CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Fruits are majorly types of food that come from plants, such as vegetable, the fleshy seed-associated structures of a plant that are edible in raw state. They are sources of natural components which are plants derived materials that perform key role in maintaining human health especially disease prevention, growth and development (Naz *et al*,2013) They are the major source of anti-oxidant and also vitamins needed by body e.g watermelon.

Watermelon (Citrullus vulgaris) being a fruit crop is said to have originated in the Kalahari Desert of Africa but cultivated more in the tropical region of the earth. Water melon is a warm season crop and is not chill-resistant. It requires a long growing season in the subtropics, but fast growing in the tropical regions. Flowering and fruit development are promoted by high light intensity and high temperature. Watermelon does not contain fat or cholesterol as it is a good source of vitamin A, B6 and C, and has a lot of fiber, and also potassium, minerals that can play important role in human metabolism. Watermelon has Lycopene which gives watermelon its antioxidant properties that helps to reduce the risk of age-related diseases the Lycopene is also responsible for the red color of the watermelon. Every part of watermelon is a known to be edible this includes the seeds, flesh, and the rinds because it is said to contain amino acids, citruline, and phenolic compounds. Watermelon contains 92% water.

Watermelon (Citrullus vulgaris) is botanically considered as the fruit belonging to the family Cucurbitaceae. (Edward *et al*, 2003).

It is a large, sprawling annual plant with coarse, hairy pinnately-lobed leaves and yellow flowers. It is grown for its edible fruit, which is a special kind of berry botanically called a pepo. The watermelon fruit has deep green smooth thick exterior rind with grey or light green vertical stripes.

The fruit's inner part is red in color with small black seeds inserted in the flesh's center third (Wehner *et al.*, 2001).

Enzymes serve in living organisms a broad range of functions. For signal transduction and cell re gulation, they are indispensable, often through kinase and phosphatases. A significant role of enzyme is the animal's digestive system.

Enzymes are biological catalysts for macromolecular purposes. They are accountable for thousands of life supporting metabolic life processes. Enzymes are extremely selective catalysts that accelerates rate and specificity of metabolic chemical reactions, ranging from food digestion to the DNA synthesis.

β-Galactosidase (Lactase) enzymes are used in industrial and therapeutic applications . In this present study, the native β-Galactosidase producers of bacteria and fungi were isolated from soil, milk, curd. β-Galactosidase family of enzyme is a glycoside hydrolase involved in the hydrolysis of the disaccharide lactose into its constituents of glucose and galactose. β-Galactosidase is a glycoside containing galactose. The organic moiety replaces the H of the OH group on galactose carbon-1.

It is a glycoside hydrolase enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides through the breaking of a glycosidic bond. β-Galactosidase include carbohydrates containing galactose where the glycosidic bond lies above the galactose

molecule. β-Galactosidase is important for organisms as it is a key provider in the production of energy and a source of carbons through the breakdown of lactose to galactose and glucose. β

Galactosidase is essential to organisms because it is a main source of electricity and a source of carbon through the breakdown of galactose and glucose lactose. β-Galactosidase is commonly used in the food sector to enhance milk products' sweetness, sol ubility, flavor and digestibility (Richmond, Gray, and Stine, 1981; Grosova *et al.,* 2008a). This enzyme has been used as a model for studying its activity in amorphous matrices (Burin and Buera, 2002). Enzymatic hydrolysis of lactose by β Galactosidase is one of the most popular technologies to produce lactose reduced milk and related dairy products for consumption by lactose intolerant people (Ladero, Santos, and García-Ochoa, 2000; Ladero *et al.,* 2001; 2002; Jurado *et al.,* 2002; Sener, Apar, and Ozbek, 2006; Haider and Husain, 2008). Sources of β Galactosidases are found in microorganisms (bacteria, fungi, yeasts), plants especially in almonds, peaches, apricots, apples and animal organs (Nagy *et al.,* 2001; Flood and Kondo, 2004; Haider and Husain, 2007a). The major industrial enzymes are obtained from Aspergillus sp. and Kluyveromyces sp. β Galactosidase from Kluyveromyces lactis is one of the most widely used enzymes (Zhou and Chen, 2001a; Jurado *et al.,* 2002; Lee *et al.,* 2003; Klewicki, 2007). β Galactosidases are widely distributed in plant tissues. These enzymes have been shown to be involved in a number of biological processes including plant growth, fruit ripening and in the hydrolysis of lactose. Molecular approaches were also used to unravel the role of β Galactosidases in fruit development and ripening (Li *et al.,* 2001; Lopez *et al.,* 2002).

1.2 STATEMENT OF RESEARCH PROBLEM

This research work is to extract, and purify and characterize beta- galactosidase in the seed of watermelon available in Nigeria and widely consumed by a large population in Nigeria. The enzyme will digest lactose in milk consumed by people who lack lactose. They will not have to depend on that extracted from bacteria, fungi, yeast, almond, peaches, and apricots.

1.3 JUSTIFICATION OF STUDY

Various study has established the fact that absorption of undigested lactose in small intestine requires the activity of this enzyme; hence, the deficiency of this enzyme leads to lactose intolerance. Lactose intolerance affects around 70% of world's adult population, while the prevalence rate of lactose intolerance is 60% in Pakistan. This study will further reveal the method of extraction, isolation and characterization of β-Galactosidase from watermelon will help in the production of lactase enzyme which aids in the reduction lactose intolerant.

1.4 AIM AND OBJECTIVES OF RESEARCH

This aim of this study is to;

1. Extract (β-Galactosidase) from the seed of *Citrullus vulgaris* and determine the activity of the crude extract enzyme.
2. Salt out the extracted enzyme through Ammonium Sulphate Precipitation andp urify through a Sephadex G-25 column
3. Characterize the β-Galactosidase from the seed of Citrullus vulgaris and determine optimum, pH, Temperature, Km and Vmax.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 WATERMELON (*CITRULLUS VULGARIS)*:

2.1.1 Origin:

Watermelons have been grown since prehistoric time, they were grown by the ancient Egyptian, and apparently cultivated thousands of years ago in Asia Minor, Russia, and the near and Middle East (Pierce, 1987). The plant was thought to be originally coming from Africa, particularly native to Central Africa, although explorers reported evidence of possible American origin. Descriptions indicated that the early American melon was of the Citron type (Ware, 1968). Early French explorers found Indians growing watermelon in the Mississippi Valley. Its culture was not known in Europe until the sixteen century (Ware, 1968). In term of world production watermelon is currently grown mainly in China, Turkey, the USSR, Egypt, the USA, Iran, Japan, Italy, Spain and Syria (Snowdon, 1990, Robinson and Decker, 2003). Gokovsky 1971) reported that Sudan is considered as the center of origin of watermelon especially Kordofan Zone, where it grows as a wild plant.

2.1.2 Classification:

The cucurbits (family cucurbitaceae) form a diverse group of species grown around the world under many conditions and for many different purposes. All cucurbits are frost sensitive, but they differ in their ability to withstand cold and heat. They are grown in low lands and mountains, in

fields, green houses, in tropical desert and temperature regions. The major cultivated types include cucumber, melon (cantaloupe or musk melon, honey dew, etc.), squash (cucurbita spp.), pumpkin, and watermelon. Despite the large differences between and within the cucurbit species, they are morphologically very similar in appearance. The germination of cucurbit vegetable seeds requires relatively warm temperature (Lorenz and Maynard, 1980), and takes place within 3 or 4 days at 25 30°C. Cucurbit seeds will continue to develop even after the fruit is removed from the vine. If fruits are harvested before they are fully mature, due to impending frost or other reasons, it is advisable to store them 1 or 2 months before extracting the seeds. Watermelon is an aggressive vining annual plant adapted to mean temperatures greater than 21°C. Watermelon differs from other economically cucurbits by having pinnatifid leaves, with 3-4 pairs of lobes which are subdivided and toothed. The hairy stems are growing up to 5 m, they are thin, angular and grooved with 2-3 branched tendrils. Dwarf watermelon varieties with reduced internode have been bred, but most commercial cultivators are highly branched vines, measuring up to 10-m in length (Pierce, 1987, Robinson and Decker, 2003).

The root system is deep, extensive and superficial (Pierce, 1987), whereas Robinson and Decker (2003) reported that the root system is relatively extensive but shallow. Petioles are 1-10 cm in length. The flowers are 2-3 cm in diameter with 3-5 stamens and hairy ovary. Watermelon is monoecious. The solitary, light yellow flowers are less showy than those of many other cucurbits. In addition to sweet there are bitter types. Among the latter there is a form of *Citrullus vulgaris* known as “Egusi”, grown in some regions of Nigeria, whose flesh is inedible and only the seed are used.

