# PREPARATION OF MALTODEXTRIN USING STARCH FROM *Musa Chilliocarpa* BACKER AND A-AMYLASE FROM *Phaseolus Vulgaris* L.

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## ABSTRACT

Banana maltodextrin was prepared by enzymatic hydrolysis of banana starch by using α-amylase from immature *Phaseolus vulgaris* L. (White Kidney bean) seeds. α-Amylase from immature *Phaseolus vulgaris* L. (White Kidney bean) seeds was isolated by ammonium sulphate precipitation ( 20-60 %) followed by Sephadex G- 100 gel filtration chromatography. The optimum pH and optimum temperature of α-amylase were 5.6 and 50 $^{o}$C, respectively. Starch was isolated from unripe *Musa chilliocarpa* Backer cv. Pheegyan (Banana) and yield percent was 43.6 %. Banana starch was characterized by SEM, XRD and TG-DTA methods. Banana maltodextrin ( yield percent of 5.5 %) was found to have a dextrose equivalent of 6 by phenol-sulphuric assay method. Low dextrose equivalent maltodextrin was characterized by SEM and some of its chemical properties were also determined.

**Keywords:** banana maltodextrin, *Musa chilliocarpa* Backer,*Phaseolus vulgaris* L., α-amylase , banana starch, dextrose equivalent

## Introduction

The white kidney bean (*Phaseolus vulgaris* L.) ( Figure 1) is a warm season annual plant, with quick growth, weak stems usually climbing and alternate compound leaves, composed of three large leaflets; the flower vary in color from white to purple. The tender pods are used as vegetable. The seeds are rich in protein. White kidney beans are extensively cultivated in all parts of Myanmar. So the white kidney beans can be a cheaper source for the alpha

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amylase which is extensively used in food, textiles and pharma industries (Nerkar *et al*., 2011). Scientific classification of white kidney bean (Kress *et al*., 2003) is described as follows:

| | | |
|---|---|---|
| Family | : | Fabaceae |
| Genus | : | *Phaseolus* |
| Binominal Name | : | *Phaseolus vulgaris* L. |
| English Name | : | Common bean, Kidney bean, Bush bean, French bean |
| Myanmar Name | : | Bo-sa-pe |

(a) (b) (c)

**Figure 1.** White kidney bean ( *Phaseolus vulgaris* L.)
(a) flower (b) plant (c) pods and seeds

Banana belongs to the family *Musaceae*, genus *Musa* and is a general term embracing a number of species or hybrids in this genus. Bananas are an important foodcrop in many countries and grown extensively in the tropical and subtropical regions. Banana is one of the vegetable fruits that grow well in the tropics (Ihekoronye and Nogddy, 1985). Banana though generally consumed as a dessert fruit, also contains high content of starch when unripe and could be exploited as a starch source. Green bananas have a large amount of starch during its unripe stage (Cordenunsi and Lajolo, 1995).

Amylases are important enzymes employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents (Adu *et al*., 2005). α-Amylase (1,4-D-glucan

glucanohydrolase, E.C.3.2.1.1) catalyzes the random hydrolysis of amylose, amylopectin and related polysaccharides to smaller oligosaccharides and glucose. α-Amylases are hydrolytic enzymes that are widespread in nature, being found in animals, microorganisms and plants (Franco *et al.*, 2000). Amylases (α- amylase) are among the most important enzymes in present-day biotechnology. α -Amylase was used as the raw material in various industries, such as food industry, detergent industry, textile industry and pharmacy industry. α - Amylase was widely used in the food industry such as baking, brewing, fruit juices, starch syrups.

Maltodextrin $(C_6H_{10}O_5)_n.H_2O$ is a mixture of saccharides with a molecular weight between polysaccharides and oligosaccharides with dextrose equivalent lower than 20 (not sweet), which is available as white powders mostly or concentrated solution (Alexander, 1992). Maltodextrin is more soluble in water than native starches, also is cheaper in comparison with other major edible hydrocolloids and its solutions have a bland flavour and smooth mouthfeel (Dokic-Baucal *et al*., 2004).

