Determination of Optimal Temperature and PH of Lipase Enzyme Extracted from Beni Seed and Bambara Nuts

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ABSTRACT
Lipase is an enzyme that catalyzes the hydrolysis of ester bonds in substrates such as phospholipids, triglycerides and cholesteryl esters. Lipase (E.C. 3.1.1.3) was extracted from germinating bambara groundnut and beniseed and assayed using 0.1M phosphate buffer pH 8. The crude lipases showed optimum activity at pH 8.0 and temperature of 50^0C for Beniseed and pH 9.0 and temperature of 40^0C for Bambara groundnut. The Km and Vmax of lipase from Beniseed was observed to be 26.3416mg and 0.0076µm mL^{-1} min^{-1} respectively while that of lipase from Bambara groundnut was observed to be 21.141mg and 0.0058µm mL^{-1} min^{-1}. Lipase from germinated Bambara groundnut and Beniseeds has various industrial applications following its thermo stability and alkaline pH making them of greater importance in processes that require minimal corrosion problems.

INTRODUCTION

Lipase (Triacylglycerol acylhydrolase)(E.C. 3.1.1.3) is an enzyme which catalyzes the hydrolysis of ester carboxyl bonds in acylglycerol to release free fatty acids and glycerol. It is capable of catalyzing etherification at waterrestricted environment. Generally, lipase has a catalytic triad which consists of amino acid: serine, histidine and aspartate or glutamate residues but the character of each lipase maydiffer. The difference in lipase character is caused by the difference in its protein structure (Lotti and Alberghina,2007) Lipases from microbial source can have their character influenced by its medium where it grows, while lipases from plant and animal sources are not influenced by their environment.Lipases from plant sources have specific properties, such as high affinity with triacylglycerol of the plant content. This property is not found in lipases from microbial sources (Huang *et al.,* 2008)Lipases have been isolated from many species of plants, animals, bacteria, fungi, and yeast. It have been widely used in the food and other industrial applications and thus there is an increasing demand in discovering new lipase sources having unusual characteristics to suit particular applications.

One of the oldest and least understood of pop cultural phrase, “Open sesame” was provided to the world by Scheherazade in her story “Ali baba and the Forty Thieves” in one Thousand and one Nights” . This phrase “open sesame” was used to open up a sealed cave where a group of thieves resided. The reason this term was used is because a ripe sesame seed pod will burst open at the slightest touch (Makni, 2010). Sesame plant and the seed known as Beniseed is tall annual herb of the pedaliaceae family, which grows extensively in Asia, particularly in Burma,

China, and India (Kan, 2007). It is also one of main commercial crops in Nigeria, Sudan and Ethiopia .

Sesame is very drought-tolerant plant due to its extensive root system. However, it requires adequate moisture for germination and early growth. While the crop survives drought as well as presence of excess water, the yields are significantly lower in either condition. Moisture levels before planting and flowering impact yield most. Most commercial cultivars of sesame are intolerant of water-logging. Rainfall later in the season prolongs growth and increases high harvest-shattering losses. Wind can also cause shattering at harvest. Sesame seeds are thought to be the world's oldest condiment, and have been an integral part of the cuisine of India, Sumer, Egypt, and the Asian subcontinent for thousands of years (Fletcher, 1988): A creation legend from Assyria tells us that one night the gods drank their fill of sesame wine, the next day they created the earth, forever intent wining the two (Esteves, 2011).. Archeological evidence shows that the use of sesame oil as a food goes back to at least 3000BCE in the Middle East, and that Babylonians were using it for a base for their perfumes as early as 2100BCE (Makni, 2010). It may seem as if sesame seeds on bread may be a new idea, but there are Egyptian paintings depicting bakers sprinkling them food, they are also medicinal used at least since the time of Husbandman's Classic of the medicinal material written over 2000 years ago. Historically, they have been used as a tonic for kidney and liver ailments, and as a mild laxative in India . Its oil seeds are sources for some phyto-nutrients such as omega-6 fatty acids, flavonoid phenolic anti-oxidants, vitamins and dietary fiber with potent anti-cancer as well as health promoting properties .

The sesame seeds plant requires well-drained sandy soil and tropical environment to flourish. It grows to about 5 feet tall and bears plenty of pink-white foxglove type flowers. The pods appear soon containing white, brown, or black seeds depending up on the cultivar type, arranged in rows inside. Each pond (2-5cm in length) is a long rectangular box like capsule with deep grooves on its sides. A pod (1 to 3in.in length) may contain up to 100 or more seeds. More than just the nutty topping on a hamburger bun, sesame seeds are full of powerful disease-fighting phytonutrients ((Milder *et al*., 2000). These versatile seeds have long been used by different cultures throughout the world, yet many people are unaware of their healthful qualities. Recent research has focused on the unique sesame lignans found in abundance in the seeds, which have shown great potential in reducing blood lipid levels and blood pressure, fighting inflammation and cancer, boosting the body's antioxidant capacity, and enhancing vitamin E bioavailability (Ghasi, 2000). A wealth of evidence reveals the power contained within these tiny seeds in helping manage some of today's prevalent health disorder's and gives us plenty of reasons to add them to our daily diet (Zohary, 2000).

Bambara nut is a seed of Africa origin used locally as a vegetable. It was first found in West Africa (NRC, 2006). The plant is leguminous and has numerous nitrogen fixing nodules on the root. Bambara nut which constitute complete food is reported to contain protein, carbohydrate and lipid and can be consumed at different stages of maturation (NRC, 2006)

The Bambara nut (seed) is an under-utilized tropical; legume that is indigenous to Africa. It grows in areas where the cultivation of other legumes such groundnut is too risky due to poor soil condition or the threat of drought. It yields as much as 3-5 tons/hectare under condition (Banford, 1984; Oyenuga, 1982) and Uwaegbule (1978) described Bambara nut as a legume

crop which contains higher crude protein than many other legume grains and therefore recommended its incorporation in livestock feeds. Carbohydrate account for approximately 45% of the total dry seed weight and has a lipid content of 6-8% (Mandava and Hoogenkamp, 1999).

1.1 JUSTIFICATION OF STUDY

The nutritional and medicinal efficacy of Beniseed and Bambara nut as raw, roasted or even baked cannot be over emphasized due to their reach content of many significant variables. It will earn many investors incomes and boost the cultivation of these plants to meet with the ever increasing demand of the consumers. The potency of these seeds will allowed favourable competition with the conventional drugs presently in circulation.Despite all these advantages, their industrial raw material is still at very low ebb.

More recently however, there is growing interest by the industries in Nigeria to look inward for the sourcing of their raw materials. For instance, the food, pharmaceutical and cosmetic industries are yarning for the replacement of wheat with low cost, highly nutritive alternatives in several applications and hence the need for this research into the activity of this enzyme for nutritional and industrial applications.

1.2 AIM AND OBJECTIVES

1.3 AIM

The aim of this research work is to establish an improved way of Beniseed and Bambara nut and facilitate their industrial applications by assaying for the activity of enzyme lipase in the samples.

