Extraction of amylase enzyme from yam | experiment

Amylolytic enzymes are widely distributed in plant tissues, e. g. in storage tissues such as seeds and tubers and in vegetative organs such as leaves. There exist two types of amylases in some species of plants, (E. C. 3. 2. 1. 1; 1-4-Î±-D-glucan glucohydrolase) and (E. C. 3. 2. 1. 2; 1-4-Î²-D-glucan maltohydrolase) amylases [Thoma, J. A., J. E. Sprandlin and S. Dygert, 1971]. Beta-amylase (-1, 4-glucan maltohyrolase, E. C. 3, 2, 1, 2) is an exoamylase that attacks the non reducing ends of starches molecules, producing a-maltose and a limit dextrin as products [Thoma, J. A., J. E. Sprandlin and S. Dygert, 1971]. In starch-enriched tissues, Î²-amylase may play a role in the mobilization of starch during germination or sprouting tubers [Greenwood, C. T. and E. A. Milne, 1968]. Many reports have been demonstrated that Î²-amylase has a great commercial value in food and beverage industries. The enzyme is useful in structural studies of starch and glycogen. Marshal and Whelan [Marshall, J. et al 1973] report on the removal of any contaminating Î²-glucosidase. The practical interest of Î²-amylase was concentrated on its capacity to produce maltose syrups from starch [Biovin, P., 1997.]. Î²-amylase has previously been purified and characterized from different types of plant sources and a few of microbial origin. In higher plants, the molecular characterization of Î²-amylase has been carried out on enzyme purified from the organs enriched in starch such as sweet potato tubers [Balls, A. K., 1948, et al], leaves [Vikso-Nelson, A., et al 1997], bulbs [Dicko, M. H., et al, 2000], seeds of various cereal species such as barley [Shinke, R. et al 1971], wheat [Trachuk, R. et al 1966], rice [Okamato, K. and T. Akazawa, 1978] and other higher plants such as soybean [Gertler, A. and Y. Birk, 1965]. On the other hand, much less information is available on the purification and characterization of Î²-amylase from root. The present study reports the

purification of Î²-amylase from Yam (Dioscorea esculenta) root to a pure state along with its characterization.

Amylase is an enzyme that breaks starch down into sugar. Amylase is present in human saliva, where it begins the chemical process of digestion. Foods that contain much starch but little sugar, such as rice and potato, taste slightly sweet as they are chewed because amylase turns some of their starch into sugar in the mouth. The pancreas also makes amylase (alpha amylase) to hydrolyse dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy. Plants and some bacteria also produce amylase. As diastase, amylase was the first enzyme to be discovered and isolated (by Anselme Payen in 1833). Specific amylase proteins are designated by different Greek letters. All amylases are glycoside drolases and act on Î±-1, 4-glycosidic bonds. It will start to denature at around 60C.

Amylase digests not only carbohydrates but also dead white blood cells. For example, when you are low in amylase you are a candidate for abscesses (inflamed areas with pus but not bacteria). If you have a toothache and are being treated with antibiotics, but it doesn’t go away, chances are you have an abscess. Amylase is involved in anti-inflammatory reactions such as those caused by the release of histamine and similar substances. The inflammatory response usually occurs in organs which are in contact with the outside world, i. e., the lungs and skin. These include skin problems such as psoriasis, eczema, hives and all types of herpes. Some lung problem including asthma and emphysema may require amylase plus other enzyme

formulas depending on the particular ailment. There are many types of amylases, but of importance are: Î±-amylase, Î²-amylase and glucoamylase.

A diagram of an amylase molecule from human saliva.

1. 1 Î² Amylase

(EC 3. 2. 1. 2) (alternate names: 1, 4-Î±-D-glucan maltohydrolase; glycogenase; saccharogen amylase) Another form of amylase, Î²-amylase is also synthesized by bacteria, fungi, and plants. Working from the non-reducing end, Î²-amylase catalyzes the hydrolysis of the second Î±-1, 4 glycosidic bond, cleaving off two glucose units (maltose) at a time. During the ripening of fruit, Î²-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit.