In Myanmar, banana is abundant because it can grow all year long. Therefore, this research focuses on the use of low cost banana to prepare maltodextrin using α- amylase from white kidney bean seeds as a route to improve the value - added raw material.

## Materials and Methods

### Sample Collection

Immature white kidney bean (*Phaseolus vulgaris* L.) seeds and unripe banana fruits (*Musa chilliocarpa*), locally known as phee–gyan– nget–pyaw, were collected from Tawma Village, Sint Kaing Township, Mandalay Region.

### Extraction of α-Amylase from Immature White Kidney Bean Seeds

Immature white kidney bean seeds (200 g) were ground in a mortar to obtain a homogeneous mixture. Into it, 500 mL of acetate buffer (pH 5.6) was added and stirred for 30 min. The mixture was filtered through two layers of muslin and the filtrate was collected. Then it was centrifuged at 3000 rpm for

15 min to obtain crude enzyme extract. The crude α- amylase enzyme extract was first brought to 20 % saturation with solid ammonium sulphate (analar) (66.24 g). After centrifugation for 15 min at 3000 rpm the supernatant was applied again with ammonium sulphate (143.4 g) to achieve 60 % saturation. The resulting enzyme precipitate was collected by centrifugation at 3000 rpm for 15 min.

Crude α-amylase (0.382 g) was dissolved in 5 mL of 0.1 M acetate buffer (pH 5.6). This solution was applied to a Sephadex G-100 gel filtration column previously equilibrated with the same buffer. The flow rate was adjusted to 12 mL/h by a mini pump and 1.5 mL fractions were collected per tube using a fraction collector. After collection, the protein content of each tube was checked by measuring the absorbance at 280 nm wavelength using a UV-visible spectrophotometer. Each tube was also measured for α-amylase enzyme activity by Nelson-Somogyi method.The fraction that had the highest activity of α-amylase enzyme was pooled. The protein content and α-amylase activity of pooled enzyme fraction were analyzed by the Biuret method and α-amylase activity was measured by Nelson-Somogyi method (Nelson and Somogyi, 1973). The pooled fraction showing the highest α-amylase activity was concentrated with acetone 1:9 ratio.

**Determination of Optimum pH of α- Amylase- catalyzed Reaction**

A 0.1 mL of 2 % (w/v) starch solution was added into a test tube containing 0.1 mL of hydrochloric acid–sodium chloride buffer solution (pH 1) and the contents were mixed well. Into another test tube 0.1 mL of enzyme solution was added. Both test tubes were kept at 30 $^{o}$C for 10 min to reach the thermal equilibrium. The former solution mixture was poured into a test tube containing 0.1 mL of enzyme solution and the contents were mixed well. Then the solution mixture was kept at 30$^{o}$C. After 10 min , the reaction was interrupted by adding 1 mL of alkaline copper reagent solution. The contents were then mixed thoroughly. The test tube was heated on a vigorously boiling water bath for 10 min. Next, the test tube was cooled under running tap water for 1 min and 1 mL of arsenomolybdate colour reagent solution was added into the test tube. After shaking vigorously, this solution was diluted to 10 mL with distilled water and mixed by inversion. The absorbance was measured at

750 nm. For blank solution, 0.1 mL of distilled water was used instead of 0.1 mL of prepared enzyme solution.

The whole of the above procedure was repeated with hydrochloric acid–sodium chloride buffers (pH 1.6 and 2.2), acetate buffers (pH 3.6, 4.6, and 5.6), phosphate buffers (pH 6.2, 7.0, and 8.0) and sodium carbonate–bicarbonate buffers (pH 9.6 and 10.6 ).