1.4 OBJECTIVES OF THE STUDY

The above aim will be achieved through the following objectives:

Establishment of method of purification of Beniseed and Bambara nut lipase enzyme.

Apply the success recorded in the laboratory experimentation to human and industrial applications.

Give appropriate recommendation on the best way of Beniseed and Bambara nut application in reducing cholesterol in human heart and its industrial usage.

CHAPTER TWO

2.0LITERATURE REVIEW

Sesame seed is considered to be one of the oldest oilseed crop known to humanity (Cowley, 1990). Sesame has many species, and most are widely used as species to the native of sub-Sahara Africa. *Sesamum indicum* the cultivated type, originated in India (Dorathea, 2010). Some report claims that Sesame was cultivated in Egypt during the Ptolemaic period, while others suggest that it records from Babylon and Assyria, dating about 4000years ago. Egyptian called it sesame, and it is included in the list of medicinal drugs in the scrolls of the Ebers papyrus dated to be over 3600 years. Archeological report from Turkey indicate that sesame was grown and pressed to extract oil for essential applications 2750 years ago in the empire of Ururtu (Serpico and White, 2000).It was a crop that could be grown by substance farmers at the edge of deserts, where no crops grow. Sesame has been called a survivor crop (Ray, 2011).

In Hindu legends and beliefs, tales are told in which sesame seeds represent a symbol of immortality and the god Mahavishni's consort Maha Sri Devi herself representing more properties of the sesame seeds, as such it is considered as the most auspicious oil next to ghee used in Hindu rituals and prayers. Sesame seed is a common ingredient in various cuisines. It is used whole in cooking for its rich nutty flavor. Tan and black sesame varieties are roasted and used for making the flavoringgomashio.

The onset of the symptoms may occur within a few minutes up to 90minutes after ingestion of sesame seed product. It was common finding that most patients had other allergic diseases such as asthma, hay fever, and eczema, and most patients also had a relative with an allergic disease. More than two thirds of the patients with sesame allergy also had food allergic reactions to other foods (Aaronov *etal.*, 2008).

For thousands of years, sesame seeds have been a source of food and oil. Sesame has one of the highest oil content of any seed, some varietals exceeding 50 percent oil content compared to soybean's 20 percent. Sesame oil is one of the most stable vegetable oils, with long shelf life, because of the high level of natural antioxidants (sesamin, sesamolin, and sesamol). Oil from the seed is used in cooking, as salad oils and margarine, and contains about 47 percent oleic and 39 percent linoleic acid. Sesame seed oil, like sunflower seed oil, is fish in omega 6 fatty acids, but lacks omega 3 fatty acids. Sesame seed is also rich in protein, at 25 percent by weight. The flour that remains after oil extraction is between 35 to 50 percent protein, has good effective carbohydrates, and contains water-soluble antioxidants (sesaminol glycosides) that provide added shelf-life to many products. This flour, also called sesame meal, is an excellent high-protein feed for poultry and livestock (Ray, 2011). The addition sesame to high lysine meal of soybean produces a well-balanced animal feed.

The relative ratio of protein and oil, as well as essential amino acids and essential fatty acids varies with sesame cultivar as well as growing conditions. In 2008, about 65 percent of the annual sesame crop was processed in oil and 35 percent was used in food. The food segment includes about 42 percent roasted sesame, 36 percent washed sesame, 12 percent ground sesame and 10 percent roasted sesame seed with salt (Oplinger, 2000).

2.1 DESCRIPTION OF THE PLANT

It is an annual plant growing 50 to 100cm (1.6 3.3ft) tall, with opposite leaves 4 to 14cm (1.6 to 5.5in) long with an entire margin; they are broad lanceolate, to 5cm (2in) broad, at the base of the plant, narrowing to just 1cm (0.4in) broad on the flowering stem. The flowers are yellow, tubular, 3 to 5cm (1.2 to 2.0in) long, with a four-lobed mouth. The flowers may vary in color with some being white, blue or purple (Putnam, 2000). If the seed is too moist, it can quickly heat up and become rancid (Ray, 2011).

2.2 DESCRIPTION OF THE SEED

Sesame fruit is a capsule, normally pubescent, rectangular in section and typically grooved with a short triangular beak. The length of the fruit capsule varies from 2 to 8cm. its width varies between 0.5 to 2cm, and the number of loculi from 4 to 12. The fruit naturally splits opens (dehisces) to release the seeds by spitting along the septa from top to bottom or by means of two apical pores, depending on the varietal cultivar. The degree of dehiscence is of importance in breeding for mechanized harvesting as is the insertion height of the first capsule (Oplinger, 2000).

Sesame seeds are small. The size, form and colors, vary with the thousands of varieties now known. Typically, the seeds are about 3 to 4 millimeters long by 2 millimeters wide and 1 millimeter thick. The seeds are ovate, slightly flattened and somewhat thinner at the eye of the seed (hilum) than at the opposite end. The weights of the seeds are between 20 and 40 milligrams. The seed coat (testa) may be smooth or ribbed. Sesame seeds come in many colours depending on the cultivar harvested. The most traded variety of sesame is off white coloured. Other common colours are buff, tan, gold, brown, reddish, gray, and black. Sesame seed is sometimes sold with its seed coat removed (decorticated). This is the variety often present on top of buns in developed economics (Ray, 2008).

2.3 ECONOMIC IMPORTANT OF SESAME SEEDS

The seeds have high oil content around 55%. Sesame is used in cooking and in preparation of salads and also finds its use in production of margarine, soap, pharmaceuticals, paints and lubricants. The residue left after the extraction of oil known as the oil seed cake which is used as cattle feed (Agarwal, 2000).

2.4 BENEFITS OF SESAME SEEDS

1. Delicious, crunchy sesame seeds are widely considered healthful foods. They are high in energy but contain many health benefiting nutrients, minerals, antioxidants and vitamins that are essential for wellness.

2. The seeds are especially rich in mono-unsaturated fatty acid oleic acid, which comprise up to 50% fatty acids in them. Oleic acid helps to lower LDL or “bad cholesterol” and increase HDL or “good cholesterol” in the blood. Research studies suggest that Mediterranean diet which is rich in mono-unsaturated fats help to prevent coronary artery disease and stroke by favoring health lipid profile.

3. The seeds are also very good source of dietary proteins with fine quality amino acids that are essential for growth, especially in children. Just 100g of seeds provide about 18g of protein (32% of daily- recommended values).

4. In addition, sesame seeds contain many health benefiting compounds such as sesamol (3, 4-methylene-dioxyphenol), sesaminol, furyl-methanthiol, guajacol (2-methoxyphenol), phenylethanthiol and furaneol, vinylguacol and decadienal. Sesamolsesaminol are phenolic anti-oxidants. Together, these compounds help stave off harmful free radicals from the body.

5. Sesame is amongst the seeds rich in quality vitamins and minerals. They are very good sources of B-complex vitamins such as niacin, folic acid, thiamin (vitamin B1), pyridoxine (vitamin B6), and riboflavin.