2.1.3 Watermelon Varieties

Citrullus vulgaris is cultivated globally in over 96 nations. Worldwide, there are approximately 1 200 varieties of watermelon ; offering consumers a broad option. Varieties of tiny bitter inedible fruits to big succulent sweet fruits vary greatly. The varieties vary in vigor, earliness and productivity; shape, color and marking of fruits; thickness and texture of rind; color, texture, flavor and sugar content of flesh; size, color and number of seeds (Purseglove, 1972). Watermelon varieties fall into three broad classes based on how the seed was developed:

- Open-pollinated,
- F1 hybrid and
- Triploid (seedless).

Over several generations of choice, open-pollinated varieties are created.

The choice can be based on output, quality and resistance to disease. Open-pollinated varieties have true-to-type seed (seed saved from generation to the next will retain the same features) and are less expensive than F1 hybrid varieties.

F1 hybrids are created from two inbred lines that have been self-fed and then crossed for several generations, with the subsequent seed being sold to growers. F1 hybrid seed will display enhanced uniformity of harvest form and time relative to open-pollinated seed and may show yield increases of as much as 20% to 40% over open-pollinated varieties cultivated under comparable circumstances. Cost and availability are the disadvantages of F1 hybrid seed. F1 hybrid seed will be as much as five to ten times as costly as open-pollinated seed, and available F1 hybrid varieties will change from year to year.

The third type is triploid or seedless watermelon. These are created by doubling the usual amount of chromosomes by generating watermelon crops and crossing them with ordinary crops of watermelon.

The resulting crops have the normal number of chromosomes one-and - a-half times. Since they have an unusual number of chromosomes, they are unable to create sustain able seed. They also produce very little pollen, so normal watermelon with triploid wat ermelon must be planted as a source of pollen.

Although triploid watermelons are referred to as seedless, they are not really seedless, but in stead have undeveloped seeds that are soft and edible. Triploid seeds will be even more expensive than F1 hybrid seeds (Boyhan, *et al.* 2008)

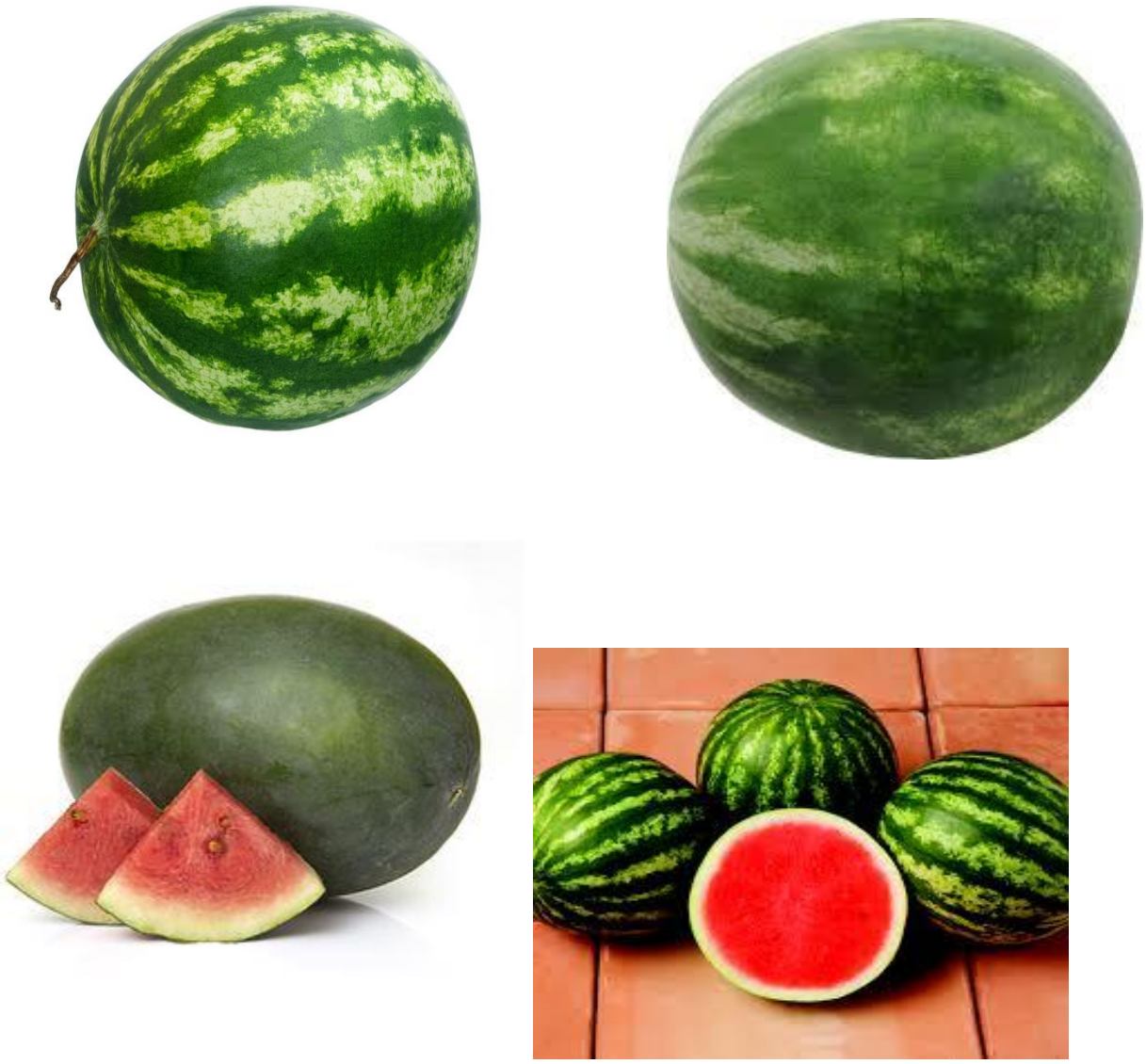

Fig 1 Different varieties of *Citrullus vulgaris*

2.1.4 Economic Importance and Uses of Watermelon

Watermelon is a significant source of water for the natives during dry periods in certain semi-desert districts; even today there are districts in Africa where it is grown for this purpose (Boswell, 2000).

Greatly oversized watermelons have no sound market value. They are too difficult to handle without damage or wastage; most customers do not want them; and they are likely to be inferior in quality to those of normal size. Modern emphasis is upon high quality of garden products rather than mere size (Boswell, 2000).

Fresh and cold, the watermelon is used almost completely as a dessert. But the rind is produced of preserves or sweet "pickles." The seeds are used in Nigeria only for planting (Boswell, 2000). In some cultures, it is popular to bake watermelon seeds and eat them (Produce, 2008).

Beer is produced of watermelon juice in Europe, or the juice for its sugar can be boiled down to heavy syrup like molasses. In Iraq, Egypt and elsewhere in Africa, melon flesh is used in some dry district as a staple food and animal feed as well as a source of water. The seeds are cooked, with or without salting, and consumed in the Old World, especially Asia. By salting or brining big parts or halves in barrels, Orientals also maintain watermelon. (Boswell, 2000)

2.1.5 Nutritional Value of Watermelon

Watermelon is 92% water and 8% sugar. It is rich in lycopene, an antioxidant that gives it its characteristic color. It is fat free (Medicine Net, 2004). Watermelon can be processed and used for juice syrups and sweets. Oil rich in vitamin D can be extracted from the seed. Their sugar content boosts our energy so that in any aspect we are positive.

High water content cleans the human organism and works well for our digestive and urinary system.

The use of watermelons in our daily diet is obviously very healthy as it has a beneficial healing i mpact on patients with coronary, liver, gold bladder and kidney. Half kg of fruit can meet our daily need in vitamin C. In addition to the 85% water content it contains 7-15% of sugar, minerals, vitamins and also small amount of proteins. Vitamins present are carotenes, vitamin B complex and traces of C vitamin. Mineral content present are potassium, magnesium, phosphorus, calcium, zinc, iron, and cuprum. It is a good source of carotenes and lycopene as well. Apart from nutrient value, it is also significant as natural medicine source (Ignjatovic, 2005).

Watermelon is rich in carotenoids. Lycopene, phytofluene, phytoene, beta-carotene, lutein, and neurosporene are some of the carotenoids in watermelon. Lycopene is the b ulk of watermelon carotenoids. The content of carotenoid differs based on the watermelon type. Depending on the variety, carotenoid content in red fleshed watermelon ranges between 37 and 121 mg/kg fresh weight, whereas lycopene ranges between 35 and112 mg/kg fresh weights (HonCod, 2008).