**Determination of Optimum Temperature of α-Amylase-Catalysed Reaction**

A 0.1 mL of 2 % (w/v) starch solution was added into a test tube containing 0.1 mL of acetate buffer (pH 5.6) solution and the contents were mixed well. Into another test tube 0.1 mL of enzyme solution was added. Both test tubes were kept at 20 ºC for 10 min to reach the thermal equilibrium. The former solution mixture was poured into a test tube containing 0.1 mL of enzyme solution and the contents were mixed well. Then, the solution mixture was kept at 20ºC. After 10 min incubation, 1 mL of alkaline copper reagent solution was added to stop the reaction. The test tube was heated in a vigorously boiling water bath for 10 min. Next, the test tube was cooled under running tap water for 1 min and 1 mL of arsenomolybdate colour reagent solution was added into the test tube. After shaking vigorously, this solution was diluted to 10 mL with distilled water and mixed by inversion. The absorbance was measured at 750 nm. For blank solution 0.1 mL of distilled water was used instead of 0.1 mL of enzyme solution.

The whole of the above procedure was repeated except that the temperature was fixed variously at 30ºC, 40ºC, 45ºC, 50ºC, 55ºC, 60ºC, 65ºC, 70ºC and 75ºC.

**Extraction of Starch from Banana**

Banana starch was isolated using the procedure of Kim*et al* (1995) with some modification. The fruits were peeled and cut into 5 to 6 cm cubes (500 g total weight), immediately rinsed in sodium sulphite solution (1.22 g / L) and macerated with the sulphite solution (1:1 ratio) for 2 min at low speed in a blender. The homogenate was consecutively sieved through 50 and

100 meshes until the washing water was clear. The solution was centrifuged at 3000 rpm for 30 min and the sediment dried in air for 48 h, and finally ground with a mortar and pestle until passing through a 100 mesh sieve. Yield percent of banana starch was 43.6 %.The resulting banana starch was stored at room temperature in a sealed container.

**Preparation of Maltodextrin by Enzymatic Hydrolysis of Banana Starch using α-Amylase**

Maltodextrin was prepared from banana starch according to the procedure of Bello- Perez *et al* (2002).

A banana starch (20 g) was mixed with 100 mL of distilled water. The suspension in distilled water was adjusted to pH 6.5 and then gelatinized in a boiling water bath for 10 min. After cooling to 60°C, α-amylase from white kidney bean seeds was added to achieve a concentration of 0.01 % (w/v). The flask was tightly covered and the hydrolysis reaction was allowed to proceed at 60°C for 15 min under mild agitation. After that, the sample was cooled down to 4°C in water-ice bath and then centrifuged at 3000 rpm for 30 min. The supernatant was recovered and the enzyme inactivated by heating in a boiling water bath for 10 min after addition of 0.1 M hydrochloric acid to reach a pH value of 4.02. After freeze drying, the maltodextrin was collected.

**Characterization of Banana Starch and Maltodextrin**

Surface morphology of prepared banana starch was investigated by a scanning electron microscope model (Jeol-JSM-5610 LV, Japan) operating at 15 kV and 550 x magnification and that of prepared maltodextrin by 15kV and 1000 x magnification at Universities' Research Center. X-ray diffraction (XRD) analysis of banana starch was carried out using X-ray diffractometer (Rigaku, Tokyo, Japan). The banana starch powder was scanned using Cu $K_\alpha$ radiation ( $\lambda$ = 1.54056 Å) at 40 kV and 40 mA. The scanning region of the angles (2θ) was from 10º to 70º. Thermogravimetric–Differential Thermal Analysis (TG-DTA) of banana starch was done by using Simultaneous TG-DTA (DTG-60H), Thermal Analyzer (Shimadzu, Japan)at Universities' Research Center.

**Determination of Dextrose Equivalent of Prepared Maltodextrin by Phenol – Sulphuric Assay Method**

Standard glucose (0.2 g) was dissolved in 20 mL of distilled water in a 100 mL volumetric flask and the volume of the solution was made up to the mark with distilled water to obtain 2000 μg/ mL of solution. From this stock solution 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mL of solutions were drawn out and placed in each 100 mL volumetric flask and diluted to the mark with distilled water to give 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 μg/ mL of glucose solutions, respectively.