6. 100g of sesame contains 97mcg of folic acid, about 25% of recommended daily intake. Folic acid is essential for DNA synthesis. When give in expectant mothers during per-conception period, it may prevent neural tube defects in the baby.

7. Niacin is another B-complex vitamin found abundantly in sesame. About 4.5mg or 25% of daily-required levels of niacin is provided by just 100g of seeds. Niacin help reduce LDL-cholesterol levels in the blood. In addition, it enhances GABA activity inside the brain, which in turn helps reduce anxiety and neurosis.

8. The seeds are incredibly rich sources of many essential minerals. Calcium, iron, manganese, zinc, magnesium, selenium, and copper. These minerals have vital role in bone mineralization, red blood cell production of cardiac and skeletal muscle activities.

Just a hand full of sesame seed a day provides enough recommended levels of phenolic anti-oxidants, minerals, vitamins and protein (Stanford Medicine, 2012).

2.5 COMPOSITIONS OF SESAME SEEDS

Sesame seeds contain more of the edible oil and proteins, with the compound lignans and some fibers. Also present in sesame seeds is phytosterols. These lignans (sesamin, sesamolin) which have 370.3mg/100g present in sesamin and sesamolin 202.7mg/100g. The chemical properties of sesame are protein 22.20%, fat 63.25%, moisture content 5.60%, and ash content 5.39% all present in the seed. The fat in the oil comprises of fatty acids with the compositions of oleic acid 35.0%, linoleic acid 35.0%, palmitic acid 7.0%, and stearic acid 3.5%. The seed is also a good source of vitamin E, K, and C (Hirata, 1996).

2.6 PHYTOSTEROLS

Plant sterols, phytosterols are cholesterol-like compounds that are found mostly in vegetable oils, nuts and legumes. There are about 44 sterols known to exist in plants. The most abundant phytosterols are, however, beta-sitosterol, campesterol, and stigmasterol. Phytosterols are not produced in the body. Thus, their sole source is diet. Phytostrols have the same function as cholesterol in the body. Cholesterol is necessary component of cell membrane and require for the synthesis of sex hormones and bile acids. However, when cholesterol is high in the blood (serum) it is associated with heart disease. Plant-base diet rich in phytosterols is known to

reduce serum total cholesterol and low density lipoprotein (LDL) cholesterol. On the other hand, diet based on animal food (meat, egg etc) contributes to elevated serum cholesterol level. For example, it was found that subjects fed with wheat germ containing high phytosterols had 42% lower cholesterol in their blood as compared to those who were fed phytosterol-free wheat germ. The mechanisms suggested on how phytosterols help reduce serum cholesterol include enhancing excretion of cholesterol, interfering with cholesterol synthesis, and competing for cholesterol acceptor sites in the intestinal walls (Chen, 2005).

2.7 SESAME OIL

Sesame oil (also known as Gingelly oil or til oil) is an edible vegetable oil derived from sesame seeds. Research also indicates that the rich presence of antioxidants and polyunsaturated fats in sesame oil could help control blood pressure. The composition of sesame oil is composed of fatty acid (palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and Eicosenoic).Despite sesame oil's high proportion (41%) of polyunsaturated (omega-6) fatty acids, it is least prone, among cooking oils with high smoke points, to turn when kept in the oil (Essential oil, 2006).

2.8 TRADITIONAL USE OF SESAME OIL

Sesame oil is reputed to penetrate the skin easily and is used for oil massage. The sesame oil, is specially used for massaging the foot. It is also used for hair and scalp massage (Essential oil, 2006).

2.9INDUSTRIAL USE OF SESAME OIL

In industry, sesame oil may be used as

1. A solvent in injected drugs or intravenous drip solutions.
2. A cosmetics carrier oil
3. Coating stored grains to prevent weevil attacks. The oil also has synergy with some insecticides.

Low grades oil used locally in soaps, paints, lubricants, and illuminants (Essential oil, 2006).

2.10 VITAMINS AND MINERALS CONTENT

Sesame oil is a source of vitamin E. (CBS News, 1994). Vitamin E is an antioxidant and has been correlated with lowering cholesterol levels. Sesame oil also contains magnesium, copper, calcium, iron, zinc, and vitamin B6. Besides being rich in vitamin E, there is insufficient research on the medicinal properties of sesame oil.

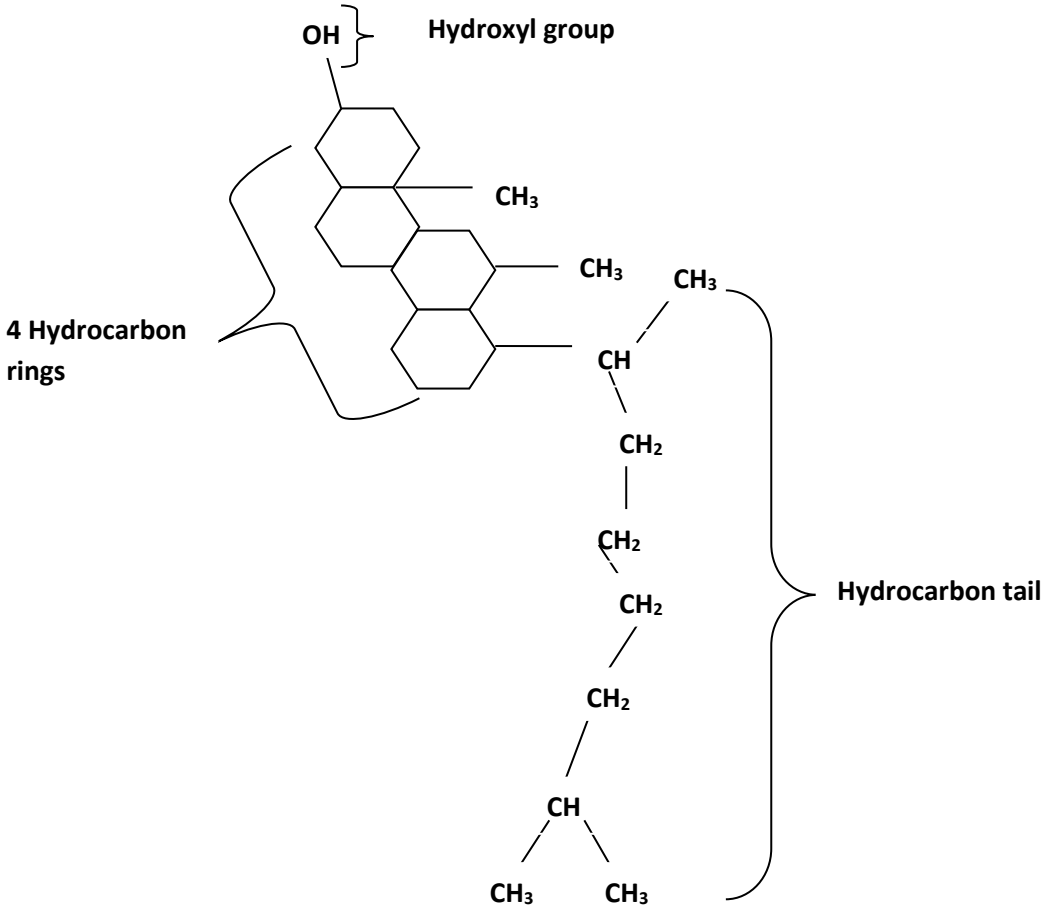
2.11 BLOOD PRESSURE

Sesame oil has a high percentage of polyunsaturated fatty acid (omega-6 fatty acids) but it is unique in that it keeps at room temperature. This is because it contains two naturally occurring preservatives, sesamol and sesamin. (Normally, only oils predominately composed of the omega-9 monounsaturated oil, like olive oil, keep at room temperature(Agarwal, 2000).

