprisingly, garlic was frowned upon by foodie snobs in the United States until the first quarter of the twentieth century, being found almost exclusively in ethnic dishes in working-class neighborhoods. But, by 1940, America had embraced garlic, finally recognizing its value as not only a minor seasoning, but as a major ingredient in recipes. Quaint diner slang of the 1920's referred to garlic as Bronx vanilla, halitosis, and Italian perfume. Today, Americans alone consume more than 250 million pounds of garlic annually. (Daniel and Maria, 2000)

Garlic seed is a bulb bearing perennial plant. It is closely related to onions, grows to a height of up to 1 foot and narrow, sword shape leaves. Garlic seed, like the onion has small, six parts, whitish flowers borne on umbels. The fruit is a capsule containing black kidney shaped seed. The bulb which has a strong characteristics oduor and taste with moisture of not less than 22% is covered with a papery skin and may be broken into constituent bulb that's called cloves.

When the cloves are filly matured in the late summer or early autumn, they are gathered and used as culinary herb. Garlic is widely cultivated in many part of the world in the manner as onion. In the spring, the cloves are set into four to six inches apart in ordinary garden soil. Garlic thrives under a wide variety of soil and climate.

South west of Asia believes that the edible bulb of garlic plant composed of sugar rich food. The storage leaves are also source of pungent oil. Their long tubular, aboveground leaves are also eaten. (Gribss A. 1990).

2.1.1 Description

Garlic resembles the onion except that it has flattered solid leaf blades and produces a composite or compound bulb, consisting of several small densities crowded, angular, bulblets or cloves enclosed within the white or pink skin of the parent bulb. Each clove is derived from the auxiliary bulb of the younger foliage leaf and consist of a protective cylindrical sheath a single thickened storage leaf and a small central bud. The leafless flowering stalks when produced is smoother, round, solid and is at first coiled. The younger flowers head is protected by a long beaked, periphery path, which is soon shed. The whitish flowers are generally intermixed with the bulbils.

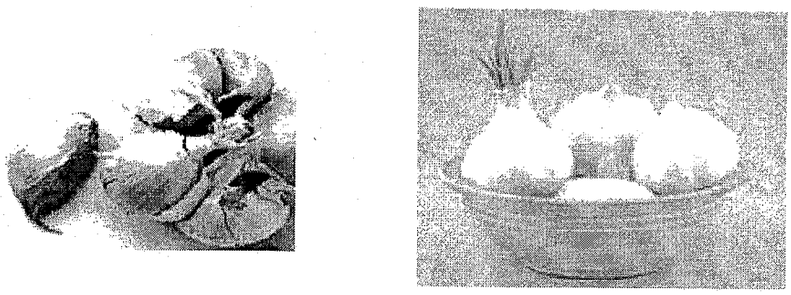


Fig 2.1: garlic bulbs

Wild garlic is an erect, strong smelling perennial up to 1m high. It has three methods of reproduction - bulbils, bulbs and seeds. Wild garlic bulbs and bulbils sprout after the first autumn rains. During winter and spring the leaves and stems develop while underground

bulbs form at the base of the plant. Heads are produced in late spring through to summer. Most heads produce bulbils only, but some heads produce both bulbils and flowers. Some seeds are formed by the flowers but reproduction by seeds is minor. When the aerial parts of the plant die in late summer the bulbils are shed on to the ground.

Stems - Cylindrical and unbranched, may produce clusters of aerial bulbils and/or flowers.

Bulbs - Up to six are formed at the base of the plant around the old bulb. Some bulbs have soft white shells, others have hard brown shells and can remain dormant in the soil for up to six years. Bulbils - Brown, smooth and shiny, similar in size and shape to wheat grains; up to 300 produced in a terminal head. (Parsons and Cuthbertson,1992)

2.1.2 Method of Propagation

Garlic is propagated vegetative through its cloves and planting is done in rows. It is a frost hardy crop, which develops underground, and the crop matures in about four to five months. Owing to its pungency, garlic is generally regarded as flavoring agent rather than as a vegetable; been used largely for flavoring soup, stew pickles and salad, it has a strong flavoring than in onion and its smell stays in the mouth for hours. Good cooks rub their salad with cloves of garlic before they put in the salad ingredients. Garlic is very popular in France especially in province where almost every dish is flavoured with garlic. The pungent substance, Allicin, is released when garlic is cut or crushed as the substrate. Allinine is converted to Allicin under the influence of enzyme Allinase. Dehydrated garlic in a pulverized or granular state is replacing the fresh bulbs for industrial and home use in many countries. (Paul, 1962)

2.1.3 Harvesting

The harvesting season in California is from May to August. Garlic bulbs are very delicate, bruise easily and losing quality. Caution must be taken during harvest to ensure gentle handling. The first harvesting step is to mow the foiled and cut off dried tops of the garlic bulbs cloves to the ground. After this the field is sprinkled with water to soften soil and the foot is mechanically undercut with a sharp blade pushed through the soil below

the garlic bulbs. They are carefully scooped up and deposited on soft ground. Bulbs are loaded into the sucks by hand after sorting out damage ones on the field. (Paul, 1962)

2.1.4 Varieties

The principle varieties mostly grow for dehydration are; California late (Pink or Italian), California early (White or Mexican) and Creole. Because of the problem of nematode transmission in the planting stock, there is an increasing tendency to be free from disease and pest as garlic is an expensive crop to grow.

2.1.5 Garlic Selection and Storage

Choose garlic heads that are firm to the touch, with no nicks or soft cloves. If you notice dark, powdery patches under the skin, pass it up because this is an indication of a common mold which will eventually spoil the flesh.

Unpeeled heads of garlic is stored in an open container in a cool, dry place away from other foods. Do not refrigerate or freeze unpeeled garlic. Properly stored garlic can keep up to three months. As garlic ages, it will begin to produce green sprouts in the center of each clove. These infant green sprouts can be bitter, so discard them before chopping the garlic for your recipe.

However, if you plant the cloves and let them sprout to a height of about six inches, you can use the sprouts like chives in salads and such.

Garlic can also be purchased as peeled whole cloves or minced, both stored in olive or vegetable oil.

Harvested garlic should be stored in a cool dry place with access to air. It may be kept for about 6 months after harvesting. (Kamenetsky et al, 2004)

2.1.6 Dehydration Method of Storage

Another method of storage which constitutes the largest method of storage is by dehydration. This method involves grinding of the garlic bulb and then store under controlled temperature of relative humidity not more than 70%.

Garlic bulbs which may consist of 6 to 36 cloves are broaden into individual cloves by passing between rubber covered rollers which exert just enough pressure to crack the bulb without crushing the cloves. The loose ''Paper shell'' is removed by screening and aspirating. The cloves are then washed in a flood washer at which the time the root shrubs are floated off. Garlic is sliced and dehydrated in a manner similar to that used for onions. After drying the pink skin, which adhered to the fresh cloves, can be removed screening and air aspiration, garlic is commercially dried to about 6.5% moisture content.

Dehydrated garlic is sold commercially as powder granules or in sliced chopped or in minced from packing similar to that used for onion. (Paul, 1962)

2.1.7 Diseases and Infection Attacked

During storage, Erwinia and Botrytis spp attack garlic and onion. Fasarium spp. causes bulb end rot. Members of pseudomonas Cepacea are frequent spoilage agent of garlic and onions aspergillums alia are specific of decay producing black mould rots. The number of micro organisms present in dried powder varies from day to day and count of viable organisms are often high that no specification can be conveyed to purchasers.

Owing t the bacteriostat properties of fresh and dried onion and garlic, their plate count are unreliable. A 0.5% concentration of potassium bisulphate in diluents overcomes the inhibition (Wei et al, 1967). The same principle has now been advocated in performing pre enrichment procedure when all spoke, cannon and are fan are cultured for the presence of salmonella (Nilsson and Andrew, 1976)

2.2 Advantages/ Importance of Garlic over Onion

The garlic uses are very wide. This ranges from a flavour in the preparation of food, as a drink in form of juice, larvicidal, pharmaceuticals and medicinal among others

2.2.1 Drinks

Garlic juice succus-Allis, Bruise garlic 80g and express the juice, the marc is mixed with water 20ml and again express the liquid. The operation is repeated until the desired

volume of the mixed juice is achieved. If 20ml of alcohol is added and then allow staying for 14 days, the product is then filtered. The constituent's composition for 20ml is 80g sucrose (6% acetic acid) and 2 to 4ml of juice can be taken as a dose.