Into ten separate test tubes, 1 mL each standard solutions (20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 μg/ mL) were added. Next, 1 mL of 5% phenol solution was added to each test tube and mixed. A blank and sample solutions were prepared with 1 mL each of distilled water and sample solution (0.1 % w/v), respectively, instead of standard glucose solution. Concentrated sulphuric acid (5 mL) was again added to each test tube so that the steam hit the liquid surface directly to produce good mixing and homogeneously. Each tube was agitated during addition of acid. After about 10min, the tubes were shaken again and placed in water- bath at 30°C for 20 min. The yellowish brown colour was stable for several hours. Calibration curve was constructed by employing 10 different concentrations of standard glucose at 490 nm. From the calibration curve the reducing sugar content in maltodextrin was calculated. Dextrose equivalent (DE) was determined by the following equation.

$$DE = \frac{\text{Reducing sugar content as dextrose}}{\text{Total solid}} \times 100$$

**Determination of pH, Moisture and Protein of Prepared Maltodextrin**

pH of banana maltodextrin was determined by a pH meter (Oyster- 15) which was previously calibrated with pH 4 and 7 buffer solutions.Moisture content was determined by oven drying method and protein content was determined by Kjeldahl digestion method**.**

## Results and Discussion

### Purification of α-Amylase

Various organic solvents (e.g., acetone, alcohol, and diethyl ether) and salt (e.g., ammonium sulphate, ammonium carbonate, etc.,) can be used to make partial purification of enzyme. In this study α - amylase was isolated from immature white kidney bean seeds by ammonium sulphate precipitation method followed by Sephadex G-100 gel filtration chromatography. Ammonium sulphate precipitation method was chosen for salt fractionation because of its high solubility in water, lack of toxicity, cheapness and lack of harmful effects on enzyme activity. It is the most commonly used reagent for salting out the proteins because of its high solubility that permits the achievement of the solutions with higher ionic strength (Voet *et al.,* 1999). Sephadex G-100 (superfine) is a new kind of gel filtration medium which combines a highly porous gel structure with excellent chemical and physical stability. It fractionates proteins in the molecular weight range of 3000 to 60,000.

α-Amylase activities, protein contents and specific activities of the enzyme solutions in each purification step are shown in Table 1. The crude extract having specific activity of 0.10 was subjected to ammonium sulphate precipitation and resulted in specific activity of 1.20 μmol $min^{-1}$ $mg^{-1}$ at the final purification step. So 12.0 fold purification was achieved. Michelena and Castillo (1984) purified α- amylase from *Aspergillus foetidus* by $(NH_4)_2SO_4$ precipitation and achieved 11fold purification.

**Table 1.** Enzyme Activity and Specific Activity at Different Purification Steps

| Purification steps | Total activity (μmol $min^{-1}$ ) | Total protein (mg) | Specific activity (μmol $min^{-1}$ $mg^{-1}$) | Purification (fold) |
|---|---|---|---|---|
| Crude extract | 781.2 | 7937.6 | 0.10 | 1 |
| After 20% ammonim sulphate precipitation | 778.5 | 2412.0 | 0.32 | 3.2 |
| After 60% ammoium sulphate *precipitation* | 149.5 | 243.3 | 0.61 | 6.1 |
| *Sephadex G-100* | 204.4 | 171 | 1.20 | 12.0 |

**Optimum pH of α-Amylase – Catalyzed Reaction**

The effect of pH on the activity of immature white kidney bean α-amylase is shown in Figure 2. The enzyme activity increased steadily from pH 1 to 5.6 and then decreased with increasing pH. Enzyme showed the optimum activity at pH 5.6 with activity of 0.5807 μmol $min^{-1}$ $mL^{-1}$. A decline in the enzyme activity was recorded on either side of pH 5.6. It was reported that optimum pH of mango α- amylase was 5.5(Yasin and Chaudhary, 1981). Khoo *et al* (1994) reported that the α-amylase enzyme was found to have maximum activity at pH 6.0. α-Amylase from apple showed the optimum activity at pH 6.8 (Kanwal *et al* 2004).