Not only is watermelon packed with thirst-quenching water and natural sweetness, it is also an excellent source of two powerful antioxidants: lycopene, and beta carotene. Lycopene is what provides watermelon it's rich, red color and is associated with decreased danger of muscle degeneration, challenges to the prostrate, and a range of other degenerative circumstances. Beta carotene is another strong antioxidant that can assist prevent free radicals from damaging your cells. (Kim, 2008).

2.1.6 *Citrullus vulgaris* seeds

Watermelon seeds protein isolate has great potential for incorporation into human food products not only as a protein supplement in diets of under nourished but as functional agent in different food products. It exhibits some good functional properties. 1995).Watermelon seeds contained nutritionally useful quantities of essential amino acids as well as minerals which made them potentially useful as food supplement (Zhang, *et. al.,* 2001 and Olaofe, *et. al.*1994). In Cameroon, watermelon seeds are ground in to a paste and added to cassava leaves and cooked into a sauce (Adam, *et. al.*,2017).

Decorticated ground watermelon seeds are used as a flavor component of gravies (Nwokolo, 1987).

2.1.7 Morphology and Physiology

Watermelon is a plant from the warm season. It is not resistant to chilling and needs a season of lengthy growth. High light intensity and elevated temperature promote the growth of flowers and fruit. Watermelon is the only economically significant cucurbit with pinnatifid (lobed) leaves ; the whole (non-lobed) leaves of all other species. With the exception of a whole-leaf (non-lobed) gene mutant regulated by the nl (non-lobed) gene, the leaves are pinnately split into three or four pairs of lobes. The habit of growing watermelon is a trailing vine. The stems are thin, hairy, angular, grooved, and at each node they have branched tendrils. The stems are extremely branched and up to 30 feet long, although with smaller, less branched stems there are dwarf kinds (dw-1 and dw-2 genes). With a taproot and many lateral roots, the roots are vast but shallow.

Watermelon has tiny, less showy flowers than other cucurbits. After seeding, flowers begin about 8 weeks. Watermelon flowers are stamina (male), ideal (hermaphroditic), or pistillate (female), generally born on the plant as it develops. Monoecious kinds are most prevalent, but there are andromonoecious (staminate and perfect) kinds, predominantly older varieties or wild accessions. The pistillate flowers have a lower ovary and correlate the size and shape of the ovary with the final size and shape of the fruit. In many variants, at every seventh node, the pistillate or ideal flowers are borne with stamina flowers at the nodes that interfere.

Watermelon fruit is round to cylindrical, up to 24 inches long, with a rind thickness of 0.4 to 1.5 inches. The endocarp (placenta) is the edible component of the fruit. This is in contrast to melon (Cucumis melo), where the mesocarp is the inedible component of the fruit. Fruit up to 262 lb. They were registered, but they generally weigh between 8 and 35 lb. Even lower watermelon fruits ranging from 2 to 8 lb in Asia. They're popular. The rind of fruit ranges from thin to thick and from brittle to hard.

As the fruit matures and the rind lightens in colour, the seeds continue to grow. If the fruit is kept in storage (in the shade or in the plant handling room) for a few days after removing it from the vine, seeds will be simpler to remove from the fruit. If the seed is left in the fruit for too long, it will germinate in situ. In watermelon plants there is no dormancy, so on one day they can be harvested, washed, dried and planted on the next day. Depending on temperature and humidity circumstances, seeds germinate in 2 days to 2 weeks. Seeds will not germinate below 60 ° F. The optimum temperature for triploid seed germination is between 85 and 90 ° F. Temperature and humidity are more critical to the germination of triploid hybrid crops, and the prevention of excess moisture is particularly important.

2.1.8 Taxonomy

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Order Cucurbitales

Family Cucurbitaceae

Scientific name *Citrullus lanatus*

Synonyms *Citrullus vulgaris*

Genus Citrullus

Species vulgaris

2.1.9 Constituents of *Citrullus vulgaris* seed

(i) The seeds of the watermelon are of some nutritional importance as they form an article of diet in certain parts of India.

(ii) They are a potent source of urease (Damodaran & Sivaramakrishnan, 1937) and a study of the individual proteins present is of obvious interest in view of the suggested protein nature of this enzyme (Sumner, 1932).From the point of view of their use in urea determinations it is also necessary to ascertain the presence among the non-protein nitrogenous constituents of the urea-producing bases arginine and canavanine.

(iii) Citrulline was isolated by Wada (1930) from water melon fruits and the possibility existed that this interesting amino-acid might be present, free or combined, in the seeds.

2.2 BETA GALACTOSIDASE ENZYME

Also known as lactase, β-D-Galactosidase was an enzyme or protein that catalyzes lactose hydrolysis. This lactose is mainly carbohydrate present in most dairy products to be ingested above the intestinal epithelium to monosaccharide glucose and galactose and has potential importance in the dairy industry. In polymers, oligosaccharides and secondary metabolites, β-Galactosidase also hydrolyzes D-galactosyl residues. β-Galactosidase belongs to the GH-A superfamily of glycoside hydrolases subfamilies 1, 2, 35 and 42. β-Galactosidase was a well-known biocatalyst that catalyzes responses to hydrolysis and transgalactosylation. In some instances it is involved in the manufacturing of prebiotic galacto-oligosaccharide (GOS), which is synthesized because of its associative activity of transglycosylase. β-Galactosidase has two enzymatic operations: it cleaves or divides the β-glycosidic bond between galactose and its organic residues and cleaves cellobiosis, calories, collateral and cellulose. On the other side, it catalyzes lactose to allolactose transgalactosylation.

There are of two types of lactases, neutral and acidic, based on their optimum pH for enzyme activity. β -Galactosidase or β -D-Galactosidase-galactohydrolase is an important enzyme industrially used for the hydrolysis of lactose from milk and milk whey for several applications. Recently, its galactosyltransferase activity, which is accountable for the synthesis of transgalactosylated oligosaccharides (TOS) acting as functional foods, has increased the significance of this enzyme, with several positive impacts on customers.

The enzyme family β-Galactosidase is a glycoside hydrolase engaged in disaccharide lactose hydrolysis in its glucose and galactose constituents. Lactase is vital in milk for digestive lactose hydrolysis. The enzyme deficiency leads intolerance to lactose. For its activity, the optimum lactase temperature is about 48 ° C (118 ° F) and has an optimum pH of 6.5. The β-Galactosidase enzyme is of industrial importance as it can be used to prevent crystallization of lactose in sweetened, condensed and frozen dairy goods such as ice creams and condensed milk and to fix whey-related issues. It is also used to avoid lactose intolerance in individuals who are deficient in lactase.

Lactic acid bacteria (LAB) used as a starting point for the manufacture of milk goods are the primary variables in the fermentation and safety of fermenting foods and also play an important part in the texture and taste of food goods (Chammas *et al.* 2006). One of the glycosidase enzymes is -Galactosidase, which is commonly used in the dairy sector and manufactured by most lactobacilli (Karasova *et al.* 2002). This enzyme hydrolyzes lactose, the principal carbohydrate in milk, into glucose and galactose that can be absorbed throughout the intestinal epithel. (Troelsen, 2005; Vasiljevic and Jelen, 2001; Heyman, 2006). Galactosidase has two enzymatic operations: one of which is accountable for lactose hydrolysis and cleaves cellobiosis, cellotriosis, cellotetrosis and, to some extent, cellulose and the other, splits-glycosides. Low β-galactosidase activity creates digestive insufficiency, often referred to as lactose intolerance (Karasova *et al.* 2002; Vasiljevic and Jelen, 2001). The symptoms of lactose intolerance such as abdominal pain and diarrhea, nausea, flatulence, and or bloating after the ingestion of lactose or lactose containing food substances which can lead to decrease quality of life, and daily activities. Treatment is relatively simple by eliminating lactose from the diet or by using of supplemental β-Galactosidase enzyme replacement (Vasiljevic and Jelen, 2001). The bacterial species presently

used by the dairy industry that manufactured Galactosidase enzyme belong to the genera of Lactobacillus and Bifid bacteria. (Movahedzadeh, et al., 2010). For three specific reasons, these bacteria have become a focus of science research (Somkuti *et al.* 1998):

a) Maldigesters of lactose may eat certain fermented milk goods with little or no negative consequences,

b) These bacteria are usually considered secure (GRAS), so without comprehensive purification, the β-Galactosidase enzyme obtained from them could be used.