Garlic has expectorants, diaphoretic disinfectant and direct properties. This can be use in dose of 2 to 8g. The juice is also use in the treatment of pulmonary condition but the administration of garlic to children is too dangerous and fatalities have been reported. The garlic juice and oil extracted prevent the hyperplasia and blood coagulation charges fat investigation in 5 healthy subjects. (Wade,1982)

2.2.2 Flavour

Onion, garlic and chive belong to the genus Alliums whose members contain important sulphur flavour compound. The compounds include thiols, sulphide, disulphide, trisulphide, and thiosulphates. Both onion and garlic are of classic example of enzymatic flavour propagation in which the initial reaction product being unstable and undergo further reaction.

The general enzymatic reaction catalyze by the genus Alliums can be formulated thus

$$R\text{-}SOCH_2\text{-}CHN_2\text{-}COOH \longrightarrow RS\text{-}SO\text{-}R + NH_3 + CH_3COCOOH$$

R represents Methyl, Propyl, 1-Propyl or Allyl. The 1-Propyl compound has been identified as the lachrymator in garlic and onion.

Allicin, the active odour principle of fresh garlic, is 2-Propenyl-2-Propene Thiosulfinate. In common with all thiosulfinates, Allicin readily disproportionate spontaneous to

The odour of various crops in the Allicin genus has been characterized according to the alkyl group of the sulfides and the sulfides are formed from the degradation of RS-SO-R (thiosulfinate) (Paul, 1962)

2.3. Application of Garlic

Garlic oil can be used in different ways such as,

2.3.1 Pharmaceuticals and Medicinal

An extract of garlic called Allicin possesses antifungal activity against Candida, Cryptococcus, Trichophyton, Epidermophyton, and Microsporum spp in vitro. An application of this slice of garlic bulb was reported to aid healing during repair of perforation of the eardrum in 17 of 18 patients at Chinese Hospital. Garlic is the most potent internal disinfectant, a food disinfectant dot a chemical disinfectant, which disinfect not merely the alimentary tract, but the whole body.

If garlic is rubbed on the sole of foot, it becomes noticeable after a few minutes in the breath; it is considered an invaluable germicide. It destroys threadworms and other worms in the intestines it is of value in disorder of stomach, constipation, colitis, colic, and in Germany it is very frequently given, and apparently with success, in the case of arteriosclerosis. A remedy which has been employed continuously for thousands of years by civilized nation cannot be treated as negligible. In the case of diarrhoeas were due to typhoid, cholera, and other serious conditions.

2.3.2 Larvicidal

The larvicidal principle of garlic, it was found out that dially, di and trisulfides compound in the seed prove active against culex mosquito. Both natural and synthesis samples poured larvicidal at 5ppm. (Paul P. C 1962)

2.3.3 Coagulation

Increase plasma fibrinogen, decrease coagulation time and blood fibrinolytic activity was associated with high fat diet, all of these enhance thrombosis. Garlic feeding prevents the rise of plasma fibrinogen and fall clothing time. Fibrinolytic activity suggests that component of garlic may prevent thrombosis disorder. (Bordia and Verma 1978)

2.3.4 Effect on Lipoproteins

The effect of garlic on lipoproteins was studied in patients with coronary heart disease. Sixty-two patients were divided randomly into groups of which group I was placed on

essential oil of garlic i.e. 0.25mg for 10 months while group II served as the control experiment. Serum lipoproteins were checked at bimonthly intervals. Lipoprotein level remained rather constant in the control group during the 10 months period. The group taking essential oil of garlic however had steady decrease of low density lipoprotein and very low density lipoprotein accompanied by a progressive rise of high density lipoprotein. Lowering of LDL has also been reported in healthy individual ingesting raw garlic. (Jain,1977)

2.3.5 Chemotherapeutic Effect

Garlic oil is used for the treatment and/or of prevention of the following diseases; heart attack, hypertension, stroke, common cold as well as other associated illness.

When taken orally, garlic oil has been found to elicit a variety of physiological effect such as stimulation of bile production, lowering of blood glucose and lipid levels as well as acceleration of wound healing, reduction of hypertension and curing of common cold (Tyler,1976). When taken alone, garlic oil was found to regulate digestion, improving appetite, and clear the respiratory track. It has a very strong bactericidal acting and hence kills germs, which produce skin infections garlic oil has been reported to increase insulin level significantly and this being in the partial treatment of diabetic mellitus(Adamu etal,1984).

Both onion and garlic oil were found to inhibit blood platelet aggregation. The inhibition of blood clot formation is am important property of the oil as these prevent the onset of thrombosis episodes such as stroke or arteriosclerosis both of which may lead to fatal consequence. Studies have attributed the prophylactic and biological activities of garlic oil and onion oil to their active principle, namely:

Di -ally disulphide (C_3H-S_5-S-C_3H_5) in garlic oil and allyl propyl disulphide (C_3H_5-S-S-C_3H_7) in onion oil

Garlic is now marketed in the form of garlic pearls which contain 0 – 25% W/W of garlic oil with a recommended dose of 1 – 2 capsules before each meal.

2.3.6 Diabetes Treatment

The disease, diabetes mellitus, is caused by the inability of the body to produce the hormone insulin, which is required for the transportation of glucose across the membrane into the cell or catabolism to produce energy. The characteristic symptoms of the disease include increase in blood glucose and lipid level as a result of increased mobilization of fat from adipose tissues as an alternative source of energy. The principle of treatment of diabetes is to lower the blood glucose oxidation for energy production and increase lipid hydrolysis.

Garlic oil is effective in treatment of diabetes because it lowers blood sugar and lipid level. This effect is attributed to the organic disulphide in the oil. Available evidence suggested that the hypolipidemic effect of garlic oil and onion oil might be due to one or a combination of the two or more of the following biochemical mechanism:

Inactivation of the active site of thiol group (-SH) of key enzymes of lipid and carbohydrates metabolism by chemical components of the oils.

Increase hydrolysis of triglycerides as a result of garlic/ onion oils induced increase in lipid activity.

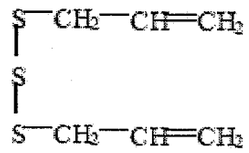
Reduce rate of triglyceride biosynthesis caused by reduce availability of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) as its utilization in metabolism of garlic/ onion oil. (Paul,1962)

2.4 Chemistry of Garlic

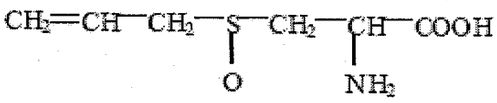
Alliin→Alliinase → Allicin

Basic information of Garlic Extract

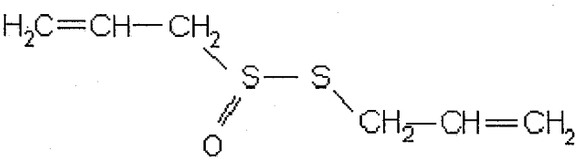
Allitridum chemical structure:



Alliin chemical structure:



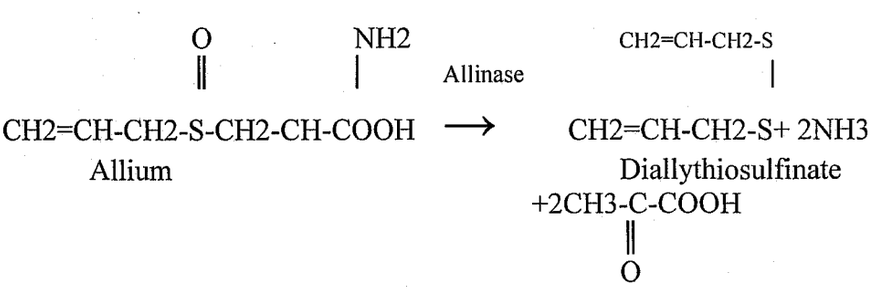
Allicin chemical structure:



SYNONYMS: 2-Propene-1-sulfinothioic acid S-2-propenyl ester Diallyl thiosulfinate