2.12 CHOLESTEROL

Cholesterol is a waxy, fat-like substance that is found in body cells. The body needs some cholesterol to make hormones, vitamin D, and substances that help digest foods. The body makes all the cholesterol it needs . However, is not water-soluble enough to dissolve in the blood. Along with fats and fat-soluble nutrients, therefore, it travels in the blood through lipoproteins such as LDL cholesterol sometimes called "bad" cholesterol and HDL cholesterol called "good" cholesterol. Cholesterol has a molecular formula of $C_{27}H_{45}OH$.

2.13 STRUCTURE OF CHOLESTEROL



The hydroxyl (OH) group is polar which makes it soluble in water. This small 2-atom structure makes cholesterol and alcohol. The alcohol, ethanol, is a much small alcohol that also has a hydroxyl group (C_2H_5OH). The 4-ring region of cholesterol is the signature of all steroid hormones (such as testosterone and estrogen). All steroids are made from cholesterol. The rings are "hydrocarbon" rings because each corner of the ring is composed of carbon atom, with two hydrogen atoms extending off the ring. The combination of the steroid ring structure and the hydroxyl (alcohol) group classifies cholesterol as a "sterol". Cholesterol is the animal sterol. Plant only make trace amounts of cholesterol, but make other sterols in larger amounts. The last region is the hydrocarbon tail. Like the steroid ring region, this region is composed of

carbon and hydrogen atoms. Both the ring and tail region are non-polar, which means they dissolve in fatty and oily substances but will not mix with water (Hirata,1996).

2.14 FORMATION OF CHOLESTEROL IN THE BODY

Gallstones form when liquid stored in the gallbladder hardens into pieces of stone-like material. The liquid, called bile is used to help the body digest fats. Bile is made in the liver, and then stored in the gallbladder until the body needs to digest fat. At that time, the gallbladder contracts and pushes the bile into a tube called a duck that carries it to the small intestine, where it helps with digestion. Bile contains water, cholesterol, fats, bile salts, and bilirubin. Bile salt break up fat, and bilirubin, gives bile and stool a brownish colour. If the liquid bile contains too much cholesterol, bile salt, or bilirubin, it can harden into stones. The two type of gallstones are cholesterol stones and pigment stones. Cholesterol stones are usually yellow-green and are made primarily of hardened cholesterol. They account for about 80% of gallstones. Gallstones compose of solid formation of cholesterol and bile salts. However, research shows that approximately 80 to 90% of all gallstones are cholesterol gallstones which form when the liver begins secreting bile that abnormally saturated with cholesterol.

Bile is an enzymatic fluid, or digestive juice, that helps the body break down foods that are high in fat. When fatty foods are eaten, bile moves through the ducks into the small intestine to aid in digestion (Chien, 2005).

2.15 HOW CHOLESTEROL IS TAKEN INTO CELLS

Cell takes up cholesterol by receptor-mediated endocytosis. Cholesterol is an essential component of all cell membranes. Most cells can, as needed, either synthesize cholesterol or acquire it from the ECF (extracellular fluid). Human cells get much of their cholesterol from the liver and, if diet is not strictly “100% cholesterol-free”, by absorption from the intestine. Cholesterol is a hydrophobic molecule and quite insoluble in water. Thus it cannot pass from the liver and/or the intestine to the cells simply dissolved in blood and ECF. Instead it is carried in tiny droplets of lipoprotein. The most abundant cholesterol carries in humans are the low-density lipoproteins or LDLs (Chee *et al.*, 2005).

The first step in acquiring LDL particles is for them to bind to LDL receptors exposed at the cell surface. These transmembrane proteins have a site that recognizes and binds to the apoliproteins B-100 on the surface of the LDL. The portion of the plasma membrane with bound LDL is internalized by endocytosis. A drop in the PH causes the LDL to separate from its receptor. The vesicle then pinches apart into two smaller vesicles: one containing free LDLs; the other containing now-empty receptors. The vesicle with the LDLs fuses with a lysosome then release free cholesterol into the cytosol. The vesicle with unoccupied receptors returns to the fuses with the plasma membrane, tuning inside out as it does so (exocytosis). In this way the LDL receptors are returned to the cell surface for reuse (Chen *et al.*, 2005).

2.16 NORMAL RANGES OF CHOLESTEROL LEVEL

Total cholesterol = ≤ 5.17mmol/l

Triglyceride = 0.45 – 1.58mmol/l

HDL-C (good) = 1.17 – 1.68mmol/l

LDL-C (bad) = ≤ 3.90mmol/l (Federal Medical Centre, Bida)

2.17 POSITIVE WAYS BY WHICH CHOLESTEROL CAN BE REDUCE IN HUMAN HEART

The followings are in the positive cholesterol can be reduce:

1. Eat fewer fat fried foods. Choose non-fat and low-fat versions of foods, if available.
2. When eating fats, select unsaturated fats. (Unsaturated fats are liquid at room temperature vegetable oils, for example. Avoid tropical oils such as palm, coconut oil and any fat that is solid at room temperature).
3. Choose fish and poultry more often than red meat.
4. Limit total amount of meat, fish poultry and low-fat cheeses to 7 ounces or less each day.
5. Regular exercise.
6. If you smoke, quit.
7. Lose extra weight.
8. Eat more soluble fiber. Good sources are fruits, beans, pea, and oats.
9. Limit egg yolks to no more than three per week. (Egg white is fat-free) (Stanford Medicine, 2012).