β-Galactosidase has a molecular weight of 520,000 Daltons and is composed of four identical subunits of MW 130,000 Daltons, each with an independent active site. Major enzymatic activities of β -Galactosidase are first, disaccharide lactose may be cleared to form glucose and galactose and then glycolysis may occur. Secondly, the enzyme can catalyze lactose transgalactosylation to allolactose, and thirdly, allolactose can be cleaved to the monosaccharides. Allolactose binds to the lacZ repressor and produces the positive feedback loop that controls the cell's β-Galactosidase quantity.

In many respects, β-Galactosidase is best recognized for its reaction with X-gal (5-bromo-4-chloro-3-indoyl-β-d-galactopyranoside), a soluble colorless compound consisting of galactose linked to a substituted indole. β-Galactose has high specificity for the galactose part of its substrates but low specificity for the remainder. Thus, X-gal is hydrolyzed, releasing the replaced indole that dimerizes spontaneously to produce an insoluble, intensely blue product. Colonies of E on growth media comprising X-gal. Because of this response, coli with active β-Galactosidase become blue. In early β-Galactosidase research, deletion of certain residues close the amino-

terminus such as 23–31 or 11–41 resulted in the tetrameric enzyme dissociating into inactive dimers. In addition, the active tetrameric form of the enzyme could be reconstituted by using peptides that included some or all of the "missing" residues (e.g., 3–41 or 3–92). The prevalent blue / white screening (with X-gal) used in cloning is based on this "α-complementation" phenomenon. It can now be rationalized in terms of the three-dimensional structure. A novel procedure was developed to measure the activity of single β-Galactosidase molecules. It depends on the conversion of the weakly fluorescent substrate resorufin β-d-galactopyranoside to the highly fluorescent product resorufin. Individual β-Galactosidase molecules produce several thousand product molecules per minute, and using a typical incubation of 15 min, the amount of product can be measured with an estimated error of about 15%.

Measurements of single molecule activity are carried out using a purpose-designed capillary electrophoresis instrument with a number of benefits. For example, a single protein molecule may be allowed to react for a desired period with a substratum and then move away from the accumulated product to a new location, allowing repeated measurement with the same protein molecule. Activity measurements can also be conducted in a single experiment for multiple protein molecules.

Molecules of β-Galactosidase both before crystallization, and from dissolved crystals, displayed a range of activity of 20-fold or greater. The pre-crystallized protein had a distribution of general activity of 38,500 900 responses per minute, while 31,600 1100 responses per minute for crystal molecules.

On the one side, it could be asserted that the variety of catalytic operations represents individual β-Galactosidase molecules ' oxidation or other such chemical alteration. During crystal growth,

additional chemical modification could also explain why the crystallized proteins have slightly reduced activity than those measured prior to crystallization.

β-Galactosidase catalyzes oxygen-glycosidic bond responses with β-D-galactopyranosides. The enzyme also responds with substrates that have other glycosidic connections, including nitrogen, sulfur and fluorine, but with significantly decreased catalytic efficacy. The enzyme is very specific to d-galactose and particularly significant are the 2, 3, and 4 positions. At these positions, the hydroxyls must each be present and catalyze the reaction in the correct orientation for the enzyme. Lactose is likely the β-Galactosidase's natural substratum, but the enzyme is promiscuous for the substrate's non-galactose portion. Assays of β-Galactosidase responses with lactose, best conducted with gas-liquid chromatography, 2 are not regularly conducted as the assay is technically cumbersome, involves consideration of the manufacturing of galactose, glucose and allolactose, and must also take into account the impacts of these goods as transgalactosidic acceptors and as a substrate for allolactose. Even lactose itself is a transgalactosidic acceptor.

2.2.1 Function of beta Galactosidase

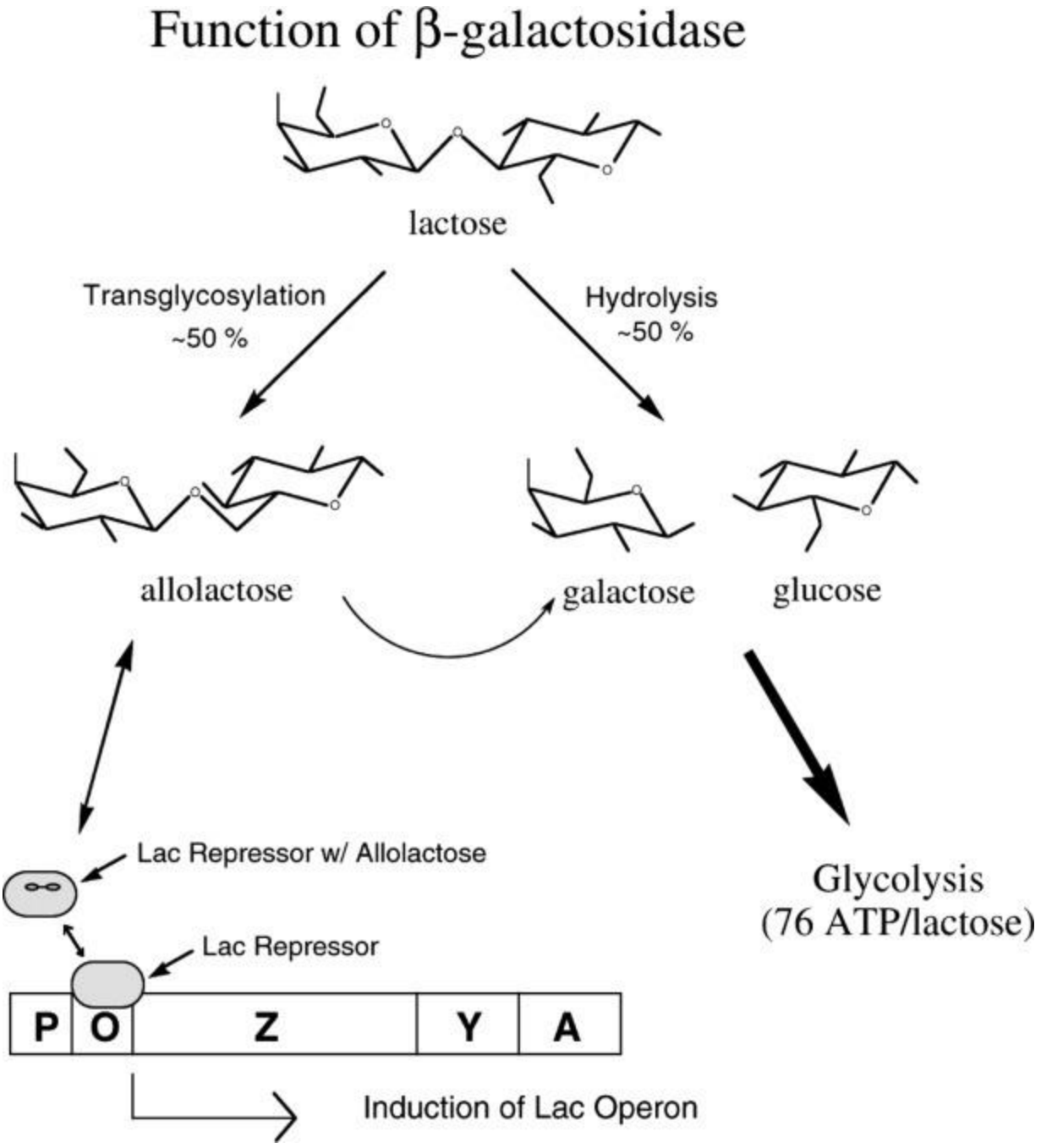

Fig 2 Function of Beta-Galactosidase

The enzyme can hydrolyze lactose into galactose plus glucose, transgalactosylate into allolactose, and allolactose can be hydrolyzed. The presence of lactose outcomes in an

allolactose synthesis that binds to the lac repressor and decreases its affinity to the lac operon. This in turn enables β-Galactosidase, the product of the lacZ gene, to be synthesized.

2.2.2 Structure of beta Galactosidase

β-Galactosidase is a tetramer of four identical polypeptide chains, each of 1023 amino acids. Initially, the crystal structure was determined in a monoclinical crystal shape with four asymmetric unit tetramers. In a orthorhombic crystal with a single tetramer in the asymmetric unit, the structure was subsequently refined to 1.7 resolution. The latter type has been used for later structural and functional research and is technically superior.