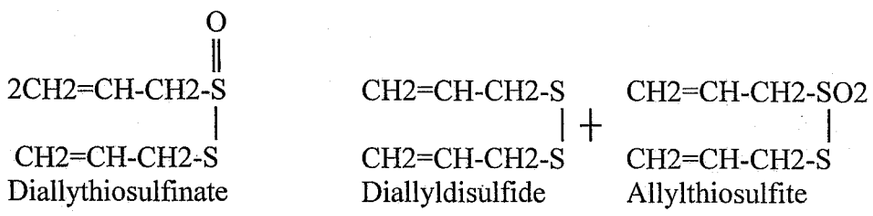
FORMULA: $C_6H_{10}OS_2$ (Lawson and Hughes, 1992)

Vegetable that belong to the genus Allium include onion, garlic, leeks and chives. Of this group of vegetable, the aroma of garlic was the first studied. This pungent aroma is derived from an odourless compound in the intact clove that when first studied was given the tribal name of Allium. This order precursor, a derivative of the amino acid, cyterine, was isolated from Garlic and identified as (+)-S-allyl-L-Cyterine sulfoxide. In the intact clove, this compound is stable but slicing or crushing the substance into contact with an enzyme Allinase that is present in Garlic. Allinase catalyses the conversion of (+)-S-allyl-L-Cyterine sulfoxide to dially thiosulfinate, ammonia and pyruvic acid as shown below.



Diallythiosulfinate (2-propenyl-2-propenethiosulfinate) had been isolated earlier from garlic oil by carallites and given the trivial name Allicin. Diallylthiosulfinate is volatile and is described as garlic like but not pleasant. Such thiosulfinate are unstable, however

and by disproportions give rise to a thiosulfinate and a disulfide. The diallyl disulfide from dially thiosulfinate is responsible for the characteritics oduor of garlic. Thus:



Analyses of volatiles from a number of Alliums were made and it was reported that the following percentages of the three sulfide radicals (methyl, propyl and allyl in the vapour from raw Alliums: 80-87% allyl, 12-19% methyl and 1% propyl in garlic.)

1% allyl, 96% propyl and 3% methyl in one variety of onion.

4% allyl, 75% propyl and 21% methyl in chives

5% allyl, 67% propyl and 28% methyl in leeks

The aroma from garlic was characterized by its high proportion of diallyl disulphide (80-89%) while chives, leeks and onion have less than 6%. A high proportion of dipropyldiosulphide characterized the aroma from onions and to a lesser extends that of chives. Chive contains more of the methyl propyl disulfide than onion. In the even milder leeks, methyl propyl disulfide accounted for more than half of the sulfide present. The presence of more than one type of allyl cysteine sulfoxide in these vegetables gives rise to mixed thiosulfinate, which accounts for the presence of mixed disulfide. (Paul,1962)

2.4.1 Classification of Garlic Products on the Market

Unlike most foods, food-processing does miraculous things to garlic. It triggers the formation of a cascade of compounds that do not already exist in raw garlic. Therefore, processing is the key to increasing the benefits of garlic and to decreasing or eliminating its toxic effects. A variety of sulfur-containing compounds formed through chemical and biological reactions, in addition to non-sulfur compounds, work synergistically (and/or antagonistically) in their contribution to the benefits of garlic internally. Based on their chemical constituents, all garlic products on the market today could be placed in one of the following four categories:

2.4.2 Garlic Oil

These products offer minute amounts of garlic essential oil in a large amount of vegetable oil. The percentage of essential oil in bulb ranges from 10.01% to 19.27%. They often express their "potencies" in theoretical amounts of raw garlic used to obtain the distilled garlic oil. There is no scientific data to show that the oil fraction represents all of the benefits of garlic. Garlic essential oil, which mainly contains several sulfides, is also the most potent source of garlic odor and causes body odor. In addition, garlic oils do not contain any of the important water-soluble compounds.

Raw garlic contains a few lipids (one or two tenth of a percent). Oil is not formed until garlic is crushed and steam distilled and then degraded down into the oily mix of sulfurous compound. The more time that elapses after crushing the more complex the compound becomes and less sulfurous they smell. The simpler sulfur compounds have the most smell and the most anti-bacterial action. The polysulfide which are among the last breakdown products of allicin have the least taste and smell and the least immediate anti-biotic effects, but are responsible for many of garlic other physiological effects. It is the allicin which is garlic's natural protection from pest and diseases. (Cavallito and Barley, 1944)

2.4.3 Garlic Powders

Chemically, there is almost no difference between the garlic flavoring powders sold at grocery stores and the garlic supplements made of garlic powder and then sold at health food stores. Often these manufacturers claim that their products deliver allicin into the body. Though no garlic supplement can contain allicin due to its instability and high reactivity, some garlic powder products contain alliin and the enzyme, alliinase, and, therefore, can produce allicin with the addition of water in a test tube (the so-called "allicin potential"). However, allicin potential is an empty promise. The environments in a test tube and in our body are totally different and there is no scientific evidence to support the idea that allicin can be produced in the stomach. Furthermore, garlic powder

contains only a residual amount of the alliin found in raw garlic, i.e., more than half of the alliin is lost during the manufacturing process. (Cavallito and Barley, 1944).

2.4.4 Garlic Oil Macerates

There are two types of oil macerate products on the market and both are packaged in soft gel capsules. One is made by simply mixing a garlic flavoring powder with vegetable oil. Its constituents are almost the same as the capsule and tablet forms of garlic powder. Another one is made by grounding raw garlic in vegetable oils. (Cavallito and Barley, 1944).

2.5 Adverse Effect of Garlic

Known adverse effects of garlic include halitosis (non-bacterial), indigestion, nausea, emesis and diarrhea.

Garlic may interact with warfarin, antiplatelets, saquinavir, antihypertensives, Calcium channel blockers, hypoglycemic drugs, as well as other medications. Consult a health professional before taking a garlic supplement or consuming excessive amounts of garlic. Garlic can thin the blood similar to the effect of aspirin.

Two outbreaks of botulism have been caused by consuming commercially produced garlic-in-oil preparations that were not properly preserved. It is especially important for home-preparation to use safe and tested food-preservation methods to retard bacterial growth, such as including sufficient salt or acidity and keeping the mixture refrigerated. It is recommended to not keep home-preparations for more than a week.

While culinary quantities are considered safe for consumption, very high quantities of garlic and garlic supplements have been linked with an increased risk of bleeding, particularly during pregnancy and after surgery and child birth. Some breastfeeding mothers have found their babies slow to feed and have noted a garlic odour coming from their baby when they have consumed garlic.[The safety of garlic supplements had not been determined for children.

The side effects of long-term garlic supplementation, if any exist, are largely unknown and no FDA-approved study has been performed. However, garlic has been consumed for several thousand years without any adverse long-term effects, suggesting that modest quantities of garlic pose, at worst, minimal risks to normal individuals. Possible side effects include gastrointestinal discomfort, sweating, dizziness, allergic reactions, bleeding, and menstrual irregularities.

Some degree of liver toxicity has been demonstrated in rats, particularly in large quantities.

There have been several reports of serious burns resulting from garlic being applied topically for various purposes, including naturopathic uses and acne treatment. On the basis of numerous reports of such burns, including burns to children, topical use of raw garlic, as well as insertion of raw garlic into body cavities is discouraged. In particular, topical application of raw garlic to young children is not advisable. Garlic and onions may be toxic to cats and dogs (Borrelli, 2007)

2.6 Vegetable Oil and Fat Extraction

In modern days, vegetable oil and fats are extracted from seeds such as groundnut, cotton seeds, soyabeans, garlic seed etc. this is carried out by either mechanical pressing or solvent extraction

2.7. Methods of Extraction

Volatile oils may be recovered from plants by a variety of methods:

(1) Distillation: distillation can de divided into the following

a. Water distillation

b. Steam distillation

c. Hydro-distillation

d. Water and steam distillation

(2) Solvent extraction : the following methods are listed under the solvent extraction

a. Maceration

b. Enfleurage

c. solvent

(3) Expression

2.7.1 Distillation

This is a method of separating mixtures based on different in their volatilities in a boiling liquid mixture. Distillation converts the volatile liquid (the essential oils) into a vapor and then condenses the vapor back into a liquid - it is the most popular, and cost effective method in use today in producing essential oils.