2.18 USES OF BAMBARA NUT

Uses of Bambara Groundnut Bambara groundnut is considered to be an underutilized crop. The low yields associated with Bambara groundnut production may be attributed to the fact that its production and crop improvement have been neglected over the past years by researchers. This neglect has occurred despite the fact that Bambara groundnut is important for small scale farmers due to its drought tolerance and commercial potential. The waning popularity of Bambara groundnut in traditional African communities can be attributed to the fact that it takes a long time to cook, it has poor milling characteristics and contains anti - nutritional factors such as tannins and trypsin inhibitors (Barimalaa and Anoghalu, 1997). However, Bambara groundnutstill plays an important role and is widely utilized in traditional dishes in several African countries such as Côted'Ivoire , Zimbabwe,Nigeria and Cameroon (Yao *et al*., 2005)

Bambara groundnut is primarily used for human consumption. The seeds are consumed at different developmental stages, either immature or fully ripe. The immature seeds can be consumed fresh, boiled, grilled, as a meal or mixed with immature groundnuts or green maize (Bamshaiye *et al.*, 2011). Mature bambara groundnut seeds are very hard, hence boiling becomes a prerequisite before any further preparation. Ripe seeds are milled to produce flour

which can be used to make biscuits and/or otherwise mixed with cereals and boiled to make porridge (Bamshaiye *et al.*, 2011). Ripe dry seeds are also roasted, broken into pieces, boiled, crushed and eaten as a relish

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Commercial canning of bambara groundnuts has been practiced in Ghana, the nuts were canned in gravy by a government factory and over 40,000 cans were produced annually (Begemann, 1986). In Zimbabwe canned Bambara beans were commercially produced for the market as 'Tulimara Nyimo Beans' and recommended for addition to soups, stews and salads. Bambara groundnut (*Vigna subterranea*) is grown for its edible seeds which are used as nutritious pulse. It is an indigenous, underutilized plant cultivated throughout the sub-saharan Africa.

According to Coudet (1984), the annual production is about 330,000 tonnes of which Africa produces half, with Nigeria as the major producing country.Presently,
improved Bambara groundnut cultivars do not exist (Linnemann and Azam-Ali, 1993). It exists as landraces which actually composed of many genotypes which are reported to result in inability to endure stresses under local agricultural systems (Zeveni, 1983). The yields are low because production and improvement of bambara groundnut have been neglected for many years by researchers, even though, the crop is important forthe small scale farmers due to its considerable commercial potentials (Bamisaye et al., 2011). It grows extensively in the Southern Guinea belt of the country (Oguntade, 1985; Enwere, 1998) where it is mostly grown as a mixed crop with maize, cowpea and groundnut (Thottapilly and Rossel, 1997). Among the underutilized plants of Africa, bambara groundnut has enough potential to warrant various sorts of investment towards its improvement. It has outstanding traits as drought tolerance, nitrogen fixation and an ability to produce yield in marginal soils.
It is a crop with a high potential for attainment of food security and poverty alleviationin Africa as it is drought resistance and highly nutritious (Boateng *et al.*, 2013).
Bambara groundnut has several production advantages. As the third most important legume after groundnut (Arachis hypogea) and Cowpea( ) in the semi-arid area of the Africa, its role in food security cannot be contested. It is composed of 65% carbohydrate and 18% protein which make it an important food crop for people who cannot affordexpensive animal protein. The fresh seeds are eaten in an unripe state as the pulse after soaking and boilingas the dry seeds are very hard. Bambara groundnutcan contribute positively and help alleviate nutritional problems, though, it is classified as an underutilized crop (Oyeleke *et al.*, 2012). The seed stores very well and it is not easily prone to attack by pests and diseases.
Despite all the advantages of the crop in food security, and it's nutritional attributes, its use as anindustrial raw material is still at very low ebb.
While many food products have been developed from soy, peanut and cowpea (e.g. milk, low spread fat, meat replacers, emulsifiers, etc.), the same cannot be said of Bambara nut.

More recently however, there is growing interest bythe industry in Nigeria to look inward for the sourcing of their raw materials. For instance, the food, pharmaceutical and cosmetic industries are yarning for the replacement of wheat with low cost, highly nutritive alternatives in several applications.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 SEEDS COLLECTION AND GERMINATION

The samples to be used for the study which were Beniseed (*Sesamum indicum*) and Bambara nut (*vigna subterrenea)* were purchased from a seller at old market Bida, Niger state. Both seeds were soaked in water for six hours and allowed to germinate under moist germination study for 24hours at room temperature (30±2ºC).

3.2 REAGENTS

- Sodium hydroxide (NaOH)
- Ethanol
- Phenolphthalein indicator

  Gum Arabic

  Olive oil

  Acetone
- 0.1M acetic acid
- 0.1M sodium acetate
- Tris buffer
- Phosphate buffer pH 7.0
- Distilled water
- Sodium benzoate

3.3 EQUIPMENT/APPARATUS

- pH meter
- Retort stand and clamp
- Burette

- White tile
- Beakers
- Conical flasks
- Volumetric flasks
- Measuring cylinders
- Water bath and shaker
- Incubator
- Weighing balance
- Refrigerator
- Blender
- Centrifuge
- Spatula

3.4 EXTRACTION OF LIPASE

Collection of Plant Materials: Beniseed (*Sesamum indicum*) and Bambara groundnut seed (*vigna subterrenea*) were bought locally from Bida Main Market Bida,Niger State Nigeria.

After 24 hours of germination, the seed coats were removed manually and 60g seed cotyledons were homogenised in chilled acetone at 4^0C. The suspension was centrifuged at 3000 rpm for 10 minutes and the residue obtained was dissolved in 100ml distilled water followed by centrifugation at 7500 rpm for 15 minutes. The supernatant was used as source of crude enzyme. The crude enzyme was stored in the refrigerator until time of assay (Michael *et al.*, 2001).

3.5 LIPASE ASSAY: TITRIMETRIC DETERMINATION OF LIPASE ACTIVITY:

Lipase activity was determined using an olive oil emulsion, which was prepared as follows: 180ml distilled water, 20ml olive oil, 0.4g of sodium benzoate, and 1g gum Arabic. The amount of fatty acids released during the reaction is determined by direct titration with NaOH to a Phenolphthalein end point.

PROCEDURE:

1. Into 3 different 250ml conical flasks 10 ml of 95% (v/v) ethanol was added and 2 to 3 drops of 1% (w/v) Phenolphthalein indicator was also added. *(This titration cocktail was used to quench the reactivity of subsamples of the reaction mixture).*

2. Into a250ml conical flask with stopper 50 ml of emulsion substrate was placed and pre-incubated for 15 min in a 37°C water bath with shaker.
3. 5ml of enzyme was added to initiate lipolysis on the emulsion substrate then timer was started with continuous shaking.
4. 5 ml reaction mixture was taken and each subsample was transferredinto separate flask containing titration cocktail prepared in step 1. Contents were swirled immediately to stop the reaction.(The quenched subsamples may be turbid. Samples may be put aside (up to 2 to 3 hours at 20°C to 22°C) for later titrimetric analysis).
5. The contents of each flask were titrated against 0.05N NaOH until a light pink color appears.
6. The volume of NaOH used in the titration was noted and used for the enzyme activity calculations. One unit of lipase is defined as the amount of enzyme required to liberate 1micromole of free fatty acid from olive oil per minute under the standard assay conditions.
7. The enzyme activity was calculated using the formula below:

$$\text{Lipase Activity} = \frac{\text{Volume of alkali consumed x Normality ofNaOH}}{\text{Time of incubation x Volume of enzyme solution}}$$

(Sadasivam and Manikam,1996).