**Optimum Temperature of α-Amylase – Catalyzed Reaction**

The effect of temperature on the activity of immature white kidney bean α-amylase is shown in Figure 3. Initially the activity increased with increase in temperature and then decreased after reaching a maximum. The

results showed that the temperature optimum of the amylase enzyme was found to be 50˚Cin acetate buffer (pH 5.6). It was observed that α-amylase lost 40.44% of its activity at 75ºC. Mohamed (2009) reported that the optimum temperature for partially purified α-amylase from Wheat *Triticum aestivum* was 50˚C. Khoo *et al* (1994) reported that the optimum temperature for purified α-amylase was 55˚C. Plant amylases (Wheat alpha -1, Pearl millet alpha-1, and Safflower seeds, mat beans) were found to have optimum temperature of 55ºC (Nerkar *et al*., 2011).

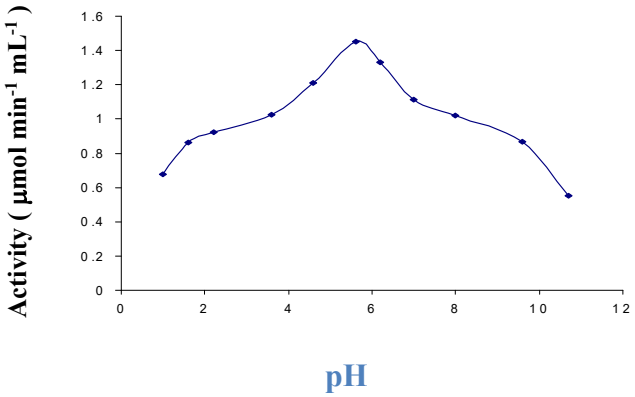


**Figure 2.** Plot of α–amylase activity as a function of pH of the solution

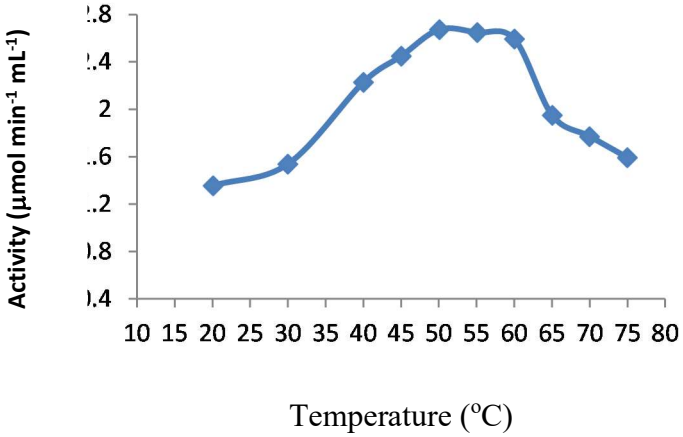


**Figure 3.** Plot of α–amylase activity as a function of temperature of the solution

**Surface Morphology of Prepared Banana Starch**

The morphology of banana starch is shown in SEM image (Figure 4). Banana starch granules were found to have irregular shapes and appeared as elongated oval. Its shape ranged from oval to irregular with the size varying from 5 to 45μm, the average size being in the range of 21 to 24 μm. The surface of the banana starch granules was found to be very smooth. So it could be indicated that isolation process was efficient and it did not cause damage to starch granules.