The downside of distillation is the fact that heat is used in this extraction method, which makes it totally unacceptable for use on very fragile material, or where the oils are extracted with great difficulty.

When this method of extraction is applied, great care has to be taken with the temperature and length of exposure of the heat to prevent damage to the oils.

Distillation can be divided into the following

(a) Water Distillation: In the manufacture of essential oils using the method of water distillation, the botanic material is completely immersed in water and the still is brought to the boil. This method protects the oils so extracted to a certain degree since the surrounding water acts as a barrier to prevent it from overheating.

When the condensed material cools down, the water and essential oil is separated and the oil decanted to be used as essential oil.

The water that is so separated in this process is also used and is marketed as "floral waters" (also called hydrosol or sweet water) - such as rosewater, lavender water and orange water.

Water distillation can be done at reduced pressure (under vacuum) to reduce the temperature to less than 100 degrees, which is beneficial in protecting the botanical material, as well as the essential oils.

(b) Steam Distillation: When steam distillation is used in the manufacture and extraction of essential oils, the botanical material is placed in a still and steam is forced over the material. The hot steam helps to release the aromatic molecules from the plant material since the steam forces open the pockets in which the oils are kept in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam.

The temperature of the steam needs to be carefully controlled - just enough to force the plant material to let go of the essential oil, yet not too hot as to burn the plant material or the essential oil.

The steam which then contains the essential oil, is passed through a cooling system to condense the steam, which forms a liquid from which the essential oil and water is then separated.

The steam is produced at greater pressure than the atmosphere and therefore boils at above 100 degrees Celsius which facilitates the removal of the essential oil from the plant material at a faster rate and in so doing prevents damage to the oil.

Some oils, like Lavender is heat sensitive (thermolabile) and with this extraction method, the oil is not damaged and ingredients like linalyl acetate will not decompose to linalool and acetic acid.

(c) Hydro- Distillation: When essential oils are extracted using hydro diffusion it is a type of steam distillation, and only varies in the actual way in which the steam is introduced into the still. With hydro diffusion the steam is fed in from the top onto the botanical material instead of from the bottom as in normal steam distillation.

The condensation of the oil containing steam mixture occurs below the area in which the botanical material is held in place by a grill. The main advantage of this method is that less steam is used, shorter processing time and a higher oil yield.

(d) Water and Steam Distillation: When steam distillation is used in the manufacture and extraction of essential oils, the botanical material is placed in a still and steam is forced over the material.

The hot steam helps to release the aromatic molecules from the plant material since the steam forces open the pockets in which the oils are kept in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam. The temperature of the steam needs to be carefully controlled - just enough to force the plant material to let go of the essential oil, yet not too hot as to burn the plant material or the essential oil.

The steam which then contains the essential oil, is passed through a cooling system to condense the steam, which forms a liquid from which the essential oil and water is then separated. The steam is produced at greater pressure than the atmosphere and therefore boils at above 100 degrees Celsius which facilitates the removal of the essential oil from the plant material at a faster rate and in so doing prevents damage to the oil. Some oils, like Lavender is heat sensitive (thermolabile) and with this extraction method, the oil is not damaged and ingredients like linalyl acetate will not decompose to linalool and acetic acid.

2.7.2 Solvent Extraction

When we talk about the broad term of solvent extraction, itdoes not only refer to chemical solvents like hexane, but also to other forms - such as solid oil and fat as well as carbon dioxide. Solvent extraction is particularly suitable for botanical material that has a very low yield of essential oil, or where it is made up of mostly resinous components and as such delivers a far finer fragrance than that of distillation. During this type of extraction, non-volatile components of the botanical material - such as waxes and pigments are also extracted and in some cases this is then removed during another process.

Under solvent extraction we list the following methods:

(a) Solvent Extraction: Essential oils can be extracted by using solvents such as petroleum ether, methanol, ethanol or hexane and is often used on fragile material such as jasmine, hyacinth, narcissus and tuberose, which would not be able to handle the heat of steam distillation. This process employs a solvent to leach out the oil and it is only practical method for recovering oil from tissues having a relatively low proportion of oil

In this process a chemical solvent such as petroleum ether is used to saturate the plant material and pull out the aromatic compounds. This renders a substance called concrete. The concrete can then be dissolved in alcohol to remove the solvent. Sometimes even the press cake left during mechanical pressing is subjected to solvent extraction to retrieve the last traces of oil. This method is quite effective but expensive. Residues of the solvent may remain in the absolute and contaminate the essential oil. The most important factor in this method is the selection of the solvent. The solvent must be selective, have a low boiling point, be chemically inert in the oil, and evaporate completely without leaving any odorous residues and must also be low – priced (Austin, 1984).

Solvent extraction can be used for oil extraction even when the oil content is too low for pressing to be employed. This process is faster and less tedious.

(b) Enfleurage: Enfleurage could be compared to certain aspects employed in maceration, but is done in a slightly different way. Glass plates in a frame (called a chassis) are covered with highly purified and odorless vegetable or animal fat and the petals of the botanical matter that are being extracted are spread across it and pressed in. The flowers are normally freshly picked before so encased in their fatty bed.

The petals remain in this greasy compound for a few days to allow the essence to disperse into the compound, where the then depleted petals are removed and replaced with a fresh harvest of petals. This process is repeated until the greasy mix is saturated with the essence, and needs to be repeated a couple of times until saturation is achieved.

When the mix has reached saturation point the flowers are removed and the enfleurage pomade - the fat and fragrant oil - then washed with alcohol to separate the extract from the remaining fat, which is then used to make soap.

(c) Maceration: With the maceration extraction method, the flowers are soaked in hot oil to have their cell membranes ruptured and the hot oil then absorbs the essence. The oil is then cleared of the botanical and decanted. This is very much the same technique used in solvent extractions, where solvents are used instead of the hot oil as used in maceration

2.7.3 Expression

Most citrus peel oils are expressed mechanically, or cold pressed. Due to the large quantities of oil in the citrus peel and the relatively low cost to grow and harvest the raw materials, citrus-fruit oil are cheaper than most other essential oils. Lemon or sweet orange oil that are obtained as by-product of the citrus industry are even cheeper. When a "cold pressed" method is referred to in the manufacture of essential oils, it basically refers to the expression method, since no heat is involved in this method.

Most nut and seed oils are also extracted using a "cold pressed" method but here oil is forced from the material under high mechanical pressure and generally produces a good

quality oil, but some manufacturers do impair this good quality by excessively refining the oil after extraction by means of chemicals or high heat.

But when we return to look at the expression method of extraction in the manufacture of essential oils, we find that most citrus essential oils are extracted this way and that three different ways are used to accomplish it.

Sponge extraction process: Most citrus essences are extracted by means of expression, and in the past were done by hand where the fruit pulp was removed, with the rind and pith then soaked in warm water to make the rind more pliable, since the pith of the fruit absorbed the water.

After the fruit has absorbed the water and become more elastic, it was inverted which helped to rupture the oil cells and a sponge placed next to the rind. It was then squeezed to release the volatile oil, which was then collected directly into the sponge. As soon as the sponge became saturated with oil, it was squeezed and the essential oil collected in a vessel and then decanted.(www.essentialoil.com)

2.8 Automatic Soxhlet Extraction

The method described by Soxhlet in 1879 is the most commonly used example of a semi-continuous method applied to extraction of lipids from foods. According to the Soxhlet's procedure, oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent, usually hexane or petroleum ether, under reflux in a special glassware.