3.6 EFFECT OF pH:

Optimum pH for lipase activity was determined covering the range (3-9) using 0.1M buffers of different pH. The buffers were: pH 3-6 (acetate); pH 7 (phosphate); pH 8-9 (Tris-HCl) (Gadge*et al.*,2011).

3.7 EFFECT OF TEMPERATURE

For optimum temperature, the enzyme assay was performed as discussed aboveexcept that incubation was done at temperatures from 20-70°C (Gadge*et al.*,2011).

3.8 EFFECT OF CONCENTRATION:

Lipase was assayed in reaction buffer (pH 8) at 24°C with different concentrations (10-120 $mgml^{-1}$) of olive oil emulsion as a substrate. The values of vmax(maximum velocity) and km (MichaelisMenten constant) were calculated from Lineweaver-Burk (LB) plot (Gadge*et al.*, 2011).

3.9 PREPARATION OF REAGENTS:

3.9.1 TO PREPARE 0.05N OF NaOH

3.9.2 PREPARATION OF 0.1M ACETATE BUFFER

To prepare 0.1M acetate buffer, 0.1M acetic acid and 0.1M sodium acetate was first prepared as follows:

- 0.1M acetic acid: -

Molarity = $\frac{\text{specific gravity X \%purity X 10}}{\text{Relative molecular mass}}$

= $\frac{1.025 \text{ X } 99.6 \text{ X } 10}{60.05}$

= 1020.9

60.05 = 17.00

Using dilution law:

$M_1V_1 = M_2V_2$

Where $M_1 = 17.00$, $M_2 = 0.1$, $V_1 = ?$, $V_2 = 1000$

$V_1 = \frac{M_2V_2}{} = \frac{0.1 \text{ x } 1000}{}$

$M_1 = 17.00$

= 5.88mls

5.9ml of glacial acetic acid was measured using a measuring cylinder and transferred into a 1000ml volumetric flask containing little amount of distilled water. The solution was then made up to the calibrated mark using distilled water.

- 0.1M sodium acetate

No of moles = $\frac{\text{Vol X molarity}}{1000}$

= $\frac{1000 \text{ X } 0.1}{}$

1000= 0.1moles

Mass in gram = no of moles X relative molecular mass

= 0.1 X 82.03

8.20g/l

Therefore 8.2g of sodium acetate was weighed using a weighing balance and transferred into a 1000ml volumetric flask. About 100ml of distilled water was added the mixture was swirled until the sodium acetate pellets were completely dissolved then distilled water was used to make the solution up to the mark.

3.9.3 PREPARATION OF 0.05N SODIUM HYDROXIDE

- 0.05N NaOH: -

Equivalent weight (W) = $\frac{Mw}{n}$

Where: n = number of electrons gained or lost per molecule i.e number of replaceable OH^- or H^+ per molecule for acids and bases.

M_w = molar weight

Equivalent weight (W) = 40/1 = 40

Mass in gram = equivalent weight (W) X normality

= 0.05 X 40g = 2g

Therefore 2g of NaOH was weighed using a weighing balance and transferred into a 1000ml volumetric flask. About 100ml of distilled water was added the mixture was swirled until the NaOH pellets were completely dissolved then distilled water was used to make the solution up to the mark.

3.9.4 PREPARATION OF TRIS-HCL BUFFER

- 0.1M Tris buffer ($C_6H_{11}NO_3$)

Mass in gram = no of moles X molar mass

= 0.1 X 121.14 = 12.114g

Approximately 12g of tris buffer was weighed and dissolved in 1000ml volumetric flask and made up to the mark with distilled water. The pH was measured using a pH meter. 500ml was measured into a beaker using measuring cylinder and 0.1M HCl solution was used to adjust the pH to 8, same was done to the other 500ml of tris buffer but this time it was adjusted to pH 9.0..the buffers were labeled accurately and stored until time of use.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