Both Î±-amylase and Î²-amylase are present in seeds; Î²-amylase is present prior to germination, whereas Î±-amylase and proteases appear once germination has begun. Cereal grain amylase is key to the production of malt. Many microbes also produce amylase to degrade extracellular starches. Animal tissues do not contain Î²-amylase, although it may be present in microrganisms contained within the digestive tract.

1. 1. 2 CARBOHYDRATE METABOLISM

Digestion of carbohydrate begins in the mouth by the action of salivary Î±-amylase. Only limited digestion of carbohydrate occurs, however, because salivary Î±-amylase is denatured in the stomach due to the low pH. Digestion begins again in the small intestine when pancreatic Î±-amylase is secreted. Starch is broken down into maltose, isomaltose, and maltotriose by Î±-amylase through the hydrolysis of Î±-1-4 glycosidic bonds. These products as

well as any other disaccharides that were ingested must be further digested to their respective monosaccharide units by brush border enzymes (maltase, isomaltase, lactase, and sucrase) before absorption. Maltose is hydrolyzed to two glucose molecules by maltase. Isomaltose is hydrolyzed to two glucose molecules by isomaltase. Lactose is hydrolyzed to one molecule of glucose and one molecule of galactose by lactase. Sucrose is hydrolyzed to one molecule of fructose and one molecule of glucose by sucrase. After absorption, glucose, galactose, and fructose are transported to the liver via the portal blood. The liver can transform galactose and fructose into glucose (Gropper et al 2005).

1. 1. 3 REACTIONS OF BETA AMYLASE

Starch + H2O in vitro breakdown of semicrystalline starch particles by beta-amylases increases significantly if they act together with glucan, water dikinase starch substrate of different sources, e. g. wheat, wheat bran, rice bran beta-amylase hydrolyzes alpha-1, 4-linkage, raw starch granules from potato, wheat, rice and corn, with the granules from rice being the best substrate, beta-amylase attacks very slowly on the starch granules, hydrolyzes corn granules efficiently at 45°C .

Beta-amylase is an exo-enzyme that catalyzes the hydrolysis of the alpha-1, 4-glucosidic linkage of the substrate liberating beta-maltose from the non-reducing end, Glu-172 and Glu-367 are catalytic residues, substrate recognition mechanism, enzyme structure beta-amylase is an inverting enzyme that hydrolyzes the alpha-1, 4-glucosidic linkage of the substrate liberating beta-maltose from the non-reducing end, catalytic mechanism, Glu-172 acts as general acid, Glu-367 acts as general base catalyzes the

hydrolysis of alpha-1, 4-glucosidic linkages of soluble starch, and liberates beta-anomeric maltose from the nonreducing ends, exo-acting enzyme, composed of two functional domains, a catalytic domain: domains A and B, and starch-binding domain: domain C, beta-amylase has three carbohydrate-binding sites aside from the active site: two in domain B named Site2 and Site3, one in domain C named Site1, roles of these sites in the catalytic reaction and raw starch-binding, beta-amylase hardly hydrolyzes raw starch from wheat, corn, potato or sweet potato, but binds to it strongly hydrolyzes the alpha-1, 4-glucosidic linkage liberating beta-maltose from the non-reducing end of substrate, enzyme/domain structure, starch binding site in domain C, catalytic mechanism starch substrate of different sources, e. g. wheat, wheat bran, rice bran starch substrate of different sources.