In this method the sample is dried, ground into small particles and placed in a porous cellulose thimble. The thimble is placed in an extraction chamber (2), which is suspended above a flask containing the solvent (1) and below a condenser (4). The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain

level it overflows and trickles back down into the boiling flask. At the end of the extraction process, which lasts a few hours, the flask containing the solvent and lipid is removed. In some device a funnel (3) allows to recover the solvent at the end of the extraction after closing a stopcock between the funnel and the extraction chamber. The solvent in the flask (1) is then evaporated and the mass of the remaining lipid is measured. The percentage of lipid in the initial sample can then be calculated. (Garcia and Anal Chem 1998)

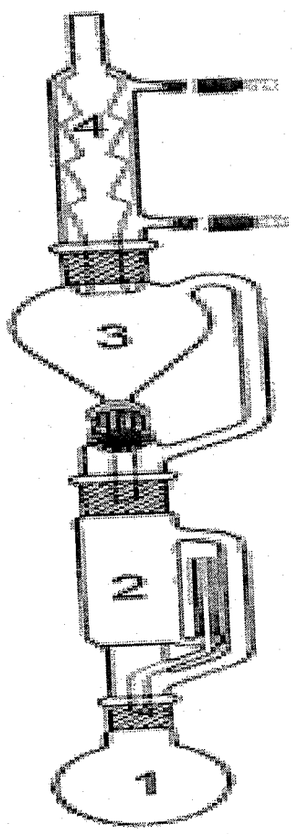


Fig2.2: Setup of Soxhlet Apparatus

2.9 The Choice of Solvent

The choice of the solvent to be used in extraction is very important. Apart from the fact that it determines the extraction rate, it also affects the quality of the product. The following are some of the factors to be considered in choosing a solvent.

Stability: the solvent should not decompose on heating. It should be relatively stable to avoid any side reaction.

Toxicity: since the product of extraction is either used by man or livestock, a toxic solvent is not proper for extraction because of the health implications.

Selectivity: the solvent must possess the ability to selectively extract the desired solute.

Recoverability: this is the ability of the solvent to be recovered after extraction has been done. A good solvent should be recoverable and recycled.

Polarity: since organic solvent dissolves only organic substances, and inorganic substances are soluble in inorganic solvents, the polarity of the solvent must therefore be taken into consideration.

Viscosity: a good solvent for extraction should have sufficiently low viscosity so that it can flow freely.

Volatility: good extraction are obtained when the solvent chosen posses relatively high volatility

Boiling point: The solvent should have a reasonably low boiling to allow to simple distillation, for solute as well as solvent purification.

The solvent should be non-corrosive

2.10 Characterization of Fats and Oils

Generally, fats and oils are characterized by carrying out analysis and test on them. i.e. to check out their quality and purification level, nutrient content and presence of toxicity as well as their identifications. Many are empirical, while others are quite specific measurement.

The characteristics can be divided into two:

The physical tests

The chemical tests

2.10.1 Physical Tests

This examines the physical properties of the oil and they are:

-Specific gravity: this is the ratio of weight substance to the weight of the same volume of water at a specified temperature.

-Boiling point: this is the temperature at which its vapour pressure becomes equal to the atmospheric pressure.

-Refractive index: this is the degree of deflection cause in a ray of light in passing from one transparent medium to another.

PH: this is used to express the degree of acidity and alkalinity of a substance. Others includes: viscosity, density etc.

2.10.2 Chemical Tests

This examines the physiochemical properties of the oil. It is the identification and measuring of the chemical composition of a substance. A chemist carrying out a qualitative analysis seeks to identify the substances in the sample. A quantitative analysis is an attempt to determine the quantity or concentration of a specific substance in the sample. Types of specialized chemical analysis performed are:

Saponification value: this is the number of milligrams of potassium hydroxide required to neutralize the fatty acids resulting from complete hydrolysis of 1g of the oil sample.

Acid value: this is the milligram of KOH required to neutralize free acid in 1g of the oil sample.

Iodine value: this is a measure of unsaturated acid present.

Others include free fatty acid.

2.11 Properties of n-Hexane

The property of n-hexane as reported in the Dictionary of Organic Compounds (1982) 5th Edition are given below

Boiling Point, Flash Point, Freezing Point, Moderate Toxic Vapour, TLV, Refractive index are 69.01^{0}C, -23^{0}C, -93.5^{0}C, 360, 1.3723 respectively

CHAPTER THREE

3.0 INSTRUMENTS/EQUIPMENTS AND MATERIALS

The apparatus, the raw materials, and chemicals used for this research work are listed in this section.

TABLE 3.0 **Instruments and Equipment**

INSTRUMENT/EQUIPMENT	MANUFACTURERS
1. Soxhlet Extraction Apparatus	Quick fit, England.
2. Mortal	England
3. Oven	Lab-line instrument Inc, England
4 .Weighing Balance	Brian Weigh England
5. Heating Mantle	England (Isopad Isomentle)
6. Thermometer	Zeal England
7. Stop Watch	Smith Germany
8. PH Meter	Corning Ltd USA
9. Visco Tester	Gladden, Japan
10. Temperature measurement(digital)	Copenhagen
11. Refractometer.	Bellingham Stanley
12. Density Bottle	Pyrex, England
13. Beaker, Conical flask, round bottom, flat bottom flask, pipette, measuring cylinder, filter paper, funnel, rotary evaporator.	Pyrex, England

TABLE 3.1 **Lists of Chemicals and Material**

CHEMICALS/ MATERIALS	MANUFACTURALS
1.Garlic Seed	Minna Niger state
2. Distilled Water	Chemistry Laboratory FUT Minna
3. Diethyl ether	May and Baker Limited England
4.KOH, NaOH, HCL, Hexane, Phenolphthalein, Ethanol	May and Baker Limited England.

3.1 Experimental Procedures and characterization of Garlic Bulb

This chapter describes the various experimental procedures and analysis carried out for the purpose of this research work.

3.1.1 Preparation of Garlic Seed for Extraction

The extraction of the garlic oil was accomplished through two stages. The stages are:

1. pretreatment stage
2. solvent extraction

Pretreatment stage: this include the various operation carried out on the garlic seed to make it ready for extraction. The operations are:

(i) Decortication of garlic seed: this is the removal of the outer layer to get the garlic seed. A knife was used to decorticate the seed.

(ii) Blending: the garlic seeds were blended with the use of electric blender in the laboratory.

(iii) Drying: the blended garlic sample was dried in an oven for 24 hours at the temperature of 80^0Cto remove the moisture. This enhances the oil extraction. The unit operation involved in the process was illustrated in the flow diagram below,

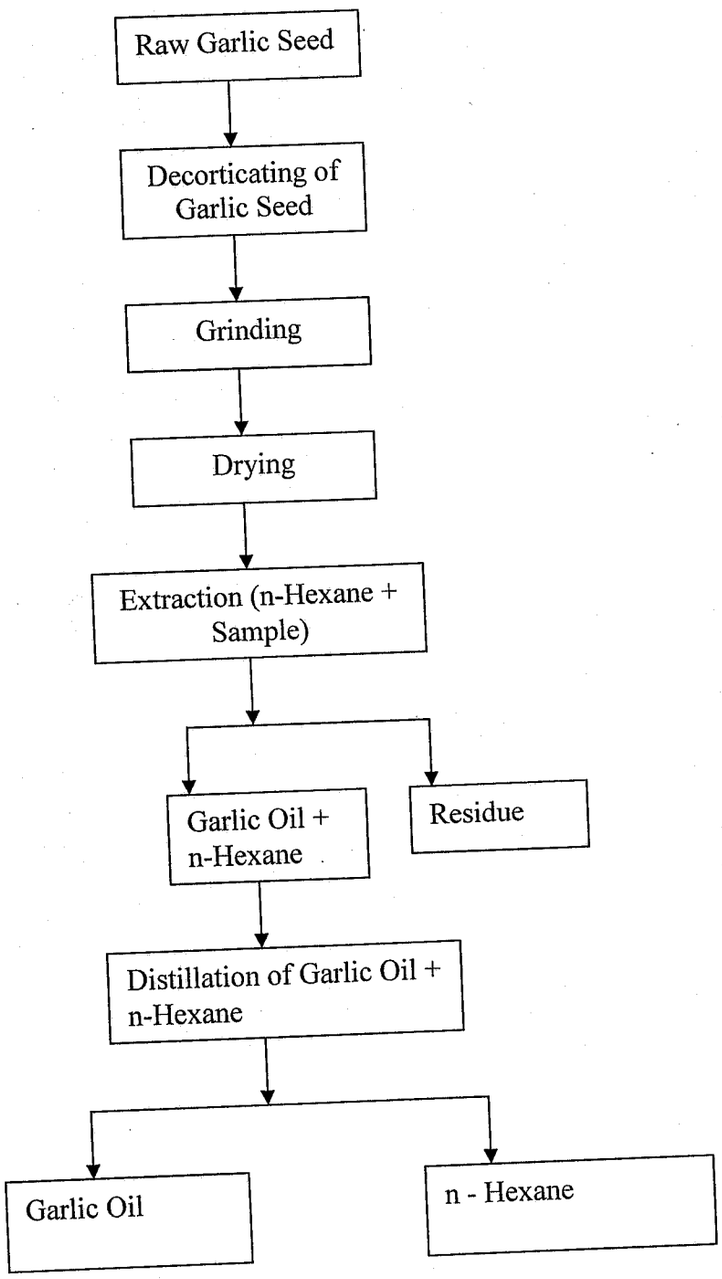


Fig. 3.1 Unit operation of the extraction of garlic oil.