Chart 4.1.1: Effect of pH on Lipase Activity on Beniseed

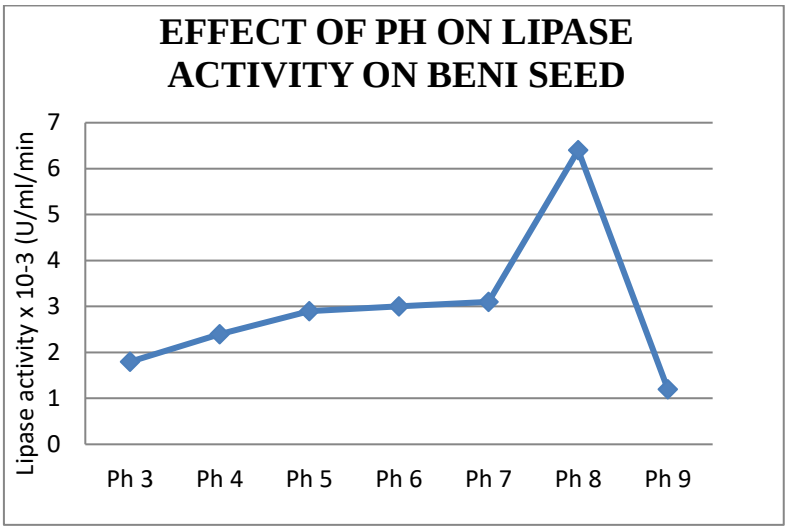


Chart 4.1.2: Effect of Velocity against SUBSTRATE on Lipase Activity of Beniseed

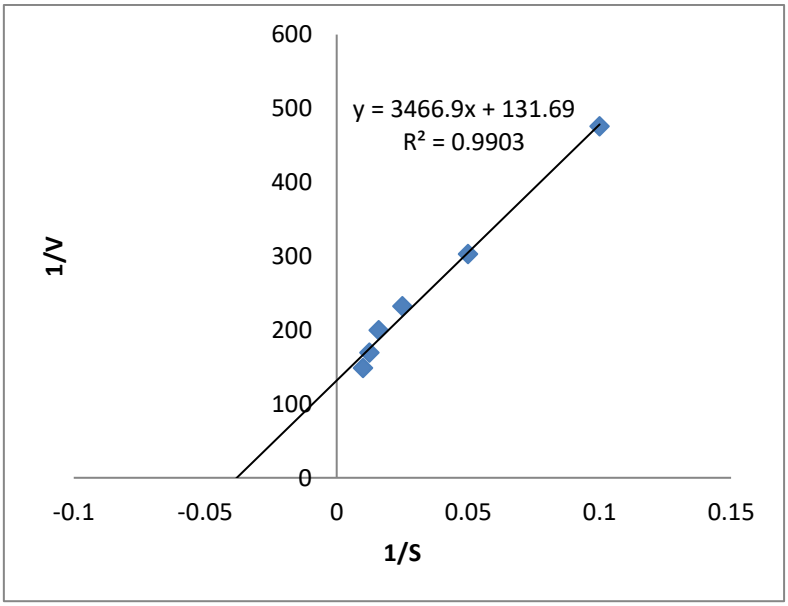


Chart 4.1.3: Effect of Temperature on Lipase Activity of Beniseed

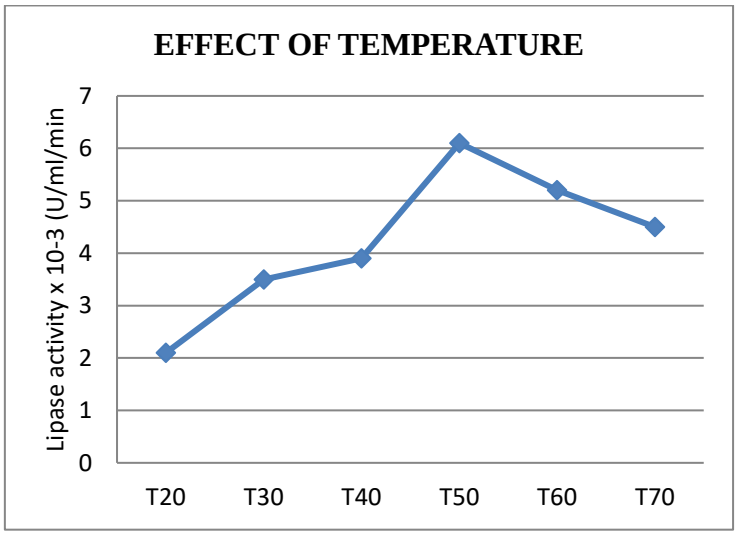


Chart 4.1.4: Effect of Substrate Concentration of Lipase Activity of Beniseed

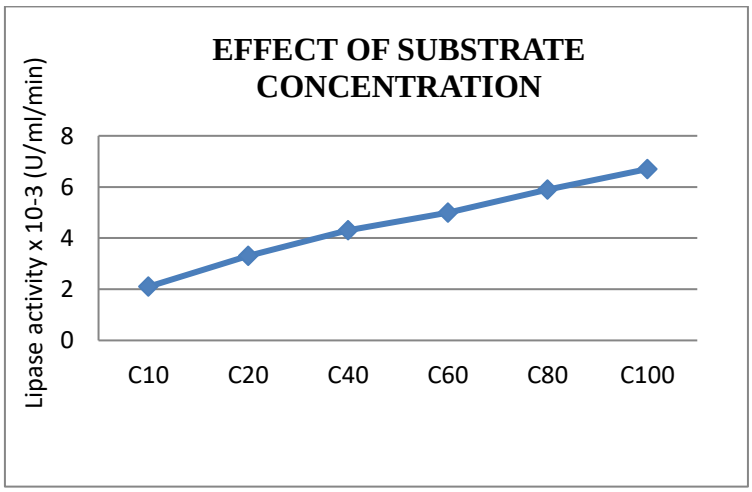


Chart 4.2.1: Effect of pH on Lipase Activity on Beniseed

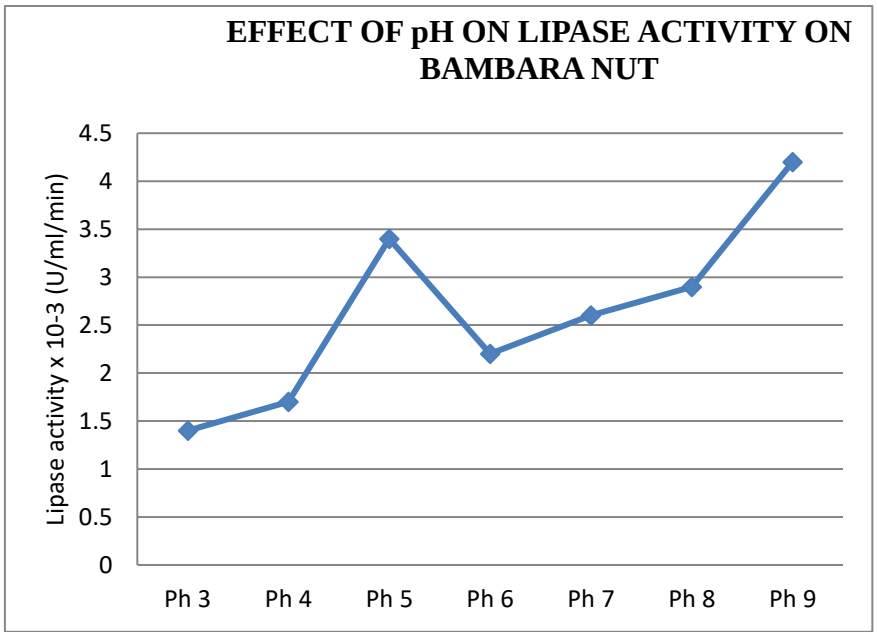


Chart 4.2.2: Effect of Velocity against Substrate on Lipase Activity of Bambara nut