The 1023 amino acids form five well defined structural domains within each monomer. The third (main) domain (residues 334–627) is a barrel called triose phosphate isomerase (TIM) or α8β8 with the active site forming a profound pit at the end of this barrel at the C-terminal. As noted below, amino acids from elsewhere in the same polypeptide chain as well as from other chains within the tetramer also contribute critical elements of the active site. There is one horizontal double axis of symmetry, one vertical and one perpendicular to the board. The two-fold horizontal axis forms the so-called "lengthy" interface, while the "activating" interface forms the vertical symmetry axis. There is one horizontal double axis of symmetry, one vertical and one perpendicular to the board. The two-fold horizontal axis forms the so-called "lengthy" interface, while the "activating" interface forms the vertical symmetry axis.

It was proposed that β-Galactosidase originated from a much easier, single-domain TIM barrel enzyme which had an expanded active-site cleft and could have cleaved expanded oligosaccharides. The subsequent incorporation of extra domains could have decreased the size

of the active-site cleft with binding disaccharide substrates to a pocket commensurate. In addition, some of these extra components may encourage inducer, allolactose, manufacturing.

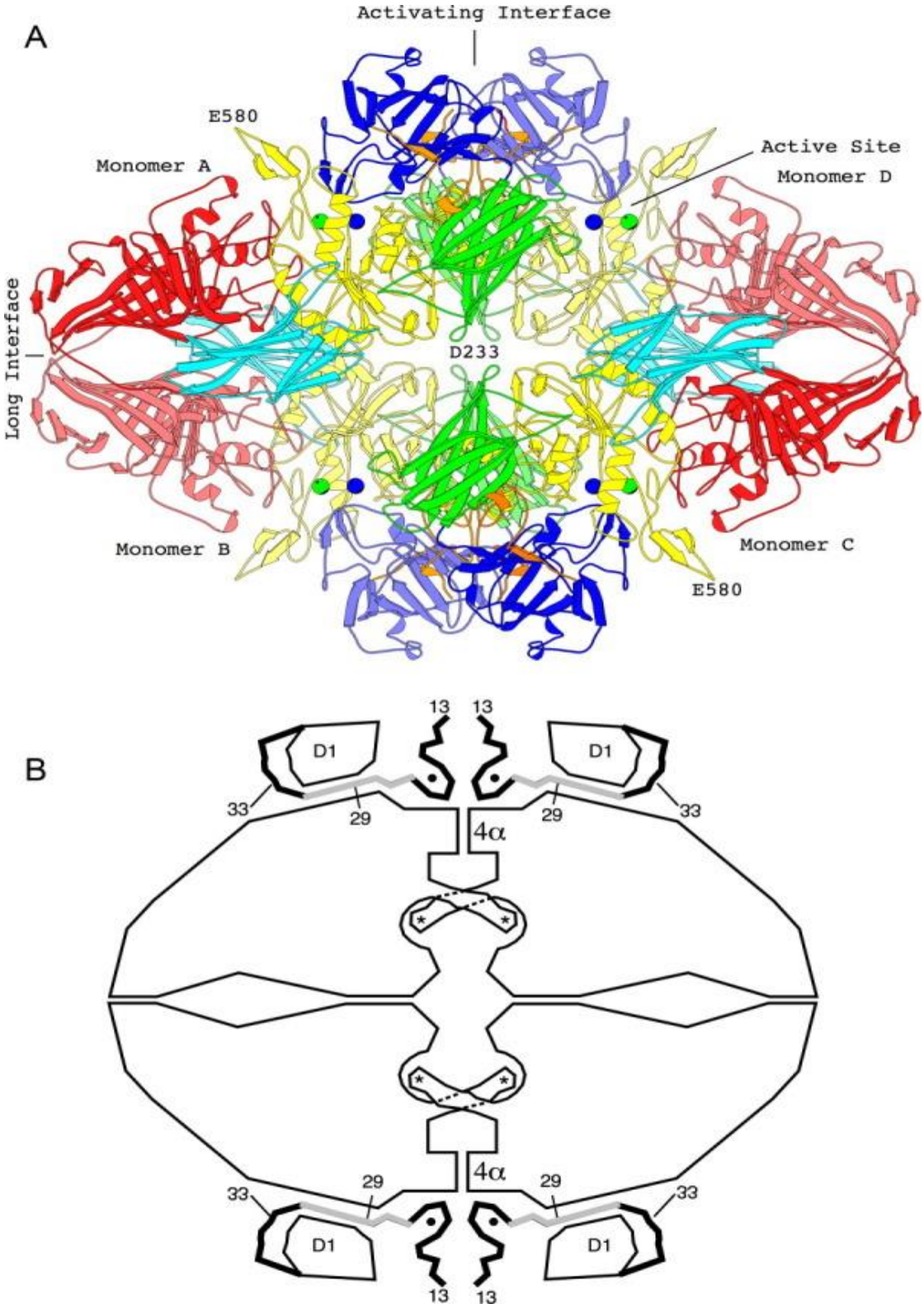

Fig 3 Structure of Beta- Galactosidase.

2.2.3 Sources of β-Galactosidase

In essence, the enzyme was omnipresent. Different organisms, including crops, livestock and microorganisms, generated β-Galactosidase. In many crops such as almonds, peaches, apricots, apples, tips of wild flowers, soy bean seeds, alfalfa and coffee, it is commonly dispersed in nature. The enzyme was also discovered in animal bodies such as dogs, rabbits, snails, calves, sheep, cows, rats in the intestine, brain, placenta and testis. In addition, lecturers have also been discovered in human saliva, primate and farm animal fetuses, rat and mouse tissues, and dogs ' plasma serum and urine. β-Galactosidase is produced by a number of microorganisms. A number of microorganisms produce β-Galactosidase. *Candida pseudotropicalis* can also be generated from yeasts such as *Kluyveromyces marxianus, Kluyveromyces lactis (Saccharomyces fragilis and Kluyveromyces fragilis),* from fungi such as *Neurospora croussa, Aspergillus foetidus, Aspergillus flavus, Aspergillus phoenicis, Aspergillus niger, Aspergillus oryzae, Mucor pusillus* and *Mucor meihei,* from bacterial cultures like *Bacillus megaterium, Bacillus coagulans, Bacillus stearco-thermophillus, Bacillus circulans, Escherichia coli, Thermus aquaticus, Streptococcus lactis, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus helveticus, Lactobacillus thermophila* . Also β-Galactosidase is produced by thermophilic lactic acid bacteria (LAB). These cultures ' β Galactosidase demonstrates elevated stability and elevated temperature activity. For the following purposes, these microorganisms are used for science research:

a) Fermented milk products have no negative or confrontational impacts on malignant lactose

b) These bacteria are generally considered safe (GRAS), so without comprehensive purification, the galactosidase enzyme obtained from them could be used.

2.2.4 Use of beta Galactosidase

The nutritional use of lactose was limited because this enzyme is lacking in a large part of the world's population and can not use lactose as a consequence of which they develop maldigestion or intolerance of lactose. Hygroscopic lactose that has the proper and powerful ability to absorb odors and flavours. Hygroscopic lactose that has the proper and powerful ability to absorb odors and flavours. This results in many frozen imperfections, such as crystallization in dairy goods, enhancement of sandy or gritty texture, and formation of deposits. This generates a potential benefit of β-Galactosidase. Enzymatic lactose hydrolysis could assimilate foods enriched with sugars from lactose. Lactase enzyme is vital for the intolerant of lactose and is also of industrial importance because it is used to prevent crystallization of lactose, to improve the solubility of milk products and also to fix the problem caused by the use and disposal of whey that would lead to environmental or ecological pollution. In addition, the implementation of β-Galactosidase could fix the issue of crystallization during storage by generating hydrolyzed milk while generating caramelized lactose owing to low dissolvability. Furthermore, β-Galactosidase's transgalactosylation property has prominent therapeutic applications such as disorder treatment and digestive supplements enhancement. It also has potential bioremediation, biosensor and diagnostic applications. The enzyme is also used as a model to study its amorphous matrix activity.

2.2.5 Characterization of beta Galactosidase

- From *Escherichia coli*

- The lactase enzyme can be characterized by Folin Wu procedure, Benedict's test, Barford's test, Molisch test, Bials test and Mucic acid test. For the confirmation of the lactase enzyme after growing the isolated cultures on Whey medium.
- From *Saccharomyces fragilisl*
- From Apricot (*Prunus armeniaca kaisa*)

2.2.6 Inhibition of Beta Galactosidase

The β-Galactosidase of *S. fragilis* Y-1109 was inhibited by several different types of compounds. Heavy metal ions inhibits β-Galactosidase completely, whereas the monovalent cation inhibits β-Galactosidase to a lesser degree. Ammonium ion, can by itself activated β-Galactosidase.