Beta-amylase hydrolyzes alpha-1, 4-linkage, raw starch granules from potato, wheat, rice and corn, with the granules from rice being the best substrate, no efficient hydrolysis of raw starch granules, very slow enzymic attack catalyzes the release of maltose from soluble starch. Malbranchea sulfureastarch substrate of different sources, e. g. wheat, wheat bran, rice bran 106. 9% of the activity with amylose, soluble starch, amylose and amylopectin are the most suitable substrates, some activity against native starch, exo-hydrolase that releases beta-maltose from the non-reducing end of alpha-1, 4-linked poly- and oligoglucans until the first alpha-1, 6-branching point along the substrate molecule is encountered, beta-amylase should be a key enzyme in starch degradation during the germination of millet seeds, enzyme activity increases during days 1-4 of germination starch substrate of different sources, e. g. wheat, wheat bran, rice bran best substrate, pure and

low quality starches, maize starch, tapioca starch maltose is the major end product, traces of maltooligosaccharides, no glucose as product.

Beta-amylase is involved in starch degradation during mango ripening, which is clearly triggered by detachment from the mother-plant starch enzyme induction upon a cold shock at 4°C leads to starch-dependent maltose accumulation, which might be required for protection of the photosynthetic electron transport chain, maltose influences the carbohydrate metabolism.

Of the components of starch, amylopectin presents the great challenge to hydrolytic enzyme systems. This is due to residues involved in 1, 6-glycosidic branch points which constitute about 4-6% of the glucose present. Most hydrolytic enzyme are specific for 1, 4-glycosidic links yet the 1, 6-glycosidic links must also cleaved for complete hydrolysis of amylopectin to glucose. Some of the most impressive recent exercises in the development of new enzymes have concerned debranching enzymes.

It is necessary to hydrolyse starch in a wide variety of processes which may be condensed into two basic classes;

1. Processes in which the starch hydrolysate is to be used by microbes or man and
2. processes in which it is necessary to eliminate starch.

In the former processes, such as glucose syrup production, starch is usually the major component of reaction mixtures, whereas in the latter processes, such as the processing of sugar cane juice, small amounts of starch which contaminate non-starchy materials are removed. Enzymes of various types

are used in these processes. Although starches from diverse plants may be utilized, corn is the world’s most abundant source and provides most of the substrate used in the preparation of starch hydrolysates.

There are three stages in the conversion of starch

- Gelatinisation, involving the dissolution of the nanogram-sized starch granules to form a viscous suspension;
- Liquefaction, involving the partial hydrolysis of the starch, with concomitant loss in viscosity; and
- Saccharification, involving the production of glucose and maltose by further hydrolysis.

Galatinisation is achieved by heating starch with water, and occurs necessarily and naturally when starchy foods are cooked. Gelatinized starch is readily liquefied by partial hydrolysis with enzymes or acids are saccharified by further acidic or enzymic hydrolysis (Chaplin, 2004).

USES OF AMYLASE

Amylase enzyme finds use in bread making and to break down complex sugars such as starch (found in flour) into simple sugars. Yeast then feeds on these simple sugars and converts it into the waste products of alcohol and CO2. This imparts flavour and causes the bread to rise. While Amylase enzymes are found naturally in yeast cells, it takes time for the yeast to produce enough of these enzymes to break down significant quantities of starch in the bread. This is the reason for long fermented doughs such as sour dough. Modern bread making techniques have included amylase enzymes (often in the form of malted barley) into bread improver thereby

making the bread making process faster and more practical for commercial use.

When used as a food additive, and may be derived from swine pancreas or mould mushroom.

Bacilliary amylase is also used in clothing and dishwasher detergents to dissolve starches from fabrics and dishes.

Workers in factories that work with amylase for any of the above uses are at increased risk of occupational asthma. 5-9% of bakers have a positive skin test, and a fourth to a third of bakers with breathing problems are hypersensitive to amylase.

An inhibitor of alpha-amylase called phaseolamin has been tested as a potential diet aid.

