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Biological Approach to Degrade Natural Starch Using Amylase.

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Abstract: Starch is the most abandoned content of any food we add to our daily meals. Which contains different polysaccharides and digestion of these starches is a good topic of research. To study the action or role of different enzymes in the process of digestion we experimented on raw starch opted from the sweet potato. Commonly sweet potato is known for its high amount of starch presence. In this research paper, we experimented with starch digestion using the α-Amylase enzyme. In the end, we calculated the activity of the amylase on starch and the total content of starch present in the sweet potato.

Index Terms **– Sweet potato, Starch, Amylase, Degradation, Glucose**

I. INTRODUCTION

Starchy substances constitute the major part of the human diet for most of the people in the world, as well as many animals. Sweet potatoes are one of the high starches containing food grown in many countries. Sweet potato is an important economic crop that can adapt successfully to a wide range of habitats, including marginal regions. Starch is a mixture of two polysaccharides, the linear molecule of amylose, which consists of polymers of glucose, and amylopectin, a highly branched molecule. It is a dicotyledonous plant belonging to the family Convolvuluses, in which there are approximately 50 genera and over 1000 species. Artificial selection of sweet potatoes, as well as the occurrence of natural hybrids and mutations, has resulted in the existence of a very large number of cultivars.

Starch can be sub-divided into cereal, legumes, palm and tuber or root starches. Tapioca (Manihotesculenta Crantz) and sweet potato (Ipomea batatas Lam) starches are examples of starch derived from roots or tubers. Granules of tuber and root starches are oval, although round, spherical, polygonal and irregular shapes also exist (Lideboom, Chang, &Tyler, 2004).Sweet potato starch can be used as an ingredient in bread, biscuits, cakes, juices, ice cream and noodles, or converted to glucose and isomerized glucose syrup. Glucose syrup is utilized in a variety of foods, such as: candies, ice-cream and jams and isomerized glucose can be used in lactic acid beverages, soft drinks, bread and many other foods.

Starch susceptibility to enzyme attack is influenced by several factors such as amylose and amylopectin content (Holm & Bjorck, 1988; Ring, Gee, Whittam, Orford, & Johnson, 1988), particle size, crystalline structure and the presence of enzyme inhibitors. Among these factors, granular structure is believed to be the most important (Zhang & Oates, 1999).

The enzymatic saccharification of raw starch has been gaining much attention in recent years as an energy saving method. In conventional methods, starch has been gelatinized at high temperature to enhance its liquification and saccharification. Several researches on enzymatic hydrolysis of raw starch have been reported for saving this high cooking cost. Amylases capable of digesting raw starch are mainly produced by microorganisms, especially fungi. Were reported as new sources of amylases which have strong activity for raw starch.. In 1986, 36% of the total net production was utilized for starch production. Sweet potato is resistant to typhoon, drought, pests and diseases, and starch productivity per ha per year is remarkably high. Due to these facts, it has been a favorable raw material for alcohol fermentation and the production of starch syrup and glucose. However, there are not sufficient reports on the properties of sweet potato starches.

This paper describes the enzymatic digestibility of the starches from eight varieties of sweet potato. Granule sizes and amylose contents are also described.

II. MATERIALS

2.1 Requirements:

a) Potato Starch
b) Distilled water
c) DNSA reagent
d) Sodium phosphate buffer (pH- 7.02)
e) α-amylase (enzyme)

2.2 Instruments:

a) Incubator
b) Weighing balance
c) Spectrophotometer
d) Thermometer
e) pH meter

2.3 Glass wares:

a) Test tubes
b) Beaker
c) Pipette

2.4 Plastic wares:

a) Measuring cylinder
b) Test tube stand

III. METHODOLOGY

3.1 Isolation of cook soluble starch:

The raw source for starch (sweet potato) was cooked and smashed in the same boiled water. The solution was filtered by filter paper. The filtered solution was centrifuged to separate the suspended particles. The supernatant will be treated as cooked soluble starch, and also the pellet was diluted with distilled water and was considered as starch.