2.2.7 Application of beta Galactosidase

- **Health**

About 75% of the world's adult population are unable to consume milk and other milk products due to lactose intolerance. This problem can be solved, if lactose present in the products was hydrolyzed by β-

Galactosidase to readily utilizable sugars like glucose and galactose. Another application of enzymatic hydrolysis of lactose is the formation of GOS. These are indigestible compounds which act as dietary fiber. They promote the growth of intestinal bifidobacteria that are essential for healthy functioning of intestine and liver.

- **Food technology**

High content and low solubility of lactose in dairy products for example frozen milks, ice-cream, condensed milk and whey spreads, leads to lactose crystallization causing grimy and gritty

texture. β-Galactosidase usage in food industry can decrease the concentrations of lactose and develop the quality or value of dairy items by increasing the creaminess, softness, digestibility and sweetness of the product.

Lactase also improves the utilization of high protein supplements containing milk.

- **Environment**

In cheese industry, production of lactose as waste causes many commercial and environmental issues. Lactose hydrolysis by β-Galactosidase found in whey can be used to make sweet syrup. The sweet syrup can be used as a source of sugar in confectionary, feedstuffs, soft drinks, sweets, baking, ice-cream, dairy desserts, molasses.

- **Biotechnological applications**

Enzyme hydrolysis of lactose is a main biotechnological method as the hydrolyzed products can be consumed by lactose maldigesters. Immobilization of β-Galactosidase in liposomes is done to improve the taste of lactose-hydrolyzed milk. Cold-active β-Galactosidase is used in food industries for producing lactose-free dairy products. The natural whey waste can be utilized as a low-priced, easy availability substrate for bacterial cell cultivation after the lactose hydrolysis by β-Galactosidase. Whey proteins recovered through ultrafiltration and hydrolyzed to deliver numerous valuable pharmaceutical products.

- **Biosensor applications**

Biosensors are essential tools in the field of agro diagnostics, immunoassays, screening of drug, forensics, and analysis of gene expression, gene toxicology analysis, and Pharmacogenomics. They have many advantages such as sensitivity, accuracy, reliability and low cost. It combines the biomolecule recognition and sensitivity of signal transducers. Various biosensors are

established by different bio recognition elements such as whole cell, antibiotics, nucleic acids and peptides.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EQUIPMENT AND REAGENTS

3.1.1 Equipment

Test Tubes, Beaker, Conical flask, Measuring Cylinder, Pipette, Cotton Wool, Aluminum Foil, Burette, Spatula, Stirring Rod, Weighing Balance, Cold Centrifuge, Water Bath, Hot Plate, UV/Visible Spectrophotometer, Bio Freezer, Warring Blender.

3.1.2 Reagents

Distilled Water, 0.1M Phosphate Buffer pH 7.0, Copper Reagent(CuSO4), Alkaline Reagent (NaOH), Folin-C, Ammonium Sulphate $(NH_4)_2$, Sephadex G-25, Glucose ($C_6H_{12}O_6$), Albumin,

3.2 COLLECTION, PROCESSING AND EVALUATION OF *Citrullus vulgaris*

3.2.1 Experimental Site:

The study was carried out at the Biochemistry laboratory of the Department of Biological Science, College of Basic and Applied Science, Mountain Top University within the Southwestern State of Nigeria

3.2.2 Collection and preparation of Fruit Material.

The fresh *Citrullus vulgaris* was obtained from Ketu market, Lagos State and was confirmed to be good and suitable for experiment, they were transported down to the experimental site where it was washed, sliced and the seeds were removed.

The seeds were rinsed with water and then air-dried by spreading them on the surface of the laboratory table at about 25^0C.

3.2.3 Preparation of Crude Enzyme

Five hundred and seventy (570) of the dried seed weighing 48.03g were poured into 100mls of 0.1M Phosphate buffer pH 7.0 and then homogenized with the use of a warring blender. This extract was regarded as the crude enzyme, which was then sieved using a clean white handkerchief, the extract was centrifuged in a refrigerated centrifuge at 4^0C at 3500rpm

3.3 PROCEDURE FOR ENZYME ACTIVIVTY

Dano milk was weighed (2.564g) and dissolved in 100ml of distilled water. This contained 1% lactose.

In 100g of Dano milk, there is 39% lactose

1% Lactose = 100g dano = 39% lactose

X g = 1% lactose

$$\frac{100g \times 1\%}{39\%} = 2.546\text{g}$$

To 4ml of the milk solution, 1ml phosphate buffer pH 7.0 was added and then 1ml of the extracted crude enzyme was added and incubated at 37^0C in a water bath for 15 minutes.

The reaction was stopped by adding 1ml of 4.2% Perchloric acid, 1ml of copper reagent and then placed in the water bath at 37^0C for 20 minutes. The solution was cooled and 1ml of Folin-C reagent, and 7ml of water were added.

One of the sample was diluted with 20ml of distilled water and the absorbance taken at 450nm.

3.4 PROCEDURE FOR PROTEIN DETERMINATION

The method of Lowry *et al* (1951) was modified for the experiment.

To 1ml of crude enzyme, 4ml of alkaline solution (NaOH and distilled water) and 1ml of copper solution (CuSO4 and distilled water) was added. The mixture was kept at room temperature for 10 minutes. 0.5ml of distilled Folin-C was added and mixed, it was left to stay for another 30 minutes. The absorbance at 750nm was read against blank.

3.5 PURIFICATION

3.5.1 PURIFICATION THROUGH AMMONIUM SULPHATE PRECIPITATION

Ammonium sulphate (70% saturation) was added to the crude extract. The mixture was left overnight in the refrigerator at 4^0C after which the supernatant was separated from the precipitate by centrifuging in a cold centrifuge at 2000rpm for 15min. The precipitate containing the protein

was dissolved in 100ml 0.1M Phosphate buffer pH 7.0 poured into a dialysis bag tied at both ends and dialyzed against distilled water overnight to remove the salt.

3.5.2 PURIFICATION THROUGH A SEPHADEX G-25 COLUMN PRINCIPLE

This is the separation on the basis of differences in molecular size by passing them down through a column containing packed gel. Small molecules can enter the gel but larger molecules are excluded from the cross –linked network, which complete separation eventually occurs. Bringing out the larger molecules before the smaller ones.

Procedure;

A known weight (4.5g) of Sephadex G-25 was dissolved in 100ml of distilled water and placed over a boiling water bath for three hours for the bead to swell. The solution was poured into a burette stocked with wool at the tip to serve as a filter. The gel was left for about 5 to 6 minutes to pack, and then eluted with 7.0 pH phosphate buffer. The length of the packed gel was measured as 26cm and the width of the burette also 3cm. To the packed gel, the dialysate from the ammonium sulphate precipitation was poured and 3mls fractions were collected into 20 labelled test tubes.

The absorbance of each fraction was read at 280nm using a Jenway 7205 UV/Visible spectrophotometer. A graph of tube number was plotted against absorbance to determine the peak, where tube 3- 12 were then polled. Nine fractions were pooled and the activity of the enzyme pooled were determined.

3.6 CHARACTERIZATION OF β-GALACTOSIDASE EXTRACTED FROM SEEDS OF *Citrullus vulgaris*

3.6.1 Optimum Temperature Determination

Optimum temperature was determined by carrying out the enzyme assay at 20^0C, 30^0C, 40^0C, 50^0C, 60^0C, 70^0C.

1ml of the dialysis and of the Sephadex dialysis purified enzyme solution was used.

3.6.2 Optimum pH Determination

Optimum pH was determined by carrying out the enzyme assay procedure with buffers of pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0.

1ml of the dialysis and of the Sephadex purified enzyme solution was used.

3.6.3 Effect of Substrate Concentration

The effect of substrate concentration on the enzyme activity was measured using 0.2%, 0.4%, 0.8%, 1.0%, 1.2%, 1.6%, 2.0%, and 4.0% lactose for enzyme assay.