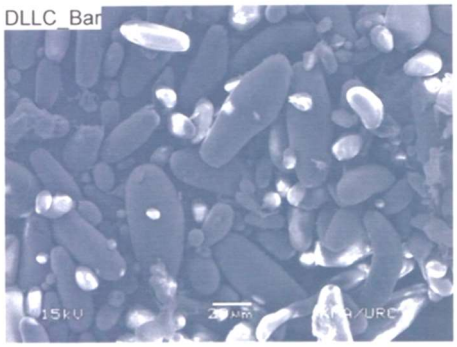


**Figure4. SEM image of banana starch granules (550× magnification)**

**X-Ray Powder Diffraction Pattern of Prepared Banana Starch**

Semi-crystalline nature of banana starch was observed in X-ray diffractogram (Figure 5) because of the presence of both sharp and diffuse diffraction peaks. Banana starch showed strong diffraction peaks at 15.3°, 17.2°, 22.1°and23.4° of 2θ. Parallel double amylopectin molecules result in the formation of crystalline regions, while amylose molecules result in the formation of amorphous regions in the starch structure.

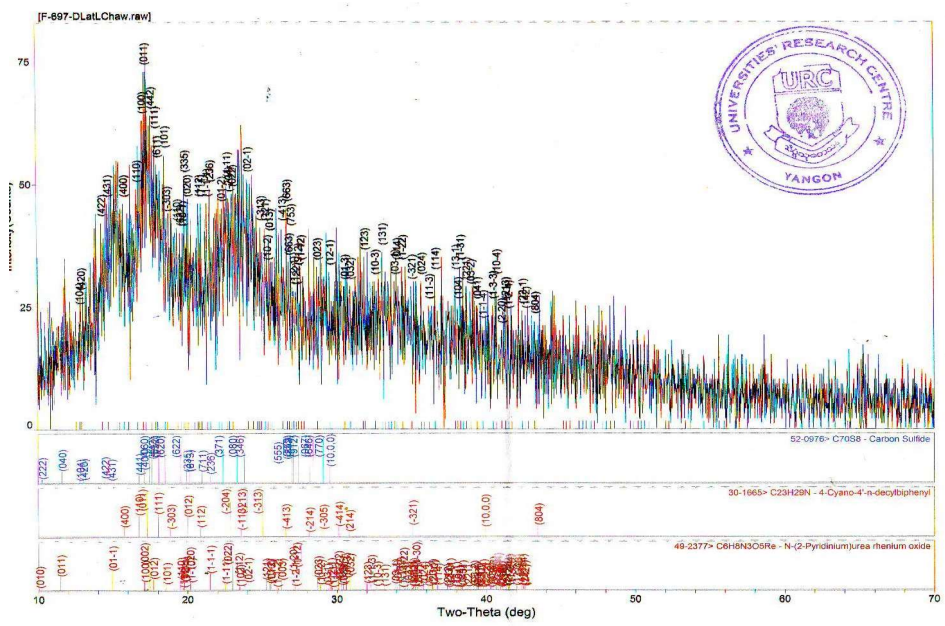


**Figure 5.** X ray diffractogram of banana starch

## Thermal Properties of Banana Starch

TG- DTA thermogram of banana starch is shown in Figure 6. It was noted that initial weight loss began at approximately 50°C and reached a constant weight plateau after losing about 16% of its initial weight. The weight loss corresponds to the loss of moisture content from the banana starch. DTA curve shows a small endothermic peak at 65.56 °C. Between 120 °C and 307 °C, banana starch was found to be thermally stable. Moreover, another weight loss was started at approximately 308°C. At this temperature the banana starch began to degrade. This temperature is the degradation temperature of banana starch. Aht-Ong and Charoenkongthum (2002) reported that the degradation temperature of banana starch was 315 °C. DTA curve shows two exothermic peaks at 357.42 °C and 482.31 °C due to degradation of starch. At the end of the experiment (503.46 °C), weight loss % was 96.945 % and thus 3.055 % residue was left.