3.2 Preparation of buffer:

To prepare 100ml of Acetate buffer (0.1M pH 5) 1.381g of sodium acetate and 0.49025g acetic acid was added and the pH will be adjusted to required value using NaOH or HCL.

3.3 Preparation of glucose standard curve:

For the estimation of enzyme activity the standard curve of glucose was performed. The glucose concentration taken was 1 mg per ml. The sugar was estimate by DNSA method taking absorbance at 540nm.

3.4 Preparation of α-amylase enzyme solution:

To prepare 10 ml of enzyme solution, 10mg of α-amylase was mixed with 10ml of distilled water.

3.5 Enzyme assay:

The reaction mixture containing 1.0 ml of 1.0% cooked soluble starch, 0.5 ml of 0.1 M acetate buffer (pH 5.0), and 0.5 ml of an enzyme solution, was incubated at 40°C for 10 min. The reducing sugars produced were assayed by the DNSA method. One unit of amylase was defined as the amount of enzyme that liberated 1 mol glucose per min.

The additions were added as follows:

Table 1: Assay sample readings

Glucose (ml)	Buffer (ml)	DNSA (ml)		Buffer (ml)	Concentration	O. D. 540 nm
0	1	1		1	0	0.0
0.2	0.8	1	Incubation	1	0.2	0.125
0.4	0.6	1	For	1	0.4	0.303
0.6	0.4	1	5 min	1	0.6	0.405
0.8	0.2	1		1	0.8	0.67
1	0	1		1	1	0.792

3.6 Digestion of raw starch:

The reaction mixture consisting of 0.5 ml of a 4% (w/v) sweet potato starch suspension 0.5 ml. of 0.1 M acetate buffer (pH 5.0) and 0.5 ml of an enzyme solution was incubated for 24 hours at RT. The amount of reducing sugars was measured by the DNSA method.

Table 2: Addition of the contents and process followed

REAGENT	TEST	BLANK
Starch	1 ml	1 ml
Amylase	1 ml	-
Incubate at room temperature for 3 min		
DNSA	2 ml	2 ml
Boiling water bath incubation for 5 min		
Amylase	-	1 ml
Distilled water	9 ml	9 ml

Cool at room temperature and measure absorbance at 540 nm against blank.

IV. RESULTS

4.1 Digestion of raw starch

Table 3: Observations for supernatant starch

Starch Solution (ml) (supernatant)	Concentration (mg/ml)	Abs. (at 540 nm)
01	01	0.09