Blood serum amylase may be measured for purposes of medical diagnosis. A normal concentration is in the range 21-101 Mol/L. A higher than normal concentration may reflect one of several medical conditions, including acute inflammation of the pancreas, macroamylasemia, perforated peptic ulcer, and mumps. Amylase may be measured in other body fluids, including urine and peritoneal fluid.

Two amylases are common to the baking industry, alpha-amylase and beta-amylase also known as alpha-1, 4-glucan glucanohydrolase and alpha-1, 4-glucan maltohydrolase.

Amylases convert starch into sugar : the Î±-amylase will cleave the starch randomly (the so called 1-4 bonds in the starch) while the Î²-amylase can only chop off two sugar units at the time at the end of the starch chain. Normally there is enough Î²-amylase present in the flour but sometimes addition of Î±-amylase is needed. The Î±-amylase will cut the starch into smaller units called dextrins and the more Î±-amylase activity there is, the better for the Î²-amylase because there are more extremities available. So the substrate for the Î²-amylase is either starch or dextrins and the product is maltose.

Alpha-amylase is an endoenzyme that attacks linkages within the molecular structure. It randomly cleaves starch chains at interior a-1, 4-glycosidic linkages producing short chains of glucose molecules or dextrins. Beta-amylase is an exoenzyme and cleaves maltose units from the non-reducing end of the starch molecule. In order for these enzymes to function, the starch granule must be ruptured so that the individual starch molecules are available for enzymatic action.

Depending upon their origin, alpha- and beta-amylases show differences in pH and temperature optima, thermostability, and other chemical stability. They do not require co-enzymes for activity, although alpha-amylase activity is enhanced by the presence of calcium.

The pH optimum for alpha-amylase is 4. 5 and it is inactivated at a pH of 3. 3 to 4. 0. This pH dependence decreases the efficacy of this enzyme in sour doughs. Beta-amylase is active across a much broader pH range, 4. 5-9. 2, with a pH optimum of 5. 3. Alpha-amylase is relatively thermostable up to

70°C, whereas beta-amylase loses about half of its activity at this temperature. Fungal amylase is the least temperature stable, followed by cereal amylase, while bacterial amylase is stable at higher temperatures. New intermediate stability enzymes have been developed that are active above the gelatinization temperature of starch (60°C), but are totally inactivated at the later stages of baking (80-90°C). The objective is to maximize the anti-staling effect without creating a gummy, sticky product.

INHIBITORS OF AMYLASE ACTIVITY

Amylase inhibitors are naturally present in many plants and protect the plant from pests by not allowing the insect to break down starch and gain energy from it. Plants may contain separate protease inhibitors as well or amylase inhibitors may play a dual role and also inhibit proteases. Protein amylase inhibitors as well as non-protein amylase inhibitors exist. Amylase inhibitors may be active against a wide variety of amylases or may be specific to certain insect amylases or mammalian amylases (Franco, et al 2002).

Structure of Proteinaceous Amylase Inhibitors

The determination of the structure of a complex between porcine pancreatic amylase and a protein amylase inhibitor isolated from bean (Phaseolus vulgaris) showed interaction between the pancreatic amylase active site and the inhibitor. Conformational changes were observed in the pancreatic amylase upon the binding of the inhibitor. The inhibitor was found to be a dimer with a disaccharide attached to one of the amino acid residues. (Bompard G., et al, 1996).

Amylase Inhibitors in yam tuber

(Shivaraj, et al,. 1979) reported that sweet potatoes do not contain amylase inhibitors while (Rekha, et al, 1999) reported the presence of amylase inhibitors in 79 of the 100 varieties tested. Cultivar differences as well as isolation procedure could account for these differing results. Before performing amylase inhibitor assays, Shivaraj and others homogenized sweet potato with water, allowed the samples to sit for 1 hour, centrifuged the samples, collected the supernatant, and then subjected the supernatant to heat treatment (80°C for 10 minutes) to destroy native amylases. Rekha and others homogenized yam tuber in a sodium phosphate buffer containing polyvinyl pyrrolidone and sodium chloride, stored the samples in the refrigerator, centrifuged the samples, and then performed trichloroacetic acid precipitation to remove native amylases. Rekha and others chose to use TCA precipitation rather than the heat treatment Shivaraj found heat treatment to be ineffective at destroying all native amylase activity.