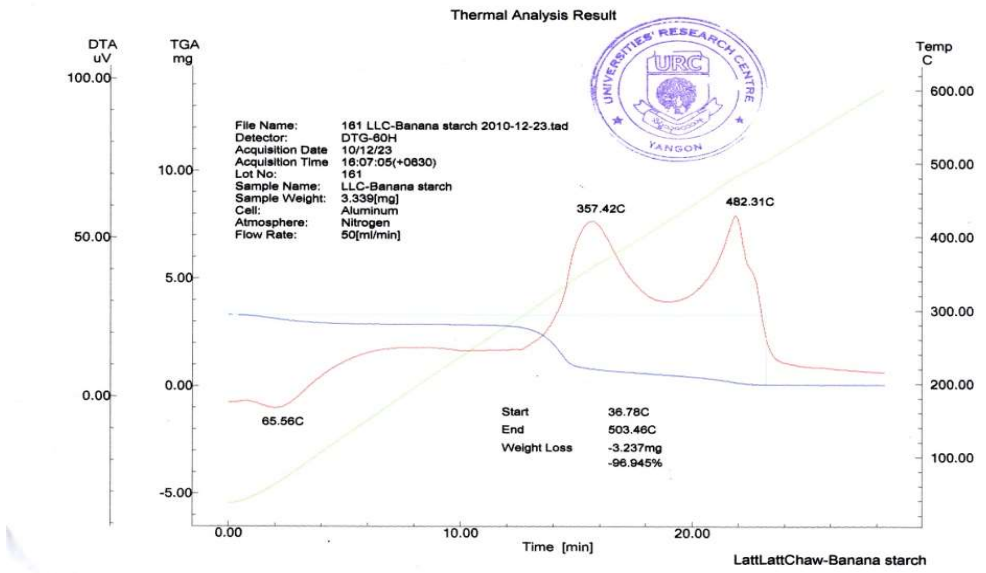


**Figure 6.** TG-DTA thermogram of banana starch

## Maltodextrin Preparation by Using α–Amylase

Nowadays, acid hydrolysis of starch is limited for maltodextrin production and particularly recommended for production of glucose syrups . Enzymatic hydrolysis of starch has distinct advantages compared to acid process. There is no need to remove salts formed during acid neutralization and due to wider pH range and lower temperatures this process is more economic and control of process is easier too (Haki and Rakshit, 2003). The action of α- amylase on granular starch consists of the following three steps:

(1) diffusion of the enzyme toward the substrate,

(2) the adsorption of the enzyme on the substrate surface and possible pores, and

(3) the catalytic event .

In this study enzyme-catalyzed conversion with α-amylase from immature white kidney bean seeds was used for the preparation of maltodextrin from banana starch. The yield percent of maltodextrins was

5.5 %. The banana maltodextrin prepared in this study was found to have white colour.

**Morphology of Banana Maltodextrin**

Scanning electron micrograph of banana maltodextrin is depicted in Figure 7. It was observed that banana maltodextrin showed particles with irregular shapes and rough surface compared to elongated oval shape and smooth surface of banana starch. The change in granular surface is due to the attack of α-amylase. The amylase either erodes the granule surface or digests channels from selected points on the surface toward the center of the granule. Five patterns of attack have been identified: pin-holes/ pepper-potting, sponge-like erosion, many medium- sized holes, distinct loci leading to single holes individual granules and surface erosion (Evers, 1979). In this study two patterns of attack were observed, *i.e,* sponge- like erosion and surface erosion.

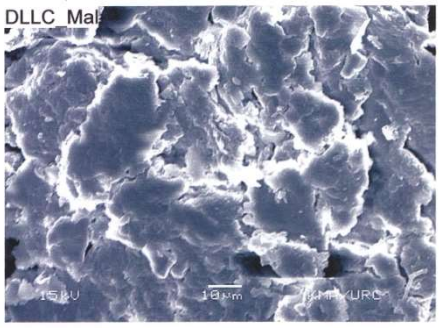


**Figure 7.** SEM image of banana maltodextrin (1000 x magnification)

**Dextrose Equivalent of Banana Maltodextrin**

Dextrose equivalent of maltodextrin was obtained by determining the reducing sugar content employing phenol-sulphuric assay method. The reaction of carbohydrate with phenol and sulphuric acid in aqueous solution gives a brown colour. This reaction can be used for the quantitative

colorimetric microdetermination of monosaccharides and their polymerization products, such as oligosaccharides and polysacchairdes (Handa,1966).