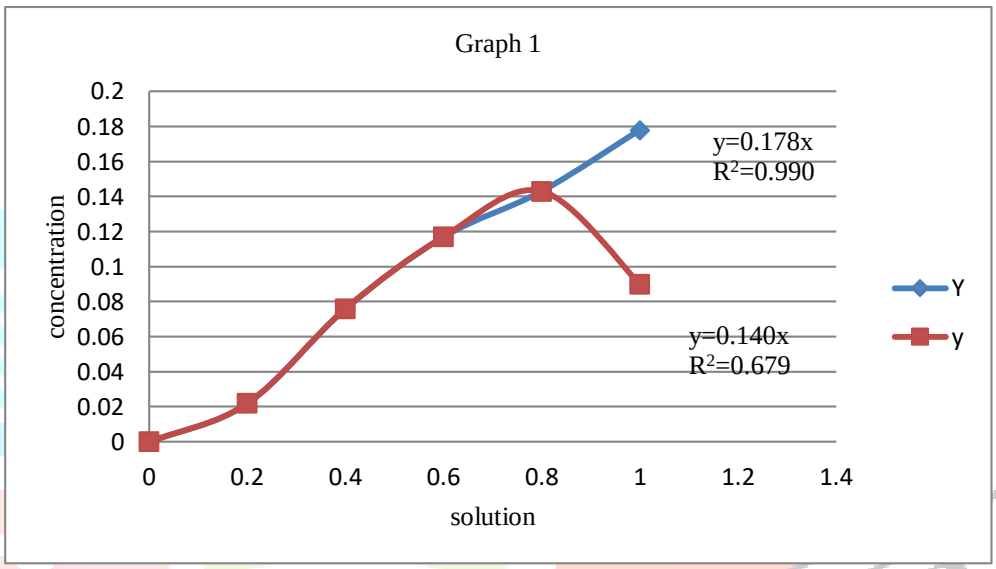

Fig 1: Graph for supernatant starch

Table 3: Observations for pellet starch

Starch Solution (ml) (pellet)	Concentration (mg/ml)	Abs. (at 540 nm)
01	01	0.168

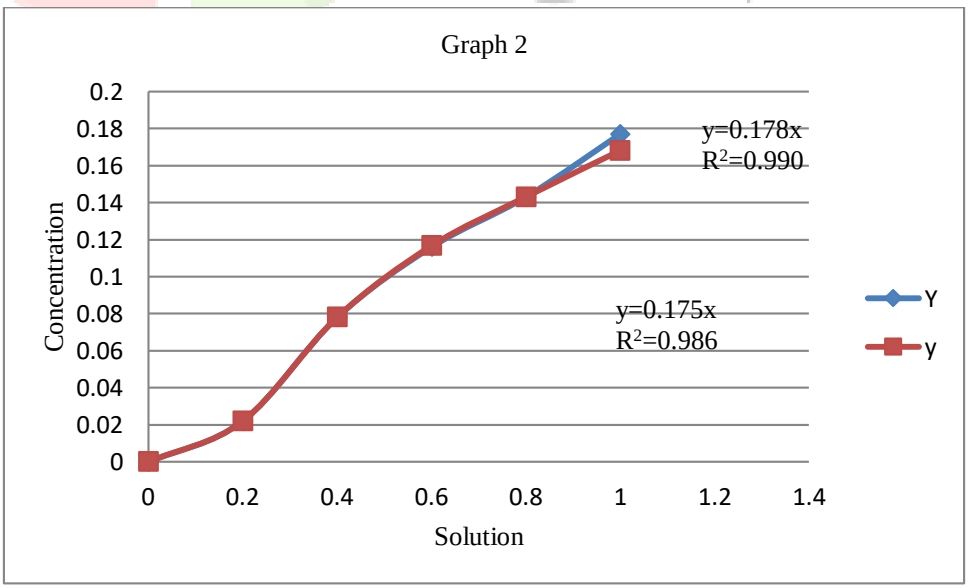

Fig 2: Graph for pellet starch

V. CALCULATIONS

5.1 Activity of α- amylase (for supernatant starch):

1 I. U. = 1 mol of glucose production/min

5.2 Concentration of sweet potato starch(x) from the graph using equation y=0.679x

Abs. (y) = 0.09nm

Conc. of sweet potato starch (x) = 0.09/0.679 = 0.1325 mg/ml

$$1\mu\text{mol of glucose produced/ml.min} = \frac{0.1325 \times 10^3}{180 \times 5 \times 2}$$

$$= 0.7361 \text{ I. U.}$$

5.3 Activity of α- amylase (for pellet starch):

1 I. U. = 1 mol of glucose production/min

5.4 Concentration of sweet potato starch(x) from the graph using equation y=0.1755x

Abs. (y) = 0.168nm

Conc. of sweet potato starch (x) = 0.168/0.1755 = 0.9573 mg/ml

$$1\mu\text{mol of glucose produced/ml.min} = \frac{0.9573 \times 10^3}{180 \times 5 \times 2}$$

$$= 0.5328 \text{ I. U.}$$

VI. DISCUSSION

Amount of starch present in pellet is more than starch content present in supernatant. There is more enzyme activity present on pallet starch than supernatant starch (because the amount of starch present is relatively less). We can increase the concentration of enzyme we get more digestion rate for the starch. Although the maximum concentration of enzyme in which it gives its maximum activity can be calculated experimentally by conducting various experiments.

As α-amylase is able to degrade starch into its monomeric and dimeric units like glucose and maltose, however as it is a biological way to degrade starch it can prove an alternative to the chemical hydrolysis of starch.

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