3.1.2 Determination of Moisture Content

Procedure: A metallic dish was dried in an oven at a temperature of 80^0C for 20min, cooled in a dessicator . Few grams of garlic seeds were taken into the dish and weighed. The dish and the sample were then dried in an oven at a temperature of 80^0C for 30min. it was quickly transferred to a dessicator to cool and weighed after drying. The weights of the garlic seeds before and after drying were noted as W_1 and W_2 respectively. The process was repeated two more times. The loss in weight of the sample during drying is the moisture content. The moisture and other volatile contents were calculated in percentage as:

$$\% \text{ of moisture content} = \frac{W1-W2}{W1} \times 100\%$$

3.2 Extraction of Oil from Garlic Seed with a Soxhlet Extraction Apparatus Using Hexane as Solvent

Procedure: 350g of dried garlic seeds were crushed in a mortar. The crushed garlic seeds were known as the sample. The empty flask and a thimble were weighed and their weights were noted as M_1 and T1. Carefully some amount of sample was transferred into the thimble and the weight was noted. The weight of sample was then noted from the difference.

The thimble with the sample was placed inside a soxhlet extractor. 300ml of n-hexane was poured into 500ml round bottom flask. The soxhlet extractor with the thimble plus the sample was filled into the flask, which was placed in an electrically connected heating mantle. The clamp was used to secure it in position. The mantle was switched on and adjusted to a temperature of 70^0C and the heat increased carefully until the solvent boiled. The solvent in the flask reached its boiling temperature and vapour of n-hexane moved in the column where the garlic seed was charged. Contact between the vapour and the condenser section of the soxhlet arrangement brought about the condensation of the n-

hexane vapour. Condensed solvent vapour collected in the thimble, and dissolved the lipid in the sample. The condensed vapour thus leached out the garlic oil, the solvent with the dissolved lipid continuously ran back into the flask. The heating and the extracting process continued for 4 hours. The thimble containing the sample was removed and the sample was replaced with a fresh one, and the thimble was placed back into the extractor. The continuous soxhlet extraction was done for 6 fresh samples. The thimble was removed from the extractor after the apparatus was disconnected. The recovery of oil was done using a rotary evaporator. This separates the two mixtures that have different boiling points. Thereby mixture the solvent that has a lower boiling point was distilled from the mixture. This process continued until the solvent was recovered and only the oil was left in the flask with small amount of solvent. The flask containing the oil was removed and dried on a hot plate set at 100^0C for 30minutes to remove some of the solvent in the oil. The weights of dried oil W_0, together with a flask were noted as M_2. Weight of the empty flask was M_1, the total weight of the sample was known as Ws and it was determined as respectively. The oil was then subjected to further characterization

Note: each time when the extractor was disconnected in the process, the temperature of the heating mantle was reduced to 30^0C, which minimized the escape of the solvent. The percentage of garlic oil extracted was calculated thus:

$$\%\ of\ oil\ yield = \frac{M_2 - M_1}{W_s}\ x\ 100\% = \frac{W_0\ x\ 100\%}{W_s}$$

3.3 Characterization of Garlic Seed Oil

The characterization of garlic oil is divided into (i)physical analysis and (ii)chemical analysis.

(i) Physical Analysis: the physical analyses carried out in this wok includes, specific gravity, boiling point, refractive index, pH, viscosity and density.

3.3.1 Specific Gravity

Procedure: An empty beaker was weighed on weighing balance and weight was noted say B_1. Then it was filled up with water and the weight was also noted, B_2. The water was poured away and the garlic oil was filled into the same container. It was also denoted as B_3. The weight of water is: $(B_2 - B_1)$ g. the weight of oil is: $(B_3 - B_1)$ g.

The specific gravity was then calculated thus

$$\frac{\textit{weight of oil}}{\textit{weight of equal vol. of water}} \times 100\%$$

3.3.2 Boiling Point of Garlic Oil

Procedure: The boiling point of garlic oil was determined by putting the oil in a beaker and a thermometer immersed in it. It was heated on a heating mantle and after 45minutes of heating, the first bubble was observed. The temperature at which this bubble was observed is the boiling point of the garlic oil.

3.3.3 pH of Garlic Oil

Procedure: The pH electrode was lowered into buffer solution for stabilization of pH value. The temperature control was adjusted and the meter indicates exact pH, which was raised and rinsed with buffer solution. The electrode was then immersed in the garlic oil. The pH and the temperature of the garlic were noted.

3.3.4 Refractive Index

Procedure: Few drops of the oil were placed in the face of the prism of refractometer and gently spread and tightly closed with the slide. An ample time was allowed for the oil and the prism to attain a steady temperature. The refractive index was then read from the demarcation line after adjusting to where it coincides with the diagonal crossing.

3.3.5 Viscosity

Procedure: Two viscosity cups were installed on tripod stands each. Equal volume of garlic oil was poured into one cup while the draining hole was blocked. Immediately it was released, digital timer was pressed off and the time was noted. The same procedure was repeated two more time using equal volume of garlic oil. All the time was noted and the average of the time was taken.

The same method was used for water, and the average time taken to drain out of the viscosity cup was also noted.

Then the viscosity of garlic oil was calculated using below expression:

$$\frac{viscosity\ of\ water}{average\ time\ taken\ to\ drain} = \frac{viscosity\ of\ oil}{average\ time\ taken\ to\ drain}$$

Where viscosity of water at 29^0C is 7.65×10^{-4}

3.3.6 Density

Procedure: Weight of an empty beaker and when it contained oil was noted. The volume of oil was also noted. The difference in weight was calculated. Using the expression mass/volume, the density of the oil was determined at 29^0C.

3.4 Chemical Analysis

The chemical analyses carried out on this work include, free fatty acid, iodine value, Saponification value, acidic value.

3.4.1 Free Fatty Acid (FFA)

Free fatty acid value is a measure of the amount of free acids present in a substance. In the analysis of oil, the FFA value is defined as the number of milligrams of KOH required to neutralize the Free Fatty Acid present in 1g of oil. The presence of Free Fatty

Acid is an indication of the lipase activity or other hydrochloric acid which results into unpleasant taste and colour of the oil.

Procedure: 25ml of diethyl ether was mixed with 25ml of ethanol in a beaker. The solution was poured on 10g of garlic oil in the flask and 1ml of phenolphthalein was added to the mixture. The mixture was then titrated against 0.1M NaOH with constant shaking for which a dark pink colour was observed and the volume of 0.1M NaOH (V_b) was noted.

$$FFA = \frac{Vol.of\ base\ (V_b)\ x\ 2.82\ x\ 100}{Weight\ of\ sample\ (W_o)}$$

Where 100ml of NaOH = 2.82g of oleic acid.

3.4.2 Determination of Acidic Value

Procedure: Acid value of garlic oil was determined by multiplying FFA value of garlic oil obtained by two (2).

$$Acid\ Value = \frac{Vol.of\ base\ (V_b)\ x\ 2.82\ x\ 100}{Weight\ of\ sample\ (W_o)}\ x\ 2$$

3.4.3Determination of Saponification Value

This is the number of Potassium hydroxide required for complete Saponification of 1g of oil. If the oil is composed of glycerides, containing long chain, high molecular weight fatty acid, the saponofication value is low. If it is composed of low molecular weight, short chain fatty acid, the value is high. From the saponification value it is possible to calculate the molecular weight of oil.

Hydrolysis under basic condition is known as saponification. This has long been established method of making soap. Either sodium hydroxide or potassium is used. The format gives sodium salt of long chain fatty acid (hard soap), while the later produces potassium salt (soft soap). In each case glycerol is the by-product.