YAM

Yam is the common name for some species in the genus Dioscorea (family Dioscoreaceae). These are perennial herbaceous vines cultivated for the consumption of their starchy tubers in Africa, Asia, Latin America and Oceania. There are many cultivars of yam. Yam (Dioscorea spp., Dioscoreaceae) is classified as monocotyledonous but is considered to be closely related to dicotyledonous plants as a second cotyledon remains undeveloped in the embryo (Lawton and Lawton, 1967). The storage organ is probably a swollen hypocotyl (Lawton and Lawton, 1969), but is often described as a swollen root. A number of species are grown widely in the

humid tropics with D. rotundata and D. cayenensis being of most importance, followed by D. alata and D. esculenta (Akoroda, 1993). These are all of African or East Asian origin, with only the minor species D. trifida being of American origin (Brücher, 1989). The tubers contain about 1-3 % protein on a dry weight basis (Coursey, 1995).

Yam is source of carbohydrate; carbohydrates are one of the three major food groups needed for proper nutrition. Amylase is the digestive enzyme needed to digest carbohydrates. Carbohydrates in food are an important and immediate source of energy for the body. Starch refers to carbohydrates found in plants (grains). Vegetables and fruits are a source of sugar and are broken down to sugar or glucose. Carbohydrates are present in at least small quantities in most food, but the chief sources are the sugars are the sugars and the sugars and the starches (Wright, 1993)

Uses of yam

Food

Yams of African species must be cooked to be safely eaten, because various natural substances in raw yams can cause illness if consumed. (Excessive skin contact with uncooked yam fluids can cause the skin to itch. If this occurs, a quick cold bath will stop the itching.) Yam is consumed in various ways, but is usually boiled and eaten. This involves cutting yam into pieces, then peeling the skin, and boiling the starchy “ meat”. This is usually consumed with palm oil (traditional way), or with other sauces. The boiled yam can also be pounded with a traditional mortar and pestle to create a thick starchy paste known as Pounded Yam. This is also eaten with traditional stews and sauces. Another method of consumption is to sun dry

the raw yam pieces. When dry, the pieces turn a dark brown color. This is then milled to create a powder known as “ elubo” in Nigeria. The brown powder can be prepared with boiling water to create a thick brown starchy paste known as “ amala”. This is also consumed with the local stews and sauces. The most common cooking method in Western and Central Africa is cooked “ boiled” yam. (Wikipedia 2003).

In India this vegetable is also called Garadu. In central part of India people cut small slices of the vegetable, deep fry them, sprinkle lots of spices on it and eat as snacks. In southern part of India, it is eaten with fish curry and is a local favorite.(Wikipedia 2003)

1. 2 TYPES OF YAM

Dioscorea rotundata and D. cayenensis

Dioscorea rotunda, the “ white yam”, and Dioscorea cayenensis, the “ yellow yam”, are native to Africa. They are the most important cultivated yams. In the past they were considered two separate species but most taxonomists now regard them as the same species. There are over 200 cultivated varieties between them. The Kokoro variety is important in making dried yam chips.

They are large plants; the vines can be as long as 10 to 12 meters (35 to 40 feet). The tubers most often weigh about 2. 5 to 5 kg (6 to 12 lbs) each but can weigh as much as 25 kg (60 lbs). After 7 to 12 months growth the tubers are harvested. In Africa most are pounded into a paste to make the traditional dish of “ pounded yam” (Kay 1987).