CHAPTER FOUR

4.0 RESULTS

4.1 Purification steps of β-Galactosidase from *Citrullus vulgaris*

	Volume (ml)	Activity	Protein Concentration	Specific Activity	Purification Fold	Yield (%)
Crude Extract	**100**	**1.7945**	**0.326**	**5.504**		**100**
Dialysate	**82.2**	**0.7306**	**0.4025**	**1.815**	**0.329**	**52.6**

Sephadex G-25	**30**	**0.857**	**0.562**	**1.419**	**0.257**	**78.8**

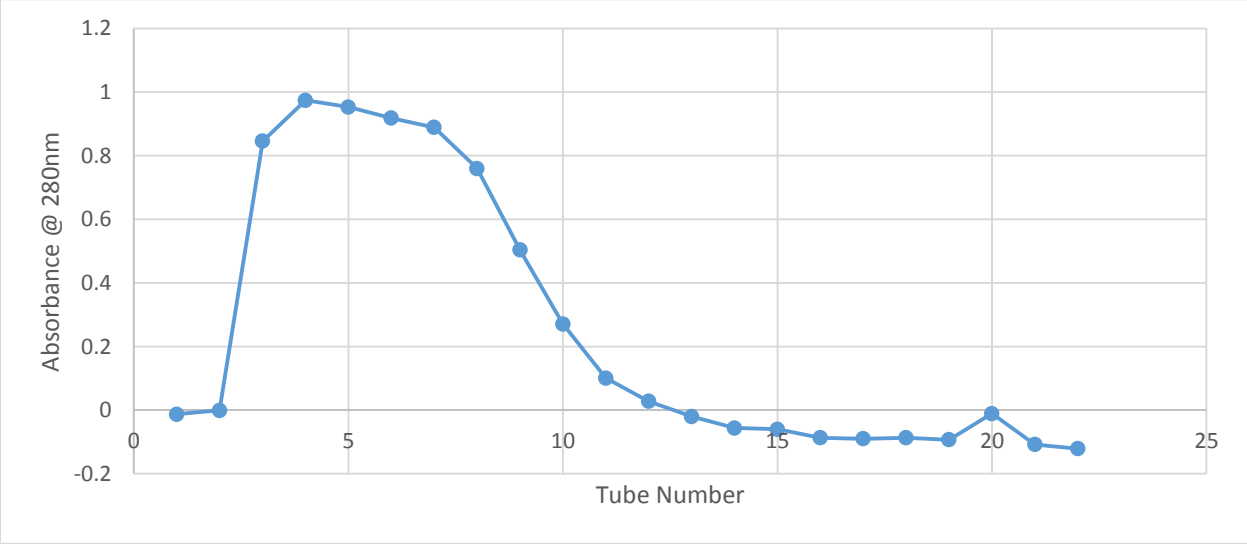

Fig 4 Elusion parttern of the beta-Galactosidase enzyme through sephadex G-25 Column Chromatography

4.2 CHARACTERIZATION OF EXTRACTED β- GALACTOSIDASE ENZYME

4.2.1 EFFECT OF TEMPERATURE

4.2.1.1 Effect of Temperature on Enzyme activity in Dialysate

The activity of the Dialysate of *Citrullus vulgaris* increased with temperature rising until reached to its maximum activity 0.058 at 40˚C, and then the activity was gradually decreased to reach 0.02at 70 ˚C as observed from Figure 4 below. Optimum temperature is 40ºC.

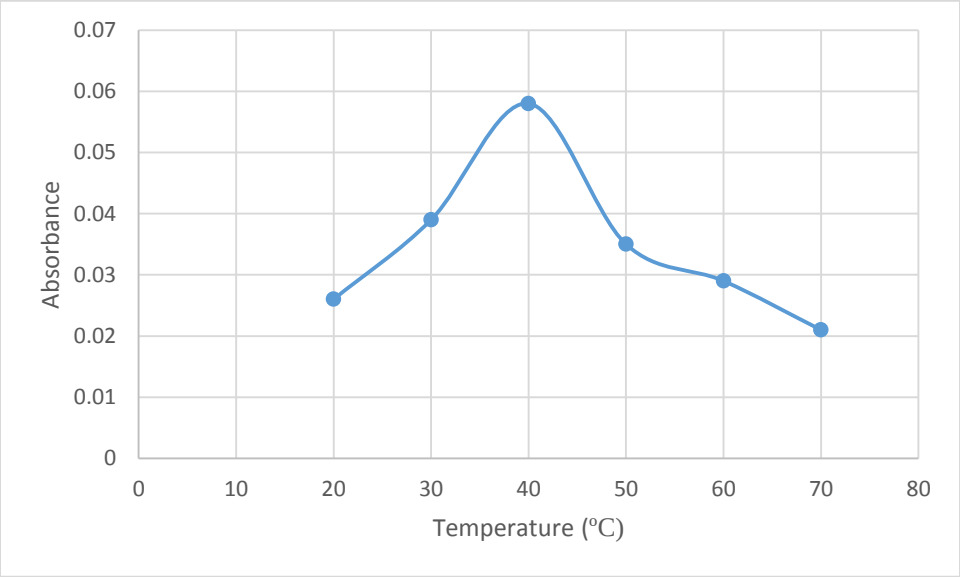

Fig 4. Effect of temperature on the activity of Dialysate

4.2.1.2 Effect of Temperature on Enzyme activity in Sephadex

The activity of β- galactosidase enzyme from *Citrullus vulgaris* increased with temperature rising until reached to its maximum activity 0.064 at 47˚C, and then the activity was gradually decreased to reach 0.03 at 70 ˚C as observed from Figure 5 below. Optimum temperature is 47ºC.

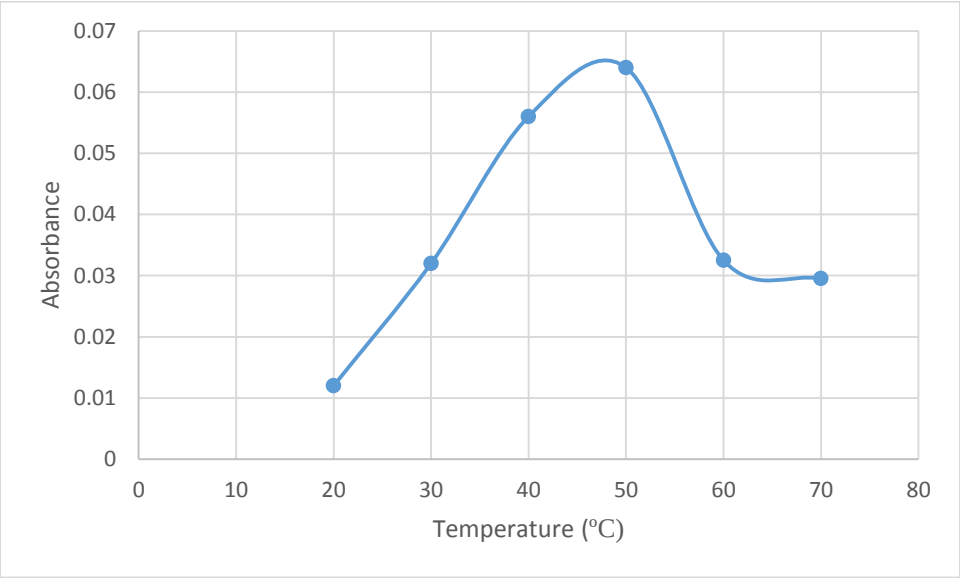

Fig 5 Effect of temperature on the activity of β -Galactosidase

4.3 Effect of pH

4.3.1 Effect of pH on enzyme activity in Dialysate

The activity of β- galactosidase enzyme from *Citrullus vulgaris* increased with pH rising until reached to its maximum activity 0.28 at pH 5.8, and then the activity was gradually decreased to reach 0.13 at pH 9.0 as observed from Figure 6 below. Optimum pH is 5.8.