Procedure: 2g of garlic oil was weighed into a conical flask. 25g of alcohol KOH was pipette and added into the conical flask. The conical flask was attached to a reflux condenser and was boiled over 30 minutes with occasional shaking. Immediately after 30min of shaking, 1ml of phenolphthalein solution was added to the mixture and was titrated while hot, against 0.5M HCL acid solution.

The mixture was observed to be light yellow. The volume of 0.5M HCL acid solution (V_1 ml) used was noted. Blank solution was used to repeat the procedure as above, i.e. the same volume of oil was replaced with distilled water (V_0 ml).

1st titration = V_1 ml.

2nd titration = V_0 ml.

$$Saponification\ value\ is\ given\ by = \frac{(V_0 - V_1)xC\ x\ 56.1}{m}$$

3.4.4 Determination of Iodine Value

Iodine value is the measurement of the unsaturation of the fatty acids of the oil and it is determined by measuring the amount of iodine absorbed by the oil. It is unitless, that is the weight of iodine absorbed per 100g of oil.

Low iodine value indicates that there is a small production of unsaturated fatty acid and therefore the oil is a solid at room temperature, while high value shows a degree of unsaturation and therefore a liquid at room temperature.

Procedure: 2g of oil was added to 5ml of CCL_4, and then dissolved in 10ml of wij's solution. The mixture was previously moisture with some potassium iodide solution. This was then left in the dark for 30min, after which 7.5ml of potassium iodide was added and mixed. The solution was titrated against 0.1M of sodium thiosulphate solution using starch as the indicator just before the end point. After the addition of other drops, the liquid looked colourless (S ml), it then turned purple.

The same procedure was repeated for blank (B ml) titration. The titre values for both (S ml) and (B ml) were noted. The iodine value was then calculated thus:

$$Iodine\ Value = \frac{126.9\,C\,x\,(V_1 - V_2)}{m}$$

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

The results obtained are shown in this section.

TABLE 4.1 Determinations of Moisture Content and other Volatile Substance

Weight of sample before drying (W_1) g	Weight of sample after drying (W_2) g	% of moisture content
5.00	3.78	24.4
5.50	4.13	24.8
10.00	7.48	25.2

TABLE 4.2 Total Weight of Sample

Number of sample	Weight of thimble (g)	Weight of sample + thimble (g)	Weight of sample (g)
1	4.13	54.13	50.00
2	4.13	54.13	50.00
3	4.13	54.13	50.00
4	4.13	54.13	50.00
5	4.13	54.13	50.00
6	4.13	54.13	50.00
7	4.13	48.73	45.44

Total Weight of Sample (W_s) = $\sum weight\ of\ sample\ used$

The percentage of oil yield = 13.93%

TABLE 4.3 Physical Properties of Garlic Oil

Properties	Description
1. Colour	Brownish yellow
2.Odour	Unpleasant smell of garlic
3 Specific gravity	1.207
4. Boiling point	142^0C
5. pH	5.6 at 29^0C
6. Refractive index	1.447
7. Viscosity at 29^0C	3.65x 10^{-3} kg/ms
8. Density at 29^0C	1.353g/cm

TABLE 4.4 Determination of Viscosity

S/no	Volume of oil(ml)	Time of flow(s)	Volume of water (ml)	Time of flow (s)
1	3.50	5.26	3.50	1.37
2	3.50	6.59	3.50	1.28
3	3.50	6.71	3.50	1.32

TABLE 4.5 Chemical properties of Garlic Oil

Properties	Description
1. Free fatty acid	56.4%
2. Acid Value	1.128mgKOH/g
3.Saponification Value	224.40mgKOH/g
4.Iodine Value	79.63.

TABLE 4.6 Free Fatty Acid Analysis (0.1M NaOH Titration values)

NaOH	Initial (ml)	Final (ml)	Result (ml)
1st	2.20	4.10	1.90
2nd	4.10	6.20	2.10
3rd	6.20	8.20	2.00
			Average 2.00

TABLE 4.7: Saponification Value Analysis (0.5M HCL)

HCL	Initial (ml)	Final (ml)	Result (ml)
1st titre	5.00	16.60	11.60 (V_1)
2nd titre	16.60	44.20	27.60 (V_0)

TABLE 4.8 Iodine Value Analysis

	Sample Titration(ml)	Blank Titration(ml)
1st titre	81.70	71.50
2nd titre	84.20	69.30
Average	82.95(V_1)	70.40(V_2)

NOTE: Detailed calculations are presented in Appendices.

4.2 Discussion of Results

The extraction of garlic oil by solvent extraction using n-hexane as solvent was carried out and the percentage yield of oil was found to be 13.93% from Table 4.2 and detailed calculations were shown in Appendix A. This is a reasonable result since the garlic seed contain one or two tenth of a percent (Cavallito and Barley, 1944). It has also been reported that mechanical pressing extracts 9.8% of garlic oil. This means solvent extraction is more effective than pressing.

In the same Table 4.2, the sample was replaced six times after which the extraction has been assumed to have completed and the time taken for each extraction was four hours. However, it is known that the concentration or the viscosity of the solvent increases with the extraction and this reduces the extraction driving force. Hence, the subsequent extractions may not be as effective as when pure solvent is employed. The colour of the garlic oil was brown

The physico-chemical analyses of the oil were carried. In Table 4.3 the physical tests are: Colour, which was observed to be brown, specific gravity, was 1.207 which conforms closely to the literature range of (1.045 – 1.060) British Pharmaceutical Code (BPC 1934). The specific gravity is used to determine the purity of oil. The oil has a boiling point of 142^0C. The refractive index of the oil was found to be 1.447 at 29^0C which is lower than those of ground nut and the soya bean oil ranging from 1.460 to 1.470 (Isha, 1992). The pH value of the oil was found to be 5.6, which is acidic and this is because the oil has been decomposed i.e. there is fatty acid. The detailed calculations of the work were shown in Appendix B.

Results of the chemical analyses are presented in Table 4.5 to Table 4.8. Percentage of free fatty acid was found to be 56.4%, This is expressed as the commonest acid present in the oil. The acid value which is two times the free fatty acid was found to be 1.128mgKOH/g; this is the measure of amount of the free fatty acid present in the oil.

The saponification value was found to be 224.40mg KOH/g. this shows that the saponification value is moderately high due to the fact that the oil contains moderately high proportion of low molecular weight fatty acid and it is moderately high compared to other oil such as ground nut oil, cotton seed and soya beans (range from 187 - 202) (Atueyi 1992). This process measures the amount of alkali, which is required to combine with the fatty acids, liberated by the hydrolysis of the salt. Iodine value of 79.63was achieved i.e. 79.63 milligram of iodine was absorbed by 100g of fat. This is a measure of the proportion of unsaturated acid present. However, there is no iodine present in oil but the test measures the amount of iodine which can be absorbed by the unsaturated acid. This is one of the properties of unsaturated organic compound and tells the reactivity of the double bonds. Iodine value gives total degree of unsaturation expressed as the percentage of iodine absorbed by the oil.

Moreover, these compounds of garlic oil could be identified by infra red ray analysis i.e. the method which is used in detecting the presence and occasion for estimating the type of certain functional groups in fatty acid. But unfortunately the material to carry out this analysis was not readily available.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

The conclusions and recommendations made in this work are presented in this section

5.1 Conclusion

The extraction of garlic oil by soxhlet apparatus using hexane as the solvent was carried out within the limit of experimental error; the oil yield was 13.93%. Physico-chemical characterization of garlic seed oil was also conducted. The analyses revealed that the oil was brown, it possesses an unpleasant odour due to the presence of allicin compound in the oil. The oil has a specific gravity of 1.207, boiling point of 142^0C, pH value of 5.6, refractive index of 1.4470, viscosity was 3.65×10^{-3} kg/ms, and density of 1.353g/cm^3.

Chemical analysis also indicates that the oil has free fatty acid of 56.4%, acid value of 1.128mgKOH/g, saponification value of 224.40mgKOH/g, and iodine value of 79.63mg. Error may occur during the taking of the readings, mixing of reagent and also due to the equipment.