Dioscorea alata

A piece of cake made with Ube (water yam). Dioscorea alata, called “ water yam”, “ winged yam” and “ purple yam”, was first cultivated in Southeast Asia. Although not grown in the same quantities as the African yams, it has the largest distribution world-wide of any cultivated yam, being grown in Asia, the Pacific islands, Africa, and the West Indies (Mignouna 2003). In the United States it has become an invasive species in some Southern states.

In the Philippines it is known as ube (or ubi) and is used as an ingredient in many sweet desserts. In Vietnam, it is called khoai má»¡ and is used mainly as an ingredient for soup. In India, it is known as ratalu or violet yam. In Hawaii it is known as uhi. Uhi was brought to Hawaii by the early Polynesian settlers and became a major crop in the 1800s when the tubers were sold to visiting ships as an easily stored food supply for their voyages (White 2003).

Dioscorea opposita

Dioscorea opposita, “ Chinese yam”, is native to China. The Chinese yam plant is somewhat smaller than the African, with the vines about 3 meters (10 feet) long. It is tolerant to frost and can be grown in much cooler conditions than other yams. It is now grown in China, Korea, and Japan.

It was introduced to Europe in the 1800s when the potato crop there was falling victim to disease, and is still grown in France for the Asian food market.

The tubers are harvested after about 6 months of growth. Some are eaten right after harvesting and some are used as ingredients for other dishes, including noodles, and for traditional medicines (Kay 1987). Air potato

Dioscorea bulbifera

Dioscorea bulbifera, the “ air potato”, is found in both Africa and Asia, with slight differences between those found in each place. It is a large vine, 6 meters (20 ft) or more in length. It produces tubers; however the bulbils which grow at the base of its leaves are the more important food product. They are about the size of potatoes (hence the name “ air potato”), weighing from 0. 5 to 2 kg (1 to 5 lbs). Some varieties can be eaten raw while some require soaking or boiling for detoxification before eating. It is not grown much commercially since the flavor of other yams is preferred by most people. However it is popular in home vegetable gardens because it produces a crop after only four months of growth and continues producing for the life of the vine, as long as two years. Also the bulbils are easy to harvest and cook (Kay 1987).

In 1905 the air potato was introduced to Florida and has since become an invasive species in much of the state. Its rapid growth crowds out native vegetation and is very difficult to remove since it can grow back from the tubers, and new vines can grow from the bulbils even after being cut down or burned (Schultz 1993).

Dioscorea esculenta

Dioscorea esculenta, the “ lesser yam”, was one of the first yam species cultivated. It is native to Southeast Asia and is the third most commonly cultivated species there, although it is cultivated very little in other parts of the world. Its vines seldom reach more than 3 meters (10 feet) in length and the tubers are fairly small in most varieties. The tubers are eaten baked, boiled, or fried much like potatoes. Because of the small size of the tubers,

mechanical cultivation is possible; which, along with its easy preparation and good flavor, could help the lesser yam to become more popular in the future (Kay 1987).

Dioscorea trifida

Dioscorea trifida, the “ cush-cush yam”, is native to the Guyana region of South America and is the most important cultivated New World yam. Since they originated in tropical rain forest conditions their growth cycle is less related to seasonal changes than other yams. Because of their relative ease of cultivation and their good flavor they are considered to have a great potential for increased production (Kay 1987).

Dioscorea dumetorum

Dioscorea dumetorum, the “ bitter yam”, is popular as a vegetable in parts of West Africa; one reason being that their cultivation requires less labor than other yams. The wild forms are very toxic and are sometimes used to poison animals when mixed with bait. It is said that they have also been used for criminal purposes (Kay 1987).

1. 3 Nutritional value

Yams are high in vitamin C, dietary fiber, vitamin B6, potassium, and manganese; while being low in saturated fat and sodium. Vitamin C, dietary fiber and vitamin B6 may all promote good health. Furthermore, a product that is high in potassium and low in sodium is likely to produce a good potassium-sodium balance in the human body, and so protect against osteoporosis and heart disease.