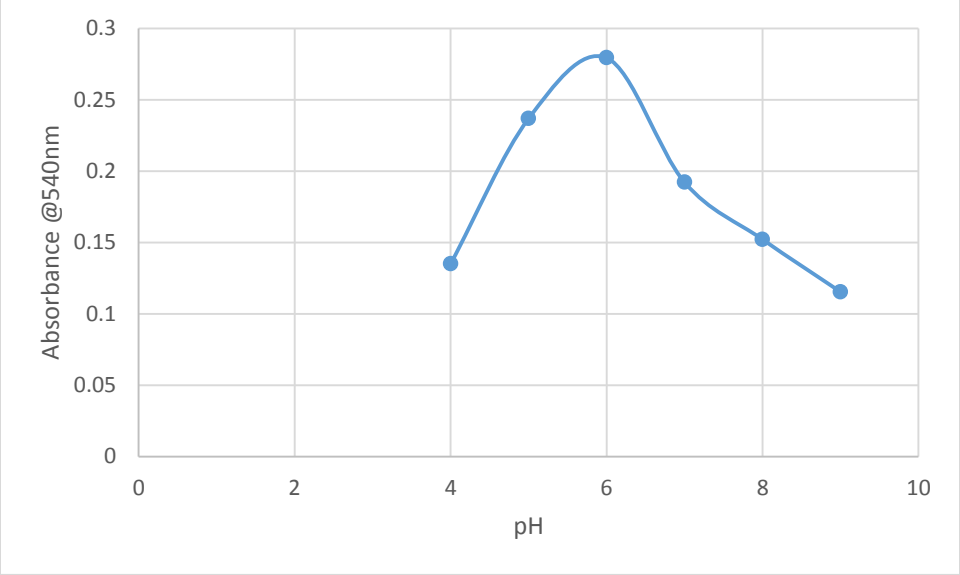

Fig 6. The effect of pH on the activity of Dialysate.

4.3.2 Effect of pH on enzyme activity in Sephadex

The activity of β- Galactosidase enzyme from *Citrullus vulgaris* increased with pH rising until reached to its maximum activity 0.34 at pH 5.6, and then the activity was gradually decreased to reach 0.13 at pH 9.0 as observed from Figure 7 below. Optimum pH is 5.6.

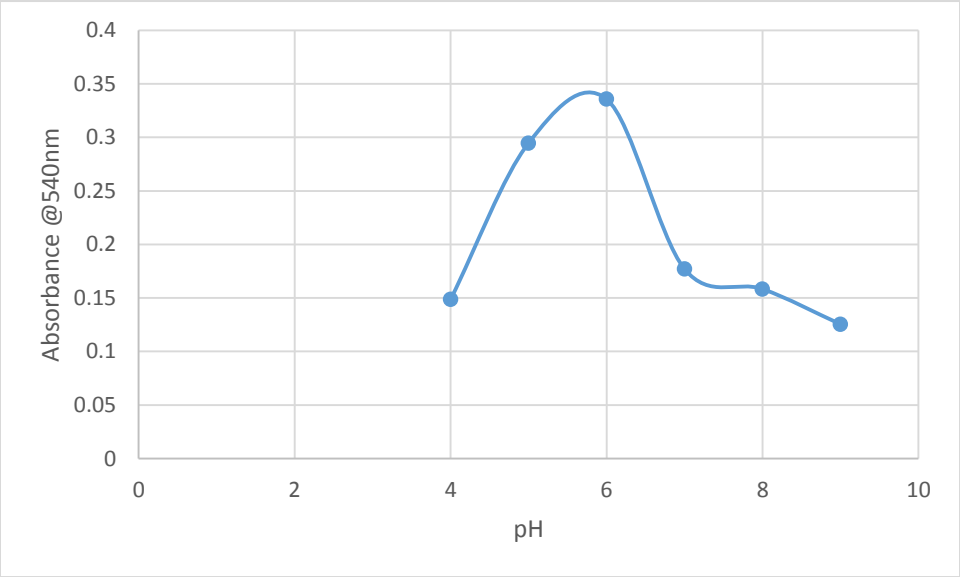

Fig 7. The effect of pH on the activity of β -galactosidase.

4.4 Effect of Substrate Concentration

4.4.1 Effect of Substrate Concentration in Sephadex

Figure 8 below shows the line-weaver Burk plot of reciprocal of velocity of β Galactosidase against reciprocal of absorbance. The result reveals that the k_m 0.1 value is and the V_{max} value is 1.667 .

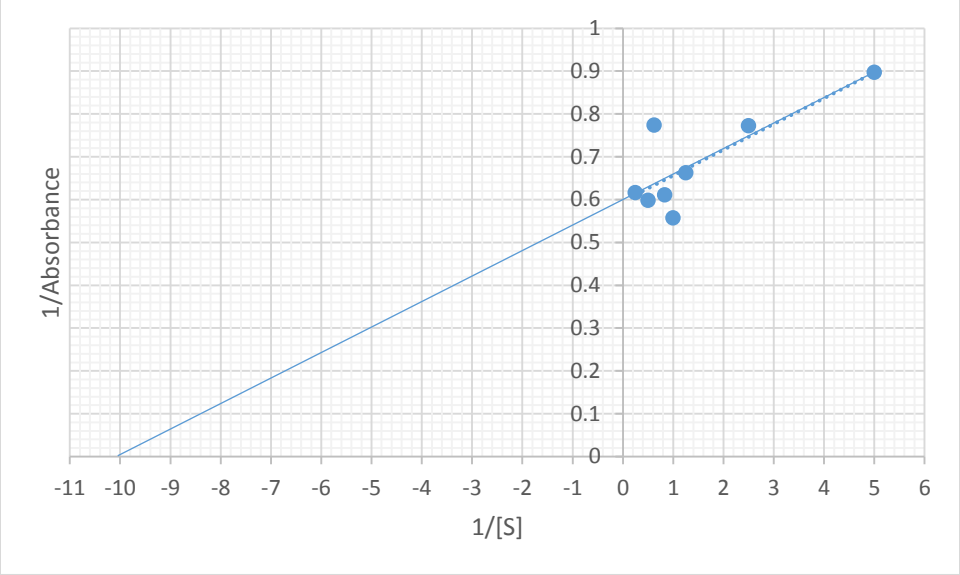

Fig 8. Effect of Substrate Concentration on Beta- Galactosidase activity.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Lactase is a member of the enzyme family β-Galactosidase. It is a glycoside hydrolase that is engaged in disaccharide lactose hydrolysis into glucose and galactose constituents. Lactase is vital in milk for digestive lactose hydrolysis. The enzyme deficiency leads intolerance to lactose. Temperature is one of the key parameters affecting enzyme activity. For its activity, the optimum temperature for β-Galactosidase is approximately 47 ° C (116.6 ° F) and has an optimum pH of 5.6 β-Galactosidase. The rate of response rises as the temperature rises. But owing to denaturation of the enzyme protein, after a certain temperature activity is reduced (Onal et al 2003). Watermelon β-Galactosidase's optimum temperature was discovered to be 47oC (Fig. 5). As is also seen from the figure, the enzyme activity was considerably decreased when the temperature was further increased. The findings compare well with past research undertaken using various β-Galactosidases by several writers. For example, Sen *et al,*. 2011. found the optimum temperature of α-Galactosidase purified from pepino with TPP (three phase partitioning) as 50ºC, also Akinwande and Kusimo 1996, and Sanad B. Al-Arrji *et al.*, 2017, who found the temperature of β- Galactosidase to be 50ºC. In general, plant β-Galactosidases show high activity in the temperature range of 37ºC – 60ºC depending to their source and also incubation time.

Another significant parameter affecting the enzyme activity is pH. Many variables, such as the incubation period, the type of buffer and its concentration and ionic strength, affect the pH optima of enzymes. As shown in the picture. 7, Optimum pH of a-Galactosidase watermelon as pH 5.6 is determined. Also, the enzyme is very active in a wide pH range of 5.0–6.0. β-

Galactosidases have distinct profiles of pH-activity based on the enzyme source. These results are correlated with prior β-galactosidases crop reports. The optimum pH values for plant-derived enzymes are generally between pH 5.0 and 7.0. This outcome is particularly crucial for the enzyme's industrial applications. Generally, β-galactosidases remain fairly stable over a wide pH range.

The crude extract was subjected to several techniques for partial purification and concluded that 70% ammonium sulfate saturation was the best method for concentration of enzyme with a specific activity 5.504U/mg proteins. After applying the purified enzyme by ammonium sulfate into Sephadex gel column one protein peak was located in the eluted fraction. Specific activity of this step was 1.419U/ml with a purification fold and enzymatic yield were 0.257 and 78.8%, respectively. The value of the optimum temperature of the sephadex differs from the value of the optimum temperature of dialysate

5.2 CONCLUSION

This study reveals that under optimized conditions, β-galactosidase was concentrated and purified from watermelon (*Citrullus vulgaris*). β-Galactosidase from *Citrullus vulgaris* has an optimum temperature 47ºC and optimum pH 5.6; Enzyme had high activity recovery at a range of 40ºC–50ºC and pH 5.0–6.0. Km and V_{max} of β-Galactosidase were calculated as 0.1 and 1.667 respectively. As a result of these, watermelon is a good source for β-Galactosidase. Biochemical properties of the enzyme have showed that, enzyme should be found a potential for various industrial applications.

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