It can also be concluded that the oil contains some of the functional groups such as esters, amides and alcohols which are the major base materials contained in the anti-hypertensive drugs like Aldomet Dopamet, Aldopa etc (Cavallity and Berley).

5.2 Recommendation

The following recommendations were made from the work carried out.

Due to the present economic situation of Nigeria, and the high price of drugs for treatment of hypertension, garlic oil should be extracted and make available for the hypertensive patients instead of procuring drugs from overseas. Since the garlic oil has been reported to treat or prevent hypertension and cancer,

Further research should be carried out to determine the compound of allicin in the garlic seed oil, using infrared analysis.

EXTRACTION AND CHARACTERIZATION OF OIL FROM GARLIC BULB

A RESEARCH PROJECT

BY

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APPENDIX A

A1 Percentage of moisture content and other volatile substances

TABLE 3.0 Percentage of moisture content and other volatile substance

Weight of sample before drying (W_1) g	Weight of sample after drying (W_2) g	%of moisture content
5.00	3.78	24.4
5.50	4.13	24.8
10.00	7.48	25.2

% of moisture content = $\frac{W1-W2}{W1} \times 100\%$

5g of sample:

Mass of sample before drying = W_1

Mass of sample after drying = W_2

$$\%\ of\ moisture\ content = \frac{W_1-W_2}{W1} \times 100$$

$$= \frac{5.0-3.78}{5.0} \times 100$$

$$= 24.4\%$$

5.5g of sample:

W_1= 5.5g

W_2 = 4.13g

$$\%\ of\ moisture\ content = \frac{W_1-W_2}{W1} \times 100$$

$$= \frac{5.50-4.13}{5.50} \times 100$$

$$= 24.8\%$$

10g of sample

W1 = 10.0g

W2 = 7.48g

$$\%\ of\ moisture\ content = \frac{W_1 - W_2}{W1} \times 100$$

$$= \frac{10.0 - 7.48}{10.0} \times 100$$

$$= 25.2\%$$

Average % of moisture content = $\frac{24.4 + 24.8 + 25.2}{3}$ = 24.8%

A2 Percentage of Garlic oil Yield by Soxhlet Extraction Equipment using n-Hexane as Solvent

Weight of empty flask (M1) = 108.1g.

Weight of thimble (filter paper) = 4.13g

TABLE 7.0 Total Weight of Sample

No. of sample	Weight of thimble (g)	Weight of sample + thimble (g)	Weight of sample (g)
1	4.13	54.13	50.00
2	4.13	54.13	50.00
3	4.13	54.13	50.00
4	4.13	54.13	50.00
5	4.13	54.13	50.00
6	4.13	54.13	50.00
7	4.13	48.73	45.44

Total Weight of Sample (W_s) = $\sum weight\ of\ sample\ used$

W_s = 345.44g

Weight of flask + oil (M_2) = 144.23g

Weight of oil (W0) = $M_2 - M_1$

=156.20g- 108.1g

= 48.10g

$$\%\ yield\ of\ oil = \frac{M_2 - M_1}{W_s}\ x\ 100\% = \frac{48.10g}{345.44g}\ x\ 100\%$$

= 13.93%

Solvent Recovered

n-Hexane boiling point = 70^0C

Volume of solvent used = 300ml

Volume of solvent recovered = 250.9ml

Volume of solvent lost = 300ml – 250.9ml

= 49.1ml

APPENDIX B

B1 Characterization of Garlic Oil by Physical Analysis

Specific Gravity

Weight of an empty container = 1.36g

Weight of container + oil = 6.37g

Weight of container + water = 5.51g

Weight of oil = 5.01g

Weight of water = 4.15g

$$Specific\ gravity = \frac{Weight\ of\ oil}{Weight\ of\ water} = \frac{5.01g}{4.15g}$$

= 1.207

Viscosity

Volume of oil = 3.5 ml

TABLE 6.0 Determination of Viscosity

S/no	Volume of oil(ml)	Time of flow(s)	Volume of water (ml)	Time of flow (s)
1	3.50	5.26	3.50	1.37
2	3.50	6.59	3.50	1.28
3	3.50	6.71	3.50	1.32

$$Average\ time\ taken = \frac{\Sigma\ \ time\ taken\ for\ oil}{3} = \frac{18.92}{3}$$

= 6.30s

$$Average\ time\ taken\ for H_2O = \frac{\sum time\ taken\ for\ H_2O}{3} = \frac{3.97}{3}$$

$$= 1.32s$$

The viscosity of water = 7.65 x 10^{-4} at 29^0C

Using the expression,

$$\frac{viscosity\ of\ water}{average\ time\ taken\ to\ drain} = \frac{viscosity\ of\ oil}{average\ time\ taken\ to\ drain}$$

$$Viscosity\ of\ oil = \frac{Viscosity\ of\ H_2O}{Average\ time\ taken\ for H_2O}\ x\ Average\ time\ taken\ for\ oil$$

$$= \frac{7.65 \times 10\text{-}4}{1.32} \times 6.30$$

$$= 3.651\ x\ 10^{-3}$$

Density

Volume of oil = 10 ml

Weight of beaker = 1.33.75g

Weight of oil + beaker = 1.47.28g

Weight of oil = 13.53g

$$Density = \frac{mass}{volume}$$

$$= \frac{13.53g}{10ml}$$

$$= 1.353g/ml$$

Boiling point = 142^0C **Refractive index** = 1.4470 at 29^0C

pH = 5.6 at 29^0C

Colour: brown colour with an unpleasant smell.

APPENDIX C

C1 Characterization of Garlic Oil by Chemical Analysis

C1.1 Free Fatty Acid

TABLE 8.0 0.1M NaOH Titration values

NaOH	Initial (ml)	Final (ml)	Result (ml)
1st	2.20	4.10	1.90
2nd	4.10	6.20	2.10
3rd	6.20	8.20	2.00

$$Average\ Vol.of\ NaOH\ used = \frac{\sum Results}{3}$$

$$= \frac{6.0}{3}$$

$$= 2.00ml$$

$$FFA = \frac{Vol.\ of\ base\ used\ x\ 2.82}{weight\ of\ sample}\ x\ 100$$

$$= \frac{2.00\ x\ 2.82}{10}\ x\ 100$$

$$= 56.4\%$$

C1.2 Acidic Value

$$Acid\ Value = \frac{Vol.\ of\ base\ used\ x\ 2.82\ x\ 2}{weight\ of\ sample}$$

$$= \frac{2.00\ x\ 2.82\ x\ 2}{10}$$

$$= 1.128mgKOH/g$$

C1.3 Saponification Value

TABLE 9.0: 0.5M HCL

Burette Readings

HCL	Initial (ml)	Final (ml)	Result (ml)
1st titre	5.00	16.60	11.60 (V_1)
2nd titre	16.60	44.20	27.60 (V_0)

$Saponification\ value\ is\ given\ by = \frac{(V_0 - V_1) x C\ x\ 56.1}{m}$ V_0 =

is the volume of 0.5mol/l hydrochloric acid solution used for blank test.

V_1 = is the volume of 0.5mol/l hydrochloric acid solution used for determination.

C = is the exact concentration of acid.

m = is the mass of tested sample.

m= 2g

$$Saponification\ Value = \frac{(27.60 - 11.60) x\ 0.5\ x\ 56.1}{2}$$

Saponification value = (27.60 – 11.60) x 0.5 x 56.1

$$= \frac{448.8}{2}$$

$$= 224.40 mgKOH/g$$

C1.4 Iodine Value

TABLE 10.0

Burette Readings

Data	Sample Titration(ml)	Blank Titration(ml)
1st titre	81.70	71.50
2nd titre	84.20	69.30
Average	82.95(V_1)	70.40(V_2)

Iodine value is given by the expression:

$$Iodine\ Value = \frac{126.9\ C\ x\ (V_1\ -\ V_2)}{m}$$

C = concentration of sodium thiosulphate used

V_1 = volume of the sodium thiosulphate solution used for blank

V_2 = volume of the sodium thiosulphate solution used for determination

M = mass of the test sample

$$Iodine\ Value = \frac{126.9\ x0.1\ x\ (82.95\ -\ 70.40)}{2}$$

$$= \frac{159.26}{2}$$

$$= 79.63$$