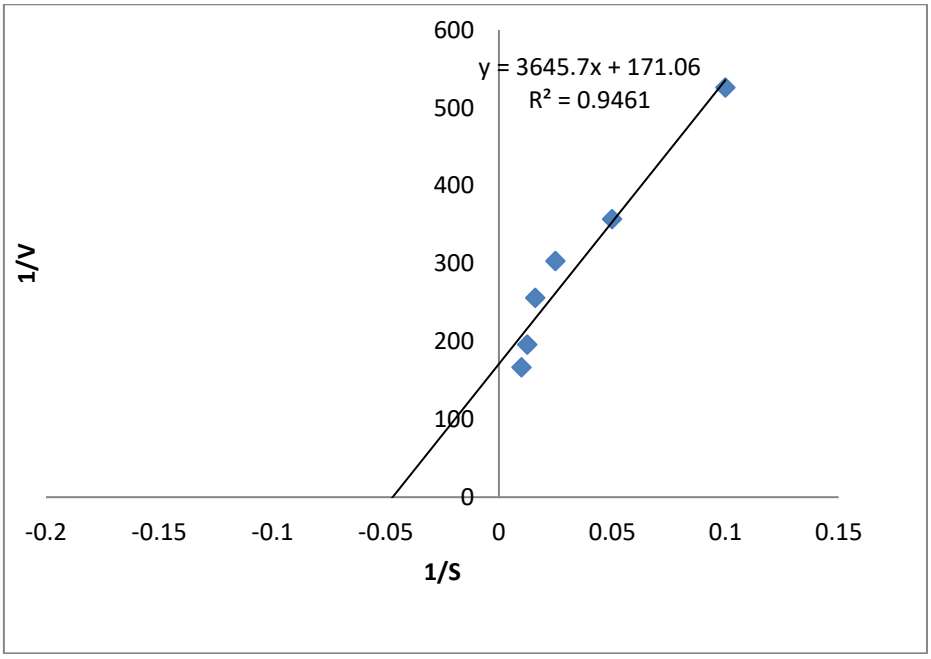


Chart 4.2.3: Effect of Temperature on Lipase Activity of Bambara nut

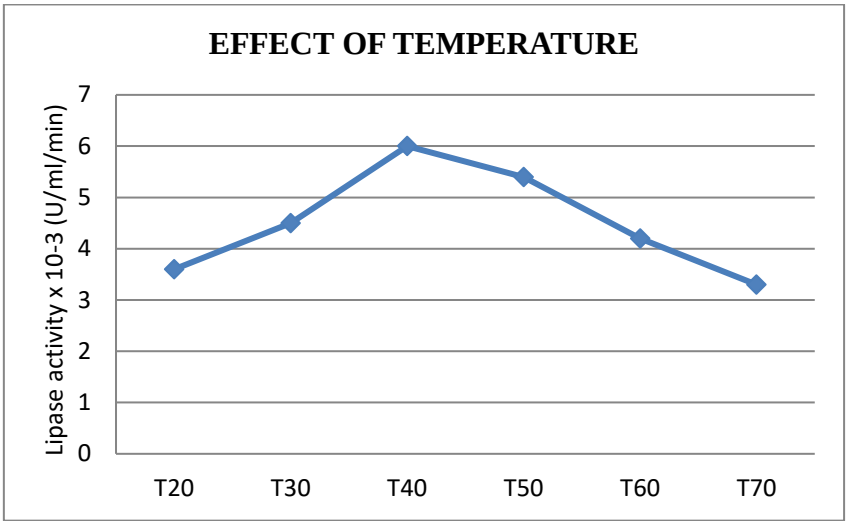
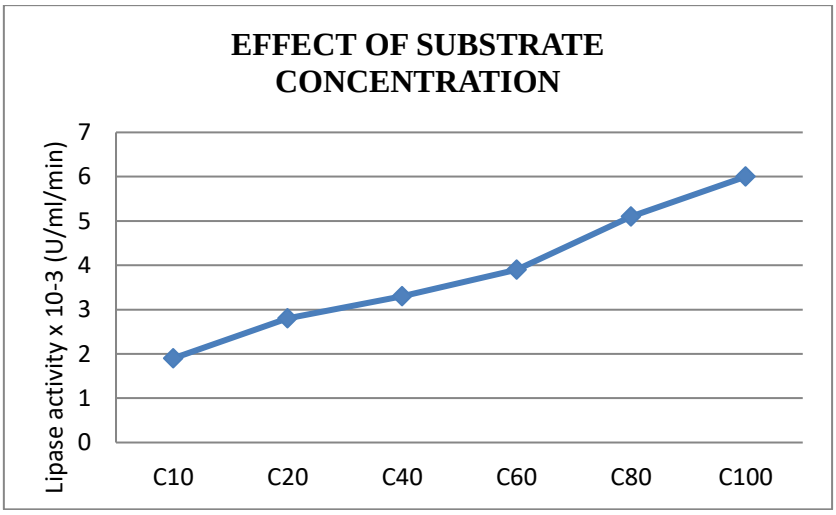


Chart 4.2.4: Effect of Substrate Concentration of Lipase Activity of Bambara nut.



4.1 DISCUSSION

Effect of temperature on lipase activity and thermo stability of the assay was performed at various temperatures of 20^0C to 70^0C and at buffer concentration of 0.1M,optimum temperature for the activity of lipase for both Beniseed and Bambara nut was achieved at the interval of T50 and T40 respectively which was T_{50}=6.1 for Beniseed and T40=6.0 for Bambara nut. The result of lipase activity at these temperature range corroborate with that obtained by Deepak *et al*., 2012 on lipase activity of Jatropha curcas and differ from that of white melon and Africaoil seed which had their optimum temperature at 30^OC(Deepark *et al*.,2012).

The enzyme activity increased with an initial increased in pH and optimum activity was noted

at pH 8for Beniseed and pH 9 for Bambara nut,this shows that lipase from these germinated seeds work maximally at alkaline medium. This result is in agreement with result of Ogueche *et al*.,2016 that characterized and purified lipase from germinated Bambara nut. The lowest pH for lipase activity in both Beniseed and Bambara nut are at pH 9 and 3respectively.Reduction in pH in both samples may be due to formation of by-products such as lactic acid and acetic acid and this findings agreed with that of Wang *et al*.,1995.

The result of velocity against substrateconcentration;at various substrate concentration velocities were determined.The maximum velocities were attained at substrate concentration of 0.1=526 and minimum concentration of 0.01substrate concentrations.This shows that as the substrate concentration increases the saturation of enzyme and velocity increases.

In both the samples,that is, Beniseed and Bambara nut, increments in substrate concentrationincreases the lipase activity.For Beniseed at C_{10} the lipase activity was 6.7 and for Bambara nut,the lipase activity was 6.0 at the substrate concentration 0f C_{10}. The lower the substrate concentration the lower the enzyme activity,hence the activity of the enzyme is a function of substrate concentration.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Crude lipase was extracted from germinated Beniseed and Bambara groundnut and partially purified using Olive oil and ethanol saturation, raising the enzyme showed optimum activity at pH 9 and pH 8 and at a temperature of 60^0C and with maximum velocities at substrate concentrations of $C_{100.}$This research contributes to the existing knowledge on lipase activity on different substrates.

5.2 RECOMMENDATION

It is recommended that purification of lipase be tried in many solvents especially the readily available ones to enhance its utilization.

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APPENDICE

BAMBARA NUT

pH	LA
pH 3	1.4
pH 4	1.7
pH 5	3.4
pH 6	2.2
pH 7	2.6
pH 8	2.9
pH 9	4.2

Table 1: showing activity profile for effect of pH on lipase from Bambara groundnut seeds.

TEMP	L A
T20	3.6
T30	4.5
T40	6
T50	5.4
T60	4.2
T70	3.3

Table 2: Showing activity profile for effect of Temperature on lipase from Bambara groundnut seeds.

CONC	LA
C10	1.9
C20	2.8
C40	3.3
C60	3.9
C80	5.1
C100	6

Table 3: Showing activity profile for effect of Concentration on lipase from Bambara groundnut seeds.

1/S	1/V
0.1	526
0.05	357
0.025	303
0.016	256
0.0125	196
0.01	166.7

Table 4: Showing activity profile for effect of Velocity against Substrate on lipase from Bambara groundnut seeds.

BENI SEED

PH	LA
Ph 3	1.8
Ph 4	2.4
Ph 5	2.9
Ph 6	3
Ph 7	3.1
Ph 8	6.4
Ph 9	1.2

Table 1: Showing activity profile for effect of pH on lipase from Beniseed.

TEMP	L A
T20	2.1
T30	3.5
T40	3.9
T50	6.1
T60	5.2
T70	4.5

Table 2: Showing activity profile for effect of Temperature on lipase from Beniseed.

CONC	LA

C10	2.1
C20	3.3
C40	4.3
C60	5
C80	5.9
C100	6.7

Table 3: Showing activity profile for effect of Concentration on lipase from Beniseed.

1/S	1/V
0.1	476.2
0.05	303
0.025	232.6
0.016	200
0.0125	169.5
0.01	149

Table 4: Showing activity profile for effect of Velocity against Substrate on lipase from Beniseed.