The calibration curve was constructed using different concentrations of standard glucose (20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 μg $mL^{-1}$) at 490 nm. A straight line ($R^2$ = 0.9984) passed through the origin implying Beer's law was obeyed (Figure 8). Dextrose equivalent was calculated from glucose content of banana maltodextrin. The dextrose equivalent was found to be 6.

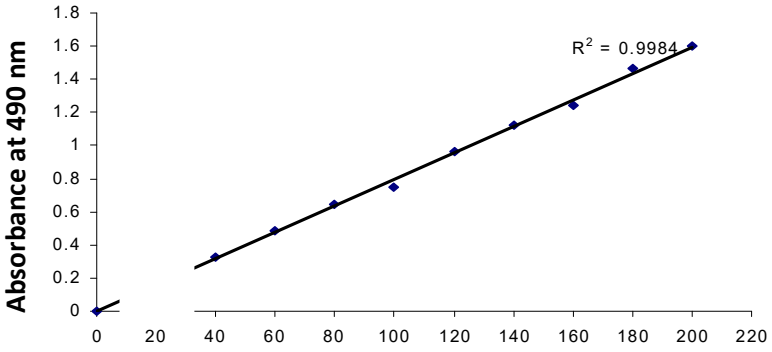


**Figure 8.** Calibration curve for standard glucose solution

## Chemical Characteristics of Maltodextrin

Table 2 shows the chemical characteristics of prepared banana maltodextrin together with the reported values (Bello-Perez *et al*., 2002). Dextrose equivalent of prepared maltodextrin was determined as 6.00. Moisture percent was 5.00 % and pH was found to be acidic, i.e., 4.02. Protein content was 0.83%. Moisture percent and pH of present work was not much different with the reported values. However, dextrose equivalent was less than reported value of 9.0. In literature, dextrose equivalent of maltodextrin is in the range of 3 to 20 ( Chronakis,1998). Protein content in prepared maltodextrin

was greater than the reported value. Regulated protein residue in maltodextrin is in the range of ≤ 0.5 to 1 % ( Food Safety Information Papers, 2009).

**Table 2.** Chemical Characteristics of Maltodextrin

| | Banana maltodextrin (Present Work) | *Reported Values |
|---|---|---|
| Dextrose equivalent | 6.00 | 9.00 |
| Moisture% | 5.00 | 5.90 |
| pH | 4.02 | 4.00 |
| Protein(%) | 0.83 | 0.10 |

*Bello-Perez *et al*., (2002)

## Conclusion

This study revealed the simple method for preparation of maltodextrin from banana starch by hydrolysis of α- amylase from immature white kidney bean seeds. α-Amylase from immature white kidney bean seeds was isolated and purified by ammonium sulphate fractionation ( 20 – 60 % ) followed by gel filtration chromatography. α-Amylase was purified 12 fold compared to the crude extract. The specific activity of α-amylase was found to be 1.20 μmol $min^{-1}$ $mg^{-1}$ of protein at final purification step. Surface morphology of prepared banana starch was elongated oval shape with smooth surface. Banana starch started to degrade at 315°C and two exothermic peaks appeared in TG-DTA thermogram indicating that degradation of banana starch. Semi-crystalline nature of banana starch was indicated by X ray diffraction analysis. Surface morphology of prepared maltodextrin was irregular shape with sponge-like erosion and surface erosion. Dextrose equivalent of prepared maltodextrin was found to be 6 indicating low dextrose maltodextrin.

## Acknowledgement

The authors would like to thank the Myanmar Academy of Arts and Science for allowing to present this paper and Professor and Head Dr DawThan Than Aye and Professor Dr Kyae Mon Lwin , Department of Chemistry,Kyaukse University for their kind encouragement.

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