Yam products generally have a lower glycemic index than potato products, which means that they will provide a more sustained form of energy, and give better protection against obesity and diabetes.

Aim and objective

The objective of this experiment is to extract the amylases mainly Î²-amylase from yam tuber and determine the enzymatic activities of the enzymes. At the end of the experiment, the amylase extracted from yam tuber can be made use of in the industries like; the pharmaceutical, plastic and textile industries among others in place of barley commonly made used of. This is even favorable considering the land mass covered by yam and also large usefulness of the yam.

CHAPTER TWO

2. 0 MATERIALS AND METHODS

2. 1 MATERIALS

Yam (Dioscoreaceae esculenta), used was from Oja Oba Market in Iwo Osun State, Ethanol, Soluble starch, 3, 5-dinitrosalicylic acid, sodium hydroxide, sodium potassium tertarate, Sephadex G200 was obtained from Pharmacia fine chemicals, Uppsala, Sweden, disodium hydrogen phosphate were products of British Drug House(BDH), poole England. The distilled water was obtained from the Department of Biochemistry, Obafemi Awolowo University, Ile Ife.

2. 2 EQUIPMENTS

Water incubator manufactured by Grant Instruments(Cambridge) Ltd, Weighing balance made in Switzerland, Centrifuge manufactured by microfield instruments England. Spectrophotometer.

2. 2 Preparation of buffer and Reagents

2. 2. 1 preparation of 0. 016 M sodium acetate buffer, pH 4. 8

To prepare acetate buffer for, 73. 10 g of sodium acetate was dissolved in 900 ml of distilled water, 4. 2 ml of acetic acid was added and then made up to 1 Litre in volumetric flask.

2. 2. 2 Preparation of 2 N sodium hydroxide

To 8 g of sodium hydroxide pellets was dissolved in 100ml of distilled water.

2. 2. 3 Preparation of colour reagent

Dinitrosalicyclic acid colour reagent, was prepared by dissolving 1. 0 g of 3, 5-dinitrosalicyclic acid in 50 ml of distilled water. 30. 0 g sodium potassium tartrate tetrahydrate was added slowly with 20 ml of 2N sodium hydroxide. It was diluted to 100 ml with distilled water.

2. 2. 4 Preparation of 1% starch

Prepared by dissolving 1. 0 g of soluble starch in 100 ml of 0. 016 M sodium acetate buffer pH 4. 8. It was boiled to dissolve and cooled, diluted to 100ml with distilled water.

2. 3 Method

The rate at which maltose is released from starch is measured by its ability to reduce 3, 5-dinitrosalicylic acid according to Bernfold(1955) . One unit

releases one micromole of Î²-maltose per minutes at 25ËšC and pH 4. 8 under the specified conditions.

2. 3. 1 Mashing and Extraction of Enzymes

With the use of mortar and pestle, 434. 52 g of yam were ground and 400ml of homogenization buffer (i. e 0. 016 M sodium acetate pH) was added and stirred, it was kept in a refrigerator for 1 hour with intermittent stirring at 10 minutes interval. It was then centrifuged at 4000 rpm for 10 minutes into components. Assay for protein and enzyme activity was then carried out after it has being stored in 70% of ammonium sulphate(212. 4 g/L).

2. 3. 2 Purification

Purification of Î²-amylase: All enzymes purification steps were carried out at room temperature. Enzyme Precipitation: The crude extract was initially fractioned by 70% (v/v) ammonium sulphate. After centrifugation at 4000 rpm for 10 minutes, the precipitated pellets were collected and re-suspended in of cold buffer. The solution was dissolved in 0. 016 M sodium acetate buffer of pH 4. 8 and layered on a Sephadex G-200 Colum (1 x 40 cm). Fractions of 5 ml were collected. The fraction was monitored for protein at 280 nM. Elution was in 0. 016 M sodium acetate buffer, pH4. 8.